

Article

Synergistic Antitumor Activity of Curcumin and the PARP1 Inhibitor PJ34 in Platinum-Sensitive and Resistant Ovarian Cancer Cells

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Simple Summary

Ovarian cancer remains one of the most lethal gynecological malignancies, largely due to the development of resistance to platinum-based chemotherapy and limited responsiveness to subsequent treatment options. Although poly(ADP-ribose) polymerase-1 (PARP1) inhibitors are clinically effective in selected patient populations, their therapeutic efficacy is often reduced in treatment-resistant disease contexts. Curcumin, a natural polyphenolic compound, has been shown to modulate multiple cancer-related signaling pathways and may enhance the activity of anticancer agents. In this study, we investigated the combined effects of the PARP1 inhibitor PJ34 and curcumin and found that this combination exhibits synergistic antitumor activity in ovarian cancer cell models, including cells with experimentally validated reduced responsiveness to platinum treatment. In addition to conventional two-dimensional assays, we employed three-dimensional tumor spheroid models and ROS-rescue experiments to better reflect tumor architecture and to functionally explore potential contributing mechanisms. Our findings show that the combination treatment markedly suppresses tumor cell proliferation and migration while promoting programmed cell death through an ROS-associated but not strictly ROS-dependent process, without inducing excessive oxidative stress. Together, these results provide preclinical, mechanistically informative proof-of-concept evidence supporting PARP inhibitor–curcumin combinations as a complementary strategy for future experimental validation in treatment-resistant ovarian cancer.

Abstract

Background/Objectives: Ovarian cancer remains a highly lethal malignancy, largely due to the development of therapeutic resistance, particularly in advanced disease. Combination strategies targeting complementary molecular pathways may enhance antitumor efficacy and help overcome resistance. The present study aimed to systematically evaluate the anticancer effects of the PARP1 inhibitor PJ34 and the natural polyphenol curcumin, administered alone and in combination, in platinum-sensitive and relatively platinum-resistant ovarian cancer models, with an emphasis on quantitative synergy assessment and functionally supported, hypothesis-generating mechanistic insight. **Materials and Methods:** Cell viability was evaluated using the MTT assay, and IC₅₀ values were derived from dose–response curves. Drug interactions were quantitatively analyzed using the Chou–Talalay method, including combination index (CI) and dose reduction index (DRI) calculations.



Academic Editor: Xavier Sastre-Garau

Received: 22 January 2026

Revised: 9 February 2026

Accepted: 12 February 2026

Published: 13 February 2026

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Intracellular reactive oxygen species (ROS) levels were measured using DCFH-DA-based assays. Cell migration was assessed using scratch-wound assays. Apoptosis was evaluated using Annexin V/PI flow cytometry, caspase-3 activity assays, and quantitative real-time PCR (RT-qPCR) analysis of apoptosis-related genes (Bax, Bcl-2, Caspase-3, Caspase-9, and p53). To further validate the findings under physiologically relevant conditions, three-dimensional (3D) tumor spheroid models were employed, and ROS involvement was functionally interrogated using N-acetyl-L-cysteine (NAC) rescue experiments to assess ROS-associated contributions rather than direct causality. Results: PJ34 and curcumin each reduced cell viability in a dose-dependent manner, whereas their combination produced a synergistic antiproliferative effect with reduced IC₅₀ values. Synergism was particularly pronounced in relatively platinum-resistant SKOV-3 cells. Combination treatment significantly enhanced regulated apoptotic cell death, as demonstrated by increased apoptotic fractions, elevated caspase-3 activity, and an increased Bax/Bcl-2 ratio, with minimal necrosis. While PJ34 moderately increased intracellular ROS levels and curcumin reduced oxidative stress, the combination was associated with the normalization of ROS levels to near-control values. In 3D tumor spheroid models, combined treatment induced marked spheroid shrinkage, loss of structural integrity, and reduced viability, indicating a preservation of synergistic cytotoxic effects beyond two-dimensional (2D) conditions. NAC pretreatment partially attenuated, but did not fully rescue, the cytotoxic effects of the combination, indicating a ROS-associated, but not exclusively ROS-dependent, mechanism of action. In addition, the combination markedly inhibited cell migration in both ovarian cancer cell lines. Conclusions: This preclinical provides evidence that combined PARP1 inhibition and curcumin treatment can exert synergistic antitumor effects in ovarian cancer models, including relatively platinum-resistant disease, through the coordinated suppression of proliferation, induction of regulated apoptosis, and inhibition of migration. The integration of quantitative synergy analysis, 3D spheroid validation, and ROS-rescue experiments provides functionally supported, hypothesis-generating mechanistic insight and supports further evaluation of PARP inhibitor–curcumin combinations as a mechanistic proof-of-concept in advanced preclinical models.

Keywords: PARP1 inhibitor; curcumin; synergy; ovarian cancer; apoptosis; oxidative stress

1. Introduction

Ovarian cancer remains the most lethal gynecological malignancy, with the majority of patients diagnosed at advanced stages (Stage III/IV) [1]. Standard treatment consists of cytoreductive surgery followed by platinum- and paclitaxel-based chemotherapy; however, the frequent development of chemoresistance and disease recurrence continues to pose major clinical challenges [2,3]. Consequently, there is a critical need for therapeutic strategies that can overcome resistance and improve long-term outcomes.

Targeting DNA repair pathways, particularly through the inhibition of ADP-ribose PARP, has emerged as a promising therapeutic approach in homologous recombination-deficient tumors [4]. PARP1 plays a central role in base-excision repair, and the pharmacological inhibition of PARP1 induces synthetic lethality in HR-defective cancers, significantly advancing ovarian cancer treatment [5,6]. PJ34, a potent and selective PARP1/2 inhibitor, is widely used as an experimental tool compound in preclinical studies to investigate the biological consequences of PARP inhibition and has demonstrated anticancer activity across multiple experimental models [7]. Although PJ34 has been reported to inhibit both PARP1 and PARP2, it is commonly used as a PARP1-preferential tool compound in

preclinical research, and the present study therefore focuses on PARP1-centered mechanisms. Moreover, while PJ34 is not clinically approved, its PARP1-selective inhibition profile provides a valuable model for understanding mechanisms that may also be relevant to clinically used PARP inhibitors, such as olaparib and niraparib, within a mechanistic proof-of-concept framework.

In addition to defects in homologous recombination, multiple molecular mechanisms have been implicated in intrinsic and context-dependent refractoriness to platinum-based chemotherapy in ovarian cancer. The dysregulation of DNA damage response signaling, aberrant activation of oncogenic pathways, impaired apoptotic control, and alterations in redox homeostasis have all been shown to contribute to poor platinum responsiveness [8]. For example, PBXIP1 has been reported to promote cisplatin resistance in ovarian cancer by enhancing survival signaling and DNA repair capacity [9]. Similarly, the elevated expression of C/EBP β has been shown to confer resistance to both platinum-based chemotherapy and PARP inhibition in ovarian cancer, mediated in part through the regulation of CDK12-dependent stress-response and survival signaling pathways (e.g., targeting CDK12 rescues C/EBP β -mediated platinum and PARP inhibitor resistance in ovarian cancer) [10]. In addition, the aberrant activation of oncogenic tyrosine kinase signaling, including anaplastic lymphoma kinase, has been implicated in cancer cell survival and resistance mechanisms, and the pharmacological targeting of anaplastic lymphoma kinase has shown promise in preclinical cancer models, underscoring the relevance of multi-targeted strategies to overcome therapeutic resistance in ovarian cancer and other malignancies [11]. Collectively, these findings highlight the multifactorial nature of platinum refractoriness and underscore the need for combination strategies that may simultaneously target DNA repair, survival signaling, and cellular stress-response pathways.

In parallel, natural compounds are increasingly explored for their potential to synergize with conventional anticancer therapies, thereby enhancing efficacy and potentially reducing treatment-associated toxicity [12]. Curcumin, the principal polyphenolic compound derived from turmeric, exhibits a broad spectrum of biological activities, including antioxidant, anti-inflammatory, and pro-apoptotic effects [13,14]. In ovarian cancer, curcumin has been reported to modulate survival signaling pathways, influence cellular redox balance, and enhance apoptotic sensitivity, which have been associated with the reversal of drug resistance [15–18]. In this context, curcumin represents an attractive potential combination partner for PARP inhibition, as it targets multiple resistance-associated processes including survival signaling pathways, redox regulation, and apoptotic sensitivity that are not directly addressed by PARP inhibitors alone. Despite these promising properties, comprehensive investigations addressing the synergistic potential and functionally supported mechanistic basis of combining a synthetic PARP inhibitor with curcumin in both platinum-sensitive and relatively platinum-resistant ovarian cancer models remain limited.

Therefore, the present study aimed to provide functionally supported, hypothesis-generating mechanistic insight into the anticancer effects of combining a synthetic PARP inhibitor with curcumin in ovarian cancer models with differential responsiveness to platinum-based chemotherapy. The key novel aspects of our investigation include: (i) the systematic and quantitative assessment of drug synergy using the Chou–Talalay method, including CI and DRI analyses, with a particular emphasis on SKOV-3 cells exhibiting experimentally validated reduced responsiveness to cisplatin; (ii) the comprehensive evaluation of multiple anticancer endpoints, including cell viability, migration, apoptosis, and redox modulation; (iii) the validation of synergistic effects in physiologically relevant 3D tumor spheroid models that better recapitulate *in vivo* tumor architecture and drug penetration barriers; and (iv) the functional interrogation of ROS involvement through rescue experiments using NAC. In addition, experimental findings were integrated with

bioinformatic network and pathway analyses to provide contextual interpretation of the observed phenotypic responses within a broader molecular framework. By employing this multi-layered approach, the present study moves beyond descriptive efficacy assessment and establishes a mechanistically informative, preclinical proof-of-concept framework for understanding the cooperative interaction between PARP inhibition and curcumin in ovarian cancer.

2. Materials and Methods

2.1. Chemicals, Reagents, and Preparation of Stock Solutions

Curcumin ($\geq 97\%$ purity) and ADP-ribose PARP1 inhibitor PJ34 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of both compounds were prepared at a concentration of 100 mM in dimethyl sulfoxide (DMSO; Sigma-Aldrich) under sterile conditions, aliquoted to avoid repeated freeze–thaw cycles, and stored at $-20\text{ }^{\circ}\text{C}$ until use. On the day of each experiment, working concentrations were freshly prepared by diluting the stock solutions in complete growth medium.

The final concentration of DMSO in all treatment conditions was maintained at or below 0.1% (v/v). Vehicle control groups containing an equivalent concentration of DMSO were included in all experiments, and preliminary control experiments confirmed that 0.1% DMSO had no detectable effect on cell viability or basal cellular responses.

The MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], fetal bovine serum (FBS), penicillin/streptomycin solution, trypsin–EDTA, and RPMI-1640 culture medium were obtained from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). All reagents were handled according to the manufacturers' instructions and were equilibrated to appropriate temperatures prior to use in cell culture experiments.

2.2. Cell Culture and Maintenance of Ovarian Cancer Cell Lines

Human ovarian adenocarcinoma cell lines OVCAR-3 (ATCC[®] HTB-161[™]) and SKOV-3 (ATCC[®] HTB-77[™]) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). OVCAR-3 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin, whereas SKOV-3 cells were maintained in Dulbecco's Modified Eagle Medium containing 10% FBS and 1% penicillin/streptomycin.

Cells were maintained in a humidified incubator at $37\text{ }^{\circ}\text{C}$ with 5% CO_2 and routinely monitored for morphology and confluency using phase-contrast microscopy. Culture media were replaced every 2–3 days, and cells were passaged at approximately 70–80% confluency using a standard trypsin–EDTA detachment protocol to maintain exponential growth and experimental consistency. Only cells within a limited passage range were used to minimize phenotypic drift.

For all experiments, cells were seeded at appropriate densities depending on the assay requirements and allowed to adhere overnight prior to treatment. Each experimental condition was performed using at least three independent biological replicates.

2.3. Cell Viability Assessment by MTT-Based Metabolic Activity Assay

Cell viability was evaluated based on cellular metabolic activity using the MTT tetrazolium reduction assay. Briefly, 5×10^3 cells from each ovarian cancer cell line were seeded into 96-well flat-bottom culture plates and allowed to adhere for 24 h under standard culture conditions. Following attachment, cells were treated with increasing concentrations of curcumin (5, 10, 25, 50, and $100\text{ }\mu\text{M}$) or PJ34 (5, 10, 25, 50, and $100\text{ }\mu\text{M}$) administered either as single agents or in combination for a total treatment duration of 48 h.

Combination treatments were designed based on fractions of the approximate IC_{50} values obtained from single-agent dose–response experiments and included $0.25 \times IC_{50}$, $0.5 \times IC_{50}$, $1 \times IC_{50}$, and $2 \times IC_{50}$ concentrations of each compound. Vehicle control wells containing 0.1% DMSO were included in all experiments.

At the end of the treatment period, 20 μ L of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] was added to each well, followed by incubation at 37 °C for 4 h to allow the formation of formazan crystals. The culture medium was then carefully removed, and 100 μ L of DMSO was added to each well to solubilize the formazan precipitate. Plates were gently agitated to ensure complete dissolution, and absorbance was measured at 570 nm using a Multiskan GO microplate reader (Thermo Fisher Scientific).

Cell viability was expressed as a percentage relative to vehicle-treated control cells, which were defined as 100% viable. Each experimental condition was performed in triplicate wells and repeated in at least three independent biological experiments.

2.4. Assessment of Cisplatin Sensitivity in OVCAR-3 and SKOV-3 Cells

To validate the relative platinum responsiveness of the ovarian cancer cell models used in this study, cisplatin sensitivity was assessed in OVCAR-3 and SKOV-3 cells using the MTT-based cell viability assay. Briefly, cells were seeded into 96-well plates at a density of 5×10^3 cells per well and allowed to adhere overnight under standard culture conditions. Following attachment, cells were treated with increasing concentrations of cisplatin (0, 2.5, 5, 10, 20, and 40 μ M) for 48 h.

At the end of the treatment period, cell viability was determined using the MTT assay as described above. Absorbance was measured at 570 nm using a microplate reader, and cell viability was expressed as a percentage relative to vehicle-treated control cells. Dose–response curves were generated by plotting cell viability against the logarithm of cisplatin concentration. IC_{50} values were calculated by nonlinear regression analysis using the “log(inhibitor) vs. normalized response (variable slope)” model implemented in GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA).

All experiments were performed in triplicate wells and repeated in at least three independent biological replicates. Statistical comparison of IC_{50} values between OVCAR-3 and SKOV-3 cells was performed using an unpaired two-tailed Student’s *t*-test, with $p < 0.05$ considered statistically significant.

2.5. Determination of IC_{50} by Dose–Response Curve Analysis

IC_{50} values were determined from cell viability data obtained at the indicated treatment time points. For each compound, viability percentages were plotted against the logarithm of drug concentration to generate dose–response curves using GraphPad Prism software (GraphPad Software, San Diego, CA, USA).

IC_{50} values, defined as the concentration required to reduce cell viability by 50% relative to vehicle-treated controls, were calculated by fitting the data to a four-parameter nonlinear regression model using the “log(inhibitor) vs. normalized response (variable slope)” equation. Curve fitting and parameter estimation were performed according to the software’s standard nonlinear least-squares regression algorithm. The resulting IC_{50} values were used to guide the selection of drug concentrations for subsequent combination and synergy analyses.

2.6. Drug Combination and Synergy Evaluation Through the Chou–Talalay Method

The interaction between PJ34 and curcumin when administered in combination was evaluated using the Chou–Talalay method, which allows quantitative determination of synergistic, additive, or antagonistic effects [15]. Combination analyses were performed using CompuSyn software (Version 1.0, ComboSyn Inc., Paramus, NJ, USA). Cell viability

data obtained from combination treatments were analyzed at a fixed dose ratio, which was determined based on the relative IC_{50} values of each compound when used as a single agent. Accordingly, combination experiments were designed using a fixed-ratio approach, and both agents were applied simultaneously at four concentration levels corresponding to $0.25 \times IC_{50}$, $0.5 \times IC_{50}$, $1 \times IC_{50}$, and $2 \times IC_{50}$, while maintaining a constant drug ratio across all combination points. Cell viability data obtained after 48 h of treatment were used for synergy analysis.

CI values were calculated across multiple effect levels to characterize the nature of the drug interaction. According to the Chou–Talalay principle, CI values < 1 indicate synergism, CI = 1 indicates an additive effect, and CI values > 1 indicate antagonism. CI and DRI values were calculated across multiple effect levels, including IC_{50} , IC_{75} , and IC_{90} , to provide a robust quantitative assessment of drug interactions. The goodness-of-fit of the median-effect model was evaluated using the correlation coefficient (r), and only datasets showing acceptable curve fitting ($r \geq 0.90$) were considered for CI and DRI interpretation. In parallel, DRI was calculated for each agent to estimate the extent to which the dose of PJ34 or curcumin could be reduced in the combination setting while maintaining a comparable inhibitory effect. CI and DRI values derived from this analysis were used to quantitatively assess the therapeutic advantage of the combined treatment strategy.

2.7. Measurement of Intracellular ROS Levels

Intracellular ROS levels were measured using the cell-permeable fluorogenic probe 2',7'-dichlorofluorescein diacetate (DCFH-DA). OVCAR-3 and SKOV-3 cells were seeded at appropriate densities and allowed to adhere overnight prior to treatment. Cells were then treated with PJ34, curcumin, or their combination for 4 h under standard culture conditions.

Following treatment, cells were incubated with DCFH-DA at a final concentration of $10 \mu M$ for 45 min at $37^\circ C$ in the dark. After incubation, excess probe was removed, and cells were gently washed with PBSto minimize background fluorescence. Fluorescence intensity, corresponding to intracellular ROS levels, was measured using a microplate reader with excitation and emission wavelengths set at 485 nm and 535 nm, respectively. ROS levels were expressed as relative fluorescence units and normalized to vehicle-treated control cells.

Each experimental condition was performed in triplicate wells and repeated in at least three independent biological experiments.

2.8. Cell Migration Assessment Through Scratch-Wound (Wound Healing) Assay

Cell migratory capacity was evaluated using a scratch-wound (wound healing) assay. OVCAR-3 and SKOV-3 cells were seeded into 6-well culture plates at a density of $3\text{--}5 \times 10^5$ cells per well and cultured until reaching approximately 90–100% confluency. To minimize the contribution of cell proliferation to wound closure, cells were serum-starved in medium containing 1% FBS for 12 h prior to scratch generation. Drug concentrations used in migration assays were selected based on preliminary MTT viability experiments and corresponded to subitotoxic IC_{20} – IC_{30} levels to minimize the contribution of cytotoxicity to wound closure inhibition.

A uniform linear scratch was created across the cell monolayer using a sterile 200 μL pipette tip. Detached cells and debris were gently removed by washing with PBS, after which fresh culture medium containing the respective treatments was added to each well. Images of the wound area were captured at baseline (0 h) and subsequently at 24 and 48 h using phase-contrast microscopy, with images acquired from the same predefined regions to ensure consistency.

Wound area measurements were quantified using ImageJ software (Version 1.53c, National Institutes of Health, Bethesda, MD, USA). Cell migration was expressed as the percentage of wound closure relative to the initial wound area, calculated according to the following formula:

$$\% \text{ wound closure} = [(Area_0 - Area_t) / Area_0] \times 100,$$

where $Area_0$ represents the wound area at 0 h and $Area_t$ represents the wound area at the indicated time point. Each experimental condition was analyzed in three independent biological replicates.

2.9. Apoptosis Analysis Through Annexin V-FITC/PI Flow Cytometry

Apoptotic cell death was quantified with flow cytometry using Annexin V-FITC and PI double staining. Following the designated treatment period, both floating and adherent cells were collected to ensure inclusion of all cell populations. Cells were harvested by trypsinization, combined with the culture supernatant, and centrifuged at $300 \times g$ for 5 min at room temperature. The resulting cell pellets were washed once with $1 \times$ Annexin V binding buffer and resuspended at a density of 5×10^5 cells per 100 μ L.

For staining, 5 μ L of Annexin V-FITC and 5 μ L of PI were added to each cell suspension, followed by incubation for 10–15 min at room temperature in the dark. After incubation, samples were immediately analyzed by flow cytometry using a BD FACSCanto II instrument (BD Biosciences, San Jose, CA, USA). For each sample, a minimum of 10,000 events were acquired to ensure statistical robustness.

Cell populations were identified based on fluorescence intensity and classified into four quadrants: viable cells (Annexin V[−]/PI[−]), early apoptotic cells (Annexin V⁺/PI[−]), late apoptotic cells (Annexin V⁺/PI⁺), and necrotic cells (Annexin V[−]/PI⁺). Total apoptotic cell fractions were calculated as the sum of early and late apoptotic populations. Each experimental condition was analyzed in at least three independent biological replicates.

Appropriate single-stain controls were used to set fluorescence compensation. Cells were first gated based on forward and side scatter parameters to exclude debris, followed by doublet discrimination using FSC-A versus FSC-H before quadrant analysis of Annexin V-FITC and PI fluorescence.

2.10. Measurement of Caspase-3 Enzymatic Activity

Caspase-3 activity was quantified using a commercial colorimetric/fluorometric caspase-3 assay kit, according to the manufacturer's instructions. Specifically, caspase-3 activity was measured using a Caspase-3 Assay Kit (Abcam, Cambridge, UK; Cat. No. ab39401). Briefly, following the designated treatment period, cells were harvested and lysed using the provided cell lysis buffer to obtain total cellular protein extracts. Protein concentrations were determined to ensure equal loading across samples.

Equal amounts of protein from each sample were incubated with the caspase-3-specific substrate DEVD-pNA in assay buffer at 37 °C for the recommended incubation period. The cleavage of the DEVD-pNA substrate by active caspase-3 resulted in the release of the chromophore p-nitroaniline (pNA), which was quantified by measuring absorbance at 405 nm using a microplate reader. Caspase-3 activity was normalized to total protein content and expressed as fold change relative to vehicle-treated control samples. Caspase-3 activity was expressed as relative activity normalized to vehicle-treated control samples.

Each experimental condition was analyzed in triplicate and repeated in at least three independent biological experiments to ensure reproducibility.

2.11. Gene Expression Analysis by RT-qPCR

2.11.1. RNA Isolation and Complementary DNA (cDNA) Synthesis

Total RNA was isolated from treated OVCAR-3 and SKOV-3 cells using either TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) or a column-based RNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturers' instructions. To eliminate potential genomic DNA contamination, all RNA samples were treated with DNase I prior to downstream applications. RNA concentration and purity were assessed spectrophotometrically using a NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA), and samples with A260/A280 ratios between 1.8 and 2.0 were considered acceptable for further analysis.

For cDNA synthesis, 1 µg of total RNA from each sample was reverse-transcribed using a commercial reverse transcription kit following the manufacturer's protocol. The resulting cDNA was diluted as required and stored at −20 °C until use in RT-qPCR analyses.

2.11.2. RT-qPCR and Relative Gene Expression Analysis

RT-qPCR was performed to analyze the mRNA expression levels of apoptosis-related genes, including Bax, Bcl-2, Caspase-3, Caspase-9, and p53. Amplification reactions were carried out using a SYBR Green-based master mix according to the manufacturer's instructions. Gene-specific primers listed in Table 1 were used for each target, and all reactions were performed under identical cycling conditions to ensure comparability.

Table 1. qRT-PCR primer sequences used in this study.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')	Amplicon Size (bp)
Bax	TTTGCTTCAGGGTTTCATCC	GACACTCGCTCAGCTTCTTG	121
Bcl-2	GGTGGGGTCATGTGTGTGG	CGGTTCAAGTACTCAGTCATCC	136
Caspase-3	GGAAGCGAATCAATGGACTCT	GGCAGGCCTGAATGATGAAG	124
Caspase-9	GGCTTTGTTTGTGTGGAGTCT	CATCTGGCTCGGGGTTACT	123
p53 (TP53)	CAGCACATGACGGAGGTTGT	TCATCCAAATACTCCACACGC	118
β-actin (ACTB)	CACCATTGGCAATGAGCGGTTC	CACCATTGGCAATGAGCGGTTC	138

β-actin was used as the internal reference gene to normalize gene expression levels and correct for sample-to-sample variation in cDNA input. Relative mRNA expression was calculated using the comparative Ct ($2^{-\Delta\Delta C_t}$) method, with gene expression levels expressed as fold change relative to vehicle-treated control samples. All qPCR reactions were conducted in technical triplicates and repeated in at least three independent biological experiments to ensure reproducibility.

Primer sequences used for RT-qPCR analysis are listed in Table 1.

2.11.3. Construction of the Integrated Target Gene Set and Bioinformatic Network Analysis

To explore the potential molecular mechanisms underlying the combined effects of PJ34 and curcumin, an integrated target gene set was established using publicly available bioinformatic databases. The primary molecular target of PJ34, PARP1, was included based on its central role in PARP inhibition. High confidence target proteins of curcumin were collected from multiple databases, including STRING, SwissTargetPrediction, PharmMapper, and STITCH, to ensure broad and reliable target coverage. For curcumin target prediction, only human (*Homo sapiens*) proteins were included, and predicted targets were filtered based on database-specific confidence criteria to reduce low-confidence associations.

The combined target gene set was subsequently subjected to protein–protein interaction (PPI) network analysis. PPI networks were constructed and visualized using Cytoscape software (Version 3.10.1, Cytoscape Consortium, San Diego, CA, USA), and hub gene identification was performed with the cytoHubba plugin to determine key regulatory nodes within the network. Protein–protein interaction data were retrieved from the STRING database using a medium-to-high confidence interaction score threshold (≥ 0.700), and disconnected nodes were excluded from downstream network analyses. Functional enrichment analyses were carried out to characterize the biological relevance of the integrated target set. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted using the DAVID platform and the clusterProfiler package to identify significantly enriched biological processes, molecular functions, and signaling pathways associated with the predicted targets.

2.11.4. GO Enrichment Analysis

GO enrichment analysis was performed to functionally characterize the integrated target gene cluster and to identify significantly overrepresented categories related to biological processes (BP), cellular components (CC), and molecular functions (MF). GO analyses were conducted using the DAVID Bioinformatics Resources and the R package clusterProfiler (version 4.0), which enable systematic annotation and enrichment assessment of gene sets.

Significantly enriched GO terms were identified based on statistical enrichment analysis, and the most representative terms within each GO category were selected for visualization. Enrichment results were displayed using enrichment factors and corresponding $-\log_{10}(p\text{-value})$ values to reflect both the magnitude and statistical significance of enrichment. These analyses were used to contextualize the predicted target genes within relevant biological functions and cellular pathways.

2.11.5. KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis was performed to identify signaling pathways significantly associated with the integrated target gene set and to elucidate their potential involvement in cancer-related biological processes. The target gene list was mapped to KEGG pathways using the KEGG REST application programming interface in conjunction with the clusterProfiler package.

Pathway enrichment was evaluated based on statistical significance and gene enrichment metrics, including p -values and the ratio of observed genes to total pathway genes. The most significantly enriched pathways were identified and ranked according to these criteria. KEGG pathway analysis was used to provide functional and signaling context for the predicted target genes and to support the interpretation of experimental phenotypic findings.

2.12. Three-Dimensional Tumor Spheroid Culture and Treatment

2.12.1. Formation of 3D Tumor Spheroids

To further validate the antitumor effects under physiologically relevant conditions that better reflect in vivo tumor architecture and cell–cell interactions, 3D tumor spheroid models were employed. Ovarian cancer cell lines were harvested during the logarithmic growth phase and resuspended in complete culture medium. Cells were seeded into ultra-low-attachment (ULA) 96-well round-bottom plates (Corning Inc., Corning, NY, USA) at a density of 3×10^3 cells per well in a final volume of 200 μL .

Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 . Under these conditions, compact and uniformly shaped spheroids formed spontaneously within 48–72 h, as confirmed through inverted light microscopy. Only spheroids exhibiting a regular spherical morphology were included in subsequent experiments to ensure experimental consistency.

2.12.2. Drug Treatment of 3D Tumor Spheroids

Following spheroid formation, culture medium was gently replaced with fresh medium containing curcumin, PJ34, or their combination (PJ34 + curcumin). Drug concentrations were selected based on IC_{50} values obtained from 2D monolayer experiments and were increased by approximately 20–30%, as commonly recommended to account for reduced drug penetration and diffusion limitations inherent to 3D spheroid systems.

The following experimental groups were established:

- Control (vehicle-treated),
- Curcumin-treated,
- PJ34-treated,
- PJ34 + curcumin-treated.

Spheroids were incubated with the indicated treatments for 72 h prior to downstream analyses. Vehicle concentrations were kept constant across all experimental groups to exclude solvent-related effects.

2.12.3. Bright-Field Imaging and Spheroid Size Quantification

Bright-field images of 3D tumor spheroids were acquired using an inverted microscope equipped with a digital camera at $10\times$ magnification. Images were captured using identical optical and acquisition settings for all experimental groups to allow direct comparison.

Spheroid size was quantified by measuring spheroid diameter using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Spheroid diameter was defined as the mean of two perpendicular measurements obtained at the widest points of the spheroid. For each experimental condition, spheroids from at least three independent biological experiments were analyzed, and mean spheroid diameter values were calculated.

2.12.4. Cell Viability Assessment in 3D Tumor Spheroids

Cell viability within 3D tumor spheroids was assessed using an ATP-based CellTiter-Glo[®] 3D Cell Viability Assay (Promega, Madison, WI, USA), specifically optimized for 3D culture systems. The assay was performed according to the manufacturer's instructions.

Briefly, an equal volume of CellTiter-Glo[®] 3D reagent was added directly to each well containing spheroids, followed by orbital shaking to ensure efficient spheroid lysis and ATP release. Luminescence was measured using a microplate reader, and viability values were expressed as a percentage relative to vehicle-treated control spheroids.

2.12.5. Live/Dead Fluorescence Staining of 3D Tumor Spheroids

To visualize treatment-induced cytotoxicity within 3D tumor spheroids, live/dead fluorescence staining was performed using Calcein-AM and Ethidium homodimer-1 (EthD-1). Following treatment, spheroids were incubated with Calcein-AM (2 μ M) and EthD-1 (4 μ M) for 30 min at room temperature in the dark.

Fluorescence images were acquired using an inverted fluorescence microscope equipped with FITC and TRITC filter sets at $10\times$ magnification. Images were obtained at the spheroid mid-plane using a single focal plane to ensure consistent depth of analysis. No post-acquisition brightness or contrast adjustments were applied, ensuring objective comparison between treatment groups.

2.13. Evaluation of ROS-Dependent Cytotoxicity Using NAC Pretreatment

To determine whether ROS contribute to the cytotoxic effects induced by PJ34, curcumin, or their combination, rescue experiments were performed using the ROS scavenger NAC (NAC; Sigma-Aldrich, St. Louis, MO, USA). 3D tumor spheroids were generated as described in Section 2.12 and allowed to form compact and uniformly shaped structures prior to treatment.

Fully formed spheroids were pretreated with NAC at a final concentration of 5 mM for 1 h at 37 °C in a humidified atmosphere containing 5% CO₂. The selected NAC concentration was based on commonly used non-cytotoxic doses reported in previous studies and preliminary optimization experiments. Following NAC pretreatment, spheroids were exposed to curcumin, PJ34, or their combination (PJ34 + curcumin) at the same concentrations used in preceding 3D spheroid experiments.

Control groups included untreated spheroids and spheroids treated with NAC alone. After treatment, spheroids were incubated for the indicated time periods prior to downstream analyses, as outlined below.

2.13.1. Intracellular ROS Measurement Following NAC Pretreatment

Intracellular ROS levels were quantified using the fluorogenic probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Following treatment, 3D tumor spheroids were enzymatically dissociated into single-cell suspensions using Accutase (Sigma-Aldrich, St. Louis, MO, USA) to ensure accurate single-cell-level analysis.

Cells were washed with phosphate-buffered saline (PBS) and incubated with DCFH-DA at a final concentration of 10 µM for 30 min at 37 °C in the dark. After incubation, cells were washed with PBS to remove excess probe and immediately analyzed by flow cytometry. Mean fluorescence intensity (MFI) values were used to quantify intracellular ROS levels. ROS measurements were normalized to untreated control samples to allow comparison across experimental conditions.

2.13.2. Cell Viability and Apoptosis Analysis Following NAC Pretreatment

Cell viability following NAC pretreatment was assessed using the ATP-based CellTiter-Glo® 3D Cell Viability Assay (Promega, Madison, WI, USA), according to the manufacturer's instructions. Luminescence signals were measured using a microplate reader and expressed as a percentage relative to untreated control spheroids.

In parallel, apoptotic cell death was evaluated by Annexin V/propidium iodide (PI) double staining followed by flow cytometric analysis. After treatment, spheroids were dissociated into single-cell suspensions, and the proportions of viable (Annexin V[−]/PI[−]), early apoptotic (Annexin V⁺/PI[−]), late apoptotic (Annexin V⁺/PI⁺), and necrotic (Annexin V[−]/PI⁺) cells were quantified. Total apoptosis was calculated as the sum of early and late apoptotic populations and compared across experimental groups to assess the impact of NAC pretreatment on PJ34- and curcumin-induced cytotoxicity.

2.14. Statistical Analysis

All quantitative data are presented as mean ± standard error of the mean (SEM) derived from at least three independent biological experiments, unless otherwise stated. Statistical comparisons among multiple experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. For comparisons of IC₅₀ values between OVCAR-3 and SKOV-3 cell lines, statistical significance was assessed using unpaired two-tailed Student's *t*-tests. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses and graphical representations were generated using GraphPad Prism software version 9.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Validation of Relative Cisplatin Responsiveness in OVCAR-3 and SKOV-3 Cells

To experimentally validate the relative platinum responsiveness of the ovarian cancer cell models used in this study, OVCAR-3 and SKOV-3 cells were exposed to increasing

concentrations of cisplatin for 48 h, and cell viability was assessed using the MTT assay. Cisplatin treatment resulted in a clear dose-dependent reduction in cell viability in both ovarian cancer cell lines.

Notably, OVCAR-3 cells exhibited a markedly greater sensitivity to cisplatin across the tested concentration range, whereas SKOV-3 cells showed a more attenuated reduction in viability. Nonlinear regression analysis of dose–response curves indicated that OVCAR-3 cells displayed a significantly lower IC_{50} value compared with SKOV-3 cells, indicating a higher platinum sensitivity. In contrast, SKOV-3 cells demonstrated significantly reduced responsiveness to cisplatin, reflected by a higher IC_{50} value ($p < 0.01$).

These findings support the interpretation that SKOV-3 cells are functionally less responsive to platinum treatment relative to the platinum-sensitive OVCAR-3 model. Accordingly, this experimental validation justifies the use of SKOV-3 cells as a relatively platinum-resistant experimental context in the present study, while avoiding assumptions of experimentally induced or clinically acquired platinum resistance (Figure S1).

3.2. PJ34 and Curcumin Synergistically Reduce Cell Viability in Platinum-Sensitive and Resistant Ovarian Cancer Cells

Cell viability in OVCAR-3 and SKOV-3 ovarian cancer cell lines was evaluated using the MTT assay following 48 h of treatment with curcumin, the PARP1 inhibitor PJ34, or their combination. OVCAR-3 cells exhibited higher sensitivity to both agents, whereas SKOV-3 cells demonstrated a comparatively less responsive phenotype, particularly in response to curcumin treatment. In both cell lines, combination treatment resulted in a markedly stronger antiproliferative effect than either compound administered alone (Figure 1).

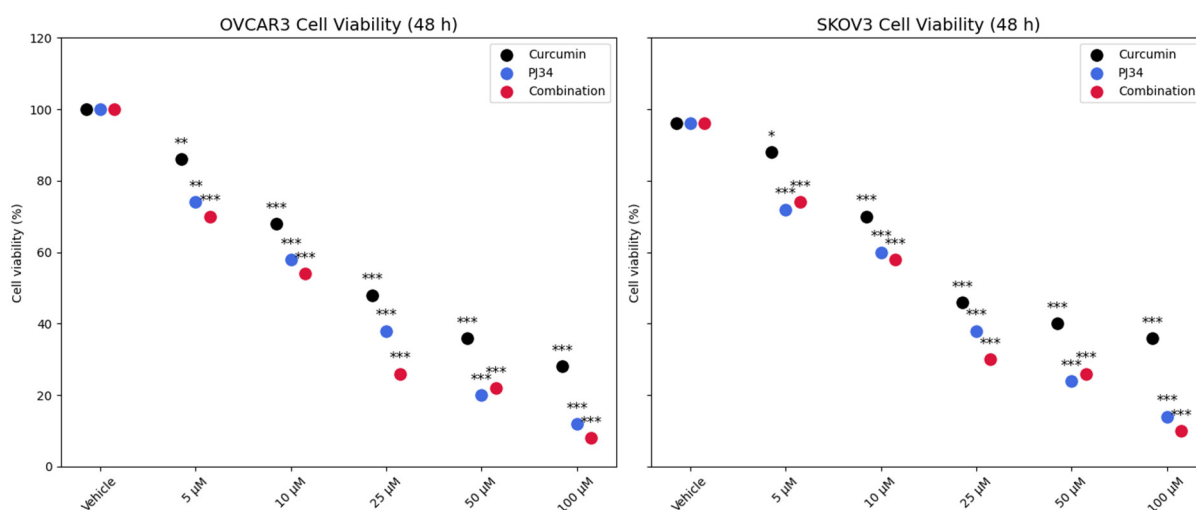


Figure 1. Effects of PJ34, curcumin, and their combination on cell viability in ovarian cancer cells. Cell viability of OVCAR-3 and SKOV-3 ovarian cancer cell lines was assessed using the MTT assay following 48 h of treatment with increasing concentrations of curcumin, the PARP1 inhibitor PJ34, or their combination. Data are expressed as mean $\pm$ SEM from at least three independent biological experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with vehicle-treated control cells).

In OVCAR-3 cells, IC_{50} values after 48 h of treatment were 18.7 μ M for PJ34 and 24.3 μ M for curcumin. At the highest tested concentration (100 μ M), PJ34 and curcumin reduced cell viability to approximately 25% and 30% of control levels, respectively. In contrast, relatively platinum-resistant SKOV-3 cells exhibited reduced sensitivity to both agents. The IC_{50} value for PJ34 in SKOV-3 cells was 28.5 μ M, while curcumin displayed a higher IC_{50} value of 42.6 μ M, consistent with a more resistant response profile (Figure 2).

IC₅₀ values were derived from nonlinear regression analysis of dose–response curves obtained from independent experiments, and statistical comparison between OVCAR-3 and SKOV-3 cells revealed significantly higher IC₅₀ values in SKOV-3 cells for both PJ34 and curcumin ($p < 0.05$).

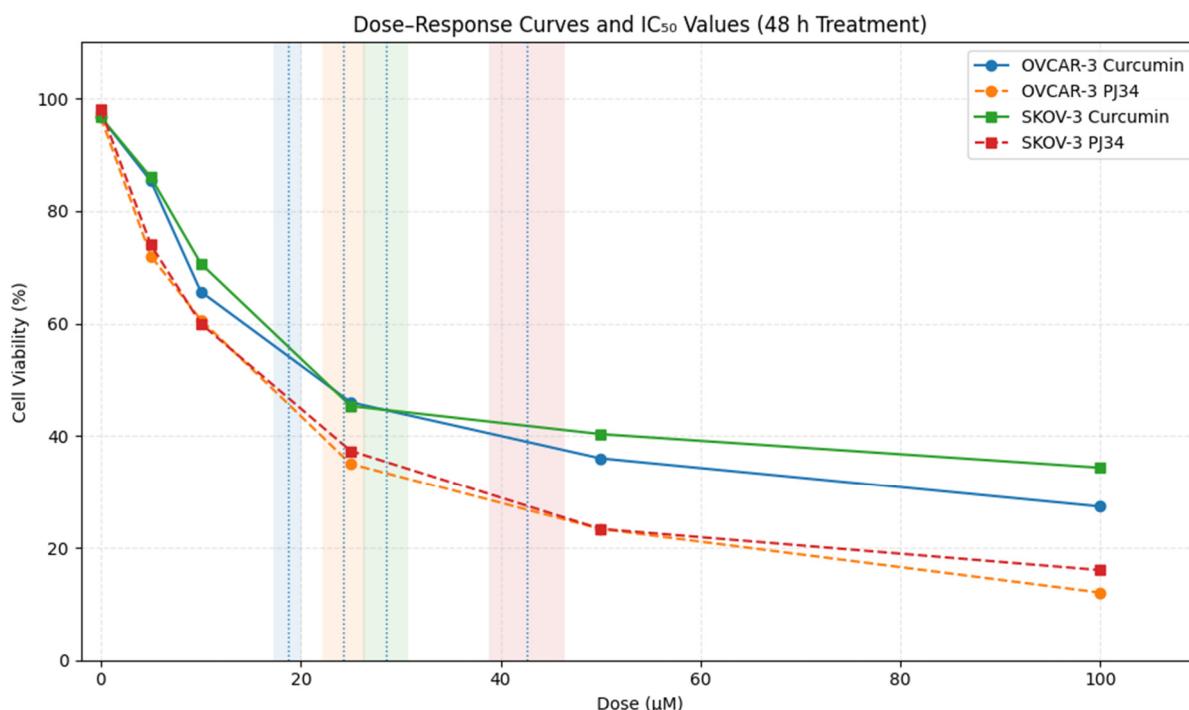


Figure 2. Dose–response curves and IC₅₀ values of PJ34 and curcumin in ovarian cancer cell lines. Dose–response curves for curcumin and the PARP1 inhibitor PJ34 were generated in OVCAR-3 and SKOV-3 ovarian cancer cells following 48 h of treatment. Cell viability data were fitted using nonlinear regression analysis to calculate IC₅₀ values. Vertical dotted lines indicate the calculated IC₅₀ values for each treatment condition, and shaded background areas correspond to the IC₅₀ region of each respective dose–response curve. OVCAR-3 cells exhibited higher sensitivity to both agents, with IC₅₀ values of 18.7 µM for PJ34 and 24.3 µM for curcumin, whereas SKOV-3 cells displayed a relatively less responsive profile (PJ34: 28.5 µM; curcumin: 42.6 µM). Data are presented as mean ± SEM from at least three independent experiments. IC₅₀ values are presented as mean ± SEM from at least three independent experiments. Statistical comparisons between cell lines were performed using unpaired two-tailed Student’s *t*-tests.

3.3. Quantitative Synergy Analyses Demonstrate Enhanced Antiproliferative Interaction Between PJ34 and Curcumin

Combination treatment using fractional concentrations based on the IC₅₀ values of each agent resulted in significantly greater antiproliferative effects in both ovarian cancer cell lines compared with single-agent treatments. Across all tested combination doses, the PJ34–curcumin combination consistently produced a stronger inhibition of cell viability than either compound alone.

Quantitative assessment of drug interactions using the Chou–Talalay method yielded CI values consistent with synergism in both cell lines. The mean CI value was 0.65 in OVCAR-3 cells and 0.52 in relatively platinum-resistant SKOV-3 cells, falling within the range indicative of synergistic interaction (CI < 1) (Figure 3A). In parallel, DRI analysis demonstrated that, in SKOV-3 cells, the effective dose of PJ34 could be reduced by approximately 2.1-fold and that of curcumin by approximately 2.8-fold when administered in combination, while maintaining comparable antiproliferative efficacy (Figure 3B).

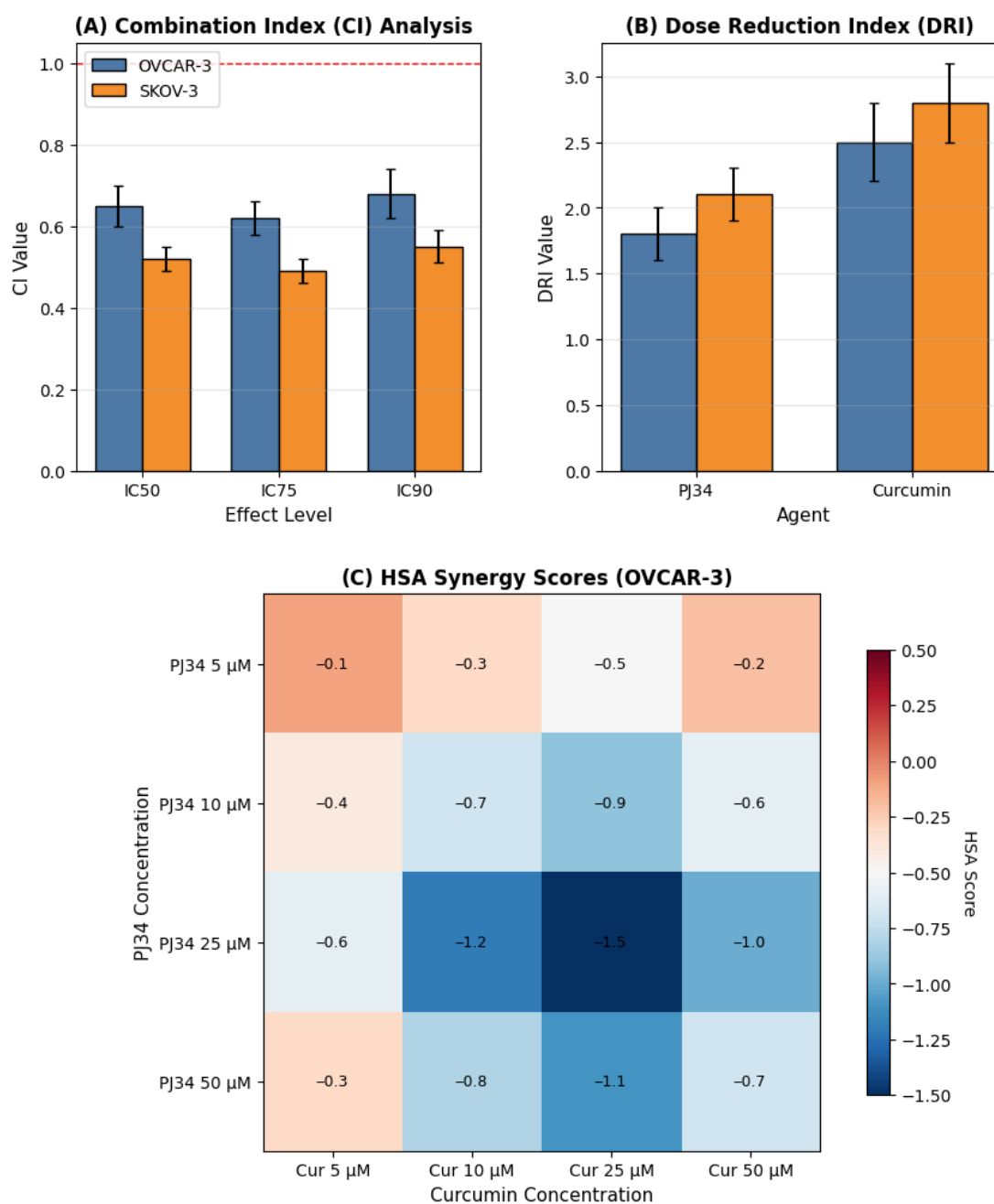


Figure 3. Multimodal quantitative analysis of the synergistic antiproliferative interaction between PJ34 and curcumin. **(A)** CI values calculated using the Chou–Talalay method in OVCAR-3 and SKOV-3 ovarian cancer cells at IC₅₀, IC₇₅, and IC₉₀ effect levels. CI values < 1 indicate synergistic interactions, CI = 1 indicates additivity, and CI values > 1 indicate antagonism. **(B)** DRI analysis illustrating the extent to which the effective doses of PJ34 and curcumin may be reduced when administered in combination while maintaining comparable antiproliferative effects. **(C)** Heatmap of synergy scores based on HSA model in OVCAR-3 cells following 48 h of treatment. Negative HSA scores (blue tones) indicate synergistic interactions, whereas positive values (red tones) indicate antagonism. Color intensity reflects the magnitude of the interaction. Dose–response curves were generated from at least three independent biological experiments, each performed in triplicate. CI and DRI values were derived from fixed-ratio combination experiments designed according to the Chou–Talalay method, using four concentration points spanning 0.25 $\times$ to 2 $\times$ IC₅₀ levels of each compound. Synergy analysis was performed using CompuSyn software (Version 1.0, ComboSyn Inc., Paramus, NJ, USA), and only datasets with acceptable median-effect curve fitting were included in CI and DRI calculations.

Synergistic interactions were further evaluated using the Highest Single Agent (HSA) model following 48 h of treatment in OVCAR-3 cells. The HSA synergy heatmap illustrated dose-dependent interaction patterns across the combination matrix, with negative HSA scores indicating synergistic effects. More pronounced synergy was observed predominantly at intermediate concentration ranges (10–25 μM), whereas lower or higher dose combinations exhibited weaker or no synergistic interactions. The numerical values displayed within each heatmap tile represent the calculated HSA synergy scores (Figure 3C).

3.4. Differential Modulation of Intracellular ROS Levels and Apoptosis by PJ34, Curcumin, and Their Combination

Intracellular ROS levels were evaluated following treatment with curcumin, PJ34, or their combination. ROS measurements were performed after 4 h of treatment. Curcumin treatment significantly reduced ROS levels in both ovarian cancer cell lines compared with vehicle-treated controls, resulting in an approximately 40% reduction in OVCAR-3 cells and a 35% reduction in SKOV-3 cells. In contrast, treatment with PJ34 led to a moderate increase in intracellular ROS levels in both OVCAR-3 and SKOV-3 cells, with elevations of approximately 50–60% relative to control values.

In the combination treatment group, curcumin partially attenuated the PJ34-induced increase in ROS levels, resulting in ROS levels that were comparable to those observed in control cells (Figure 4). Notably, despite this normalization of intracellular ROS levels, the combination treatment remained associated with markedly enhanced apoptotic responses relative to single-agent treatments.

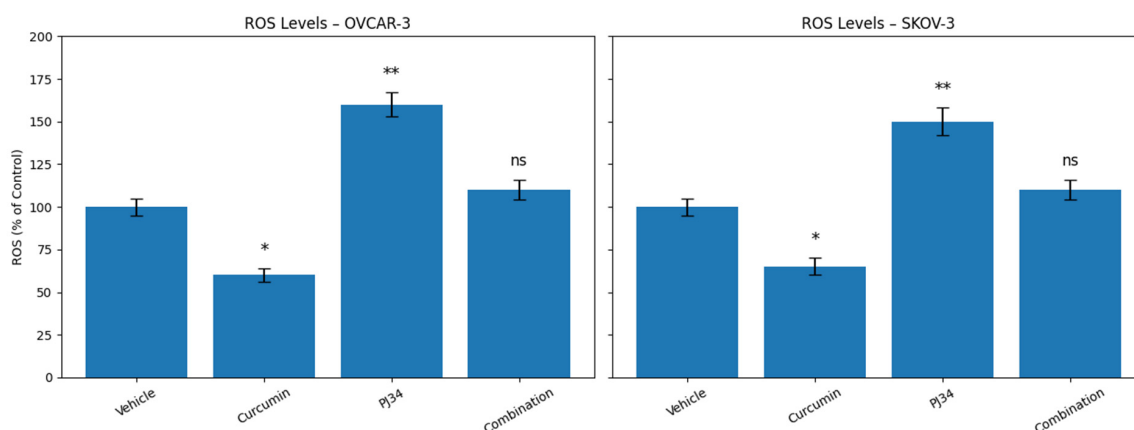


Figure 4. Effects of PJ34, curcumin, and their combination on intracellular ROS levels in ovarian cancer cells. Intracellular ROS levels were measured in OVCAR-3 and SKOV-3 ovarian cancer cells following 4 h of treatment using a DCFDA-based fluorescence assay. Curcumin treatment significantly reduced intracellular ROS levels in both cell lines compared with vehicle-treated controls, whereas treatment with the PARP1 inhibitor PJ34 resulted in a moderate increase in ROS levels. In the combination treatment group, intracellular ROS levels were comparable to, or close to, those observed in control cells. CI and DRI values were calculated from viability data obtained from three independent biological experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$ vs. vehicle control; ns, not significant).

To further examine the relationship between oxidative stress and apoptosis, total apoptotic cell fractions (Q2 + Q3) were evaluated together with intracellular ROS levels across treatment groups. Despite marked differences in ROS modulation by PJ34 and curcumin, the combination treatment induced the highest apoptotic response while maintaining ROS levels close to control values in both OVCAR-3 and SKOV-3 cells. These findings are consistent with a partial dissociation between ROS levels and apoptosis induction (Figure 5).

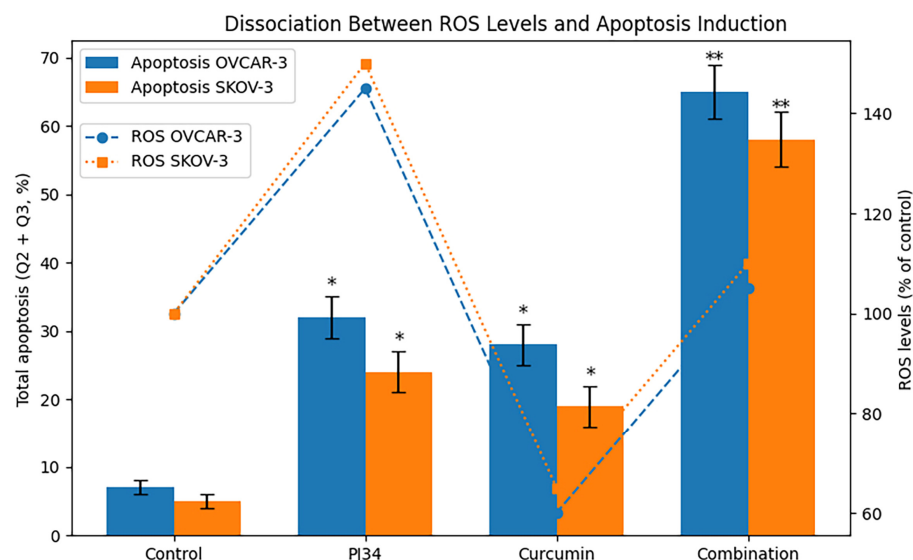


Figure 5. Dissociation between intracellular ROS levels and apoptosis induction in ovarian cancer cells. Bar graphs represent total apoptotic cell fractions (Q2 + Q3) in OVCAR-3 and SKOV-3 ovarian cancer cells following treatment with PJ34, curcumin, or their combination. Line plots indicate intracellular ROS levels expressed as percentage of vehicle-treated control cells and are shown on the secondary y-axis. While PJ34 and curcumin differentially modulated ROS levels, the combination treatment induced the highest apoptotic response without a corresponding increase in ROS, consistent with a partial dissociation between oxidative stress and apoptosis induction. Data are presented as mean $\pm$ SEM from at least three independent biological experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$ vs. control).

To further compare the magnitude of apoptotic responses between ovarian cancer cell lines, effect size analysis was performed by normalizing treatment-induced apoptosis to the respective control levels. Despite its resistant phenotype, SKOV-3 cells exhibited a pronounced apoptotic effect size following combination treatment, which was comparable to, and in some conditions approached or exceeded, that observed in OVCAR-3 cells (Figure 6).

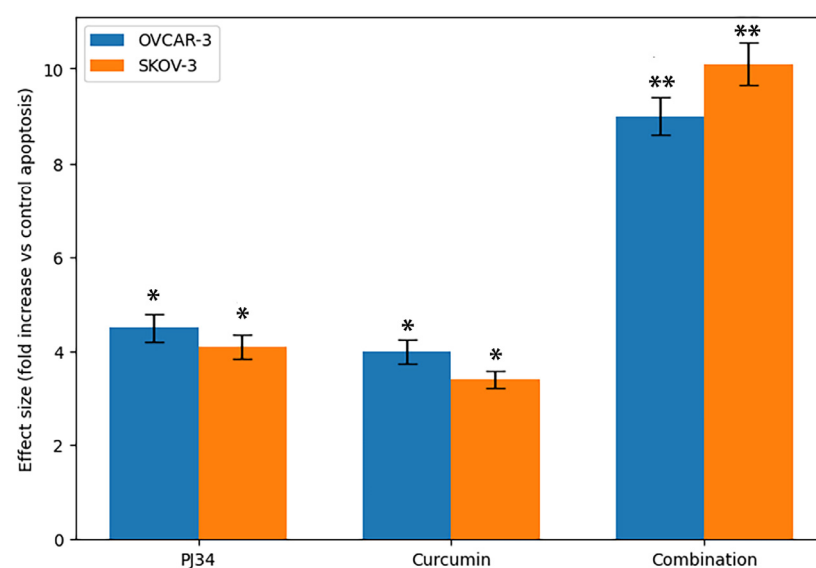


Figure 6. Effect size of PJ34, curcumin, and their combination on apoptosis induction in ovarian cancer cells. Bar graphs represent the effect size of apoptosis induction in OVCAR-3 and SKOV-3 ovarian cancer cells following treatment with PJ34, curcumin, or their combination. Effect size was

calculated as the fold increase in total apoptotic cell fractions (Q2 + Q3) relative to vehicle-treated control cells for each cell line. Data are presented as mean $\pm$ SEM from three independent biological experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$ compared with control.

3.5. Combination Treatment Markedly Suppresses Cell Migration in OVCAR-3 and SKOV-3 Cells

Cell migratory capacity was evaluated using the scratch-wound assay following 48 h of treatment. Treatment with either PJ34 or curcumin alone resulted in a noticeable reduction in cell migration in both ovarian cancer cell lines. Quantitative analysis demonstrated that both PJ34 and curcumin significantly inhibited wound closure compared with vehicle-treated control cells ($p < 0.05$). At IC_{30} concentrations, PJ34 inhibited wound closure by approximately 55% in OVCAR-3 cells and 45% in SKOV-3 cells, while curcumin reduced migration by approximately 50% and 40%, respectively.

Combination treatment using IC_{20} concentrations of both PJ34 and curcumin produced the most pronounced inhibitory effect on cell migration. After 48 h, the combination reduced wound closure by approximately 85% in OVCAR-3 cells and 78% in SKOV-3 cells, indicating a substantially stronger suppression of migratory activity compared with single-agent treatments (Figure 7). The inhibitory effect of the combination treatment on cell migration was statistically significant relative to vehicle-treated controls in both cell lines ($p < 0.01$). Importantly, these concentrations were selected based on MTT viability data and corresponded to subitotoxic conditions, under which no major loss of cell viability was observed at 24–48 h. Therefore, the observed inhibition of wound closure is unlikely to be solely attributable to reduced cell number and is consistent with an impairment of migratory behavior under these experimental conditions.

A. Representative wound closure (48 h) B. Quantitative analysis of wound closure

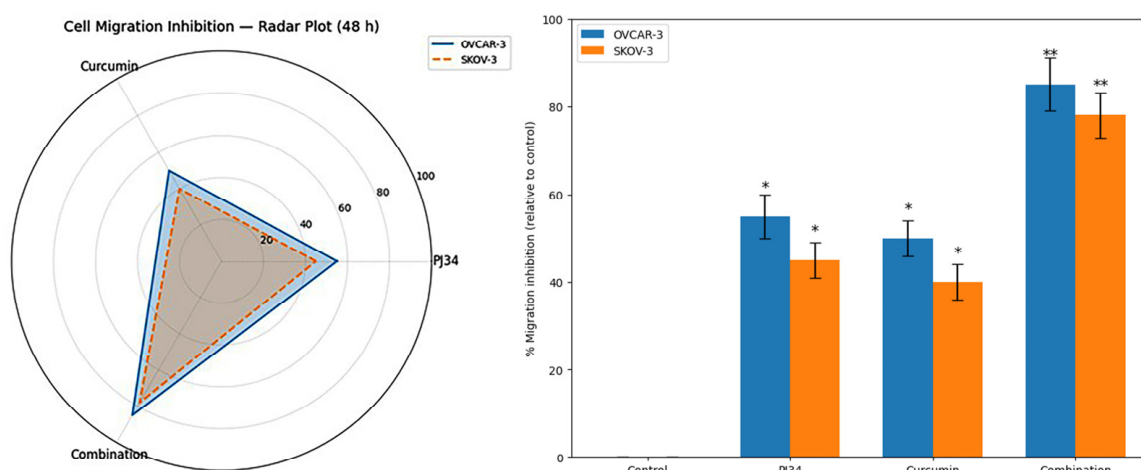


Figure 7. Effects of PJ34, curcumin, and their combination on ovarian cancer cell migration. (A) Representative wound closure images showing the effects of PJ34, curcumin, and combination treatment on cell migration in OVCAR-3 and SKOV-3 ovarian cancer cells after 48 h, as assessed using the scratch-wound assay. (B) Quantitative analysis of wound closure expressed as percentage migration inhibition relative to vehicle-treated control cells. Both PJ34 and curcumin significantly reduced migratory capacity in OVCAR-3 and SKOV-3 cells, whereas the combination treatment was associated with the greatest degree of migration inhibition in both cell lines. Data are presented as mean $\pm$ SEM from at least three independent biological experiments ($n \geq 3$). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$ vs. control).

3.6. Combination Treatment Robustly Increases Apoptotic Cell Populations in OVCAR-3 and SKOV-3 Cells

Apoptotic cell death was quantified after 48 h of treatment using Annexin V-FITC/PI staining followed by flow cytometric analysis. In OVCAR-3 cells, the baseline apoptotic rate, defined as the sum of early and late apoptotic populations, was $7.2 \pm 1.1\%$ in the control group. Treatment with PJ34 or curcumin at their respective IC_{50} concentrations increased the total apoptotic fraction to $32.5 \pm 3.4\%$ and $28.8 \pm 2.9\%$, respectively. Combination treatment resulted in a substantially higher increase in apoptosis, with total apoptotic cells reaching $65.3 \pm 4.7\%$.

Across all treatment groups in OVCAR-3 cells, the proportion of necrotic cells remained low ($<10\%$), indicating that the observed increase in Annexin V-positive populations predominantly reflected apoptotic rather than necrotic cell death.

In relatively platinum-resistant SKOV-3 cells, the basal apoptotic rate was $5.8 \pm 0.9\%$ in control conditions. Single-agent treatment induced lower levels of apoptosis compared with OVCAR-3 cells, with apoptotic rates of $24.1 \pm 2.5\%$ for PJ34 and $19.7 \pm 2.1\%$ for curcumin. In contrast, combination treatment was associated with a marked elevation in the apoptotic fraction to $58.6 \pm 5.1\%$ (Figure 8).

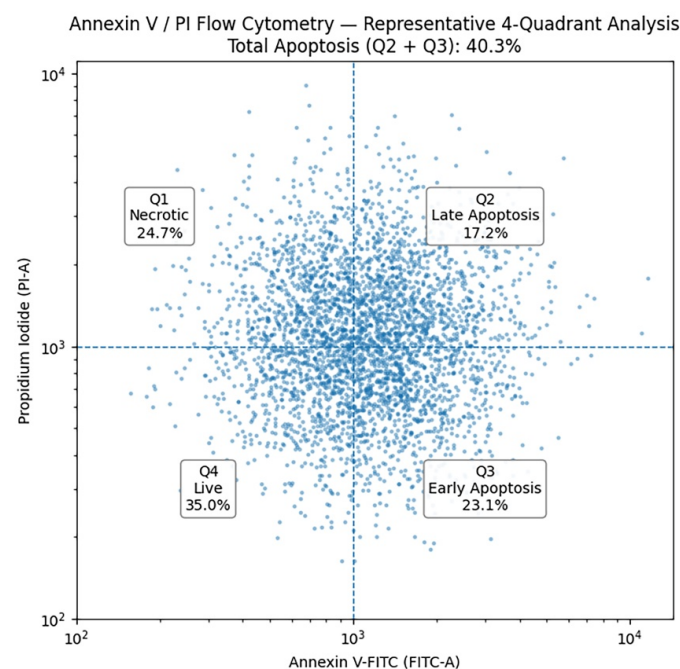


Figure 8. Representative Annexin V-FITC/PI flow cytometry analysis demonstrating apoptotic cell distribution. Representative four-quadrant scatter plot of Annexin V-FITC versus PI staining used to quantify apoptosis after 48 h of treatment. Cells were classified into four populations: Q4 (Annexin V[−]/PI[−]), viable cells; Q3 (Annexin V⁺/PI[−]), early apoptotic cells; Q2 (Annexin V⁺/PI⁺), late apoptotic cells; and Q1 (Annexin V[−]/PI⁺), necrotic cells. Total apoptosis was calculated as the sum of early and late apoptotic populations (Q2 + Q3). Data shown are representative of three independent biological experiments. Quantitative apoptosis values were derived from all biological replicates. Percentages indicate the distribution of cells within the gated population and sum to 100%.

Separate quantification of early and late apoptotic populations revealed a qualitative difference between single-agent and combination treatments. While PJ34 or curcumin alone predominantly increased early apoptotic cells, the combination treatment was associated with a pronounced shift toward late apoptosis in both OVCAR-3 and SKOV-3 cells (Figure 9). The increase in late apoptotic cell fractions observed following the combination treatment

was statistically significant compared with control cells in both OVCAR-3 and SKOV-3 cell lines ($p < 0.001$).

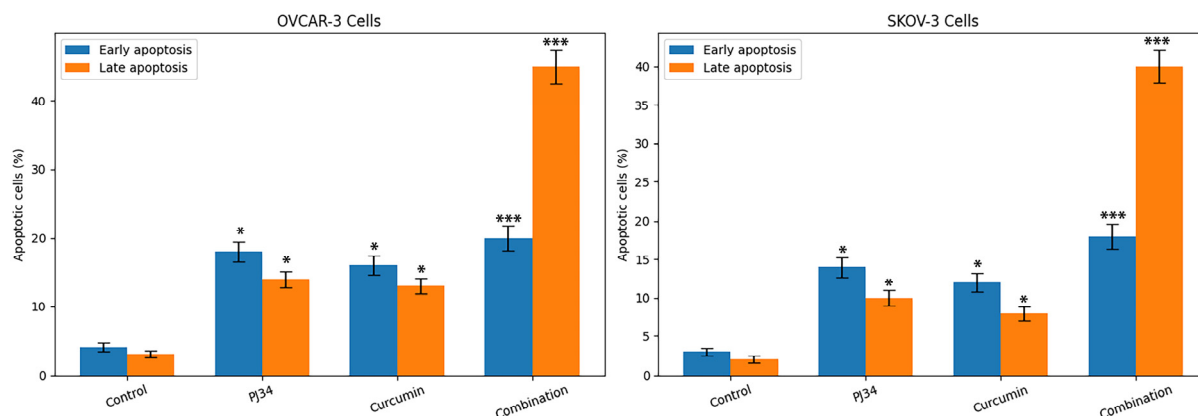


Figure 9. Differential distribution of early and late apoptotic populations induced by PJ34, curcumin, and their combination in ovarian cancer cells. OVCAR-3 (**left panel**) and SKOV-3 (**right panel**) cells were treated with PJ34, curcumin, or their combination for 48 h, and apoptosis was analyzed through Annexin V-FITC/PI staining followed by flow cytometry. Early apoptotic cells (Annexin V⁺/PI[−]) and late apoptotic cells (Annexin V⁺/PI⁺) were quantified separately and expressed as percentages of total cells. Single-agent treatments primarily increased early apoptotic populations, whereas the combination treatment resulted in a pronounced increase in late apoptotic cells in both cell lines, consistent with a shift toward later apoptotic stages. Data are presented as mean $\pm$ SEM from three independent biological experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, *** $p < 0.001$ versus vehicle-treated control cells.

To further evaluate the relationship between biochemical activation of apoptosis and apoptotic cell death, the association between caspase-3 activity and total apoptotic cell fraction (Q2 + Q3) was analyzed. A strong positive correlation was observed between caspase-3 activity and total apoptosis in both OVCAR-3 and SKOV-3 cells, consistent with an association in which increased caspase-3 activity was accompanied by higher apoptotic cell rates (Figure 10).

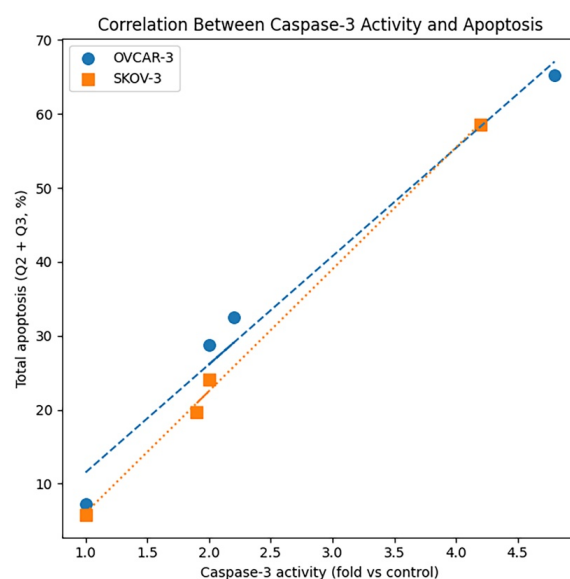


Figure 10. Correlation between caspase-3 activity and total apoptosis in ovarian cancer cells. Scatter plots illustrate the relationship between caspase-3 activity (expressed as fold change relative to control) and total apoptotic cell fractions (Q2 + Q3) in OVCAR-3 and SKOV-3 cells following 48 h of treatment.

Each data point represents the mean value obtained from independent experiments. Dashed lines indicate linear trend lines illustrating the positive association between caspase-3 activation and apoptotic cell death. Data points represent mean values derived from three independent biological experiments.

3.7. Combination Treatment Induces Maximal Caspase-3 Activation in OVCAR-3 and SKOV-3 Cells

Caspase-3 activity was evaluated as a key marker of apoptotic execution following treatment with PJ34, curcumin, or their combination. In both OVCAR-3 and SKOV-3 ovarian cancer cell lines, the highest levels of caspase-3 activity were observed in the combination treatment group. Treatment with PJ34 or curcumin alone resulted in a moderate increase in caspase-3 activity, corresponding to approximately 2.0–2.5-fold elevations relative to control levels.

In contrast, combination treatment produced a substantially greater activation of caspase-3, increasing enzymatic activity by approximately 4.8-fold in OVCAR-3 cells and 4.2-fold in SKOV-3 cells compared with vehicle-treated controls (Figure 11). Combination treatment resulted in a significantly higher caspase-3 activity compared with control and single-agent treatments ($p < 0.01$). Importantly, the caspase-3 activity assay provides a functional readout of caspase-3 activation at the protein level, as increased enzymatic activity reflects proteolytic processing of caspase-3 into its active form. Therefore, although cleaved caspase-3 protein levels were not directly assessed through immunoblotting, the marked increase in caspase-3 activity observed following combination treatment functionally supports, but does not directly confirm, activation of the apoptotic execution machinery.

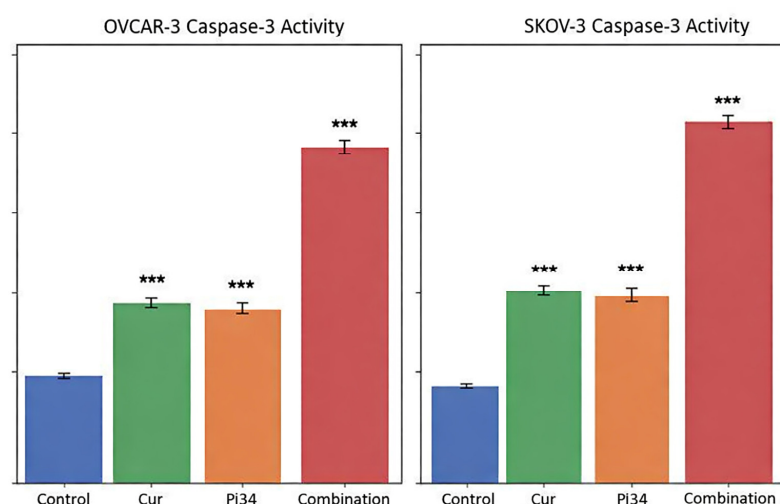


Figure 11. Effects of PJ34, curcumin, and their combination on caspase-3 activity in ovarian cancer cells. Caspase-3 activity was measured in OVCAR-3 and SKOV-3 ovarian cancer cells after 48 h of treatment using a fluorometric assay. Treatment with PJ34 or curcumin alone resulted in a significant increase in caspase-3 activity compared with vehicle-treated control cells, whereas combination treatment was associated with the highest levels of caspase-3 activity in both cell lines. Data are presented as mean $\pm$ SEM from three independent biological experiments and are expressed as fold change relative to control values. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (***) $p < 0.001$ vs. control).

3.8. Combination Treatment Shifts Apoptosis-Related Gene Expression Toward a Pro-Apoptotic Profile

RT-qPCR analysis revealed marked alterations in the expression of genes involved in apoptotic regulation, with all values normalized to vehicle-treated control cells. In the combination treatment group, the expression of the pro-apoptotic gene Bax (Bcl-2-associated X protein) was increased by approximately 3.5-fold in OVCAR-3 cells and 3.1-fold in SKOV-3 cells. Similarly, expression of the tumor suppressor gene p53 was elevated by approximately 2.8-fold in both ovarian cancer cell lines following combination treatment.

In contrast, expression of the anti-apoptotic gene Bcl-2 was strongly downregulated in the combination group, with reductions of approximately 70–80% relative to control levels. In addition, mRNA expression levels of caspase-3 and caspase-9 were significantly increased in cells treated with the combination compared with single-agent treatments and controls (Figure 12), consistent with a transcriptional profile associated with pro-apoptotic signaling.

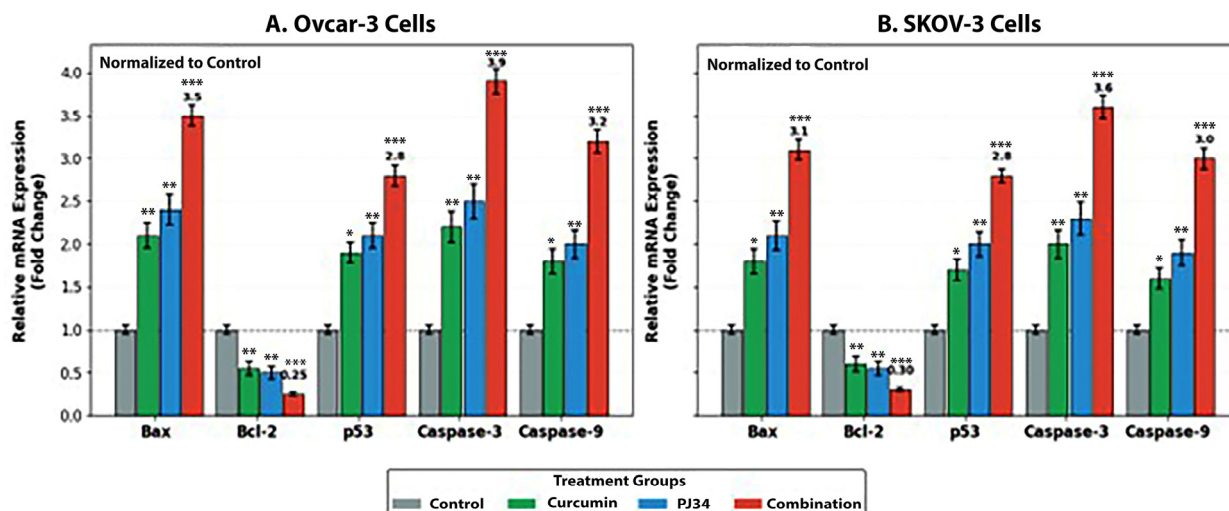


Figure 12. Effects of PJ34, curcumin, and their combination on apoptosis-related gene expression in ovarian cancer cells. Relative mRNA expression levels of Bax, Bcl-2, p53, caspase-3, and caspase-9 were analyzed using RT-qPCR in OVCAR-3 (A) and SKOV-3 (B) ovarian cancer cells following 48 h of treatment with PJ34, curcumin, or their combination. Gene expression levels were normalized to an internal reference gene and expressed as fold change relative to vehicle-treated control cells. Combination treatment was associated with increased expression of pro-apoptotic genes and reduced expression of the anti-apoptotic gene Bcl-2 in both cell lines. Data are presented as mean $\pm$ SEM from three independent biological experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control).

To further integrate pro- and anti-apoptotic signaling, the Bax/Bcl-2 ratio was calculated. The combination treatment resulted in the highest Bax/Bcl-2 ratio in both OVCAR-3 and SKOV-3 cells, significantly exceeding control and single-agent treatments, consistent with a shift toward a pro-apoptotic balance (Figure 13).

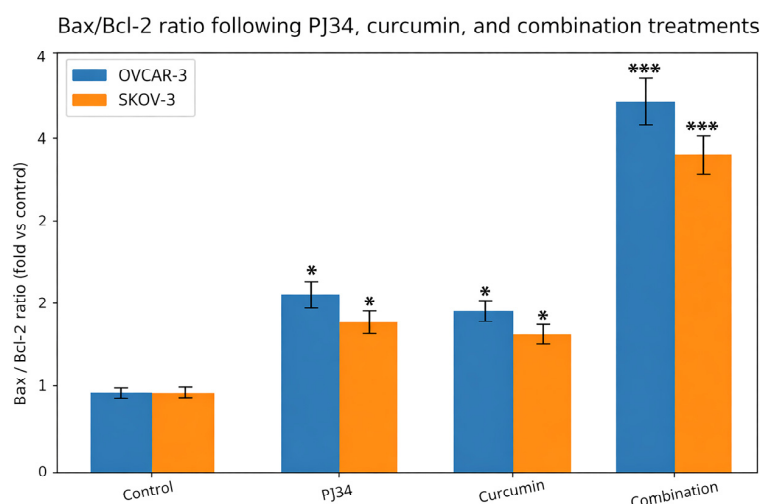


Figure 13. Bax/Bcl-2 ratio following PJ34, curcumin, and combination treatments in ovarian cancer cells. The Bax/Bcl-2 ratio was calculated based on RT-qPCR expression data and expressed as fold

change relative to vehicle-treated control cells in OVCAR-3 and SKOV-3 cell lines. Combination treatment produced the highest Bax/Bcl-2 ratio in both cell lines, consistent with a shift toward a pro-apoptotic balance. Data are presented as mean $\pm$ SEM from three independent biological experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, *** $p < 0.001$ versus vehicle-treated control cells).

3.9. Bioinformatics Analyses

3.9.1. Protein–Protein Interaction (PPI) Network Analysis

A target gene cluster consisting of 125 proteins was constructed by combining PARP1, the primary molecular target of PJ34, with 124 high-confidence curcumin-associated target proteins obtained from public databases. Protein–protein interaction (PPI) analysis of this gene set was performed using the STRING database, and the resulting interaction network was visualized and analyzed using Cytoscape software (Version 3.10.1, Cytoscape Consortium, San Diego, CA, USA). Hub gene analysis was conducted using the cytoHubba plugin, which ranks nodes based on topological parameters such as degree and centrality. This analysis identified TP53, AKT1, TNF, IL6, CASP3, JUN, EGFR, MYC, ESR1, and HIF1A as the top hub genes within the network (Figure 14). These genes exhibited the highest levels of connectivity, indicating their central positions in the integrated PJ34–curcumin target network. It should be emphasized that the identified hub genes were derived from *in silico* network analysis and do not imply direct modulation by PJ34 or curcumin in the present experimental system. Rather, these hub nodes represent highly connected regulatory points that may provide biological context for the observed cellular phenotypes. Notably, the identified hub genes showed conceptual concordance with the experimentally observed cellular phenotypes. Central nodes such as TP53 and CASP3 were associated with apoptosis regulation, aligning with the increased p53 expression, caspase-3 activity, and apoptotic cell fractions observed following combination treatment. Similarly, AKT1 and EGFR, which are linked to cell survival and migration pathways, were consistent with the pronounced antiproliferative and antimigratory effects detected *in vitro*. Accordingly, the alignment between predicted hub genes and experimental phenotypes should be interpreted as contextual and supportive rather than as evidence of direct molecular regulation, supporting the biological relevance of the integrated target network.

3.9.2. GO and KEGG Pathway Enrichment Analyses

GO enrichment analysis was performed to characterize the functional annotation of the integrated target gene cluster. In the Biological Process (BP) category, the most significantly enriched terms included negative regulation of apoptosis, negative regulation of cell proliferation, response to ROS, positive regulation of DNA templated transcription, and positive regulation of the MAPK signaling pathway (Figure 15).

In the Molecular Function (MF) category, enriched terms were predominantly associated with cytokine activity, DNA binding, transcription factor binding, and protein kinase activity.

KEGG pathway enrichment analysis identified several signaling pathways that were significantly overrepresented among the target genes (Figure 15). The most enriched pathways included Pathways in cancer ($p = 1.2 \times 10^{-15}$), PI3K–Akt signaling pathway ($p = 3.5 \times 10^{-12}$), p53 signaling pathway ($p = 4.7 \times 10^{-9}$), and Apoptosis ($p = 6.1 \times 10^{-8}$). Additional significantly enriched pathways included the FoxO signaling pathway, HIF-1 signaling pathway, and Chemical carcinogenesis—ROS pathway, which together provide functional context for the predicted target network rather than evidence of direct pathway activation.

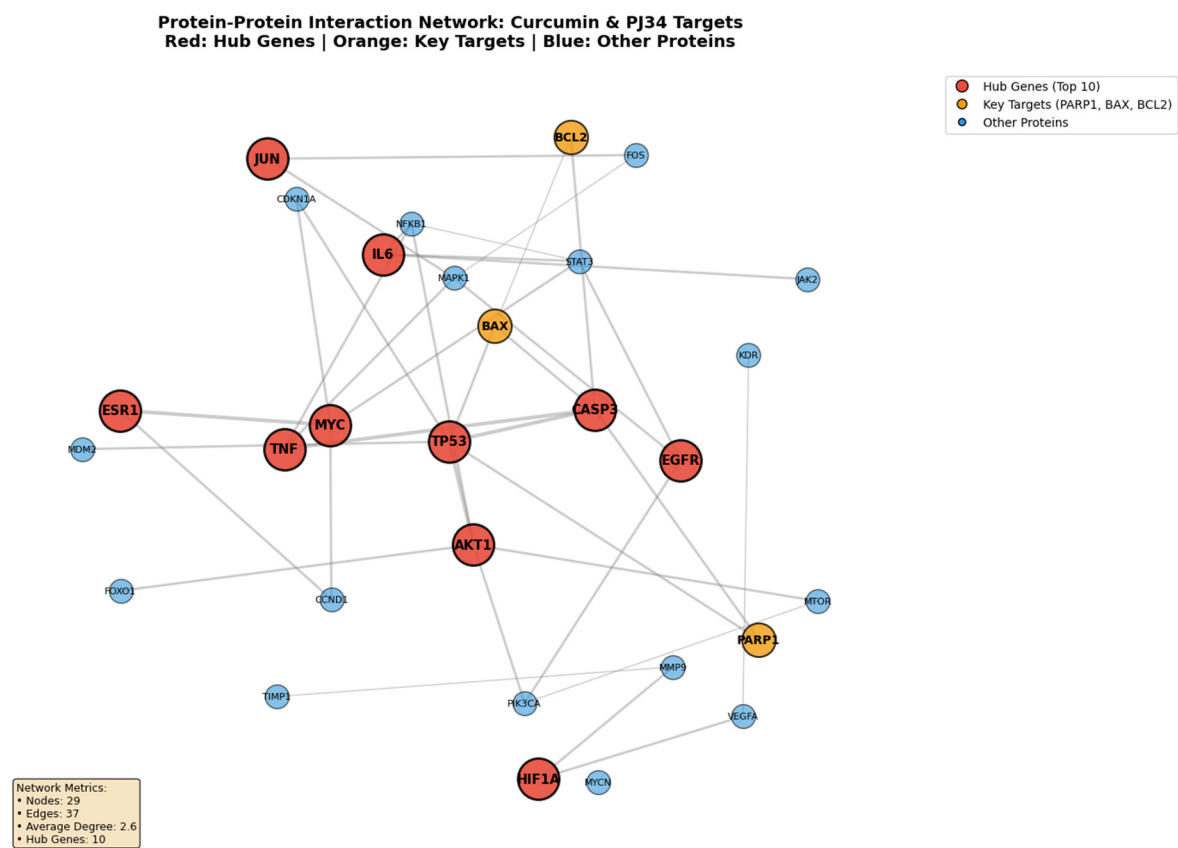


Figure 14. Protein–protein interaction (PPI) network of curcumin- and PJ34-associated target genes. The protein–protein interaction network was constructed using the STRING database based on an integrated target gene set consisting of PARP1 and high-confidence curcumin-associated targets. Network visualization and hub gene identification were performed using Cytoscape and the cytoHubba plugin. Hub genes identified by topological analysis (TP53, AKT1, TNF, IL6, CASP3, JUN, EGFR, MYC, ESR1, and HIF1A) are shown as enlarged red nodes, indicating high connectivity within the network. Orange nodes represent selected key targets, and blue nodes represent other interacting proteins. The PPI network represents an in silico analysis derived from curated database interactions rather than experimental replication. Accordingly, the functional relevance of the identified hub genes should be interpreted as contextual and supportive, serving to provide biological context for the experimentally observed apoptotic, antiproliferative, and antimigratory phenotypes, rather than evidence of direct molecular modulation.

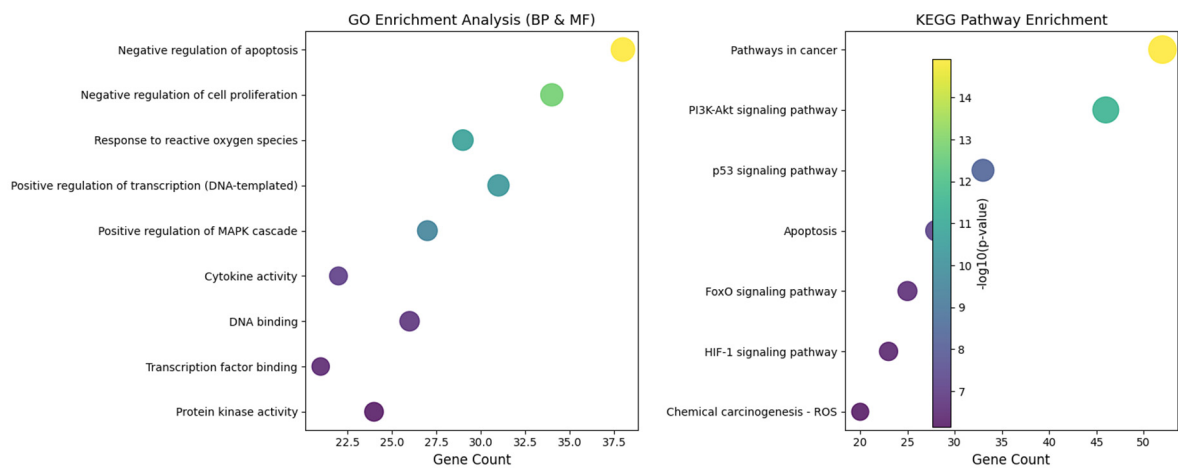


Figure 15. GO and KEGG pathway enrichment analysis of the integrated target gene set of curcumin and PJ34. GO enrichment analysis was performed on the integrated target gene set obtained by combining

124 high-confidence curcumin-associated targets with PARP1, the primary molecular target of PJ34. Significantly enriched GO terms were classified into Biological Process (BP) and Molecular Function (MF) categories. In the BP category, enriched terms included negative regulation of apoptosis, negative regulation of cell proliferation, response to ROS, positive regulation of DNA-templated transcription, and positive regulation of the MAPK signaling pathway. The MF category was enriched in terms related to cytokine activity, DNA binding, transcription factor binding, and protein kinase activity. KEGG pathway enrichment analysis identified significantly enriched signaling pathways, including Pathways in cancer, PI3K–Akt signaling pathway, p53 signaling pathway, Apoptosis, FoxO signaling pathway, HIF-1 signaling pathway, and Chemical carcinogenesis—ROS. Enrichment significance is shown as $-\log_{10}(p\text{-value})$, and bubble size represents the number of genes associated with each term or pathway. GO and KEGG enrichment analyses represent *in silico* functional annotation derived from curated target gene sets, rather than experimentally replicated datasets. Accordingly, the enriched terms and pathways should be interpreted as providing contextual biological insight into the observed experimental phenotypes, rather than as evidence of direct pathway modulation.

3.10. Effects of PJ34 and Curcumin in a 3D Tumor Spheroid Model

3.10.1. Formation and Morphological Alterations of 3D Tumor Spheroids

Under control conditions, ovarian cancer cells formed compact, spherical, and well-defined 3D tumor spheroids with smooth borders. Treatment with curcumin resulted in a moderate reduction in spheroid size while largely preserving spheroid integrity. PJ34 treatment induced a more pronounced decrease in spheroid size, accompanied by partial loss of spheroid compactness. Notably, combined PJ34 and curcumin treatment was associated with marked spheroid shrinkage and loss of structural integrity, manifested by irregular borders and fragmented morphology (Figure 16A).

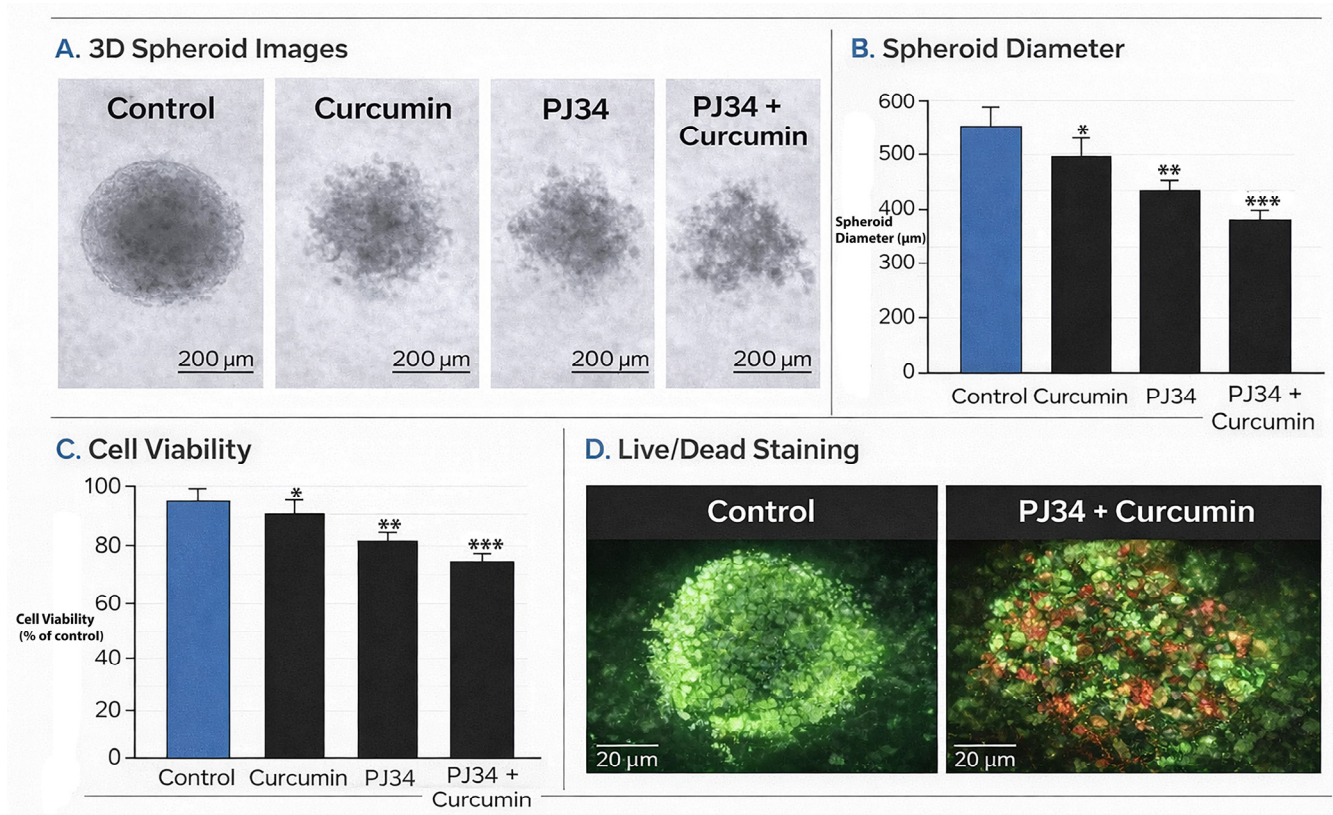


Figure 16. 3D tumor spheroid model confirms synergistic cytotoxicity of PJ34 and curcumin. (A) Representative bright-field images of 3D tumor spheroids formed from ovarian cancer cells cultured in ultra-low-attachment plates under control conditions or treated with curcumin, PJ34, or their combination (PJ34 + curcumin). Control spheroids exhibit compact, spherical morphology with

smooth borders, whereas single-agent treatments induce partial spheroid disorganization and size reduction. Combined PJ34 and curcumin treatment was associated with pronounced spheroid shrinkage and loss of structural integrity. Scale bar: 200 μm . **(B)** Quantitative analysis of spheroid diameter (μm). Spheroid size was significantly reduced following curcumin and PJ34 treatments compared with control, with the most pronounced decrease observed in the PJ34 + curcumin group, consistent with enhanced growth inhibition in the 3D model. **(C)** Cell viability of 3D tumor spheroids expressed as percentage of control. Viability was modestly reduced by curcumin alone and more strongly decreased by PJ34 treatment. Notably, combined PJ34 and curcumin treatment produced the greatest reduction in cell viability, consistent with an enhanced cytotoxic effect in 3D culture conditions. **(D)** Live/dead fluorescence staining of representative spheroids. Viable cells are shown in green (Calcein-AM), and non-viable cells are shown in red (Ethidium homodimer-1). Control spheroids display predominantly green fluorescence, reflecting high viability, whereas PJ34 + curcumin-treated spheroids show extensive red fluorescence and disrupted architecture, consistent with increased cell death following combination treatment. Scale bar: 20 μm . Data are presented as mean $\pm$ SEM ($n = 3$). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control).

3.10.2. Quantitative Reduction in Spheroid Size Following Combination Treatment

Quantitative analysis of spheroid diameter demonstrated significant differences among treatment groups. Compared with control spheroids, both curcumin and PJ34 treatments significantly reduced spheroid size. The most pronounced reduction was observed in the PJ34 + curcumin group, which exhibited a significantly smaller spheroid diameter than either single-agent treatment (Figure 16B). These findings are consistent with the preservation of the antiproliferative effects observed in 2D cultures within a 3D tumor spheroid context.

3.10.3. Suppression of Cell Viability in 3D Tumor Spheroids

Cell viability analysis revealed that both curcumin and PJ34 reduced spheroid viability relative to control spheroids. Importantly, combined PJ34 and curcumin treatment resulted in the strongest reduction in spheroid viability, significantly exceeding the effects observed with either agent alone (Figure 16C). This result is consistent with the extension of the synergistic cytotoxic interaction between PJ34 and curcumin to a 3D tumor spheroid setting.

3.10.4. Live/Dead Fluorescence Staining Reveals Enhanced Cell Death Following Combination Treatment

Live/dead fluorescence staining demonstrated that control spheroids predominantly exhibited green fluorescence, reflecting a high proportion of viable cells. Spheroids treated with either curcumin or PJ34 displayed increased red fluorescence, consistent with enhanced cell death. In contrast, spheroids treated with the PJ34 + curcumin combination exhibited extensive red fluorescence and a marked reduction in green signal, consistent with a pronounced loss of cell viability and disruption of spheroid integrity (Figure 16D).

3.11. NAC Pretreatment Attenuates PJ34- and Curcumin-Induced ROS Generation

Treatment of 3D tumor spheroids with PJ34 resulted in a significant increase in intracellular ROS levels compared with control spheroids, whereas curcumin alone reduced oxidative stress. In the combination treatment group, intracellular ROS levels were elevated relative to control but remained lower than those observed with PJ34 treatment alone, consistent with a partial normalization of ROS levels. Notably, pretreatment with NAC markedly reduced ROS levels in PJ34 + curcumin-treated spheroids, as evidenced by a significant decrease in DCF fluorescence intensity. NAC alone did not induce a significant change in ROS levels relative to control spheroids (Figure 17A).

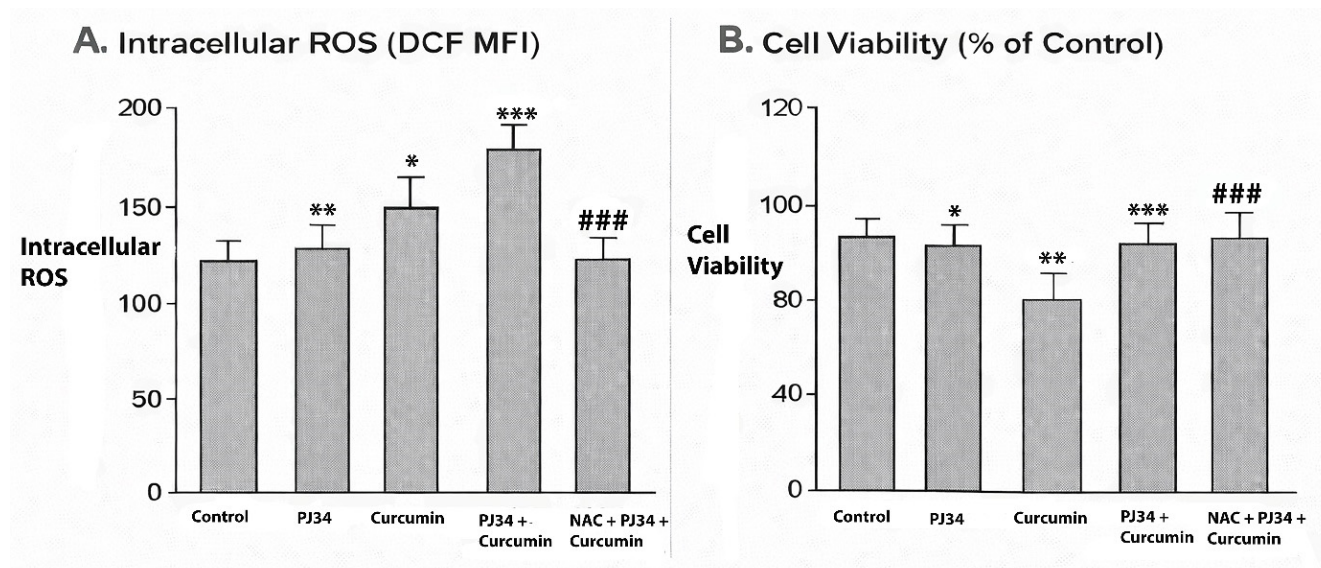


Figure 17. NAC pretreatment attenuates PJ34- and curcumin-induced ROS generation and cytotoxicity. (A) Intracellular ROS levels were quantified through flow cytometric analysis of DCF fluorescence and expressed as mean DCF MFI. Treatment with PJ34 resulted in a significant increase in intracellular ROS levels compared with control cells, whereas curcumin alone reduced oxidative stress. In the combination treatment group, ROS levels were elevated relative to control but remained lower than those observed with PJ34 treatment alone, consistent with partial normalization of ROS levels. Pretreatment with the ROS scavenger NAC significantly reduced ROS accumulation in PJ34 + curcumin-treated cells. (B) Cell viability was assessed and expressed as percentage of control. PJ34 and curcumin treatments significantly reduced cell viability, with the most pronounced cytotoxic effect observed in the PJ34 + curcumin combination group. NAC pretreatment was associated with a partial recovery of cell viability in the combination-treated group, although viability did not fully return to control levels. Data are presented as mean $\pm$ SEM from at least three independent biological experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control; ### $p < 0.001$ vs. PJ34 + curcumin).

3.11.1. ROS Scavenging Partially Reverses PJ34 + Curcumin-Induced Cytotoxicity

Consistent with the reduction in intracellular ROS levels, NAC pretreatment significantly attenuated the cytotoxic effects of PJ34 and curcumin combination treatment in 3D tumor spheroids. Spheroids pretreated with NAC exhibited significantly higher cell viability and reduced apoptotic fractions compared with spheroids treated with PJ34 + curcumin alone (Figure 17B). However, NAC pretreatment did not fully restore cell viability to control levels, supporting a partial, but not complete, rescue effect.

3.11.2. ROS Contributes to, but Does Not Fully Account for, PJ34 + Curcumin-Induced Cell Death

Although ROS scavenging significantly reduced both ROS accumulation and apoptosis, the incomplete reversal of cytotoxicity suggests that ROS contributes to, but does not solely mediate, PJ34 + curcumin-induced cell death. These findings are consistent with a multifactorial, ROS-associated mechanism of action underlying the synergistic cytotoxic effects of PJ34 and curcumin in the 3D tumor spheroid model.

4. Discussion

The present study provides evidence that the combined treatment of PJ34, a selective PARP1 inhibitor, and curcumin, a pleiotropic natural polyphenol, is associated with a synergistic anticancer effect in both OVCAR-3 and SKOV-3 ovarian cancer cell models with differential responsiveness to platinum-based chemotherapy. This work extends the

existing literature on curcumin-based combination strategies by providing a rigorously quantified and functionally informed evaluation of combination efficacy. First, we present systematic and quantitative evidence of drug synergy using the Chou–Talalay method in conjunction with DRI analysis, thereby offering a pharmacologically relevant assessment of the cooperative interaction between PJ34 and curcumin. Second, particular emphasis was placed on SKOV-3 cells exhibiting experimentally validated reduced responsiveness to cisplatin, a context in which the synergistic interaction was more pronounced than in the platinum-sensitive OVCAR-3 model. Third, experimental phenotypic findings were integrated with bioinformatic network and pathway analyses, allowing contextualization of the observed cellular responses within a broader molecular framework. Collectively, this multilevel approach provides a more comprehensive interpretative framework than prior studies reporting combination effects without rigorous synergy quantification or systems-level integration. Importantly, the conclusions of the present study are supported by convergent functional and molecular readouts, including apoptosis assays, caspase-3 activity measurements, and redox modulation analyses, while explicitly acknowledging the absence of direct protein-level validation.

Both PJ34 and curcumin individually reduced cell viability in a dose- and time-dependent manner; however, Chou–Talalay analysis clearly demonstrated synergism ($CI < 1$) across multiple effect levels in both ovarian cancer cell lines. Importantly, this synergistic interaction was more pronounced in SKOV-3 cells, which exhibit an experimentally validated reduced responsiveness to platinum-based chemotherapy and aggressive biological behavior. This observation is particularly relevant, as PARP inhibition alone often exhibits a limited efficacy in ovarian cancer contexts characterized by poor platinum responsiveness and the absence of BRCA mutations [19].

The differential platinum responsiveness of the ovarian cancer cell models used in this study is well supported by the previous literature. Large-scale comparative analyses have consistently demonstrated that SKOV-3 cells exhibit a reduced sensitivity to cisplatin relative to OVCAR-3 cells. For example, Domcke et al. reported an approximately 11-fold higher cisplatin IC_{50} value in SKOV-3 cells compared with OVCAR-3 cells, highlighting a substantial functional difference in platinum response. Similarly, Beaufort et al. identified SKOV-3 as a less cisplatin-responsive model and OVCAR-3 as a cisplatin-sensitive model based on standardized in vitro drug response profiling. These findings are further supported by pharmacogenomic data from the Cancer Cell Line Encyclopedia, in which SKOV-3 cells display a lower cisplatin AUC value indicative of relative resistance, whereas OVCAR-3 cells show a more sensitive response profile. Collectively, these studies, together with our experimental cisplatin sensitivity data, support the use of SKOV-3 as a relatively platinum-resistant model in comparison with the platinum-sensitive OVCAR-3 cells, while acknowledging the molecular heterogeneity of ovarian cancer cell lines [20–23].

The observed dose reduction indices (DRI) support the translational relevance of the combination by indicating that effective antiproliferative responses can be achieved at lower drug concentrations in vitro. Such dose reduction is critical for minimizing off-target toxicity, a recognized limitation of PARP inhibitors in clinical settings [24]. Curcumin's ability to sensitize resistant cancer cells has previously been attributed to its multitargeted inhibition of survival and stress-response pathways, including PI3K/Akt and NF- κ B signaling [25], which is consistent with the synergistic effects observed in this study.

ROS play a dual role in cancer biology, functioning both as signaling mediators and as inducers of oxidative damage. In the present study, PJ34 treatment moderately increased intracellular ROS levels, likely reflecting impaired DNA repair and metabolic stress following PARP1 inhibition [26]. In contrast, curcumin significantly reduced ROS levels, consistent with its well-established antioxidant properties [27]. Notably, combination treatment

normalized ROS levels to near-control values. Rather than representing a contradictory outcome, this finding indicates that excessive oxidative stress is not the primary driver of apoptosis and that ROS contributes to, but does not solely account for, the cytotoxic effects induced by the combination. These data are consistent with a model in which PJ34 and curcumin act through complementary mechanisms, with PARP inhibition inducing DNA damage and metabolic stress, while curcumin may modulate survival pathways and increase apoptotic sensitivity without a reliance on excessive oxidative stress. Such apoptosis induction occurring in the absence of excessive oxidative stress could represent a therapeutic advantage, as high ROS levels can activate compensatory survival pathways and contribute to treatment resistance [28]. Importantly, the normalization of intracellular ROS levels in the combination group supports the interpretation that apoptosis induction is not driven by excessive oxidative stress but rather reflects coordinated apoptotic signaling in which ROS is contributory but not the sole determinant.

Importantly, the synergistic antitumor effects observed in 2D monolayer cultures were consistently reproduced in a 3D tumor spheroid model, which more closely recapitulates *in vivo* tumor architecture, cell–cell interactions, and drug penetration barriers. In this physiologically relevant system, combined PJ34 and curcumin treatment induced a marked spheroid shrinkage, loss of structural integrity, and reduced cell viability, demonstrating that the synergistic cytotoxic interaction is preserved beyond conventional 2D conditions. Notably, ROS scavenging by N-acetyl-L-cysteine partially attenuated, but did not fully rescue, the cytotoxic effects of the combination, suggesting that ROS contributes to, but does not solely account for, the observed antitumor activity. Collectively, these findings are consistent with an ROS-associated yet multifactorial mechanism of action, in which PARP1 inhibition, the coordinated modulation of redox homeostasis, and the activation of apoptotic signaling pathways may cooperatively contribute to synergistic tumor cell death in ovarian cancer cells. Notably, combination treatment induced a clear and statistically significant reduction in viability in 3D tumor spheroids, demonstrating that the synergistic cytotoxic effect observed in 2D monolayer cultures is preserved under diffusion-limited, 3D conditions, albeit with reduced magnitude.

Cell migration assays further demonstrated that while PJ34 and curcumin individually inhibited wound closure, their combination produced a marked suppression of migratory capacity in both ovarian cancer cell lines. The particularly strong inhibition observed in SKOV-3 cells is noteworthy given their high metastatic potential. These findings are consistent with previous reports implicating PARP1 in cytoskeletal remodeling and epithelial–mesenchymal transition (EMT) [29], as well as studies showing that curcumin suppresses migration by targeting EGFR, focal adhesion kinase (FAK), and matrix metalloproteinases [30]. In this context, the bioinformatic identification of EGFR, MYC, and HIF1A as hub genes provides supportive contextual insight, as these factors are central regulators of invasion, angiogenesis, and hypoxia-driven tumor progression, but does not imply direct modulation of these pathways in the present experimental system.

Flow cytometric analysis revealed that combination treatment induced a marked increase in apoptotic cell populations compared with either agent alone, while necrosis remained minimal. This pattern is consistent with a regulated, programmed mode of cell death, which is therapeutically favorable. The observed increases in caspase-3 activity and caspase-9 expression are compatible with an engagement of the intrinsic apoptotic pathway, although direct causal relationships at the protein cleavage level were not assessed in this study. These findings are further supported by a significant shift toward a pro-apoptotic gene expression profile, including the suppression of Bcl-2 and upregulation of Bax, a key determinant of mitochondrial outer membrane permeabilization [31]. Importantly, the ability of the combination treatment to enhance apoptotic responsiveness in SKOV-3 cells is

particularly relevant, as impaired apoptotic signaling is a well-recognized feature of ovarian cancer cells exhibiting reduced responsiveness to platinum-based chemotherapy [32].

Our bioinformatic analyses further provide contextual support for the experimental findings. Hub gene analysis identified TP53, AKT1, and CASP3 as central nodes within the integrated target network, indicating the potential involvement of interconnected survival and apoptotic pathways. GO enrichment analysis highlighted biological processes related to apoptosis regulation, proliferation control, oxidative stress response, and transcriptional regulation, while KEGG pathway analysis revealed a significant enrichment of PI3K-Akt, p53, FoxO, HIF-1, and apoptosis signaling pathways. These pathways are known to interact closely in ovarian cancer progression and therapeutic resistance [33–35]. Importantly, the enrichment of pathways related to chemical carcinogenesis and ROS is concordant with the controlled redox modulation observed in the combination group, supporting a coordinated rather than excessive oxidative stress response [28].

Given the predictive nature of *in silico* target identification, the bioinformatic findings should be interpreted as supportive and hypothesis-generating in nature, rather than as evidence of direct molecular modulation. Accordingly, additional experimental validation will be required to confirm the functional involvement of specific hub genes in this system.

In summary, the present study demonstrates that the combination of PJ34 and curcumin exerts synergistic antiproliferative, pro-apoptotic, and anti-migratory effects in ovarian cancer cell models with differential responsiveness to platinum-based chemotherapy. By integrating quantitative synergy analysis with phenotypic assays and bioinformatic pathway modeling, we provide a mechanistically informed framework supporting the cooperative involvement of complementary pathways, including PARP1-dependent DNA repair, p53-mediated apoptotic signaling, and PI3K–Akt survival signaling. While the bioinformatic predictions require further functional validation, they offer valuable hypothesis-generating insights that may inform the rational design of targeted combination strategies. Future studies should focus on validating these interactions in more complex experimental models and addressing pharmacological challenges associated with curcumin delivery to further explore the translational potential of this combination [36].

Although PJ34 is not a clinically approved PARP inhibitor, it was intentionally employed in this study as a well-established mechanistic tool compound to interrogate PARP1-dependent cellular pathways. Accumulating evidence indicates that PJ34 functions as a potent and relatively selective experimental probe capable of inducing robust, cancer-selective cell death responses, rather than acting as a nonspecific PARP inhibitor [37]. Previous studies have demonstrated that PJ34 selectively eradicates human cancer cells in xenograft models and promotes DNA damage-associated apoptotic responses, particularly when combined with genotoxic agents such as cisplatin or temozolomide [38]. In addition, PJ34 has been shown to disrupt mitochondrial integrity and to reveal distinct PARP1 dysfunction-associated cell death mechanisms, underscoring its value for the mechanistic dissection of PARP1-related vulnerabilities in cancer cells [39]. Importantly, PJ34 shares the core mechanism of PARP1 catalytic inhibition with clinically approved PARP inhibitors, including olaparib and niraparib. However, clinically used PARP inhibitors exhibit additional pharmacological properties—most notably PARP trapping activity and broader selectivity across PARP family members—which may influence therapeutic efficacy and toxicity profiles. In this context, the use of PJ34 enables the focused interrogation of PARP1-dependent signaling in a simplified experimental setting, without confounding effects related to PARP trapping or pan-PARP inhibition. Accordingly, while the present findings provide mechanistically informed, preclinical insight into how PARP1 inhibition cooperates with curcumin to enhance apoptotic cell death, extrapolation to the clinical setting should be undertaken with appropriate caution. Future studies employing clini-

cally approved PARP inhibitors will be essential to validate and extend the translational relevance of these observations.

Several limitations should be acknowledged when interpreting the translational relevance of this study. While the present work provides functionally supported mechanistically informative insight through quantitative synergy analysis, apoptosis profiling, ROS modulation, rescue experiments, and validation in 3D tumor spheroid models, a direct protein-level validation of key signaling events was not performed. Specifically, markers of PARP activity, DNA damage (e.g., γ H2AX), and apoptotic execution (e.g., cleaved caspase-3 and cleaved PARP) were not assessed using Western blotting or related proteomic approaches, which represents a limitation of the study. Due to budgetary and time constraints, protein-based analyses could not be conducted within the scope of this work. Importantly, however, the mechanistic interpretations presented here are supported by convergent functional, transcriptional, and rescue-based evidence, including caspase-3 activity measurements and ROS modulation analyses, and should therefore be regarded as mechanistically informed rather than molecularly definitive. In addition, PJ34 was intentionally employed as a well-established PARP1-preferential tool compound to interrogate PARP-dependent cellular pathways in combination with curcumin. Although PJ34 is not clinically approved, its use provides preclinical proof-of-concept insight relevant to clinically used PARP inhibitors such as olaparib, niraparib, and rucaparib, which share a common core mechanism of PARP enzymatic inhibition. Nevertheless, clinically approved PARP inhibitors exhibit additional pharmacological properties, including PARP trapping and broader PARP family selectivity, which may influence therapeutic outcomes. Accordingly, the direct extrapolation of these findings to the clinical setting should be undertaken with caution, and further experimental validation using clinically approved PARP inhibitors and more advanced preclinical models will be required.

Recent advances in diarylheptanoid and curcumin chemistry have substantially expanded the understanding of curcumin as a chemically versatile scaffold rather than a fixed natural product. These studies have demonstrated that strategic structural modifications can address key limitations of native curcumin, including chemical instability, rapid metabolic degradation, and suboptimal drug-like properties. In particular, contemporary synthetic approaches and structure–activity relationship analyses have shown that curcumin-derived analogs can be generated with improved stability, enhanced biological potency, and greater suitability for mechanistic interrogation and preclinical development [40].

Within this framework, the present study contributes a biologically grounded proof-of-concept by demonstrating that curcumin can function as an effective cooperative partner to PARP1 inhibition in ovarian cancer models with differential platinum responsiveness. While the current work does not address chemical optimization directly, the observed functional synergy between curcumin and PARP inhibition provides a rational biological context in which chemically optimized curcumin analogs may be further evaluated. Accordingly, recent developments in curcumin and diarylheptanoid chemistry offer a clear translational pathway for extending the biological findings reported here toward the future mechanistic refinement and preclinical validation of PARP inhibitor–curcumin-based combination strategies.

Moreover, curcumin is well known to exhibit a poor bioavailability due to its low solubility, rapid metabolism, and systemic elimination, which has historically limited its clinical efficacy [41,42]. This pharmacokinetic limitation, while not addressed in the present *in vitro* study, constitutes a recognized barrier to clinical translation. Although the present work extends beyond conventional 2D monolayer cultures by incorporating 3D tumor spheroid models, these models still represent a simplified approximation of the *in vivo* tumor microenvironment and do not fully recapitulate its cellular and molecular

complexity. Future studies should therefore evaluate combinations of clinically approved PARP inhibitors with bioavailability-optimized curcumin formulations in more advanced preclinical models, including refined 3D systems and in vivo models, to more accurately assess translational potential.

Figure 18 provides an integrative, hypothesis-driven graphical overview of the experimental and bioinformatic findings of this study, intended to conceptually summarize the proposed cooperative interactions underlying the combined effects of PJ34 and curcumin in ovarian cancer cells. As illustrated, PJ34-mediated PARP1 inhibition is associated with DNA damage and moderate oxidative stress, while curcumin is proposed to modulate key survival signaling pathways (including PI3K–Akt and NF- κ B) and exert antioxidant activity. When combined, these complementary actions are functionally associated with a synergistic interaction characterized by enhanced apoptosis, reduced proliferation, and a marked inhibition of cell migration, achieved at reduced drug doses (CI < 1; increased DRI). Importantly, the schematic emphasizes that the combination is associated with the normalization of intracellular ROS levels, rather than the induction of excessive oxidative stress, supporting the interpretation that apoptosis induction is not driven by ROS overload but reflects coordinated apoptotic signaling in which ROS plays a contributory, non-dominant role. The lower panel integrates in silico network analysis, highlighting TP53, AKT1, CASP3, EGFR, and HIF1A as central hub genes that provide biological context linking the observed phenotypic outcomes to interconnected apoptotic signaling, survival pathways, redox homeostasis, and migration-related processes. Together, this schematic should be interpreted as a conceptual synthesis of functional assays and bioinformatic predictions, rather than a definitive signaling map, and serves to contextualize the multi-level evidence supporting a cooperative antitumor interaction between PJ34 and curcumin, particularly in ovarian cancer models exhibiting reduced responsiveness to platinum-based chemotherapy.

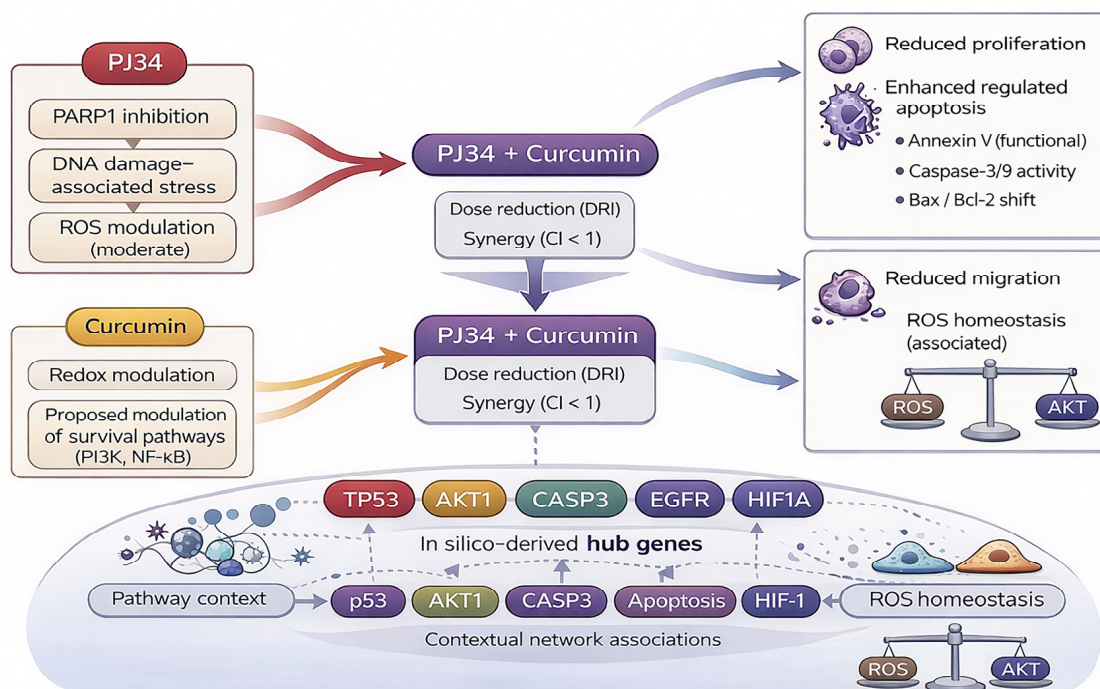


Figure 18. Hypothesis-driven conceptual model integrating functional and in silico findings of PJ34 and curcumin combination treatment in ovarian cancer cells. This schematic provides a conceptual, hypothesis-driven integration of the experimental and bioinformatic findings of the present study and does not represent a confirmed signaling pathway. The upper panels summarize functional phenotypic outcomes observed following combined treatment with PJ34 and curcumin, including

reduced proliferation, enhanced regulated apoptosis (as assessed by Annexin V positivity, caspase-3/9 activity, and Bax/Bcl-2 expression shifts), and reduced cell migration, achieved at reduced drug doses as reflected by quantitative synergy metrics ($CI < 1$; increased DRI). PJ34-mediated PARP1 inhibition is shown as being associated with DNA damage-related cellular stress and moderate ROS modulation, while curcumin is depicted as proposed to modulate redox balance and survival-associated signaling pathways. Importantly, the combination treatment is associated with ROS homeostasis rather than excessive oxidative stress, consistent with experimental findings indicating that ROS contributes to, but does not solely drive, apoptosis induction. The lower panel integrates *in silico*-derived hub genes (TP53, AKT1, CASP3, EGFR, and HIF1A) identified through protein–protein interaction and enrichment analyses. These elements are presented to provide biological context and network-level associations linking the observed phenotypic responses to interconnected apoptotic, survival, redox, and migration-related processes. All depicted interactions should be interpreted as supportive and hypothesis-generating, based on integration of functional assays and bioinformatic predictions, rather than as evidence of direct molecular regulation.

5. Conclusions

In conclusion, this study demonstrates that combined treatment with the PARP1 inhibitor PJ34 and curcumin elicits a robust and reproducible synergistic antitumor effect in ovarian cancer cell models with differential responsiveness to platinum-based chemotherapy, including relatively platinum-resistant SKOV-3 cells. Through integrated functional assays, quantitative synergy analysis, bioinformatic modeling, validation in 3D tumor spheroids, and ROS-rescue experiments, we demonstrate functionally that this combination suppresses proliferation, enhances regulated apoptotic cell death, and markedly inhibits migratory capacity.

Importantly, the observed synergy is achieved at reduced drug doses and is not associated with excessive oxidative stress. Instead, the functional interrogation of ROS contribution supports an ROS-associated, multifactorial mode of action, functionally consistent with PARP1-dependent DNA damage responses, the modulation of redox homeostasis, the activation of intrinsic apoptotic signaling, and the attenuation of survival-associated pathways such as PI3K–Akt signaling. These processes are proposed to cooperatively contribute to the enhanced antitumor response observed with the combination, rather than representing a single dominant causal mechanism. This profile distinguishes the combination from single-agent treatments and is particularly relevant in ovarian cancer cell models exhibiting reduced responsiveness to platinum-based chemotherapy, where impaired apoptotic signaling and increased migratory capacity are commonly observed phenotypic features.

While remaining preclinical and hypothesis-generating, the inclusion of advanced *in vitro* models and functional rescue experiments moves this work beyond descriptive efficacy assessment and provides mechanistically informed proof-of-concept evidence for PARP inhibitor–curcumin combination strategies. Future studies employing clinically approved PARP inhibitors, bioavailability-enhanced curcumin formulations, and *in vivo* models will be essential to further assess translational potential.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cancers18040620/s1>, Figure S1: Comparative cisplatin sensitivity in OVCAR-3 and SKOV-3 ovarian cancer cells.

Author Contributions: A.E.G.: Conceptualization, Methodology, Investigation, Data curation, Visualization, Software, Writing—original draft preparation, Writing—reviewing and editing, Supervision. M.C.T.: Methodology, Investigation, Visualization, Software, Validation, Data curation, Writing—original draft preparation. İ.Ö.: Conceptualization, Methodology, Investigation, Data curation, Visualization, Software, Writing—original draft preparation, Writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All experimental data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare that they have no competing interests.

Abbreviations

PARP1	Poly(ADP-ribose) polymerase 1
PJ34	Synthetic PARP1 inhibitor
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
IC ₅₀	Half-maximal inhibitory concentration
CI	Combination index
DRI	Dose reduction index
ROS	Reactive oxygen species
DCFH-DA	2',7'-Dichlorofluorescein diacetate
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
PBS	Phosphate-buffered saline
ATCC	American Type Culture Collection
RT-qPCR	Quantitative Real-Time PCR
PPI	Protein–protein interaction
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
HSA	Highest Single Agent
ANOVA	Analysis of variance
SEM	Standard error of the mean
SD	Standard deviation

References

1. Siegel, R.L.; Giaquinto, A.N.; Jemal, A. Cancer Statistics, 2024. *CA Cancer J. Clin.* **2024**, *74*, 12–49. [[CrossRef](#)]
2. Lheureux, S.; Braunstein, M.; Oza, A.M. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. *CA Cancer J. Clin.* **2019**, *69*, 280–304. [[CrossRef](#)] [[PubMed](#)]
3. Torre, L.A.; Trabert, B.; DeSantis, C.E.; Miller, K.D.; Samimi, G.; Runowicz, C.D.; Gaudet, M.M.; Jemal, A.; Siegel, R.L. Ovarian cancer statistics, 2018. *CA Cancer J. Clin.* **2018**, *68*, 284–296. [[CrossRef](#)] [[PubMed](#)]
4. Lord, C.J.; Ashworth, A. PARP inhibitors: Synthetic lethality in the clinic. *Science* **2017**, *355*, 1152–1158. [[CrossRef](#)] [[PubMed](#)]
5. González-Martín, A.; Pothuri, B.; Vergote, I.; DePont Christensen, R.; Graybill, W.; Mirza, M.R.; McCormick, C.; Lorusso, D.; Hoskins, P.; Freyer, G.; et al. Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N. Engl. J. Med.* **2019**, *381*, 2391–2402. [[CrossRef](#)]
6. Moore, K.; Colombo, N.; Scambia, G.; Kim, B.-G.; Oaknin, A.; Friedlander, M.; Lisysanskaya, A.; Floquet, A.; Leary, A.; Sonke, G.S.; et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N. Engl. J. Med.* **2018**, *379*, 2495–2505. [[CrossRef](#)]
7. Jagtap, P.; Szabó, C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat. Rev. Drug Discov.* **2005**, *4*, 421–440. [[CrossRef](#)]
8. Yang, L.; Xie, H.J.; Li, Y.Y.; Wang, X.; Liu, X.X.; Mai, J. Molecular mechanisms of platinum-based chemotherapy resistance in ovarian cancer (Review). *Oncol. Rep.* **2022**, *47*, 82. [[CrossRef](#)]
9. Bugide, S.; Gonugunta, V.K.; Penugurti, V.; Malisetty, V.L.; Vadlamudi, R.K.; Manavathi, B. HPIP promotes epithelial-mesenchymal transition and cisplatin resistance in ovarian cancer cells through PI3K/AKT pathway activation. *Cell. Oncol.* **2017**, *40*, 133–144. [[CrossRef](#)]
10. Tan, J.; Dong, W.; Wang, D.; Tu, A.; Wang, Z.; Wu, X.; Zhang, F.; Zhu, Y.; Ren, L.; Ai, Y.; et al. Targeting CDK12 rescues C/EBP β -mediated platinum and PARP inhibitor resistance in ovarian cancer. *Cell. Signal.* **2025**, *139*, 112328. [[CrossRef](#)]

11. Kielbowski, K.; Żychowska, J.; Becht, R. Anaplastic lymphoma kinase inhibitors—a review of anticancer properties, clinical efficacy, and resistance mechanisms. *Front. Pharmacol.* **2023**, *14*, 1285374. [\[CrossRef\]](#)
12. Özdemir, İ.; Baş, D.D.; Öztürk, Ş.; Karaosmanoğlu, Ö.; Tuncer, M.C. Rosmarinic acid inhibits the proliferation of ovarian carcinoma cells by activating the p53/BAX signaling pathway. *Histo. Histopathol.* **2025**, *40*, 1415–1423.
13. Karaosmanoğlu, Ö.; Kamalak, Z.; Özdemir, İ.; Öztürk, Ş.; Tuncer, M.C. Apoptotic effect of thymoquinone on OVCAR3 cells via the P53 and CASP3 activation. *Acta. Cir. Bras.* **2024**, *39*, e399224. [\[CrossRef\]](#)
14. Kunnumakkara, A.B.; Bordoloi, D.; Padmavathi, G.; Monisha, J.; Roy, N.K.; Prasad, S.; Aggarwal, B.B. Curcumin, the golden nutraceutical: Multitargeting for multiple chronic diseases. *Br. J. Pharmacol.* **2017**, *174*, 1325–1348. [\[CrossRef\]](#)
15. Giordano, A.; Tommonaro, G. Curcumin and Cancer. *Nutrients* **2019**, *11*, 2376. [\[CrossRef\]](#)
16. Özdemir, İ.; Zaman, F.; Doğan Baş, D.; Sari, U.; Öztürk, Ş.; Tuncer, M.C. Inhibitory effect of Curcumin on a cervical cancer cell line via the RAS/RAF signaling pathway. *Histol. Histopathol.* **2025**, *40*, 327–334. [\[PubMed\]](#)
17. Montopoli, M.; Ragazzi, E.; Frolidi, G.; Caparrotta, L. Cell-cycle inhibition and apoptosis induced by curcumin and cisplatin or oxaliplatin in human ovarian carcinoma cells. *Cell Prolif.* **2009**, *42*, 195–206. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Bayat Mokhtari, R.; Homayouni, T.S.; Baluch, N.; Morgatskaya, E.; Kumar, S.; Das, B.; Yeger, H. Combination therapy in combating cancer. *Oncotarget* **2017**, *8*, 38022–38043. [\[CrossRef\]](#)
19. Turk, A.A.; Wisinski, K.B. PARP inhibitors in breast cancer: Bringing synthetic lethality to the bedside. *Cancer* **2018**, *124*, 2498–2506. [\[CrossRef\]](#)
20. Domcke, S.; Sinha, R.; Levine, D.A.; Sander, C.; Schultz, N. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat. Commun.* **2013**, *4*, 2126. [\[CrossRef\]](#)
21. Beaufort, C.M.; Helmijr, J.C.; Piskorz, A.M.; Hoogstraat, M.; Ruigrok-Ritstier, K.; Besselink, N.; Murtaza, M.; van IJcken, W.F.J.; Heine, A.A.J.; Smid, M.; et al. Ovarian cancer cell line panel (OCCP): Clinical importance of in vitro drug response. *Mol. Cancer* **2014**, *13*, 199.
22. Adams, K.M.; Wendt, J.R.; Wood, J.; Olson, S.; Moreno, R.; Jin, Z.; Gopalan, S.; Lang, J.D. Cell-intrinsic platinum response and associated genetic and gene expression signatures in ovarian cancer. *Cancer Gene Ther.* **2025**, *32*, 985–996. [\[CrossRef\]](#)
23. Mansour, M.; van Ginkel, S.; Alata, M.; Bani, I.; Elhussin, I. Molecular signature of cisplatin resistance in ovarian cancer identifies therapeutic opportunities for re-sensitization. *J. Cancer* **2026**, *17*, 49–58. [\[CrossRef\]](#)
24. Herencia-Ropero, A.; Llop-Guevara, A.; Staniszewska, A.D.; Domènech-Vivó, J.; García-Galea, E.; Moles-Fernández, A.; Pedretti, F.; Domènech, H.; Rodríguez, O.; Guzmán, M.; et al. The PARP1 selective inhibitor saruparib (AZD5305) elicits potent and durable antitumor activity in patient-derived BRCA1/2-associated cancer models. *Genome Med.* **2024**, *16*, 107. [\[CrossRef\]](#)
25. Kabir, M.T.; Rahman, M.H.; Akter, R.; Behl, T.; Kaushik, D.; Mittal, V.; Pandey, P.; Akhtar, M.F.; Saleem, A.; Albadrani, G.M.; et al. Potential Role of Curcumin and Its Nanoformulations to Treat Various Types of Cancers. *Biomolecules* **2021**, *11*, 392. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Murata, M.M.; Kong, X.; Moncada, E.; Chen, Y.; Imamura, H.; Wang, P.; Berns, M.W.; Yokomori, K.; Digman, M.A. NAD⁺ consumption by PARP1 in response to DNA damage triggers metabolic shift critical for damaged cell survival. *Mol. Biol. Cell* **2019**, *30*, 2584–2597. [\[CrossRef\]](#)
27. Mashozhera, N.T.; Reddy, C.S.; Ranasinghe, Y.N.; Natarajan, P.; Reddy, U.K.; Hankins, G. Curcumin-Induced Molecular Mechanisms in U-87 MG Glioblastoma Cells: Insights from Global Gene Expression Profiling. *Molecules* **2025**, *30*, 2108. [\[CrossRef\]](#)
28. Trachootham, D.; Alexandre, J.; Huang, P. Targeting cancer cells by ROS-mediated mechanisms. *Nat. Rev. Drug Discov.* **2009**, *8*, 579–591. [\[CrossRef\]](#)
29. Pu, H.; Horbinski, C.; Hensley, P.J.; Matuszak, E.A.; Atkinson, T.; Kyprianou, N. PARP-1 regulates epithelial-mesenchymal transition (EMT) in prostate tumorigenesis. *Carcinogenesis* **2014**, *35*, 2592–2601. [\[CrossRef\]](#)
30. Tsai, J.R.; Liu, P.L.; Chen, Y.H.; Chou, S.H.; Cheng, Y.J.; Hwang, J.J.; Chong, I.W. Curcumin Inhibits Non-Small Cell Lung Cancer Cells Metastasis through the Adiponectin/NF- κ B/MMPs Signaling Pathway. *PLoS ONE* **2015**, *10*, e0144462. [\[CrossRef\]](#)
31. Díaz, P.; Sandoval-Bórquez, A.; Bravo-Sagua, R.; Quest, A.F.G.; Lavandero, S. Perspectives on Organelle Interaction, Protein Dysregulation, and Cancer Disease. *Front. Cell Dev. Biol.* **2021**, *9*, 613336. [\[CrossRef\]](#)
32. Deng, C.; Hu, F.; Zhao, Z.; Zhou, Y.; Liu, Y.; Zhang, T.; Li, S.; Zheng, W.; Zhang, W.; Wang, T.; et al. The Establishment of Quantitatively Regulating Expression Cassette with sgRNA Targeting BIRC5 to Elucidate the Synergistic Pathway of Survivin with P-Glycoprotein in Cancer Multi-Drug Resistance. *Front. Cell Dev. Biol.* **2022**, *9*, 797005. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Zhou, Z.; Wei, B.; Liu, Y.; Liu, T.; Zeng, S.; Gan, J.; Qi, G. Depletion of PARP10 inhibits the growth and metastatic potential of oral squamous cell carcinoma. *Front. Genet.* **2022**, *13*, 1035638. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Vousden, K.H.; Prives, C. Blinded by the light: The growing complexity of p53. *Cell* **2009**, *137*, 413–431. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Toprak, V.; Özdemir, İ.; Öztürk, Ş.; Yanar, O.; Kizildemir, Y.Z.; Tuncer, M.C. Thymoquinone Enhances Doxorubicin Efficacy via RAS/RAF Pathway Modulation in Ovarian Adenocarcinoma. *Pharmaceutics* **2025**, *17*, 536. [\[CrossRef\]](#)
36. Subramani, P.A.; Narala, V.R. Challenges of curcumin bioavailability: Novel aerosol remedies. *Nat. Prod. Commun.* **2013**, *8*, 121–124. [\[CrossRef\]](#)

37. Visochek, L.; Atias, D.; Spektor, I.; Castiel, A.; Golan, T.; Cohen-Armon, M. The phenanthrene derivative PJ34 exclusively eradicates human pancreatic cancer cells in xenografts. *Oncotarget* **2019**, *10*, 6269–6282. [[CrossRef](#)]
38. Cseh, A.M.; Fabian, Z.; Quintana-Cabrera, R.; Szabo, A.; Eros, K.; Soriano, M.E.; Gallyas, F.; Scorrano, L.; Sumegi, B. PARP inhibitor PJ34 protects mitochondria and induces DNA-damage-mediated apoptosis in combination with cisplatin or temozolomide in B16F10 melanoma cells. *Front. Physiol.* **2019**, *10*, 538. [[CrossRef](#)]
39. Cohen-Armon, M. The modified phenanthridine PJ34 unveils an exclusive cell-death mechanism in human cancer cells. *Cancers* **2020**, *12*, 1628. [[CrossRef](#)]
40. Sudarshan, K.; Yarlagadda, S.; Sengupta, S. Recent Advances in the Synthesis of Diarylheptanoids. *Chem. Asian J.* **2024**, *19*, e202400380. [[CrossRef](#)]
41. Elena, S.; Anjana, S.; Cat Tuong, D.T.; Chin, S.; Moniruzzaman, M.; Huy Doanh, B.; Karthikeyan, A.; Min, T. Curcumin's Therapeutic Potential in Ovarian Cancer: Current Insights and Future Perspectives. *Food Sci. Nutr.* **2025**, *13*, e71099. [[CrossRef](#)] [[PubMed](#)]
42. Tomeh, M.A.; Hadianamrei, R.; Zhao, X. A review of curcumin and its derivatives as anticancer agents. *Int. J. Mol. Sci.* **2019**, *20*, 1033. [[CrossRef](#)] [[PubMed](#)]

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