Synergistic Effect of Bazedoxifene and PARP Inhibitor in the Treatment of Ovarian Cancer Regardless of BRCA Mutation

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Abstract. *Background/Aim:* Poly (ADP-ribose) polymerase inhibitors (PARPis) are one of the targeted therapies proven to treat breast cancer gene (BRCA)-mutant ovarian cancer. Because most ovarian cancers are BRCA wild-type, it is necessary to extend the usage of PARPis. In the present study, we combined the PARPi, talazoparib, and the IL-6 inhibitor, bazedoxifene, for the treatment of human ovarian cancer cells. *Materials and Methods:* The human ovarian cancer cell lines, SKOV3, UWB1.289 (BRCA1-null) and OV75, were treated with talazoparib and bazedoxifene, as monotherapy or combination treatment. The effects of treatment on cell viability, migration, growth and colony formation were examined. Western blot was used to investigate pathways that may be involved in the antitumor effects of the two agents. *Results:* The combination of talazoparib and bazedoxifene showed synergistic inhibition of cell viability, cell migration, cell growth, and cell colony formation on all the studied cell lines. The expression of p-AKT, c-myc, p-ERK, ER α was inhibited, and γ -H2AX expression was induced. *Conclusion:* Combined inhibition of PARP and IL-6 may be an efficacious treatment for ovarian cancer, independently of BRCA mutation status.

Ovarian cancer is the most lethal gynecological cancer and the fifth leading cause of cancer-related death in women in the USA (1). In 2019 alone, there were approximately 22,530 new

cases of ovarian cancer resulting in 13,980 deaths (2). The standard treatment for ovarian cancer is radical surgery with adjuvant chemotherapy and taxane combined with platinum as the first line approach (3). Surgical techniques and chemotherapy have been well-studied in the effort to improve long-term survival of ovarian cancer (4). However, the five-year survival rate has not increased significantly in the past two decades (3). Thus, there is urgent need to investigate new pharmacologic interventions with a tolerable side effect profile, and targeted therapy is a promising approach (5).

One of these targeted therapies, oral PARPis, have already been used as maintenance therapy in the treatment of ovarian cancer (6). The mechanism of PARPis was first reported as synthetic lethality in cancer cells with BRCA mutations (7-9). PARPis prevent the repair of DNA single-strand breaks (SSB), hence promoting the conversion of SSB to double-strand breaks (10). BRCA1 and BRCA2 are essential players in DSB repair through homologous recombination (HR) (7-9). Therefore, cancer cells with BRCA loss-of-function mutations would only have low-fidelity repair pathways, resulting in an increase in genomic instability, ultimately leading to tumor-specific cell death (7-10). PARPis have been established as an anticancer therapy in patients with BRCA mutations, including talazoparib, which was approved by the Food and Drug Administration (FDA) in 2018 (11).

However, the majority of ovarian cancer patients do not have BRCA mutations (12). Thus, new studies have been focused on the possibility of extending the use of PARPis for patients without BRCA mutations. It has been reported that in addition to DNA repair pathways, PARP inhibitors also activate other pathways such as phosphoinositide 3-kinase (PI3K)/AKT, estrogen, cyclinD1, nuclear factor kappa B (NF- κ B), extracellular signal-regulated kinase (ERK), and pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF α), interleukin (IL)-6, interferon- γ (IFN γ), E-selectin, and intercellular adhesion molecule (ICAM-1) (13). It has been previously demonstrated that the combination of

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PARPis with AKT inhibitors or CyclinD1 silencing led to greater inhibitory effects on cancer cells (14, 15).

IL-6 is a pro-inflammatory cytokine secreted by many cell types, including tumor cells (16), stimulating the generation and growth of tumors. IL-6 is secreted by most ovarian cancer cells and promotes tumor cell growth, migration, and invasion (4, 17, 18). High levels of IL-6 can result in chemoresistance and are a marker for poor prognosis in ovarian cancer patients (19-21). Therefore, inhibiting IL-6 is a promising targeted therapy for ovarian cancer cells.

The IL-6/JAK/STAT3 is the main pathway activated by IL-6, which binds to the IL-6 receptor and GP130 to form a signaling complex, leading to tyrosine phosphorylation of STAT3 (p-STAT3) and activation of downstream target genes like c-Myc (22, 23). However, many other pathways can also be activated by IL-6, such as PI3K/AKT, MAPK/ERK, Wnt, and COX-2 (24). Moreover, IL-6 can also increase estrogen secretion, which in turn can induce higher IL-6 levels. Bazedoxifene, initially established as a third-generation selective estrogen receptor modulator, was found in our previous studies to inhibit IL-6/GP130 (25-29). We have previously shown that bazedoxifene has antitumor activity in breast cancer (23) and colon cancer (30) cells, while having a favorable side effect profile.

Together, PARPis and IL-6 inhibitors have the potential for therapeutically significant antitumor activity through interplay across several pathways, suggesting synergistic effects on ovarian cancer cells, therefore allowing usage of PARPis for a wider patient population. Hence, in the present study, the PARPi, talazoparib, and the IL-6 inhibitor, bazedoxifene, were investigated as a combination therapy for ovarian cancer, both BRCA-null and wild-type ovarian cancer cells, *in vitro*.

Materials and Methods

Chemicals. Talazoparib was purchased from MedKoo Biosciences (#204710) and azedoxifene acetate was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany; #198481-33-3). Both were stored in DMSO (dimethyl sulfoxide; Sigma-Aldrich, Merck KGaA) as 20 mM at -20°C. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (#298-93-1), and the stock concentration was stocked in ddH₂O as 5mM at -20°C. Primary and secondary antibodies were purchased from Cell Signaling Technology.

Cell lines. Human ovarian cancer cell lines SKOV3 and UWB1.289 were purchased from American Type Culture Collection (Manassas, VA, USA). The OV75 cell line was kindly provided by Dr. Jocelyn Reader (University of Maryland School of Medicine Division of Gynecologic Oncology, Baltimore, MD, USA). The OV75 cell line was cultured in HOSE Media (31). The UWB 1.289 cells, a BRCA1-null cell line (32), were cultured in a 1:1 mixture of RPMI-1640 medium (Corning Inc., Corning, NY, USA; cat. no. 10-040-CV) with mammary epithelial cell growth medium (MEGM, Lonza Pharma &

Biotech Inc., Basel, Switzerland; cat. no. CC-3150) with 3% fetal bovine serum (FBS, Sigma-Aldrich; Merck KGaA; cat. no. F2442). The SKOV3 cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM, Corning, Inc.; cat. no. 10-013-CV) supplemented with 10% FBS and 1% penicillin/streptomycin (ThermoFisher Scientific Inc., Waltham, MA, USA; cat. no. 15140122). The medium was replaced twice a week. All cell lines were maintained in a humidified atmosphere with 5% CO₂ at 37°C.

Measurement of interleukin-6. Cells were seeded in a 6-well plate at a density of 70% per well. The cell-free culture supernatant was collected the next day. IL-6 levels were detected in the supernatant using enzyme linked immunosorbent assay (ELISA) at OD=450 nm (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands). Each assay detects cytokine concentrations as low as 5±7 pg/ml.

Western blot analysis. The ovarian cancer cells were seeded in 10-cm plates at a cell density of 70% and treated with talazoparib (0.5 µM), bazedoxifene (2.5 µM), combination treatment, or DMSO at 37°C overnight. Protein was extracted using ice cold lysis buffer (Cell Lysis Buffer, Cell Signaling Technology, Inc. cat. no. 9803S; 0.5% 0.2 M PMSF, 0.5% 0.2 M NaF, 0.5% 0.2 M NaPP, 0.5% 0.1 M Na₃VO₄ and 4% 25X CP1). Protein concentration was determined using the Microplate BCA Protein Assay kit (Thermo Fisher Scientific, Inc.; cat. no. 23252). Proteins were separated by 10% SDS-PAGE gels and transferred to PVDF membrane under 350 mA for 110 min. After blocking in 5% milk for 1 h at room temperature, membranes were incubated in primary antibodies overnight at 4°C. After washing with Tris-buffered saline with 0.1% Tween-20 3 times for 15 min, membranes were incubated with secondary antibody for 1.5 h. Blots were imaged with the Storm scanner (Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA) and Amersham Imager 680 (GE healthcare Inc, Mariborough, MA, USA).

All primary antibodies were purchased from Cell Signaling Technology, stored at -20°C, and diluted 1:1000 in 5% milk: p-STAT3 (Y705) (rabbit mAb, #9131S), STAT3 (rabbit mAb, cat. #4904S), p-AKT (rabbit mAb, #4060S), AKT (rabbit mAb; #4691S), phospho-histone H2A.X (rabbit mAb, #2577S), phospho-p44/42 MAPK (Erk1/2) (rabbit mAb, #4370S), p44/42 MAPK (Erk1/2) (rabbit mAb, #4569S), c-Myc (rabbit mAb, #13987S), Estrogen Receptor α (D8H8) (rabbit mAb, #8644S), GAPDH (rabbit mAb, #2118S). The secondary antibody was diluted 1:10000 in 5% milk (Cell Signaling Technology, Anti-rabbit IgG HRP-linked antibody, #7074).

MTT cell viability assay. Ovarian cancer cells were seeded in 96-well microtiter plates with 3,000 cells and 100 µl medium per well. Cells were treated with different drugs: talazoparib (0.1 µM for UWB1.289; 0.5 µM for SKOV3; 0.5 µM for OV75), bazedoxifene (5 µM for UWB1.289; 2.5 µM for SKOV3; 2.5 µM for OV75), combination treatment and DMSO after overnight incubation. After a 72-h incubation at 37°C, 20 µl MTT was added to each well for an additional 4-h incubation. Then, 150 µl of DMF solubilization solution was added into each well and incubated overnight in a shaker, protected from light, at room temperature. The absorbance was read at OD=595 nm using an EL808 Ultra Microplate Reader (BioTek, Winooski, VT, USA). The DMSO cells were set at 100% and the cell viability of drug-treated cells was determined relative to DMSO cells. Then, the combination index (CI) was determined

by CompuSyn software (www.combosyn.com). The CI values indicate additive effect when equal to 1, an antagonistic effect when >1 , and a synergistic effect when <1 , based on the theorem of Chou-Talalay (33).

siRNA transfection. The SKOV3 and UWB1.289 cells were seeded in 96-well plates with 4,000 cells per well and transfected with GP130 siRNA (Cell Signaling Technology, #6582s) or negative control siRNA (Cell Signaling Technology, #6568s) at a final concentration of 50 nM using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, #11668019) according to the manufacturer's instructions. Cells were transfected for 48 h and subsequently processed for the MTT assay, after which, cells were treated with DMSO or talazoparib (1.0 μ M for UWB1.289; 0.5 μ M for SKOV3) for 72 h.

Wound healing/cell migration assay. SKOV3 cells were seeded in a 6-well plate and cultured overnight until adherent and 100% confluent. A 100- μ l pipette tip was used to make a straight scratch in the monolayer of each well. Then, cells were washed with PBS twice and treated with talazoparib (0.5 μ M), bazedoxifene (2.5 μ M), combination treatment, or DMSO in culture medium. Images were captured at 0 h as well as after incubation at 37°C for 17 h, when the wound of DMSO-control well healed. All images were captured by a Leica microscope with Leica Application Suite X (LAS X) software platform. Inhibition of migration was measured using ImageJ software (<http://rsb.info.nih.gov/ij/>) and calculated according to the formula: $100 - [(final\ area/initial\ area) \times 100\%]$ (34).

Cell growth assay. The same number of cells were seeded in 24-well plates (SKOV3, 2×10^3 cells per well; UWB1.289, 5×10^3 cells per well), cultured overnight at 37°C, and treated with talazoparib (0.005 μ M for UWB1.289; 0.5 μ M for SKOV3), bazedoxifene (2.5 μ M for UWB1.289; 2.5 μ M for SKOV3), combination treatment, or DMSO. Cell number was counted on days 2, 4, 6 and 8 after treatment. At last, we plotted the growth curves and analyzed them.

Colony-forming assay. The same number of cells were seeded in 6-well plates (SKOV3: 2×10^4 cells per well, UWB1.289: 5×10^3 cells per well), cultured overnight at 37°C, and treated with talazoparib (0.001 μ M for UWB1.289; 1 μ M for SKOV3), bazedoxifene (5 μ M for UWB1.289; 5 μ M for SKOV3), combination treatment, or DMSO. After a 48-h treatment, cells were incubated in fresh medium for 2-3 weeks (UWB1.289 for 3 weeks; SKOV3 for 2 weeks). Then, cells were washed with PBS, fixed with methanol for 30 min, and stained with toluidine blue for 1.5 h. The colonies were counted under a microscope (Olympus IX81, Tokyo, Japan).

Statistical analysis. Data were analyzed using GraphPad Prism 7 software (GraphPad Software Inc., San Diego, CA, USA) and all quantitative are expressed as mean $\pm$ standard deviation (SD) of three independent experiments. Statistical significance was assessed using one-way and two-way ANOVA test with Tukey's test. Significance was set at $p < 0.05$. One (*), two (**), and three (***) asterisks are used to indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Results

IL-6 levels in human ovarian cancer cells. IL-6 levels were measured in ovarian cancer cells SKOV3, UWB1.289, and OV75 using ELISA. As shown in Figure 1, ovarian cancer

Figure 1. Interleukin (IL)-6 levels in human ovarian cancer cells.

cells expressed high levels of IL-6 (SKOV3, 191 pg/ml; UWB1.289, 1259 pg/ml; OV75, 4113 pg/ml).

Bazedoxifene combined with talazoparib synergistically inhibited cell viability of human ovarian cancer cells. The effects of bazedoxifene and talazoparib on ovarian cancer cell viability were confirmed and compared to the combination treatment. As shown by MTT assay (Figure 2A), ovarian cancer cell viability was inhibited by talazoparib, bazedoxifene alone, as well as by the combination treatment in UWB1.289 [bazedoxifene (B), 87.3%, B vs. DMSO (D), $p = 0.0033$; talazoparib (T), 77.0%, T vs. D, $p < 0.0001$; B+T, 60.5%, B vs. B+T, $p < 0.0001$, T vs. B+T, $p = 0.0006$], SKOV3 (bazedoxifene, 54.4%, B vs. D, $p < 0.0001$; talazoparib, 78.6%, T vs. D, $p < 0.0001$; B+T, 42.9%, B vs. B+T, $p = 0.0003$, T vs. B+T, $p < 0.0001$) and OV75 cells (bazedoxifene, 53.5%, B vs. D, $p < 0.0001$; talazoparib, 75.4%, T vs. D, $p = 0.0009$; B+T, 36.2%, B vs. B+T, $p = 0.0081$, T vs. B+T, $p < 0.0001$). Moreover, combination treatment induced greater inhibition of cell viability compared to monotherapy. Furthermore, the combination index (CI) of the two drugs was found to be <1 (UWB1.289, CI=0.4381; SKOV3, CI=0.80235; OV75, CI=0.35628), demonstrating a synergistic effect of the two drugs.

According to our previous work, bazedoxifene is an inhibitor of IL-6, which requires IL-6 receptor and GP130 binding to form a signaling complex that activates target genes (22, 23, 35). Thus, GP130 knockdown can inhibit cell viability. GP130 was knocked down by siRNA transfection, followed by MTT assay. The results showed that the cell viability of ovarian cancer cells was inhibited by GP130 knockdown (Figure 2B). When talazoparib was added, the combination treatment demonstrated a greater inhibitory effect than monotherapy in UWB1.289 [GP130-siRNA,

Figure 2. Effects of talazoparib, bazedoxifene, and their combination on cell viability. MTT assays were performed to evaluate cell viability. Cell viability of ovarian cancer cells was synergistically inhibited by the bazedoxifene and talazoparib combination (A). Transfection with GP130 siRNA followed by MTT assays (B). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

64.4%, GP130-siRNA(G) vs. Control-siRNA(C), $p = 0.0002$; talazoparib, 71.9%, T vs. C, $p = 0.0009$; T+G: 46.4%, T vs. T+G, $p = 0.0016$, G vs. T+G, $p = 0.0132$] and SKOV3 cells (GP130- siRNA, 80.5%, G vs. C, $p = 0.0054$; talazoparib, 80.7%, T vs. C, $p = 0.0057$; T+G: 55.9%, T vs. T+G $p = 0.0010$, G vs. T+G $p = 0.0012$).

Bazedoxifene combined with talazoparib inhibited cell migration of human ovarian cancer cells. The effects of bazedoxifene and talazoparib on cell migration were investigated using the wound healing assay on the SKOV3 cell line, but not UWB1.289, due to its monolayer phenotype unsuitable for this assay. Our results (Figure 3) showed that both bazedoxifene and talazoparib monotherapy inhibited cell migration of SKOV3 and the combination of these two drugs induced a greater inhibitory effect than monotherapy (SKOV3: bazedoxifene, 65.2%, B vs. DMSO, $p < 0.0001$; talazoparib, 61.5%, T vs. DMSO, $p < 0.0001$; B+T: 22.4%, B vs. B+T, $p < 0.0001$, T vs. B+T, $p < 0.0001$).

Bazedoxifene combined with talazoparib inhibited cell growth of human ovarian cancer cells. Given that the combination of bazedoxifene and talazoparib synergistically inhibited the cell viability of ovarian cancer cells, we further analyzed the effect of the two drugs on cell growth. Our results (Figure 4) showed that either bazedoxifene or

talazoparib inhibited cell growth of ovarian cancer cells and the combination of the two drugs demonstrated a greater inhibitory effect than monotherapy in UWB1.289 (DMSO, 265.7×10^3 ; bazedoxifene, 81.3×10^3 , B vs. D, $p < 0.0001$; talazoparib, 50.3×10^3 , T vs. D, $p < 0.0001$; B+T: 9.0×10^3 , B vs. B+T, $p < 0.0001$, T vs. B+T, $p < 0.0001$) and SKOV3 cells (DMSO, 114.3×10^3 ; bazedoxifene, 63.0×10^3 , B vs. D, $p < 0.0001$; talazoparib, 82.0×10^3 , T vs. D, $p < 0.0001$; B+T: 29.7×10^3 , B vs. B+T, $p < 0.0001$, T vs. B+T, $p < 0.0001$).

Bazedoxifene combined with talazoparib inhibited cell colony formation of human ovarian cancer cells. Cell colony formation assay was performed to analyze the effect of talazoparib and bazedoxifene on colony formation in ovarian cancer cells, an important stem cell behavior which plays a significant role in tumor recurrence and metastasis (36, 37). The results (Figure 5) showed that talazoparib and bazedoxifene each individually inhibit colony formation of the ovarian cancer cells. The combination of the two drugs demonstrated a greater inhibitory effect than monotherapy of either drug (Figure 5) (UWB1.289: bazedoxifene, 49.4%, B vs. D, $p < 0.0001$; talazoparib, 30.0%, T vs. D, $p < 0.0001$; B+T: 7.2%, B vs. B+T, $p < 0.0001$, T vs. B+T, $p < 0.0001$; SKOV3: bazedoxifene, 45.9%, B vs. D, $p < 0.0001$; talazoparib, 32.1%, T vs. D, $p < 0.0001$; B+T: 25.5%, B vs. B+T, $p = 0.0018$, T vs. B+T, $p = 0.0186$). Thus, talazoparib and

Figure 3. Effects of talazoparib and bazedoxifene monotherapy and combination treatment on cell migration. Wound-healing assays were performed to detect the migration ability of SKOV3 cells after talazoparib, bazedoxifene, combination, or DMSO treatment. *** $p < 0.001$ and **** $p < 0.0001$.

bazedoxifene combination treatment has the potential to prevent ovarian cancer recurrence and metastasis.

Bazedoxifene combined with talazoparib synergistically inhibited the expression of targeted genes in human ovarian cancer cells. In order to investigate the possible pathways of interaction between talazoparib and bazedoxifene, western blot analysis was performed by us. According to our results (Figure 6), the expression of p-AKT, c-Myc, p-ERK and ER α was inhibited by the combination of the two drugs, while γ H2AX expression, which is a biomarker for DNA double-strand breaks (38), was induced. The p -values of DMSO groups compared with B+T groups were less than 0.05 (SKOV3: p-AKT, $p = 0.0436$; c-Myc, $p = 0.0087$; p-ERK, $p = 0.0003$; ER α , $p = 0.0332$; γ H2AX, $p < 0.0001$. UWB1.289: p-AKT, $p = 0.0179$; c-Myc, $p < 0.0001$; p-ERK $p = 0.001$; ER α , $p < 0.0001$; γ H2AX, $p = 0.0158$).

Discussion

Ovarian cancer is one of the most common tumors in women in USA and has a high recurrence and mortality rate (3). More than half of patients are diagnosed with advanced ovarian cancer and there are approximately 75% of ovarian cancer patients will relapse due to intrinsic and acquired chemotherapy resistance, which lead to cancer recurrence. Thus, it is necessary to develop new treatment methods and drugs. Targeted therapies have been studied in ovarian cancer, demonstrating improved therapeutic efficacy and minimized side effects (39). Therefore, ovarian cancer research has been focused on discovering new targeted therapies (40).

PARPs are new class of antitumor therapy agents; they lead to the formation of double-strand breaks at replication forks, due to an accumulation of DNA single-strand breaks

Figure 4. Effects of talazoparib, bazedoxifene and drug combination on cancer cell growth, $**p<0.01$ and $****p<0.0001$. The cell growth of ovarian cancer cells was synergistically inhibited by combination treatment with bazedoxifene and talazoparib.

(41). PARPis have already been used as maintenance therapy in the treatment of BRCA-mutant ovarian cancer with good efficacy and a favorable side effect profile as well (5, 6, 42, 43). However, the majority of patients present with BRCA-proficient disease and are not eligible for this important class of drug (44). Therefore, researchers have been focusing on the use of PARPis in ovarian cancer with wild-type BRCA status.

During the last decade, the clinical use of PARPis has been further expanded to ovarian cancer patients without BRCA mutations. Olaparib (45) and niraparib (46) were approved by FDA as the maintenance therapy for complete or partial platinum-sensitive ovarian cancer, irrespective of the the BRCA status. Lynparza (47) and rucaparib (48) were also approved as maintenance treatment regardless of BRCA status. Additionally, it has been found that the combination of DNA methyltransferase 1 inhibitor (DNMTi) and talazoparib inhibited breast and ovarian cancer cells harboring either wild-type or mutant BRCA (44). Cyclin D1 silencing

or AKT inhibitors have been shown to sensitize BRCA1 wild-type ovarian cancer cells to olaparib (14, 15), and the combination of carboplatin and olaparib demonstrated synergistic effect beyond BRCA-dependent synthetic lethality in high-grade serous ovarian cancer cell lines (49). Taken together, all these findings demonstrate that the combination of PARPis with other drugs/inhibitors may expand their application in ovarian cancer with wild-type BRCA status. However, DNMTi (44), cyclin D1 silencing and AKT inhibitors (14, 15) have not yet been approved by the FDA, while the use carboplatin is limited due to its side effects and drug resistance (50).

Bazedoxifene has been approved by the FDA as a third-generation selective estrogen receptor modulator for the prevention of postmenopausal osteoporosis, showing minimal side effects (26). Moreover, it acts as an IL-6 inhibitor that downregulates the IL6/STAT3 pathway and downstream target genes, such as p-AKT, c-Myc, p-ERK (4, 18). Bazedoxifene also inhibits the expression of ER α , which

Figure 5. Cell colony formation assay after treatment with varying concentrations of talazoparib and bazedoxifene monotherapy, combination treatment, or DMSO. The combination of bazedoxifene and talazoparib synergistically inhibited colony formation of ovarian cancer cells. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.0001$.

interplays with the IL-6 pathway (29). According to previous studies, bazedoxifene has shown antitumor activity in many cancer types, such as breast cancer (23), colon cancer (30), head and neck cancer (25) and gastrointestinal cancer (51). However, there are few studies on its application in ovarian cancer.

In the present study, the combination of talazoparib and bazedoxifene was studied in BRCA wild-type as well as BRCA-null ovarian cancer cell lines, *in vitro*. Talazoparib, is a novel FDA-approved PARP inhibitor, which additionally exhibits tumor growth suppression activity through other pathways, such as sex hormone, ERK, and AKT pathways (13). The results showed that cell viability, growth, migration and colony formation were inhibited by talazoparib and bazedoxifene monotherapy, while combination treatment demonstrated a greater inhibitory effect than monotherapy; these findings were also confirmed by western blot. Cell stemness is related to tumor recurrence and metastasis (36,

37) and cell migration is a crucial step in cancer cell metastasis (52, 53). Therefore, bazedoxifene and talazoparib combination treatment may have the potential to prevent ovarian cancer cell recurrence and metastasis.

Conclusion

The combination of IL-6 and PARP inhibitors induced synergistic inhibition of cell viability, cell growth, migration, and colony formation in ovarian cancer cells, regardless of BRCA mutation status. In addition, γ -H2AX expression was induced by the two drugs, which demonstrates that combination treatment induced double-strand breaks. Thus, this IL-6 inhibitor and PARPi combination has the potential to be a novel combination targeted therapy against ovarian cancer regardless of BRCA mutation status. Further research is warranted to investigate this drug combination as a candidate for targeted anti-cancer therapies.

Figure 6. Bazedoxifene combined with talazoparib synergistically inhibited the expression of IL-6 pathway and DNA repair downstream target genes in human ovarian cancer cells. SKOV3 and UWB1.289 cells were treated with talazoparib and bazedoxifene monotherapy, combination treatment, or DMSO. Levels of p-STAT3, p-AKT, p-ERK, c-Myc and γ-H2AX expression were determined by Western blot analysis (B+T group/DMSO control). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Ruijie Zhang and Jiayuh Lin conceived the original idea. Jiayuh Lin supervised the project. Ruijie Zhang carried out the experiment and wrote the manuscript with support from Jiayuh Lin. Tiffany Wang contributed to data analysis and editing the manuscript.

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