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An *ex vivo* assay of XRT-induced Rad51 foci formation predicts response to PARP-inhibition in ovarian cancer

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Abstract

Objective—*BRCA*-positive ovarian cancer patients derive benefit PARP inhibitors.

Approximately 50% of ovarian cancer tumors have homologous recombination (HR) deficiencies and are therefore “*BRCA*-like,” possibly rendering them sensitive to PARP inhibition. However, no predictive assay exists to identify these patients. We sought to determine if irradiation-induced Rad51 foci formation, a known marker of HR, correlated to PARP inhibitor response in an ovarian cancer model.

Methods—Ovarian cancer cell lines were exposed to PARP-inhibitor ABT-888 to determine effect on growth. Rad51 protein expression prior to irradiation was determined *via* Western blot. Cultured cells and patient-derived xenograft tumors (PDX) were irradiated and probed for Rad51 foci. *In vivo* PDX tumors were treated with ABT-888 and carboplatin; these results were correlated with the *ex vivo* ionizing radiation assay.

Results—Three of seven cell lines were sensitive to ABT-888. Sensitive lines had the lowest Rad51 foci formation rate after irradiation, indicating functional HR deficiency. Approximately 50% of the PDX samples had decreased Rad51 foci formation. Total Rad51 protein levels were consistently low, suggesting that DNA damage induction is required to characterize HR status. The *ex vivo* IR assay accurately predicted which PDX models were sensitive to PARP inhibition *in vitro* and *in vivo*. ABT-888 alone reduced orthotopic tumor growth by 51% in A2780ip2 cell line, predicted to respond by the *ex vivo* assay. Three PDX models' response also correlated with the assay.

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Conflict of interest statement

None of the authors have a conflict of interest.

Conclusions—The *ex vivo* IR assay correlates with response to PARP inhibition. Analysis of total Rad51 protein is not a reliable substitute.

Keywords

Ovarian cancer; PARP inhibitor; Homologous recombination deficiency; Rad51

Introduction

Epithelial ovarian cancer is the most lethal gynecologic malignancy in developed nations, with an estimated 14,270 deaths in the United States alone in 2014 [1]. Even though initial treatment with a standard platinum/taxane regimen is effective in about 80% of women diagnosed with advanced stage disease, median progression-free interval is only approximately 18 months, and median 5-year survival is approximately 40% [2,3].

Over the past decade, great strides have been made in understanding the genetic and molecular basis of cancer. In particular, hereditary cancer syndromes have been very important in informing how specific mutations can give rise to cancer and how those mutations can be selectively therapeutically targeted. Hereditary breast and ovarian cancers caused by mutations in the *BRCA1* and *BRCA2* genes, which are important for homologous recombination (HR)-mediated DNA repair when double-stranded DNA breaks (DSB) are encountered [4,5], are a prime example of this. BRCA-deficient cells are dependent on single-strand break (SSB) DNA-repair pathways. Poly(ADP-ribose)-polymerase (PARP) inhibitors take advantage of this dependence, causing apoptosis through synthetic lethality in cells defective in HR, either through BRCA deficiency or other genetic abnormalities.

An important protein in DNA repair and HR is Rad51 [6]. When complexed with several other proteins including BRCA1 and BRCA2, Rad51 facilitates DNA exchange between sister chromatids at damaged sites, including those induced by irradiation [6–9]. Embryonic lethality is observed in Rad51 knockout mice after exposure to radiation [10, 11], suggesting that it is essential in the repair of DSBs. Rad51 foci formation is therefore diminished in cells that have a defect in HR.

Data recently published by The Cancer Genome Atlas (TCGA) group suggests that as many as 50% of patients with high grade serous ovarian cancer have defects in members of the HR pathway [12]. It is postulated that these patients may benefit from PARP inhibitor therapy, similar to patients with *BRCA* mutations. However, an abnormality discovered by mutation analysis or expression profiling does not always translate to functional compromise. A functional assay identifying defective HR may be more accurate in predicting response to PARP inhibition. Such a functional assay might then be utilized to determine whether a biomarker that is clinically feasible to analyze could be used to predict response in the clinical setting. The goal of this study was to determine whether Rad51 foci formation, a well-known functional marker of HR, could identify ovarian cancers that would respond to PARP inhibitor therapy.

Materials and methods

Established ovarian cancer cell lines and patient-derived xenografts

Established human ovarian carcinoma cell lines A2780ip2, SKOV3ip1, HeyA8, ES2, SKOV3TRip2, A2780cp20, and HeyA8MDR were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Hyclone, Logan, UT). The taxane-resistant lines HeyA8MDR and SKOV3TRip2 were maintained in media with paclitaxel 150 ng/ml. All experiments were conducted with cells that were at 70–80% confluence and less than 20 passages from stock. Stock cell lines were confirmed to be the assumed genotype by microsatellite marker testing.

Patient-derived xenografts (PDX) were established from freshly-collected omental tumor nodules. IRB approval was obtained and patients were consented prior to surgery. Omental tumor nodules from newly diagnosed, untreated patients were excised at the time of primary tumor reductive surgery and processed immediately. Under standard anesthesia and sterile conditions, four separate 2 mm² tumor sections were implanted in a subcutaneous manner in severe combined immunodeficiency (SCID) mice (NCI-Frederick). Five mice were used per patient sample. When nodules were 0.75 cm in width, they were randomized to treatment as described below. 5 mm adjacent samples were isolated and snap frozen in liquid nitrogen and stored at –80 °C. The PARP inhibitor ABT-888 was kindly provided by AbbVie Pharmaceuticals.

Proliferation assay

To examine the sensitivity of each of the established ovarian cancer cell lines to ABT-888 alone, cells were plated at a density of 2000 cells/well in a 96-well plate. After allowing for attachment overnight, the cells were exposed to increasing concentrations of ABT-888 in triplicate. Cells were allowed to grow for four days, at which time viability was assessed with 0.15% MTT (Sigma). The IC₅₀ of drug was determined by finding the dose at which 50% of cells were killed, determined by the formula $[(OD_{450MAX} - OD_{450MIN}) / 2 + OD_{450MIN}]$. In separate experiments, cells were exposed to increasing concentrations of carboplatin in combination with fixed ABT-888 doses (determined *a priori* to be the IC₂₅ and IC₅₀ doses) to determine if sublethal doses of ABT-888 could sensitize cells to platinum agents. Synergy was assessed by the curve shift analysis [13] and calculation of the Combination Index based on the Chou–Talalay modification of Loewe's additivity model [14].

Rad51/IR-induced ex vivo assay

HR competency through Rad51 activation was determined in both established ovarian cancer cell lines and primary ovarian cancer samples after challenging with ionizing radiation (IR). Cells were plated on 6-well plates and exposed to 4 Gy or mock IR. Briefly, PDX tumor samples were mechanically dissociated in 1:5 phosphate buffered saline (PBS) and 0.25% trypsin (Hyclone, Logan, UT) using a sterile scalpel. Dissociated samples were plated on 60 mm tissue culture dishes for 1 h to obtain a purer population of tumor cells. The remaining supernatant was removed, and attached cells were trypsinized. Cancer cell concentration was determined by manual counting of a sample treated with trypan blue. $5 \times$

10⁴ cells per well were plated on collagen-coated coverslips and allowed to grow for 48 h. Cell lines were trypsinized when 70–80% confluent, replated on collagen-coated coverslips at 5 × 10⁴ cells/well of a 6-well plate, and allowed to grow for 48 h. Plates were then exposed to 4 Gy using an X-ray irradiator (Kimtron Inc., Woodbury, CT). 8 h later (based on optimization in previous studies [15,16]), the cells were rinsed with PBS and fixed with 70% ethanol. Cells were then blocked and incubated with anti-Rad51 antibody (Santa Cruz Biotech, Dallas, TX, dilution 1:500). Anti-rabbit Alexa Fluor 594-conjugated antibody (Invitrogen, Grand Island, NY) at a 1:2000 dilution was used as the secondary antibody. DAPI (4',6-diamidino-2-phenylindole, dihydrochloride) (Invitrogen) was employed for nuclear staining. Coverslips were then mounted on slides and examined for Rad51 foci with fluorescence microscopy (Carl Zeiss, Thornwood, NY). Total cells were counted, and those with ten or more Rad51 foci were considered positive as previously described [15,16].

Western blot

To determine if Rad51 foci formation after IR induction correlated to baseline Rad51 protein levels, Western blot analysis was performed on both PDX and cell line samples. Primary tumor samples corresponding to each PDX were used. 1 mm sections were shaved from each snap frozen sample, then manually dissociated in modified radioimmunoprecipitation assay (RIPA) lysis buffer with a protease inhibitor cocktail (Roche, Mannheim, Germany). Immunoblot analysis was conducted *via* standard technique [17] using anti-Rad51 antibody (Santacruz Biotech, Dallas, TX) at 1:1000 dilution overnight at 4 °C. After washing with PBS/1% Tween-20 (PBS-T), blots were incubated with 0.3 µl IRDye goat anti-rabbit secondary antibody (Li-Cor Biosciences, Lincoln, NE) in 5 ml Odyssey blocking buffer (Li-Cor) at room temperature for 1 h. Blots were washed again in PBS-T, then processed with the Odyssey CLx Infrared Imaging System (Li-Cor). To ensure equal sample loading, blots were incubated with mouse-anti-β-actin antibody at 1:20,000 dilution overnight at 4 °C, washed and exposed to 0.3 µl IR Dye goat anti-mouse secondary in 5 ml Odyssey blocking buffer (Li-Cor). The development process was the same as detailed above.

Cell lines at 80% confluence were subjected to the same immunoblot analysis with anti-Rad51 antibody but at a dilution of 1:500 at 4 °C overnight. After washing the blot was then incubated with 1:2000 anti-rabbit secondary antibody (Cell Signaling Technology, Danvers, MA) in PBS-T for 1 h at room temperature. After washing with PBS-T, the immunoblots were processed with a Xerox immunoblot developer (Norwalk, CT). Mouse anti-β-actin antibody was used as a sample loading control. The development process was the same as described above.

Two *in vivo* models were used to study the effects of the PARP inhibitor ABT-888 on tumor progression. All protocols were approved by the Institution Animal Care and Use Committee at the University of Alabama at Birmingham. Mice were cared for in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines. The first model was a cell-line based orthotopic mouse model. Female athymic nude mice (nu-nu) were obtained from the National Cancer Institute Frederick Cancer Research and Development Center (Frederick, MD). SKOV3ip1 and A2780ip2 intraperitoneal (IP) tumors were established by injection of 1 × 10⁶ cells suspended in 200 µl of serum-free RPMI

media. Seven days post-injection, mice in the orthotopic ovarian cancer cell line model were stratified into 4 treatment groups: (1) control, (2) ABT-888 alone, (3) carboplatin alone, and (4) carboplatin plus ABT-888. Each group was comprised of 10 mice for a total of 40 mice per cell line. Carboplatin 90 mg/kg was administered weekly by IP injection. ABT-888 200 mg/kg/day or an equal volume of saline was administered by oral gavage divided in twice daily doses. Mice were treated until animals in the control group showed significant tumor-related morbidity or mortality. Morbidity was defined as ascites limiting movement, limited voluntary enteral intake, or evidence of decreased blood flow. At that time, all mice were sacrificed and tumor was collected from the abdomen. Implants were collected and weighed in aggregate. Samples were stored in formalin, Optimal Cutting Media (Sakura, Leiden, Netherlands), RNAlater (Qiagen, Venlo, Netherlands), and snap frozen in liquid nitrogen.

The second *in vivo* model used was the PDX model. Tumors were isolated and implanted into mice as described above. Once subcutaneous tumors developed to a size of approximately 0.75 cm in maximal diameter, the mice were stratified into two treatment groups: (1) control treatment and (2) ABT-888 alone. ABT-888 200 mg/kg/day was administered by oral gavage divided in twice daily doses. Tumors were measured biweekly, with the primary endpoint being tumor volume, calculated by the formula $(\text{length} \times \text{width}^2) / 2$. The mice were treated for 60 days and then sacrificed. Tumors were collected and stored in the same fashion as above.

Statistical analysis

Two-way ANOVA analysis was used to compare Rad51 foci after radiation exposure. Pearson's correlation was used to compare Rad51-staining among primary tumor, PDX, and irradiated samples. A two-tailed Student's *T*-test assuming unequal variance was used to compare changes in tumor mass between untreated, ABT-888 alone, chemotherapy alone, and chemotherapy plus ABT-888. All datasets were normally distributed. Differences between samples were considered statistically significant at $p < 0.05$.

Results

Cell viability with PARP inhibition alone and in combination with carboplatin

The sensitivity of each cell line to PARP inhibition was determined using ABT-888 alone and in combination with carboplatin. Of the cell lines tested with single-agent PARP inhibition, A2780ip2 showed the greatest response (IC_{50} 8 μ M), while A2780cp20 and ES2 showed an intermediate sensitivity (55 μ M and 39 μ M, respectively, Fig. 1A, statistically more resistant than A2780ip2, $p < 0.05$). Response to PARP inhibition in A2780ip2 was not unexpected given its known *PTEN* mutation [18]. The SKOV3ip1, SKOV3TR, HeyA8, and HeyA8MDR cell lines were significantly less sensitive to single-agent ABT-888, with IC_{50} s greater than 100 μ M ($p < 0.05$, Fig. 1B). Interestingly, previously-published profiles of these cell lines demonstrate that all are *BRCA* wild-type, but only HeyA8 did not contain a mutation in at least one member of the HR family (Table 1). Therefore based on mutation status alone, SKOV3ip1 and SKOV3TRip2 would have been mistakenly assumed to be sensitive to PARP inhibition. Based on the previously reported *BRCA*ness signature [19], A2780ip2, A2780cp20, and ES2 would all have been assessed as fitting a *BRCA*ness profile.

Because the synthetic lethality of PARP inhibition is dependent on some level of DNA damage, possible PARP inhibition-induced sensitization to carboplatin in cell lines was next examined. In the presence of clinically viable doses of ABT-888, the same cell lines that had some level of single-agent PARP inhibitor toxicity were also sensitized to carboplatin. A2780ip2, A2780cp20, and ES2 all had carboplatin IC₅₀ levels reduced (reduced 2.6–7.6-fold) using both IC₂₅ and IC₅₀ levels of ABT-888 for the corresponding cell line (Fig. 1C,D,E). Lowe's additivity model demonstrated a Combination Index less than 1, suggesting synergy (IC = 0.22 in A2780ip2, 0.55 in A2780cp20, and 0.48 in ES2). However, for SKOV3ip1, in which ABT-888 did not have significant single-agent activity, ABT-888 did not sensitize cells to carboplatin (IC = 0.98, Fig. 1F).

Baseline expression of Rad51 protein in unexposed samples

Prior reports have proposed using low Rad51 foci as an assessment tool for HR status [20]. However, because this test uses immunofluorescence, it is not amenable to testing conventionally collected formalin-fixed paraffin-embedded samples. To examine if baseline total Rad51 protein could be used as a surrogate for functional response to PARP inhibition, Western blot analysis for Rad51 was performed. Protein analysis showed that Rad51 expression was prominent in all cell lines (Fig. 2A). Although levels varied, there was no discernible correlation between expression and *in vitro* response to PARP inhibition — the lowest levels of expression were noted in A2780ip2 (the greatest responder *in vitro*) and SKOV3ip1 (a non-responder).

Rad51 foci with radiation exposure

Due to low baseline levels of Rad51, rates of Rad51 foci formation after irradiation were then examined to determine which established ovarian cancer cell lines had defects in HR. Plated cells were exposed to ionizing radiation and subsequently probed for Rad51 foci formation by immunofluorescence (Fig. 2B,C). All cell lines had very few cells (<5%) with Rad51 foci formation without irradiation. However, after irradiation Rad51 foci formation was induced in all cell lines. Interestingly, SKOV3ip1 cells demonstrated the greatest induction of Rad51 foci after radiation (48% of cells, Fig. 2C). ES2 and A2780cp20 cells had an intermediate response at 20–28%, and A2780ip2 cells had the least response, at 11%. These rates of Rad51 foci formation correlated perfectly with the degree of response to single-agent PARP inhibitor therapy and sensitization to carboplatin (demonstrated in Fig. 1). A low induction of Rad51 foci formation functionally demonstrated defective HR and accurately predicted response to PARP inhibition.

Treatment of xenografts with ABT-888 and carboplatin

To determine if the noted *in vitro* response correlated with *in vivo* response, an orthotopic model was utilized examining the “PARP-resistant” SKOV3ip1 and “PARP-sensitive” A2780ip2 cell lines. One week after IP inoculation with cells, treatment was initiated with 1) vehicle, 2) ABT-888 alone, 3) carboplatin alone, or 4) combined ABT-888 and carboplatin. As predicted by the functional HR assay and *in vitro* results, treatment with ABT-888 alone did not have an effect on tumor mass in the SKOV3ip1 orthotopic xenograft (Fig. 3A). However, in A2780ip2, there was a 51% reduction in mean tumor mass, although the

difference was not statistically significant due to the variability in tumor size ($p=0.27$) (Fig. 3B). Carboplatin was effective alone in both cell lines, with a reduction of 92% in SKOV3ip1 and 80% in A2780ip2 ($p=0.001$ and 0.03 , respectively). The addition of ABT-888 to carboplatin did not improve on carboplatin alone in SKOV3ip1. In A2780ip2, combination therapy led to an additional 42% reduction in tumor mass over carboplatin alone, though again this was not statistically significant. This is likely due to the high variability in orthotopic growth in these cell lines. Therefore an additional *in vivo* model was examined.

Rad51 foci in first generation PDX tumors

Despite their common use, the SKOV3 and A2780 cell lines from which the above models were derived have recently been shown to poorly reflect expression profiles of papillary serous cancers, as defined by the TCGA dataset [21]. In order to explore the relationship between HR functionality and response in a model that more closely resembles patient tumors, a PDX model was utilized in SCID mice. This model has been demonstrated to have similarity to the patient tumors from which they were derived, at least through the first three generations [22]. Tumors were allowed to grow, and then initially harvested for assessment of Rad51 expression and induction with irradiation ($n=8$). Among the eight PDX tissue samples, only one had a detectable level of total Rad51 protein (Fig. 4A). This was in stark contrast to what was noted in established ovarian cancer cell lines, in which total Rad51 was prominently expressed in all lines. A query of the TCGA ovarian dataset [12] using cbioportal.org revealed that Rad51 is more than 2-fold downregulated at the mRNA level in just 9 of 316 cases (2.8%), and upregulated in 2 cases (0.6%). Setting a threshold of 1.5-fold increase or decrease, 21/316 cases (6.6%) had downregulation and 12 cases (3.8%) had upregulation. There was no significant trend in survival based on Rad51 over- or under-expression. Therefore total Rad51 expression is likely not a good predictor of HR functionality.

The IR *ex vivo* assay was then performed on PDX tumors to determine if exposure to irradiation produces variable responses in Rad51 foci formation, as they did in cell lines. Two PDX samples (146 and 150) had moderate amounts of Rad51 foci formation at baseline, on the order of 20–30% (Fig. 4B). Exposure to irradiation accentuated the difference, and revealed HR functionality in some samples not notable without irradiation, particularly in PDX 144 and 155. PDX tumors 135, 152 and 157 formed Rad51 foci at the lowest rates (less than 10%), suggesting a defect in HR proficiency. Interestingly, patient 157 had negative *BRCA* testing, suggesting the presence of some other mechanism of defective HR. Two PDX tumors, 136 and 155, had intermediate levels of HR functionality, at 10–25%.

To determine whether response to therapy *in vivo* could be predicted by the IR-induced *ex vivo* assay, three PDX models were examined: PDX 157, predicted to respond due to very low induction of Rad51 foci (2.0% of cells), PDX 136, with an intermediate induction of Rad51 foci (13.1% of cells), and PDX 144 with a predicted resistance profile due to high induction of Rad51 foci (78% of cells). Once implanted tumors had developed to approximately 0.75 cm in maximal diameter, treatment was initiated with either 1) vehicle

(n= 3 mice per PDX model) or 2) ABT-888 (n=2mice per PDX model). Over the 60-day treatment course, ABT-888 treated PDX 157 had an average tumor volume reduction of 93% compared to a 36% average increase in tumor volume in control mice (p = 0.003, Fig. 5A). PDX 136, which was predicted to have a moderate response based on the Rad51 assay, showed stabilization of tumor volume with single-agent ABT-888 while control tumors continued to grow (p = 0.004, Fig. 5B). PDX 144 tumors grew at equal rates with control or ABT-888, confirming resistance. Based on these results, the IR-induced *ex vivo* assay accurately predicted response to single-agent ABT-888 therapy.

Discussion

Several trials have demonstrated objective response to PARP inhibitor therapy in ovarian cancer patients with known *BRCA 1/2* mutations [23,24]. However, the clinical impact of PARP inhibition would be significantly limited if only *BRCA 1/2* mutation carriers were offered therapy, as only 15–20% of women who develop ovarian cancer have germline mutations [12,25]. It has been recognized that *BRCA* proteins are only part of a complex mechanism of DNA repair, and that defects in other proteins involved in homologous repair may lead to the same functional deficiency.

Although PARP inhibitor therapy benefit is still noted for women who are either unselected for the mutation or mutation status is unknown, the effect is less pronounced [26]. Indeed, Gelmon et al. showed that *BRCA* mutation-negative ovarian cancer patients had a response rate of 24% to the PARP inhibitor olaparib, as opposed to a response rate of 41% in *BRCA* positive patients [27]. Clearly, other pathways may be influenced by PARP inhibition, but the need for a predictive assay is apparent. To that end there have been several approaches. Mukhopadhyay and colleagues isolated and cultured cells from ascitic fluid of ovarian cancer patients [28]. They then exposed these cells to a PARP inhibitor and probed the cultures for γ H2AX and Rad51 foci as markers of HR. They found that cells identified as HR deficient by decreased Rad51 foci formation were also sensitive to PARP inhibition. However, it is unknown if ascites-derived cells exhibit the same pathophysiology as cells derived from primary tumor or metastatic implants. Furthermore, they were not able to determine if there was a correlation between response in patients or solid tumors in an *in vivo* model.

Recently Pennington and colleagues examined 390 ovarian, primary peritoneal, and fallopian tube carcinoma samples to determine the rate of mutations in selected HR genes [29]. They found that 31% of unselected patients harbored a somatic or germline HR mutation, which was associated with both platinum sensitivity and improved overall survival. These characteristics are similar to patients with germline *BRCA* mutations, which suggests that these patients may respond similarly to PARP inhibition. As the authors note, PARP inhibition may be useful in a wider range of patients than the traditional cohort of high grade serous cancers, but a predictive assay is necessary to determine who will derive the most benefit.

Our results suggest that it is possible to predict PARP inhibitor response based on *ex vivo* characterization. While we did find some *in vitro* correlation of HR competency and Rad51

formation in established ovarian cancer cell lines, our most intriguing findings involved the association between the heterotopic PDX mouse model and predicted response based on the IR-induced *ex vivo* assay. Clearly this method requires significant refinement to be clinically relevant, but the possibility remains that determining true response is achievable. Correlation between the IR-induced *ex vivo* assay and efficacy of PARP inhibition in the PDX model suggests that with further work, a genetic signature that predicts HR deficiency could be a clinically feasible way to select which patients would respond to PARP inhibitor therapy.

Expanding the concept of synthetic lethality beyond patients with germline *BRCA* mutations is an exciting avenue of inquiry. True advancement in the treatment of ovarian cancer has been slow after the incorporation of platinum agents into first-line therapy, and PARP inhibition may prove to be useful in a variety of settings, from upfront to recurrent to maintenance therapy [30]. However, from the standpoint of both toxicity and health care resources, patients need to be screened for biologic eligibility of these agents. Identifying an effective *ex vivo* assay to help predict the potential utility of PARP inhibitors in epithelial ovarian cancer patients should be a high research priority.

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HIGHLIGHTS

- Homologous recombination (HR) defects are common in ovarian cancer, suggesting a role for PARP inhibitors.
- No predictive assay for HR defects exists, but Rad51 is a reliable marker for HR.
- An *ex-vivo* IR assay using Rad51 foci formation accurately predicts PARP-inhibitor response.

Fig. 1. Single-agent response to ABT-888. (A) IC₅₀ for cell lines that showed single-agent sensitivity; maximum concentration 1000 μM. (B) Nonresponding cell lines, maximum concentration 1000 μM. (C–F) Combination ABT-888 and carboplatin in A2780ip2, A2780cp20, ES2, and SKOV3ip1.

Fig. 2. Rad51 expression (A) Western Blot for Rad51 in established ovarian cancer cell lines. (B) Representative image of immunofluorescence staining of Rad51 foci 8 h after radiation exposure. (C) Comparison of percentage cells positive for Rad51 foci after radiation in cell lines. Note: ** = $p < 0.05$.

Fig. 3.

In vivo treatment with ABT-888 alone and with carboplatin in (A) SKOV3ip1 and (B) A2780ip2 cell lines. Treatment groups are vehicle, PARP inhibitor (ABT-888), carboplatin (Carbo), and PARP inhibitor and carboplatin (ABT + Carbo).

Fig. 4.

Patient derived xenografts (A) Western blot for Rad51 in PDX. (B) Comparison of percentage cells positive for Rad51 foci after radiation exposure in xenografts. Note: ** = $p < 0.001$.

Fig. 5.

Patient xenograft (PDX) response to ABT-888 compared to vehicle in three patient-derived xenografts: (A) PDX 157 with defective HR by the *ex vivo* assay, (B) PDX 136 with an intermediate level of Rad51 foci induction, and (C) PDX 144 with intact HR.

Table 1

Reported mutations and DNA amplifications in common ovarian cancer cell lines.

	SKOV3			A2780			HeyA8			ES2		
	Mutation	CNA	Mutation	Mutation	CNA	Mutation	Mutation	CNA	Mutation	Mutation	CNA	CNA
BRCA1	WT [21]	Amplified [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	None [31]
BRCA2	WT [21]	None [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	None [31]
ATM	Mutated [31,32]	None [31]	Mutated [31]	Mutated [31]	None [31]	WT [31]	WT [31]	None [31]	Mutated [31]	Mutated [31]	None [31]	None [31]
ATR	WT [33]	None [31]	WT [31]	WT [31]	None [31]	WT [31]	WT [31]	Amplified [31]	WT [31]	WT [31]	None [31]	None [31]
EMSY	Equivocal [21,32]	None [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	None [31]
FANCD2	Mutated [32]	Amplified [31]	WT [31]	WT [31]	None [31]	WT [31]	WT [31]	None [31]	WT [31]	WT [31]	None [31]	None [31]
RAD51c	WT [31]	None [31]	WT [31]	WT [31]	None [31]	WT [31]	WT [31]	None [31]	WT [31]	WT [31]	Amplified [31]	Amplified [31]
PTEN	WT [21]	None [31]	Mutated [21,31,32]	Mutated [21,31,32]	None [31]	WT [21]	WT [21]	None [31]	WT [21]	WT [21]	None [31]	None [31]