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# Radiosensitization by the PARP inhibitor olaparib in BRCA1-proficient and deficient high-grade serous ovarian carcinomas☆

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## HIGHLIGHTS

- Olaparib radiosensitizes both BRCA1-deficient and proficient HGSOC.
- Olaparib-mediated radiosensitization is more in BRCA1-deficient HGSOC than BRCA1-proficient HGSOC.
- Olaparib inhibits PARP activity, induces more DNA damage and apoptosis when combined with radiotherapy.
- Olaparib combined with radiotherapy delay tumor growth and prolong survival in HGSOC xenograft mouse model.

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## ABSTRACT

*Objective.* Approximately 15–25% of high-grade serous ovarian carcinomas (HGSOC) harbor BRCA1/2 mutations. Inhibition of Poly (ADP-ribose) polymerase (PARP) is synthetically lethal to cells and tumors with BRCA1/2 mutation. Our goal was to investigate the radiosensitizing effects of PARP inhibitor olaparib in HGSOC with different BRCA1 status.

*Methods.* The radiosensitizing effects of olaparib were tested on BRCA1-proficient and deficient HGSOC by clonogenic survival and tumor growth assays. The effects of olaparib and radiation on DNA damage, PARP activity, and apoptosis were determined.

*Results.* BRCA1-deficient HGSOC cells were more sensitive to RT alone and exhibited significantly higher levels of olaparib-mediated radiosensitization compared to BRCA1-proficient cells. Furthermore, when combined with RT, olaparib inhibited DNA damage repair and PARP1 activity, increased apoptosis, decreased growth of HGSOC xenografts and increased overall host survival. The growth-inhibitory effects of the combined olaparib and RT treatment were more pronounced in mice bearing BRCA1-deficient tumors compared to BRCA1-proficient tumors.

*Conclusions.* These results provide a preclinical rationale for improved treatment modalities using olaparib as an effective radiosensitizer in HGSOC, particularly in tumors with BRCA1-deficiencies.



## 1. Introduction

Ovarian carcinoma (OC) is the second most common gynecological cancer and the fifth leading cause of cancer-related deaths in women [1]. In the US, approximately 22,240 new cases will be diagnosed in 2018. While early diagnosis and treatment can result in high cure rates, survival rates for stage II or higher HGSOC can be as low as 28%, as most patients with advanced HGSOC will develop recurrence within 18 months [2]. OC carries a poor prognosis and is represented by resistance to chemotherapy which still remains a major factor for the mortality in OC patients over the past decade. Hence, novel therapeutic strategies are needed to increase the survival rate of patients which currently stands at staggering low 30–40%. Radiation therapy (RT) is a treatment option in OC patients with isolated relapses or oligometastatic disease [3, 4], but doses are limited by concerns for late gastrointestinal toxicity including stricture and bowel obstruction in patients who have undergone multiple surgeries and

☆ Data StatementAll data generated or analyzed during this study are original and included in this published article (and its Supplementary Information files).

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chemotherapeutic regimens [5, 6]. Therefore, for these patients it would be ideal to improve the therapeutic window by combining RT with a radiosensitizer.

When DNA is damaged, PARP senses damaged bases, binds to DNA single-strand breaks (SSBs) and activates the base excision repair (BER) pathway by recruiting additional repair factors [7, 8]. PARP1 accounts for >90% of SSBs repair activity and is the most extensively studied family member [9–11]. Moreover, PARP functions in other repair pathways, including homologous recombination (HR), non-homologous end joining (NHEJ)and alternative microhomology-mediated end joining repair (Alt-EJ) [12–15]. Therefore, combining PARP inhibitors (PARPi) together with other DNA damaging agents such as radiation or platinum chemotherapy is hypothesized to result in increased sensitivity of cancer cells due to impaired DNA repair. RT can induce both SSBs and double-strand breaks (DSBs) of DNA, however, in the presence of PARPi, SSBs are prevented to be repaired through BER and the remaining SSBs are converted to DSBs during DNA replication. Therefore, in this situation, HR and NHEJ/Alt-EJ mechanisms for DSBs repair are likely to be most relevant for cells to survive.

Importantly, two landmark papers demonstrated a dramatic increase in lethality when cell lines with homozygous deletion or inactivation of BRCA1/2 were treated with PARPi [16, 17]. This work ushered in the concept of "synthetic lethality" in which chemical agents inhibiting a specific pathway are synthetically lethal with a mutation or genetic lesion which blocks a salvage or alternative pathway that is required for survival. Since then, multiple studies and clinical trials using PARPi in tumors with BRCA1/2 genetic lesions have confirmed these findings [18, 19]. Women with germline BRCA1/2 mutations have a 40–60% lifetime risk to develop HGSOC, and approximately 15–25% of HGSOC harbor BRCA1/2 mutations [20, 21], making HGSOC a particularly attractive target for PARPi.

The effects of PARPi have been investigated in preclinical and clinical trials as a monotherapy or combined with chemotherapy and shown promising results [15, 22]. This led us to hypothesize that PARPi could potentially be used as a radiosensitizer to enhance the therapeutic index of radiotherapy in HGSOC. Olaparib (AZD-2281), an FDA-approved inhibitor of PARP1, PARP2 and PARP3 [23], with good tolerability in phase II studies [24–26], has entered phase III clinical trials [27]. Therefore, the present study aims to evaluate the radiosensitizing effect of olaparib using in vitro and in vivo HGSOC models with different BRCA1 status. We found that olaparib produced highly significant radiosensitization in BRCA1-deficient HGSOC and modest radiosensitization in BRCA1-proficient HGSOC. Analysis of the contributions of DNA repair and apoptosis to the radiosensitization revealed that BRCA1-deficient cells incur significantly more DNA damage and apoptosis in vitro and in vivo. Therefore, we believe that our results support further clinical trials with olaparib and radiotherapy for HGSOC as well as other solid malignancies involving BRCA mutations.

## 2. Methods and materials

### 2.1. Compounds and irradiation

The PARPi olaparib (AZD2281) was provided by AstraZeneca and was dissolved in DMSO to a stock concentration of 10 mM. Cells and mice bearing flank tumors were irradiated in an X-RAD 320iX Irradiator (Precision X-ray, Inc. CT, USA), with a dose rate of 0.99 Gy per minute.

### 2.2. Cell culture

SKOV3, OVCAR3, UWB1.289, UWB1.289+BRCA1 cell lines were purchased from the American Type Culture Collection (ATCC). OVCAR8 cell line was a gift from Dr. David M. Livingston, Harvard Medical School and authenticated through STR genetic testing by ATCC. SKOV3 and OVCAR3 are BRCA1 wide-type cell lines; OVCAR8 has substantially decreased expression of BRCA1 due to hypermethylation of its promoter region; UWB1.289 has germline BRCA1 mutation within exon 11 (2594delC), which leads to a stop at codon 845 of BRCA1. UWB1.289 + BRCA1 is a stable cell line derived from UWB1.289, in which wild-type BRCA1 was restored [28, 29]. SKOV3 and OVCAR8 cells were maintained in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS), and penicillin-streptomycin (Gibco). OVCAR3 cells were maintained in RPMI-1640 medium supplemented with 20% FBS, 0.01 mg/ml bovine insulin and penicillin-streptomycin. UWB1.289 and UWB1.289 + BRCA1 cells were cultured in 50% RPMI-1640, 50% MEBM basal medium (Lonza, #CC-3151), MEGM SingleQuot additives (Lonza, #CC-4136, without gentamycin-amphotericin B), 3% FBS, and penicillin-streptomycin. All cells were cultured at 37 °C humidified 5% $CO_2$ atmosphere.

### 2.3. Immunoblot analysis

Immunoblotting was performed as previously described [30]. For detecting γ-H2AX, 2× SDS-PAGE sample buffer (62 mM Tris-HCL, PH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue, containing freshly added 5% β-mercaptoethanol) was used. The following primary antibodies were used for immunoblotting: BRCA1 (Cell Signaling, # 9025), PARP1 (Cell Signaling, # 9532), Ku80 (Cell Signaling, # 2753), γ-H2AX (Cell Signaling, # 2577), β-tubulin (Cell Signaling, # 2146), PAR (Trevigen, # 4336-BPC-100), cleaved-caspase3 (Cell Signaling, # 9664), cleaved-caspase9 (Cell Signaling, # 9505) and β-actin (Sigma-Aldrich, # A3853). The secondary antibodies used were anti-rabbit and anti-mouse HRP from Thermo Scientific.

### 2.4. Real-time quantitative PCR

RNA was isolated with TRIzol Reagent (Ambion) and reverse-transcribed into cDNA using TaqMan reverse transcription reagents (Applied Biosystems, Roche, # N8080234). Real-time quantitative PCR was performed on the resulting cDNA templates using Power SYBR Green Master Mix (Applied Biosystems, # 4367659). Data was analyzed using the QuantStudio 6 Flex Real-Time PCR System. A list of primers used is available in Supplementary Table S1. Relative mRNA levels were quantified using the standard curve method. Experiments were done in triplicate and are represented as mean ± SD.

### 2.5. Clonogenic survival assay

Clonogenic survival assay was conducted as previously described [31]. Briefly, to assess the radiosensitizing effect of olaparib, cells were seeded in triplicate in 6-well plates and allowed to adhere overnight. Cells were pretreated with DMSO (0.1%) or olaparib (1 μM) for 4 h, irradiated by increasing doses of radiation (0–6 Gy) and incubated for an additional 24 h. Then, fresh media without drug were replaced and cells were incubated for 9–10 days. Colonies were stained with crystal violet and counted. Drug cytotoxicity in the absence of radiation was calculated as the ratio of surviving fraction (SF) of olaparib-treated cells relative to untreated controls. Radiation survival data were normalized to unirradiated control under the same conditions. Dose enhancement factor (DEF) was calculated as the ratio of the dose with radiation alone divided by the dose with radiation and PARPi needed to cause 0.1 SF. A value significantly >1 indicates radiosensitization [32].

### 2.6. FITC-Annexin V apoptosis assay

The induction of apoptosis caused by irradiation, olaparib and their combination was analyzed by flow cytometry using the FITC Annexin V apoptosis detection kit I (BD Biosciences, # 556547) according to the manufacturer's instructions. Cells were pretreated with DMSO (0.1%) or olaparib (1 μM) for 4 h, followed by mock or 4Gy of radiation and incubated for 24 or 48 h. After incubation, cells were collected and washed with cold PBS. Cells were resuspended in 1× Annexin V binding

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