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Alterations in DNA Damage Response Pathways Underlying Acquired Resistance to Olaparib: Evidence from In Vitro Models

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ABSTRACT
DDR is a multifaceted system of cellular pathways that detect signal and repair DNA damage to preserve genome reliability. This mechanism is crucial in cancer biology, as deficiencies in DDR pathways often direct to tumor development and influence treatment responses. A PARPi, Ola, is frequently used for targeted OC, particularly in patients with BRCA2-mutated HR repair defects. OR often develops and reduces the drug's therapeutic efficacy. This research investigates the way the combination of Ola with medications targeting the ATM/WEE1 pathway increases its cytotoxicity in OROC cells with BRCA2 degeneration mutations. The antitumor effects of Ola alone and combined with these inhibitors are estimated in OC cell lines that are either sensitive (PEO1, PEO4) or resistant (PEO1-OR) to Ola. The BRCA2MUT PEO1 cells are used to create the PEO1-OR cell line. The appearance of 15 proteins linked to the DDR is adjusted using antibody microarrays. The findings showed that in both sensitive and resistant OC cells, Ola combined with ATMi or WEE1i dramatically decreased cell feasibility, and clonogenic survival, and triggered apoptosis, as evidenced by caspase-3/7 activation. The face of DDR-connected proteins concerned in DNA repair and cell cycle regulation changed significantly as a result of these combo therapies. The Ola-induced overexpression of proteins that control cell destiny following DNA damage is reversed in PEO1-OR cells when ATMi and WEE1i are present. Overall, OR to PARPi is successfully reversed by the addition of ATM or WEE1 inhibitors, which also changed the expression of important DDR-related proteins and had anti-proliferative effects on BRCA2MUT OROC cells. These results provide a potential method to circumvent OR in OC therapy by shedding light on the cellular response to a combination of Ola and ATM/WEE1 pathway inhibitors.

Keywords: *Olaparib, PARP Inhibitor, Ovarian Cancer, DNA Damage Response, ATM/WEE1 Inhibitors, BRCA2 Reversion, Drug Resistance.*

1. INTRODUCTION

OC is a common and fatal cancer of the reproductive system. Numerous OC cases are identified in the complex phases due to a lack of indications in the early periods and effective analyzing measures for pre-cancerous injuries [1]. Paclitaxel in conjunction with carboplatin is the first-line chemotherapy strategy for complex phase OC. Nevertheless, 70% of individuals with medium and severe points who received operation and chemotherapy lived < 5years following analysis [2]. Tumor-linked treatment has attained significant achievement and is progressively becoming a baseline action tactic due to its

exactness, efficiency, and comparatively low toxicity. There are several effective targeted managements for OC treatment, with PARPi being the most common and hopeful therapy [3]. PARPi has demonstrated a strong benefit in BRCA-mutated OC by reducing BER and progressing DNA-SSB to DSB by impeding the HR repair trail, resulting in genomic insecurity [4]. BRCA1 and BRCA2 are two essential tumor recessive genes that repair DNA DSB via the HR path. Traditional researches indicate that BRCA1 appearance is inversely related to sequence-free endurance and long-term endurance in epithelial OC [5]. Whilst both PARP and BRCA1/2 are suppressed, cell death arises dramatically. As a result, PARPi is significantly less efficient as mono-therapy for BRCA wild-type OC individuals. According to the OC patient genetic material plot, roughly 59% of all epithelial OC and 50% of all HGSOC yet express BRCA1/2 [6]. Several researches have shown that rising DNA breaks in malignancy cells and hindering HR restore paths are the primary processes for enhancing the therapy efficiency of Ola in BRCA1 wild-type OC [7]. PK catalyzes glycolysis's last permanent step, which increases the adaptation of $C_3H_5O_6P$ to $C_3H_4O_3$ and controls the instability of $C_{23}H_{38}N_7O_{17}P_3S$ into the $C_6H_8O_7$ -supported phase. The four PK subtypes are M1, M2, R, and L, with PKM2 being the common typically over-articulated malignancy [8]. Specifically, OC has been demonstrated to exhibit metabolic alteration to aerobic glycolysis, allowing it to preserve an elevated regenerative ability and endure beneath port-reliant settings [9]. Nevertheless, whether PKM2 presents an important function in the modulation of Ola sensitivity in OC cells has to be determined. Handling methods for BRCA wild-type OC individuals require additional investigation [10]. The research aims to evaluate whether combining the PARP inhibitor Ola with ATM or WEE1 inhibitors enhances cytotoxicity in OR OC cells with BRCA2 degeneration mutations. By analyzing the effects of these combination therapies on cell viability, clonogenic survival, apoptosis induction, and DDR-related protein expression, the research also aims to identify a potential tactic to beat OR and enhance treatment effects in BRAC2-mutated OC.

2. RELATED WORKS

This section summarizes the existing research based on DDR-targeted therapies, PARPi resistance mechanisms and combinational approaches in OC treatment.

Biegała et al., (2023) observed the OR in OC by creating an OR cell line (PEO1-OR) using BRCA2MUT PEO1 cells. Whole-exome sequencing revealed resistance through BRCA2 secondary mutations that restore HR repair. ATR/CHK1 inhibitors have poor efficacy, although combined therapy sensitized resistant cells. One of the limitations was partial drug sensitivity.

Biegała et al., (2023) evaluated the arrangement of Ola, a PARP inhibitor, with ATRi/CHK1i (ceralasertib and MK-8776) in OC cells that were resistant to Ola. The combination increased anticancer efficacy, overcoming resistance and changing DDR-related protein expression. Limitations include the necessity for additional in vivo validation of findings.

Biegała et al., (2025) improved the efficacy of Ola in resistant OC. A patient-derived xenograft model was used to examine the results of ATRi/CHK1i in arrangement with Ola. The findings revealed synergistic tumor growth inhibition. Limitations include the need for a better knowledge of resistance mechanisms in vivo before broadening clinical application.

Gajan et al., (2021) examined the efficacy of PARPi and platinum-based medicines in TNBC with BRCA1/2 deletion. The research discovered that OR TNBC cells retain cisplatin sensitivity despite altered DNA repair mechanisms using Ola and cisplatin combinations. However, the findings were limited to certain TNBC cell lines, making wide applicability dubious.

Chiappa et al., (2022) investigated the molecular pathways underlying PARPi resistance in OC. The research developed OR cell lines in both HR-scarce and HR-capable environments. The results demonstrated MDR1 overexpression and HR reactivation in HR-deficient cells. Ola was found to synergize with ATR, Chk1, and Wee1 inhibitors. Limitations include the need for more clinical validation.

Cahuzac et al., (2022) investigated PARPi resistance in PC by using Ola-sensitive and resistant PC cell lines. Microarray examination revealed 195 positive-synchronized and 87 negative-synchronized genes, with pathways associated with DNA damage, cell cycle, and implicated auto-phage. ROCK2, BRCC3, and ATG2B were chosen for validation. Limitations include the necessity for more in-vivo validation.

Bouberhan et al., (2023) investigated therapeutic methods for epithelial OC with chemotherapy, PARPi, and novel DDRi like ATRi, CHK1i, and WEE1i. It emphasized preclinical reasons, clinical advancements, and the efficacy of solo and combination therapies. Current treatments have produced inadequate or short-lived results.

Sun et al., (2023) explored the role of MARVELD1 in DDR and how it interacted with PARP1. MARVELD1 acetylates PARP1, resulting in a positive feedback loop critical for genomic stability. MARVELD1 knockout animals exhibit genomic instability, and the MARVELD1-PARP1 connection caused resistance to genotoxic medicines, reducing PARP inhibitor efficacy in colorectal cancer. Limitations include the need for additional in vivo analysis.

Chiappa et al., (2024) determined whether inhibiting Polo-like kinase 1 (PLK1) was sensitizing tumor cells to Ola in OC. Onvansertib and Ola were examined in vitro and in vivo, and the results revealed synergistic effects, DNA damage, and enhanced survival in OR mice. Limitations include a lower efficacy in wild-type models.

Staniszewska et al., (2022) examined the effect of Ola coupled with ICB on anti-tumor efficacy and the immunological background in BRCA1/2 mutant animals. The combination demonstrated enhanced anti-tumor effectiveness, higher immune cell populations, and immune pathway regulation. Limitations include the use of preclinical models and patient sample analysis.

Significant gaps remain, despite progress in understanding the DDR pathways in acquired OR. The particular molecular processes behind resistance were not entirely understood, and the significance of non-canonical DDR pathways was understudied. Furthermore, the lack of robust biomarkers restricted early identification of resistance, and effective combination techniques to combat resistance that necessitate additional research. Integrating multi-omics methods and functional research was critical for closing these gaps and enhancing treatment options for HGSOC.

3. METHODOLOGY

Chemicals and cell lines were carefully chosen to research OR mechanisms in HGSOC cells. The approach describes the creation of the PEO1-OR resistant cell line, followed by a variety of experimental evaluations, such as MTT and colony formation assays, qRT-PCR, apoptosis detection, and DAR profiling. Statistical analysis is performed to ensure the precision of the effects and validate the effects of combination therapy.

3.1 Chemicals

PARPi, ATMi, and WEE1i have been acquired from Selleck Chemicals. The inhibition stocks are generated in 100% DMSO and kept at -80 °C for 6 months. Gibco supplied RPMI 1640 culture media, HT-FBS, and trypsin-$C_{10}H_{16}N_2O_8$. Solvents and chemicals are sourced from Sigma-Aldrich.

3.2 Cell lines

HGSOC cell lines are procured from ECACC and processed for cryopreservation after the improvement and culture growth. The PEO1-OR cell line is generated from PEO1 via continuous Ola treatment. HGSOC cell lines are grown as mono-layers in RPMI 1640 culture media with GlutaMAX, HEPES, and 10% HI-FBS. HEPES without $C_3H_3NaO_3$ is also provided with 10% HI-FBS. Cell lines are grown at 37 °C in humid conditions with 5% CO_2.

a) Development of PEO1-OR cell line

The PEO1-OR cell line is produced from parental PEO1 cells by gradually increasing Ola concentrations (10-80 µM) over 20 days. The initial doses are calculated using the IC_{50} value from a dose-response curve. To ensure long-term resistance, cells are grown without Ola for 72 days. The MTT assay is used to measure resistance regularly, with IC_{50} values comparing PEO1-OR to PEO1. Following the establishment, the cell line is grown, cryopreserved, and frozen in liquid nitrogen. Fresh cultures are revived every 2-3 months for biological tests in an Ola-free medium.

3.3 Experimental analysis

This section describes the experimental methods used to evaluate Ola and ATM/WEE1 inhibitors in OC cells. MTT and colony formation assays evaluated cell viability and proliferation. qRT-PCR examined DDR gene expression, whereas caspase-3/7 tests and Annexin V-FITC/PI staining assessed apoptosis. The expression of DDR-related proteins is investigated utilizing an antibody microarray. These approaches explain the biological response to combination therapy, and allow a better understanding of their impact on overcoming OR in OC cells.

a) Cell viability assay

The MTT technique that assesses responses to the tested inhibitors is completed using a cell viability assay. PEO1, PEO1-OR, and PEO4 cells are planted at varying densities in 96-well plates for 2- and 5-day treatments, then incubated at 37 °C with 5% CO_2. Following treatment, MTT solution is further for 4h, and the Formazan crystals are suspended in DMSO. The saturation is calculated at 580 nm and 720 nm utilizing a micro-plate reader. Corrected absorbance values are used to calculate virtual cell feasibility as a proportion of natural controls. The experiment is conducted four times (n=4), with three scientific replicates per plate. The CDI is used to assess medication interactions.

b) Colonoscopy assay

Cells (8×10^3 cells/well) are equally distributed on a 24-well plate and treated with various medicines for 7 days. Following treatment, the culture medium is isolated, and the cells are quietly rinsed twice with PBS. The cells are then set with 10% formalin for two hours. After fixation, a 0.1% crystal violet solution is applied to stain the plate, which is then incubated for 2 hours. Finally, the stained colonies are quantified by computing absorbance at 550 nm with a microplate reader.

c) qRT-PCR Analysis

The research used qRT-PCR to profile the gene expression of ATR, CASP3, CHEK1, and PARP1 in OC cells. After 48 hours of management with Ola and ATM/WEE1 inhibitors, cells are collected. Total RNA is then removed utilizing the mmRNA-IT with phenol. The RNA quality and quantity are assessed utilizing absorbance controls at 230, 260, and 280 nm employing a BioTek Eon™ spectrophotometer, and samples are kept at −80°C. cDNA production is executed utilizing the

high-ability cDNART Kit with RNase suppressor in a PTC-200 DNA Engine® Cycler. qRT-PCR is performed using predesigned TaqMan™ Gene Expression Assays on a Rotor-Gene Q 5plex HRM system. Gene expression is estimated using the $2^{-\Delta\Delta Ct}$ technique, with findings provided as mean ± SD from 3-4 sovereign researches, each completed in replacement or triplicate for accuracy.

d) Caspase 3/7 activity assay

Apoptosis is assessed using CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit in OC cells. Cells are sowed, incubated, and treated with inhibitors or combinations for 48 hours, with 2.5 µM CPT as an optimistic organization. Cells are harvested, blemished with caspase-3/7 discovery solvent and SYTOX™ Dead Cell Stain, and analyzed using a BD™ LSR II flow pyrometer, gathering 10,000 occasions per second.

e) Cell apoptosis

The Annexin V-FITC Apoptosis Kit is employed to perceive cell apoptosis. Treated cells are dissolved in obligatory buffer and blemished with 5 µl Annexin V-FITC/5 µl PI for 20 min in the shade. The fluorescence signals are employed to identify apoptotic and necrotic cells. A FACS Calibur flow cytometer is used to determine the proportion of apoptotic cells.

f) DNA damage response array

The RayBio® C-Series Human DDR Antibody Array is utilized in the research to compare the phase of 17 DDR-connected proteins in PEO1, PEO4, and PEO1-OR OC cells. Cells are soaked with Ola and ATM/WEE1 inhibitors for 48 hours before being lysed and extracted for protein. The BCA assay is used to determine protein content and antibody arrays are processed with biotinylated antibodies and HRP-streptavidin before being detected with the Azure 300 imaging system. The experiment included two independent biological replicates (n = 4) as well as technical replicates to assure accuracy.

3.4 Statistical analysis

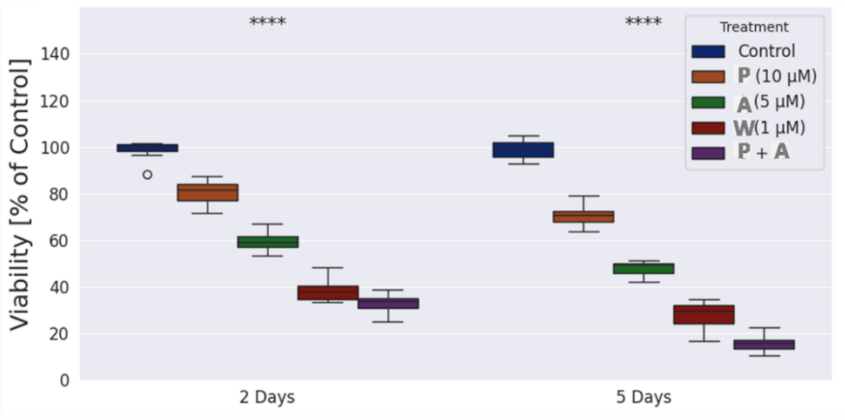
The data is evaluated with IBM SPSS 26.0, which uses the Kolmogorov-Smirnov test to measure ordinariness and Levene's test to determine variance uniqueness. Continuous variables such as cell viability, clonogenic survival, and apoptosis levels are evaluated among cohorts utilizing one-way ANOVA, and the Games-Howell post-hoc examination for pair-wise evaluations where variances differ. Changes in DDR-related protein appearance stages are evaluated utilizing one-way ANOVA. A p-value < 0.05 specifies numerical consequence.

4. RESULT

This research investigates the results of combining Ola with ATMi and WEE1i in HGSOC cells. The findings include investigations into cell viability, clonogenic survival, apoptosis, caspase activation, gene expression, dose-response correlations, and DNA damage response. Experiments are carried out on PEO1, PEO1-OR, and PEO4 cell lines to assess cytotoxic and apoptotic effects. The findings shed light on the impact of dual-targeted therapy on OROC cells. According to the result, PARPi (P), ATMi (A), and WEE1i (W) are assessed individually, while combination treatments are represented as P+A for PARPi with ATMi and P+W for PARPi with WEE1i.

4.1 Cell viability assay

The research evaluated that integrating Ola with ATM/WEE1 pathway inhibitors (ATMi/WEE1i) increases cytotoxicity in PEO1-OR cells. Statistical significance is evaluated using Kolmogorov-Smirnov test and one-way ANOVA. HGSOC cells are delighted for 2 and 5 days with 10 µM Ola, 5 µM ATMi, and 1 µM WEE1i, while PEO1-OR cells are regarded with 555 µMola, as depicted in Figure 1.



a) PE01

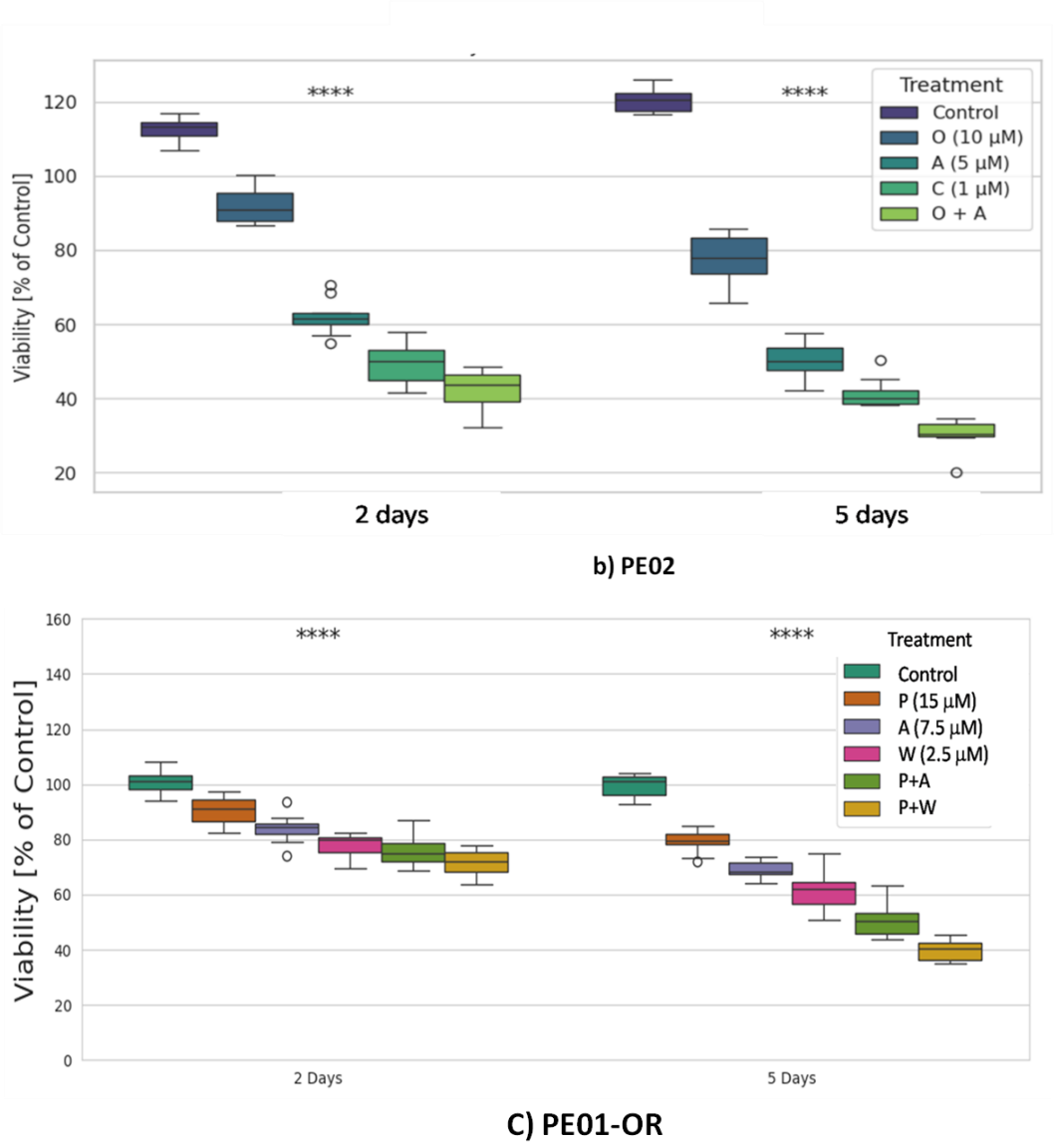


Figure 1: Effect of Olaparib and ATM/WEE1 Inhibitor Combinations on OC Cell Viability

Cell viability significantly decreased over 2 and 5 days in all treatment groups, as shown in Figure 1. The combination therapies (P+A, P+W) had the strongest cytotoxic effects ($p < 0.0001$). Ola, ATM, or WEE1 inhibitors are examples of single-agent treatments that decrease viability, although their effectiveness is lower than that of dual inhibitor combos, suggesting partial resistance when used alone. The prolonged effect of combination therapies is further highlighted by the time-dependent decline in viability. These findings point to a potential treatment approach to improve PARPi efficacy by indicating that the addition of ATM or WEE1 inhibitors successfully reverses OC cells' OR.

4.2 Clonogenic survival

The capacity of a cell to multiply and establish colonies following therapy is measured by clonogenic survival. The long-term survival of cell lines is assessed using a colony formation test. The data is evaluated using one-way ANOVA. The ability of a cell to divide into colonies, which indicates survival and proliferation, is measured by colony formation efficiency. This research evaluated how OC cell lines are affected by Ola, ATM, and WEE1 inhibitors. Increased cytotoxicity and possible efficacy in overcoming OR in the PEO1-OR cell line are indicated by decreased efficiency in treated cells, particularly when combination inhibitors are used, as demonstrated in Figure 2.

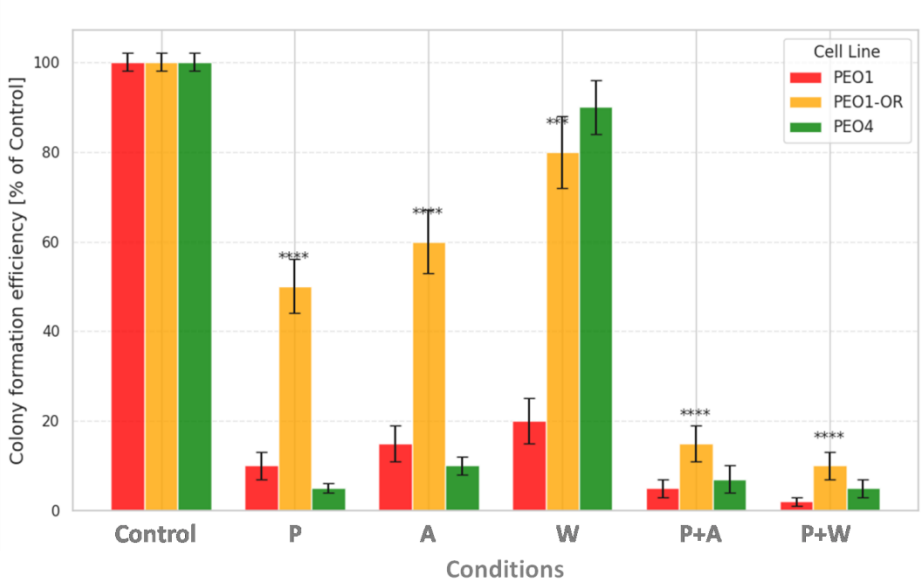


Figure 2:Effect of Olaparib and ATM/WEE1 Inhibitor Combinations on Colony Formation Efficiency in OC Cell Lines

The results show that the OC cell lines exhibit considerably lower colony formation efficiency when Ola is combined with ATM (P+A) or WEE1 (P+W) inhibitors. The most significant effects are shown in the cell lines. The cell line is susceptible to Ola and exhibits marked sensitivity to combination treatments, especially P+W, suggesting a strong synergistic or additive effect, even though single treatments with Ola (P), ATM inhibitor (A), or WEE1 inhibitor (W) reduce colony formation to varying degrees. The combination therapies are significantly altering the PEO4 cell line, indicating either different resistance mechanisms or varying sensitivity. Overall, the findings show that OR in OC cells, especially in the PEO1-OR cell line, is overcome by combining Ola with ATM or WEE1 inhibitors.

4.3 Cell apoptosis

The efficiency of treatments (P, A, W, P+A, P+W) on distinct cell lines (PEO1, PEO1-OR, and PEO4) is evaluated by measuring apoptosis. Using annexin V-FITC/PI labelling, and flow cytometry, the quantity of dead cells is measured. Statistical significance is examined using one-way ANOVA. The effectiveness of each therapy in inducing apoptosis across the various cell lines is assessed by comparing these percentages to untreated control cells, as represented in Figure 3. Figure 4 represents the dot plots of annexin V- with the shown proportions of apoptosis.

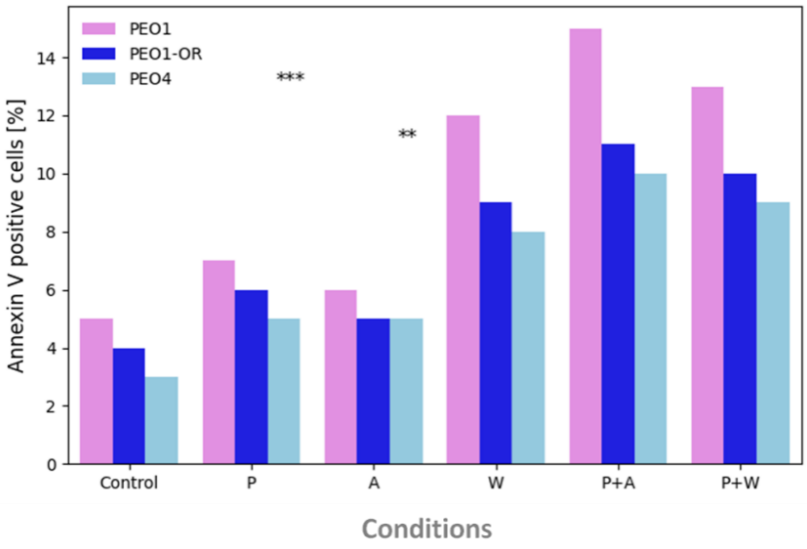
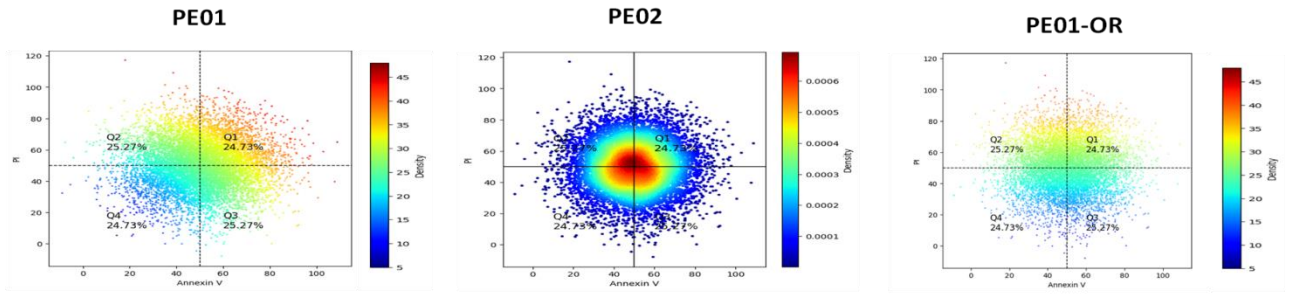
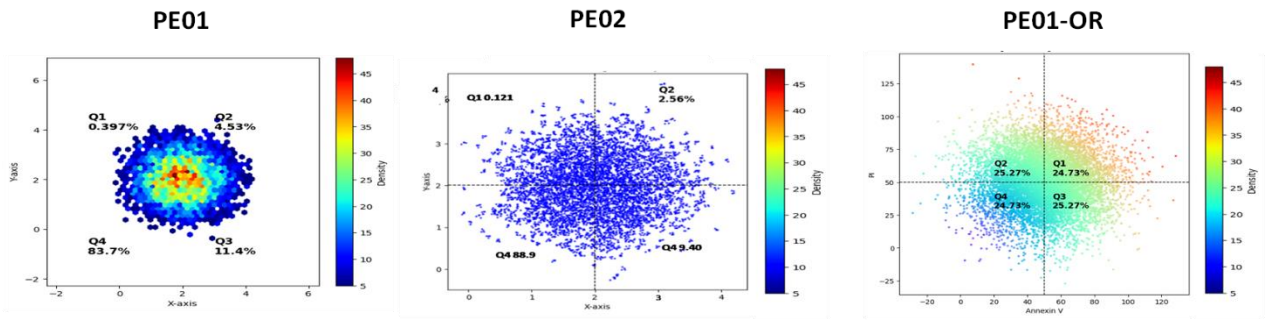


Figure 3:Comparative Analysis of Apoptosis Induction across Cell Lines under Various Treatments

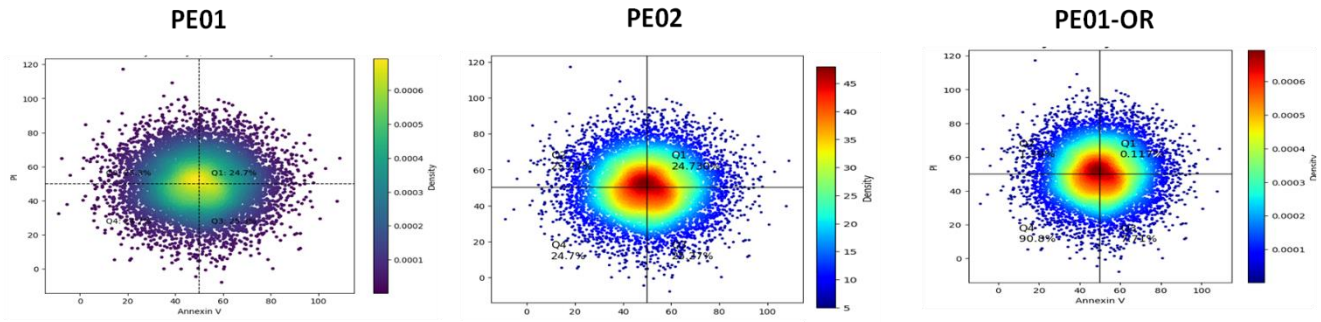
Annexin V-positive cells (%) for cell lines are shown in Figure 3 for each of the treatments (P, A, W, P+A, P+W). As evidenced by increased annexin V positivity, treatment W exhibits a substantial increase in apoptosis for PEO1. Interestingly, P+A and P+W treatments further promote apoptosis, especially in PEO1, indicating that combinatorial approaches are superior to single therapies. In comparison to PEO1-OR and PEO4, PEO1 is the most sensitive to treatment-induced apoptosis. This demonstrates how combined therapy helps specific cell lines overcome resistance and enhance their apoptotic response. Statistical significance is analyzed using Games-Howell post-hoc examination.



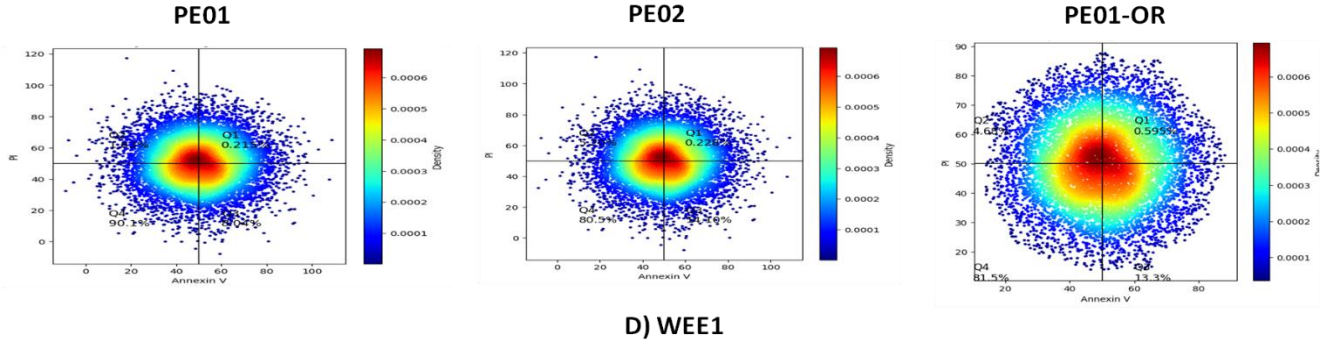
a) Control



b) PARPi



C) ATM



D) WEE1

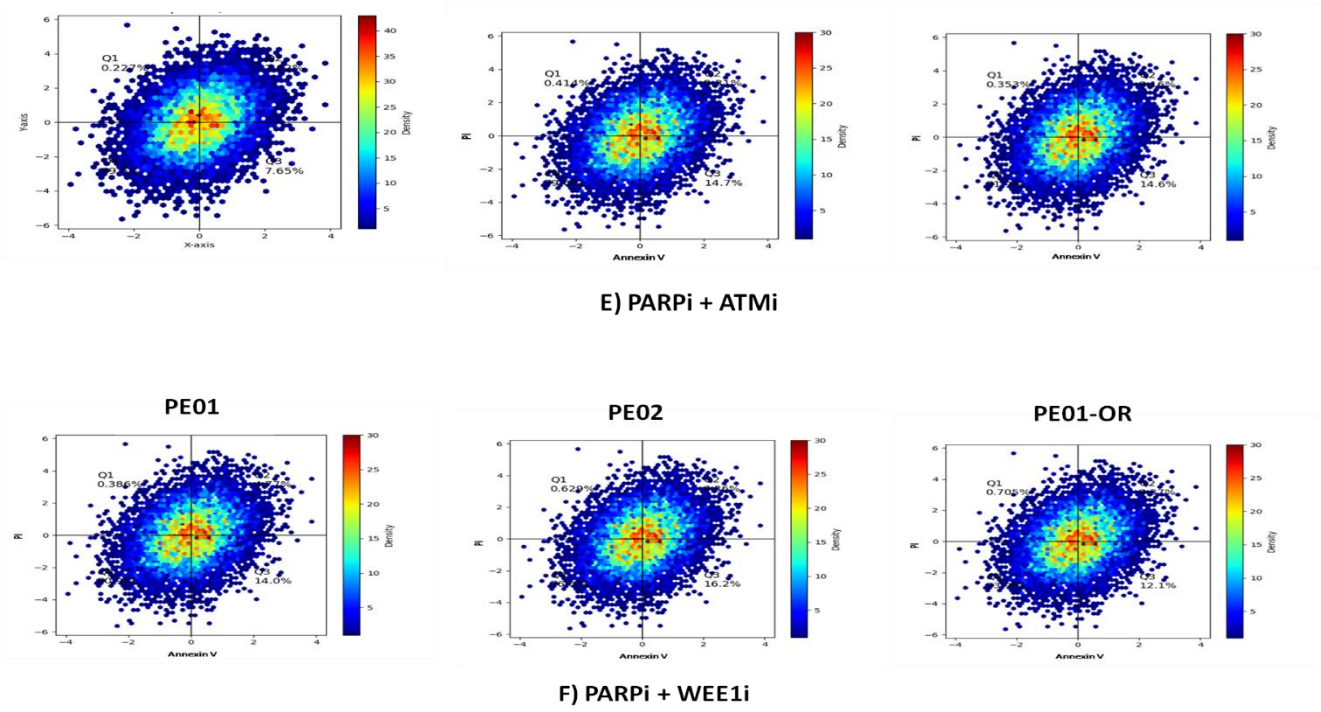


Figure 4:Apoptotic Responses across Different Treatments a)Control, b) PARPi, C) ATM, d) WEE1, e) PARPi + ATM and PARPi + WEE1.

The results show that under different treatment circumstances, both early and late apoptosis significantly increased. Increased apoptotic cell populations are observed in treatments employing PARPi and ATMi, especially when used in combination (PARPi + ATMi). In contrast to individual treatments, combination therapies have the potential to increase therapeutic efficacy and provide a promising approach to targeted cell death. This shows that combination therapies are more effective in triggering apoptosis through both early and late phases.

4.4 Caspase 3/7 activity

Through the detection of the cleavage of particular substrates, which signifies cell death, caspase 3/7 activity quantifies apoptosis. In this research, OC cells treated with Ola and ATM/WEE1 inhibitors had their apoptosis measured. Figure 5 shows that the effectiveness of treatment is confirmed by the correlation between apoptosis and caspase 3/7 activity.

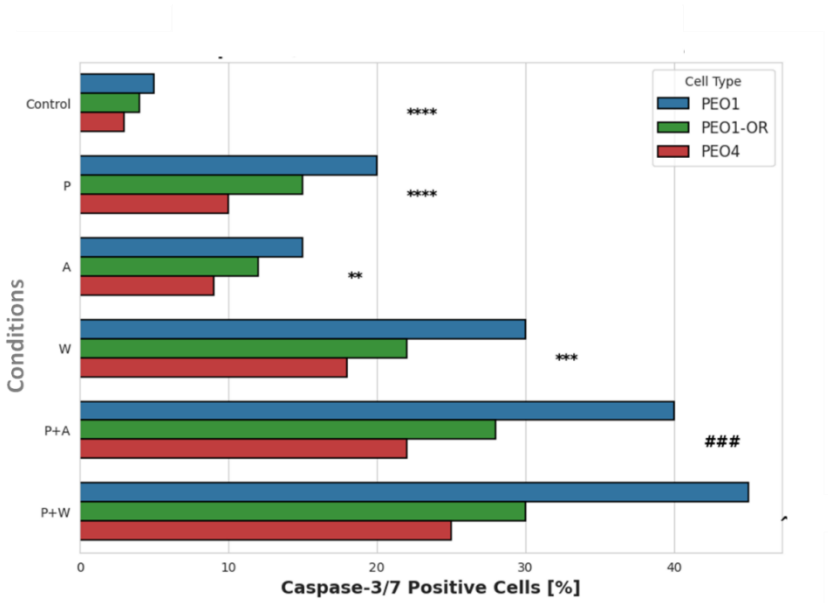


Figure 5: Caspase-3/7 Activation across Different Treatment Conditions in Cell Lines

The results show the proportion of Caspase-3/7 optimistic cells for cell lines under different treatment circumstances (P, A, W, P+A, P+W). Strong apoptosis induction is suggested by notable increases in Caspase-3/7 activity, especially for PEO1 under P, W, and P+W treatments. Particularly in PEO1 cells, combination therapies (P+A, P+W) typically show increased apoptosis, suggesting increased therapeutic efficacy through combined treatment approaches. The significant rise in Caspase-3/7 activity under W and P+W treatments demonstrates how well they work to induce cell death.

4.5 qRT-PCR analysis

The expression phases of genes linked to apoptosis are assessed using qRT-PCR analysis in a selection of cell lines and treatment scenarios. This approach advances the knowledge of the mechanisms underlying different treatments by assessing how well they induce apoptosis. Figure 6 demonstrates the analysis of CASP3 mRNA expression.

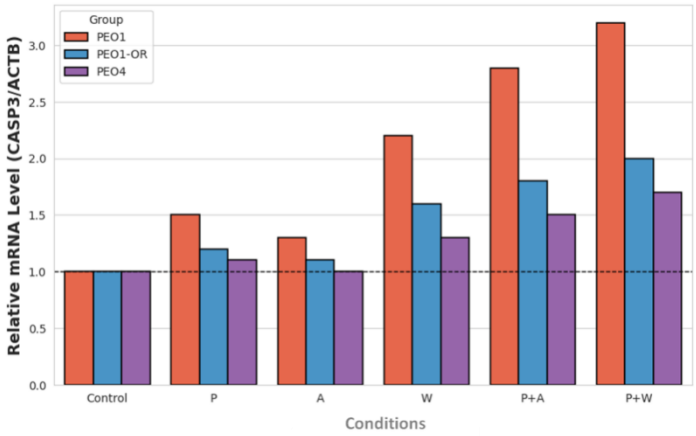


Figure 6:Comparative Analysis of CASP3 mRNA Expression across Different Conditions

The results show that P+A and P+W combo treatments considerably increase CASP3 expression, particularly in the PEO1 cell line, suggesting a strong apoptotic response. Significant CASP3 over-expression is also shown in treatment W alone, indicating that it is a useful apoptotic inducer. The increased expression of CASP3 in combination treatments suggests that they work in concert to promote apoptosis more successfully than when used in isolation. This result highlights the benefit of combinatorial methods in triggering the expression of genes linked to apoptosis, which makes them viable tactics for enhancing treatment effectiveness against resistant cell lines.

4.6 Dose-response estimation

Measuring the biological reaction of cells to different medication concentrations is known as dose-response data. The concentration of a medicine needed to inhibit 50% block of a certain biological function or response in comparison to a control is known as the absolute IC_{50} value. Cell viability is plotted against drug concentration to find the midpoint of the inhibition curve, which yields IC_{50} values. One-way ANOVA is employed to estimate the dose-response among the treatment cohorts with IC_{50} values, as denoted in Figure 7.

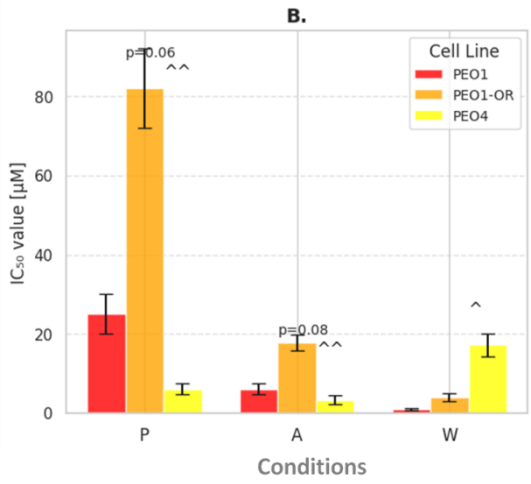


Figure 7: IC_{50} Analysis of Cell Lines across Various Treatments

The cell lines' varying sensitivity to treatments P, A, and W is shown by the IC_{50} values. In contrast to PEO1 and PEO4, which demonstrate higher sensitivity, PEO1-OR shows the highest IC_{50} value following P treatment, indicating stronger resistance. All cell lines show decreased IC_{50} values after treatment A, suggesting increased effectiveness. The sensitivity of treatment W is moderate. The nearly significant p-values point to possible treatment-specific variations that need more research.

4.7 DNA Damage Responses

The DDR antibody array is utilized to identify 15 DDR-linked proteins in PEO1, PEO4, and PEO1-OR OC cells. Protein expression changes following treatment with PARPi, ATM-i, and WEE1-i are measured by densitometric analysis. ATM/WEE1 inhibitors reduced DDR changes in resistant cells, lowering DNA repair protein overexpression associated with OR. Figure 8 demonstrates the DDR modulation and PARPi resistance, with heatmaps comparing DDR signaling in sensitive and resistant OC cells.

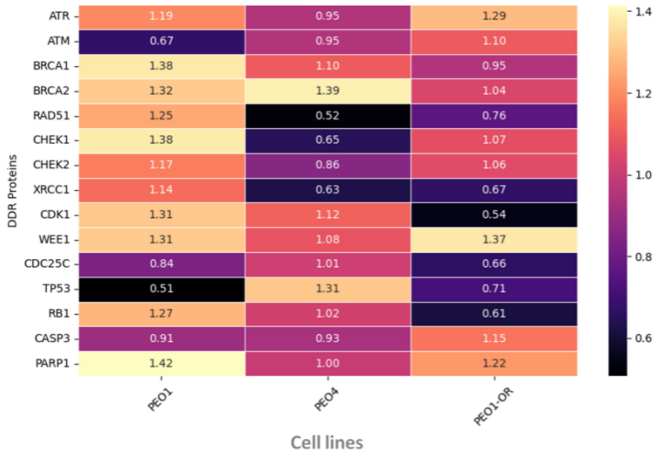


Figure 8: Differential expression of DDR proteins across cell lines

The outcome highlights a variety of DDR pathway activities by showing the variable appearance of DDR proteins across cell lines. Strong homologous recombination repair mechanisms are suggested by elevated BRCA1, RAD51, and PARP1 in PEO1, whereas improved checkpoint signaling and cell cycle regulation are indicated by greater CHEK2 and RB1 levels in PEO1-OR. PEO4 is moderately expressed in the majority of proteins. These differences suggest varied biological reactions to DNA damage, offering information about unique weaknesses and possible targets for DDR-targeted therapies.

4.8 BCA assay

The BCA test measures protein concentration via Cu^{2+} reduction and bicinchoninic acid complex production at 562 nm. Statistical significance is evaluated using Levene's test. It allowed for reliable protein quantification in OC cells treated with Ola, ATM, and WEE1 inhibitors. This standardization is critical for reliable DDR protein expression analysis, which helped assess OR mechanisms and the impact of combination therapy. Figure 9 evaluates the difference in DDR protein appearance across dissimilar cell lines.

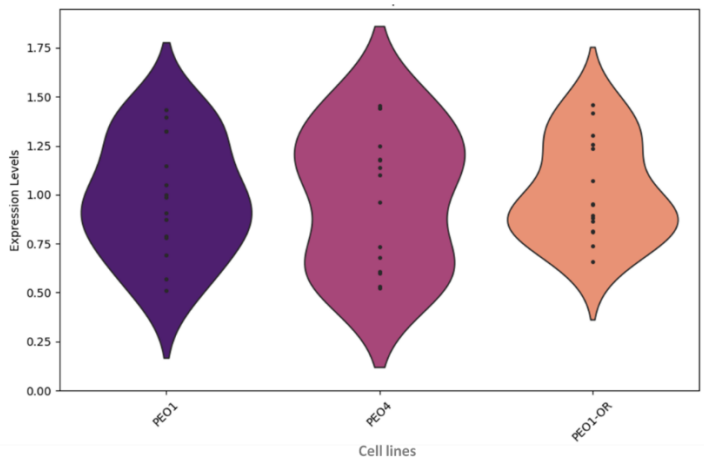


Figure 9: Variation in DDR Protein Expression across Cell Lines

The distribution of DDR protein expression levels among the three cell lines PEO1, PEO4, and PEO1-OR is shown in the result. In contrast to PEO1 and PEO4, PEO1-OR shows a wider range of expression levels, suggesting more variability and heterogeneity. This pattern implies that PEO1-OR has modified DNA damage response (DDR) mechanisms and a drug-resistant cell line. Adaptive responses that improve survival under treatment pressure are connected to such diversity. The variations in expression profiles emphasize how crucial it is to look into DDR pathways to comprehend resistance mechanisms and enhance therapeutic approaches.

5. DISCUSSION

The objective of this research was to measure the potential of combining Ola with ATMi/WEE1i to overcome OR in OC cells by measuring cell viability, clonogenic survival, apoptosis, caspase 3/7 activation, gene expression, dose-response, DDR, and protein quantification in cell lines. The results showed that combination treatments (P+A, P+W) significantly reduced cell viability, inhibited clonogenic survival, and increased apoptosis, with stronger annexin V staining and caspase 3/7 activity, indicating increased apoptotic induction. qRT-PCR revealed CASP3 overexpression in combination-treated cells, whereas dose-response analysis revealed that ATM/WEE1 inhibitors sensitized resistant cells to Ola. DDR analysis revealed unique protein expression patterns, implying that combination therapy modulates DDR pathways to overcome resistance, and BCA protein quantification supported the validity of DDR protein expression analysis. Overall, these findings showed that ATMi/WEE1i improve Ola efficacy by increasing apoptosis, decreasing cell survival, and modifying DDR pathways, indicating their potential as a therapeutic method for overcoming resistance in treatment.

6. CONCLUSION

Analyzing DDR pathways in OR helps to find resistance mechanisms, prospective biomarkers, and therapeutic targets. Understanding DDR changes helps to optimize patient classification, develop combination therapies, and overcome drug resistance, ultimately increasing treatment efficacy and clinical outcomes in HGSOC. The research observed the combination of Ola with ATMi/WEE1i overcomes OR in OC cells by analyzing cell viability, clonogenic survival, apoptosis, caspase activation, gene expression, dose-response, DDR, and protein quantification in the cell lines. The research discovered that combination treatments (P+A, P+W) dramatically reduced cell viability, inhibited clonogenic survival, and increased apoptosis, as evidenced by annexin V staining and caspase 3/7 activation. DDR research revealed that combo therapy alters DDR pathways, making resistant cells susceptible to Ola. The research's shortcomings include its in vitro nature, the lack of in vivo confirmation, and potential variances in patient-derived tumor responses. Finally, the findings demonstrate that ATMi/WEE1i improved Ola efficacy by boosting apoptosis and changing DDR pathways, indicating a possible therapeutic option for overcoming OR in OC that warrants further clinical exploration.

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Acronym	Abbreviation
PARPi	Poly (ADP-ribose) Polymerase Inhibitors
OC	Ovarian Cancer
BRCA2	Breast Cancer 2, Early Onse
HR	Homologous Recombination
ATMi	Ataxia Telangiectasia Mutated Inhibitor
WEE1i	WEE1 inhibitor
OR	Olaparib-resistant
PEO1	Parental Endometrioid Ovarian Cancer 1
PEO4	Parental Endometrioid Ovarian Cancer 4
PEO1-OR	Parental Endometrioid Ovarian cancer 1- Olaparib-resistant
ola	Olaparib
BRCA2MUT	BRCA2 Mutation

DNA	Deoxyribonucleic Acid
DDR	DNA Damage Response
BER	Base Excision Repair
SSB	Single-Strand Breaks
DSB	Double-Strand Breaks
HGSOC	High-grade ovarian cancer
PK	Pyruvate kinase
$C_3H_5O_6P$	Phosphoenolpyruvate
$C_3H_4O_3$	Pyruvate
$C_{23}H_{38}N_7O_{17}P_3S$	Acetyl-CoA
$C_6H_8O_7$	Tricarboxylic acid
Sk	Shikonin
ATR	Ataxia-Telangiectasia and Rad3-Related
CHK1	Checkpoint Kinase 1
MK-8776	Merck-8776
TNBC	Triple-Negative Breast Cancer
MDR1	Multidrug Resistance Protein 1
PC	Prostate Cancer
ROCK	Rho-associated coiled-coil-containing protein kinase
BRCC	BRCA1-BRCA2-Containing Complex
ATG2B	Autophagy Related 2B
MARVELD1	MARVEL Domain Containing 1
PLK1	Polo-like Kinase 1
ICB	Immune checkpoint blockade
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
qRT-PCR	quantitative Reverse Transcription Polymerase Chain Reaction
DMSO	Dimethyl sulfoxide
RPMI 1640	Roswell Park Memorial Institute 1640
HI-FBS	Heat-inactivated FBS
EDTA	Ethylenediaminetetraacetic Acid
Trypsin- $C_{10}H_{16}N_2O_8$	Trypsin-EDTA
ECACC	European Collection of Authenticated Cell Cultures
GlutaMAX	L-alanyl-L-glutamine
HERPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

$C_3H_3NaO_3$	Sodium pyruvate
V-FITC/PI	Annexin V- Fluorescein Isothiocyanate /Propidium Iodide
CO2	Carbon dioxide
PBS	Phosphate-Buffered Saline
mmRNA-IK + phenol	nirvana miRNA Isolation Kit with phenol.
cDNA	Complementary DNA
PTC	Phenylthiocarbamide
RT	Reverse Transcription
CBT	Camptothecin
FACTS	Fluorescence-Activated Cell Sorting
HRP	Health-Related Practices
ANOVA	Analysis of variance
IC_{50}	Half Maximal Inhibitory Concentration

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