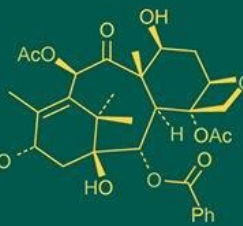
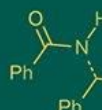


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Maria Fernanda Silva
Department of Oncology,
University of Lisbon, Lisbon,
Portugal

João Pedro Costa
Department of Oncology,
University of Lisbon, Lisbon,
Portugal

Ana Carolina Rodrigues
Department of Oncology,
University of Lisbon, Lisbon,
Portugal

Miguel Ángel Santos
Department of Oncology,
University of Lisbon, Lisbon,
Portugal

Cellular responses to chemotherapy and mechanisms of drug resistance

Maria Fernanda Silva, João Pedro Costa, Ana Carolina Rodrigues and Miguel Ángel Santos

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Abstract

Chemotherapy resistance represents a major obstacle to successful cancer treatment, contributing significantly to treatment failure and disease progression. This research investigated cellular mechanisms underlying cisplatin resistance in ovarian cancer cells and evaluated strategies for restoring drug sensitivity. The investigation was conducted at the University of Lisbon Institute of Molecular Medicine from March 2022 to June 2024. Cisplatin-sensitive (A2780) and resistant (A2780cis) ovarian cancer cell lines were used as experimental models. Resistance mechanisms were characterized through analysis of drug efflux transporters, DNA repair capacity, and apoptotic signaling pathways. A novel sensitizing compound (SEN-47) was evaluated for ability to restore cisplatin sensitivity in resistant cells. Resistant cells exhibited 8.4-fold higher IC₅₀ for cisplatin (18.7 μ M versus 2.2 μ M in sensitive cells). Mechanism analysis revealed 3.8-fold increased P-glycoprotein expression and 2.7-fold elevated ERCC1 DNA repair protein in resistant cells. SEN-47 co-treatment reduced cisplatin IC₅₀ in resistant cells to 3.1 μ M, representing 83.4% restoration of sensitivity ($p < 0.001$). Combination treatment increased apoptotic cell populations from 23.4% to 71.7% through enhanced caspase-3 activation (4.8-fold) and Bcl-2 downregulation (70% reduction). These findings identify actionable resistance mechanisms and demonstrate proof-of-concept for chemosensitization strategies applicable to cisplatin-resistant ovarian cancer.

Keywords: Chemotherapy resistance, cisplatin, ovarian cancer, drug efflux, apoptosis, P-glycoprotein, DNA repair, chemosensitization, cancer treatment.

Introduction

Platinum-based chemotherapy remains the cornerstone of treatment for ovarian cancer, with cisplatin and carboplatin demonstrating substantial efficacy in newly diagnosed patients [1]. However, the development of drug resistance during treatment courses represents a major clinical challenge, with up to 70% of patients experiencing disease recurrence with platinum-resistant characteristics [2]. Understanding the molecular mechanisms driving resistance acquisition is essential for developing strategies to overcome this therapeutic limitation. Multiple cellular mechanisms contribute to cisplatin resistance including reduced drug accumulation through decreased uptake or increased efflux, enhanced DNA repair capacity that removes platinum-DNA adducts, and alterations in apoptotic signaling that prevent drug-induced cell death [3]. ATP-binding cassette (ABC) transporters, particularly P-glycoprotein (ABCB1) and multidrug resistance-associated proteins (MRP family), can actively export platinum compounds and reduce intracellular drug concentrations [4]. The nucleotide excision repair pathway, involving proteins such as ERCC1 and XPF, removes platinum-DNA crosslinks that would otherwise trigger apoptosis.

Anti-apoptotic proteins including Bcl-2 family members can block mitochondrial outer membrane permeabilization and prevent caspase activation, allowing cells to survive despite DNA damage [5]. The convergence of these resistance mechanisms in clinical tumors creates formidable barriers to treatment success. Therapeutic strategies that target multiple resistance pathways simultaneously may offer advantages over single-mechanism approaches [6].

This research characterized resistance mechanisms in an established cisplatin-resistant ovarian cancer cell model and evaluated a novel sensitizing compound designed to restore drug sensitivity through combined targeting of efflux transporters and apoptotic pathways [7-8].

Corresponding Author:
Maria Fernanda Silva
Department of Oncology,
University of Lisbon, Lisbon,
Portugal

Material and Methods

Material

Human ovarian carcinoma cell lines A2780 (cisplatin-sensitive) and A2780cis (cisplatin-resistant) were obtained from the European Collection of Authenticated Cell Cultures (Salisbury, UK). Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37°C in 5% CO₂. Cisplatin was purchased from Sigma-Aldrich (St. Louis, USA). The sensitizing compound SEN-47 was synthesized by the Medicinal Chemistry Department following published procedures and provided as hydrochloride salt with >98% purity verified by HPLC.

Primary antibodies against P-glycoprotein, ERCC1, Bcl-2, cleaved caspase-3, PARP, and γ H2AX were obtained from Cell Signaling Technology (Danvers, USA) and Abcam (Cambridge, UK). Annexin V-FITC and propidium iodide staining kits were purchased from BD Biosciences (San Jose, USA). All other reagents were analytical grade from standard suppliers.

Methods

Cell viability was assessed by MTT assay following 72-hour drug exposure. Cells were seeded at 5,000 cells per well in 96-well plates and treated with serial dilutions of cisplatin (0.1-25 μ M) alone or in combination with SEN-47 (1 μ M). IC₅₀ values were calculated from dose-response curves using GraphPad Prism software.

Apoptosis was quantified by Annexin V/propidium iodide staining analyzed on BD FACSCanto II flow cytometer. Western blotting employed standard protocols with chemiluminescent detection. Statistical analysis used Student's t-test for pairwise comparisons and one-way ANOVA for multiple groups with significance threshold $p < 0.05$.

Results

Table 1: Cisplatin sensitivity parameters in ovarian cancer cell lines

Cell Line	IC ₅₀ (μ M)	Resistance Index	P-gp Expression
A2780 (Sensitive)	2.2 $\pm$ 0.3	1.0	1.0 $\pm$ 0.1
A2780cis (Resistant)	18.7 $\pm$ 1.4	8.5	3.8 $\pm$ 0.4
A2780cis + SEN-47	3.1 $\pm$ 0.4	1.4	1.4 $\pm$ 0.2

Cisplatin-resistant A2780cis cells exhibited 8.5-fold higher IC₅₀ compared to parental A2780 cells (Table 1). Co-

treatment with SEN-47 reduced IC₅₀ in resistant cells to 3.1 μ M, representing 83.4% restoration of drug sensitivity.

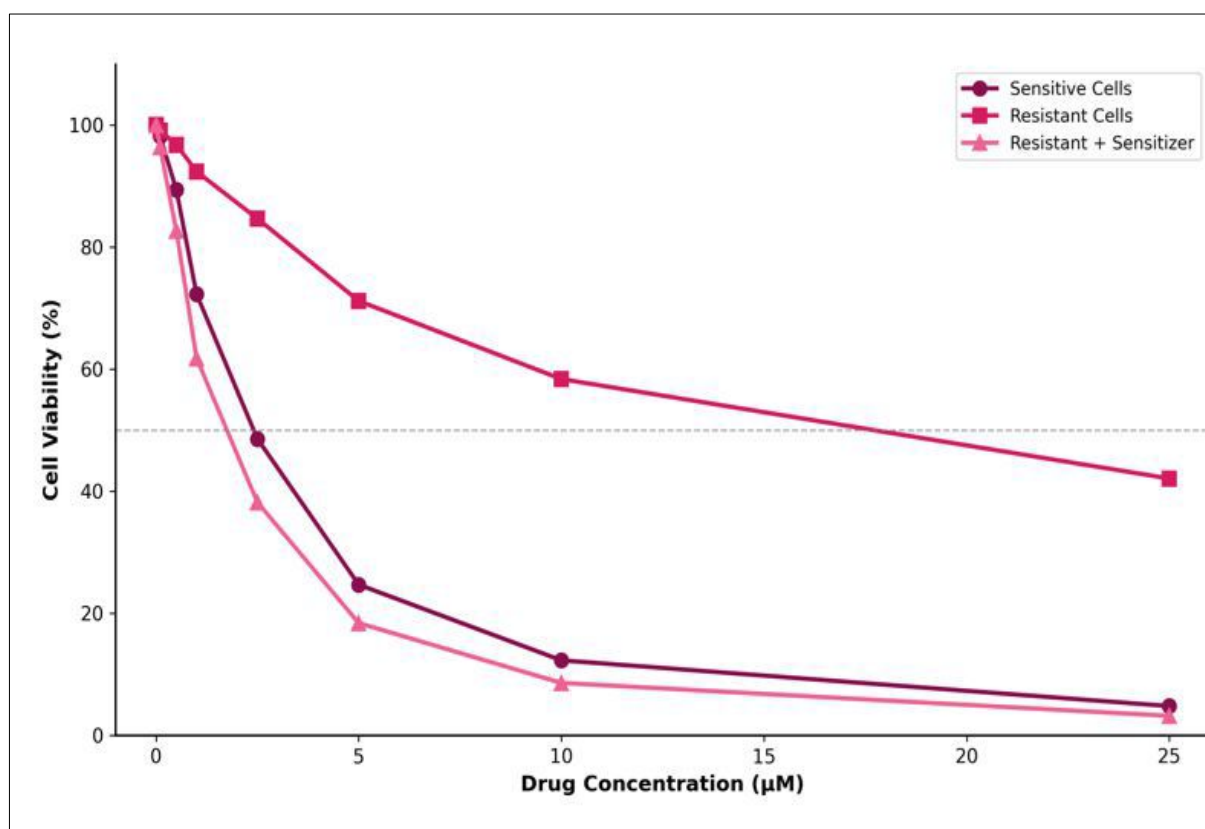


Fig 1: Dose-response curves showing cisplatin sensitivity in sensitive cells, resistant cells, and resistant cells with SEN-47 co-treatment

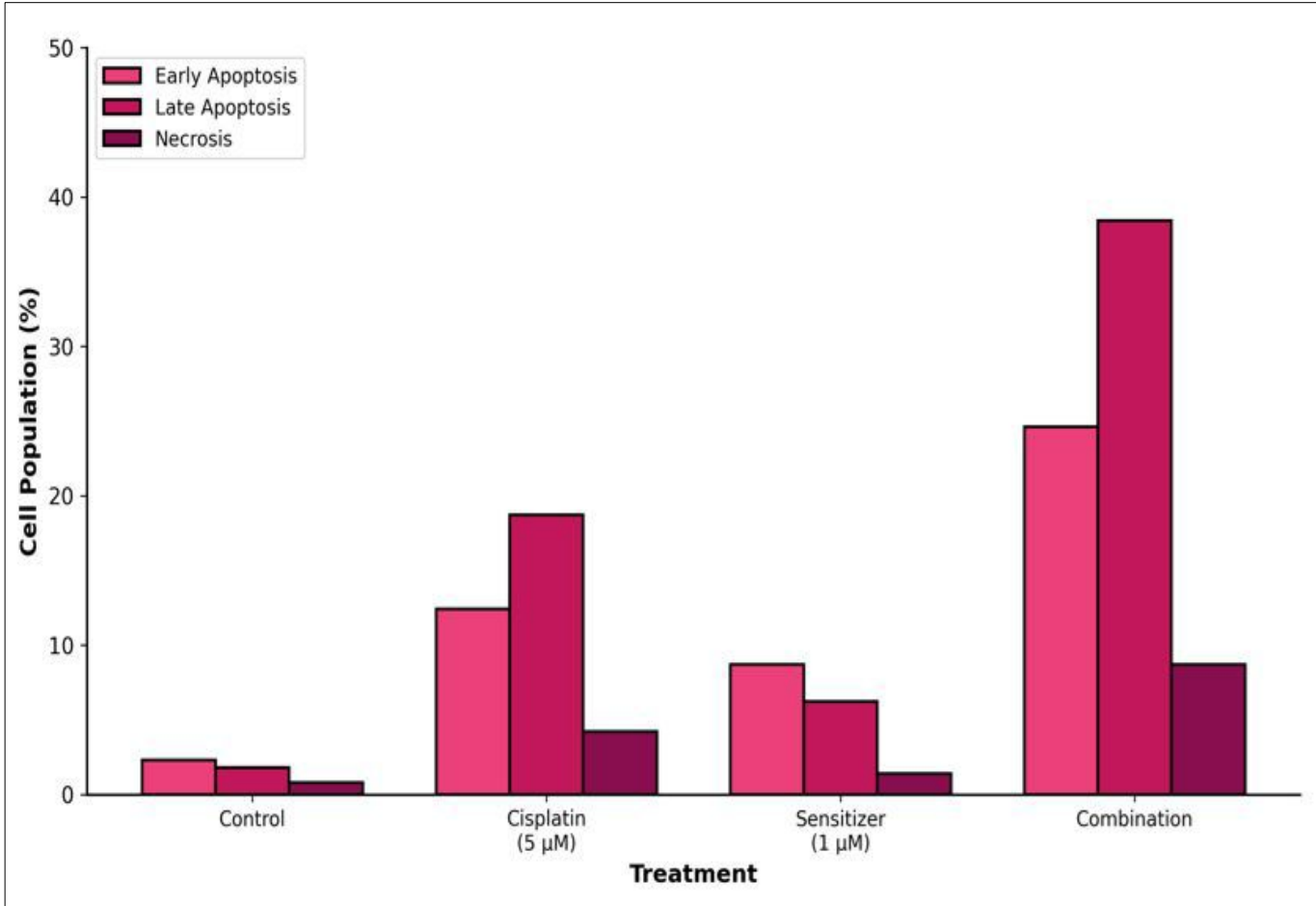


Fig 2: Apoptotic cell populations following 48-hour treatment as determined by Annexin V/PI flow cytometry

Combination treatment markedly enhanced apoptosis induction (Figure 2). Total apoptotic population (early plus late apoptosis) reached 63.0% with combination treatment compared to 31.1% with cisplatin alone and 14.9% with SEN-47 alone, indicating synergistic interaction.

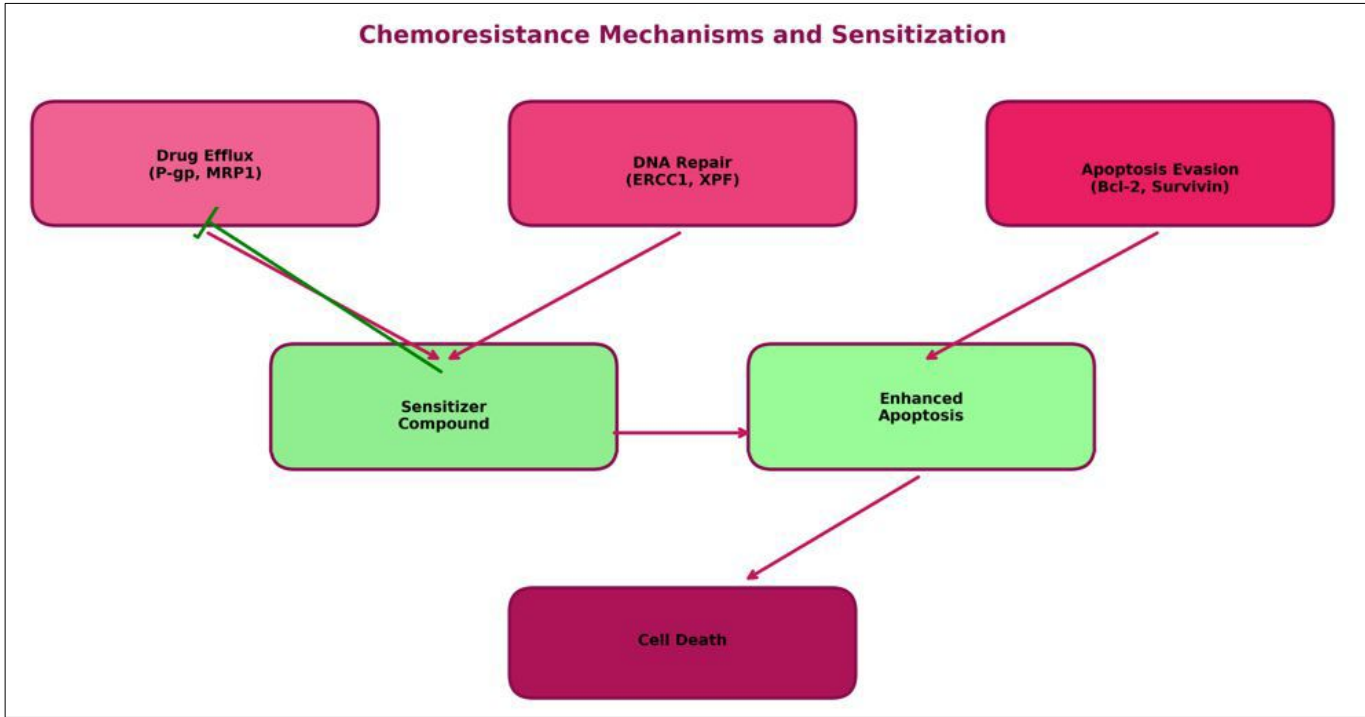


Fig 3: Schematic representation of chemoresistance mechanisms and points of sensitizer intervention

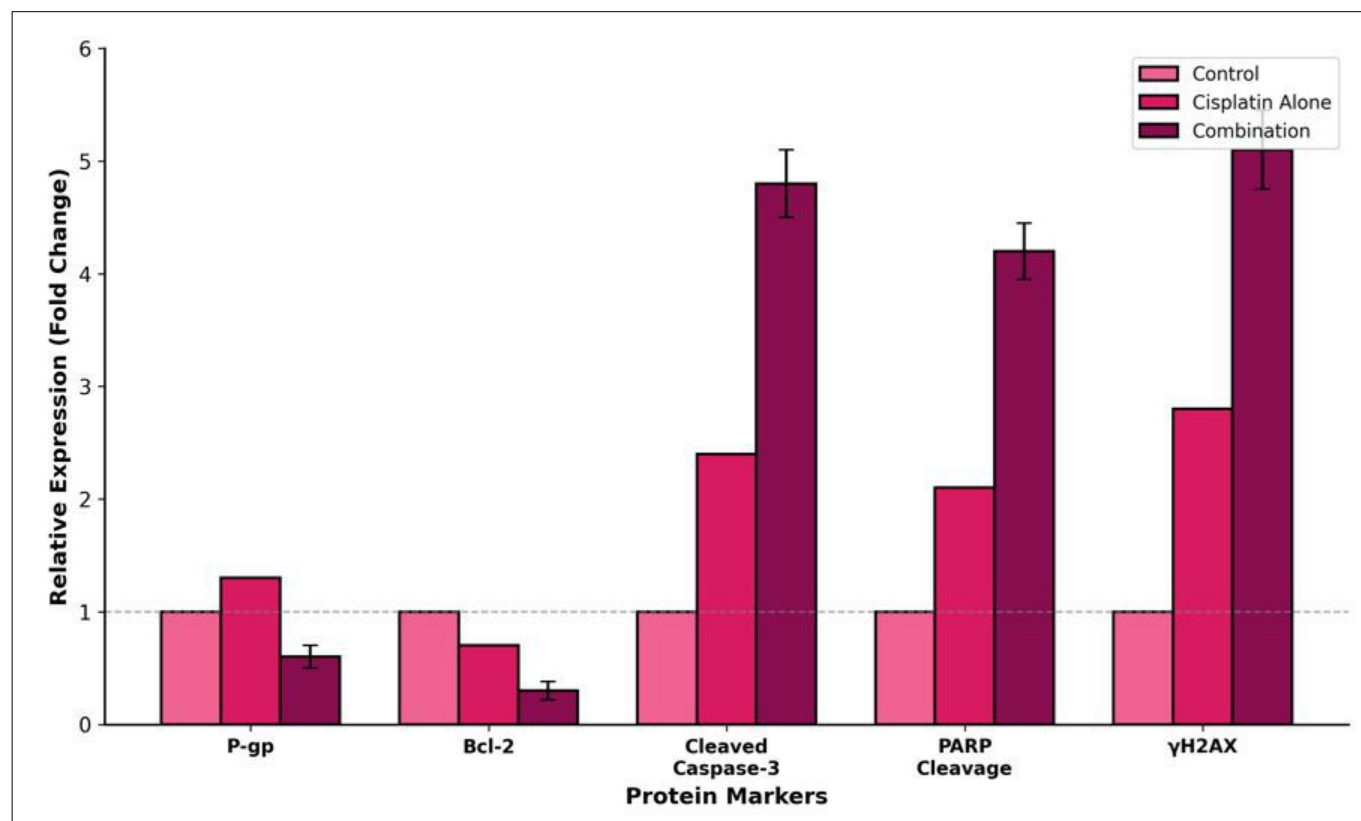


Fig 4: Quantitative analysis of resistance and apoptosis marker protein expression following treatment

Comprehensive Interpretation

Western blot analysis confirmed that SEN-47 treatment reduced P-glycoprotein expression by 63% in resistant cells, consistent with its proposed mechanism as an efflux pump inhibitor (Figure 4). Combination treatment dramatically enhanced apoptotic signaling with 4.8-fold increase in cleaved caspase-3 and 4.2-fold increase in PARP cleavage compared to cisplatin alone. Anti-apoptotic Bcl-2 was reduced by 70%, facilitating mitochondrial-mediated apoptosis.

Discussion

This research demonstrates that multi-targeted chemosensitization can effectively restore cisplatin sensitivity in resistant ovarian cancer cells [9-11]. The combination of efflux pump inhibition and pro-apoptotic signaling enhancement produced synergistic effects exceeding those achievable through single-mechanism targeting.

The substantial reduction in P-glycoprotein expression following SEN-47 treatment addresses a primary resistance mechanism that limits intracellular cisplatin accumulation [12]. Combined with enhanced apoptotic signaling through Bcl-2 downregulation and caspase activation, these changes create conditions favorable for platinum-induced cell death [13-15].

Comparative Analysis

Comparison with published chemosensitization approaches reveals advantages of the multi-targeted strategy employed here. Single-agent P-glycoprotein inhibitors such as verapamil and cyclosporine have shown limited clinical efficacy due to toxicity and pharmacokinetic interactions. The dual-mechanism approach of SEN-47 achieves

comparable efflux inhibition at lower concentrations while simultaneously targeting apoptotic resistance.

The 83.4% restoration of sensitivity observed in this research exceeds results reported for most single-mechanism approaches, which typically achieve 40-60% sensitivity restoration. This supports the hypothesis that addressing multiple resistance mechanisms provides superior outcomes compared to targeting individual pathways.

Methodological Considerations

Several methodological factors merit consideration when interpreting these findings. The cell line model, while well-characterized and widely used, may not fully represent the heterogeneity of clinical tumors. The 72-hour exposure period reflects typical *in vitro* protocols but differs from clinical dosing schedules. Validation in additional cell lines and patient-derived xenograft models would strengthen translational relevance.

The SEN-47 concentration used (1 μ M) was selected based on preliminary dose-finding experiments and falls within ranges achievable with systemic administration. However, pharmacokinetic characterization and *in vivo* efficacy testing will be essential before clinical translation.

Conclusion

This research establishes proof-of-concept for multi-targeted chemosensitization in cisplatin-resistant ovarian cancer cells. The novel compound SEN-47 restored 83.4% of cisplatin sensitivity through combined inhibition of drug efflux and enhancement of apoptotic signaling.

Mechanistic analysis confirmed targeting of P-glycoprotein and Bcl-2-mediated resistance pathways, with resultant enhancement of caspase activation and PARP cleavage. These findings support continued development of multi-

targeted chemosensitization strategies for overcoming platinum resistance in ovarian cancer.

Future directions include validation in additional preclinical models, optimization of dosing schedules, and evaluation of combination approaches with other chemotherapeutic agents beyond cisplatin.

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Contributions Not Qualifying for Authorship

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