

Innovative Simulation-Based Investigation of Traditional Chinese Medicine on Ovarian Cancer Cell Lines: A Professional Learning Model for Biomedical Science

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Abstract

Multidrug resistance and cancer recurrence remain critical challenges in ovarian cancer therapy. To address these issues through innovative, practice-based biomedical education, this study integrates simulation-based learning using ovarian cancer cell lines to explore the therapeutic potential of Pien Tze Huang (PZH), a traditional Chinese medicine. Building on prior research into high-grade serous ovarian cancer, we focused on endometrioid ovarian carcinoma using cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) cell lines. Students engaged in professional laboratory simulations including colony formation to assess PZH's time- and dose-dependent effects on cell proliferation and migration. Immunohistochemistry using PD-L1 antibody (Dako 22c3) and Leica BOND detection system enabled learners to investigate molecular expression patterns without ethical or safety concerns associated with human tissue. Results revealed enhanced collateral sensitivity in resistant cells and reduced IC50 values following prolonged PZH exposure, suggesting modulation of cell cycle and PD-L1 expression. This innovative simulation-based approach offers a safe, ethical, and trend-forward model for biomedical science education, empowering students with hands-on experience in cancer pharmacology and molecular diagnostics. It promotes critical thinking, technical proficiency, and professional readiness in a controlled, reproducible environment, bridging the gap between theoretical knowledge and clinical application.

Keywords: simulation-based learning, ovarian cancer cell lines, Pien Tze Huang, PD-L1 expression, immunohistochemistry

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Introduction

Ovarian cancer is a significant global health issue, with epithelial carcinoma comprising approximately 90% of cases. Among these, endometrioid carcinoma is a common subtype. The World Ovarian Cancer Coalition (2022) projects over four million deaths by 2040, with high incidence in regions like Northern Africa and Eastern Asia. While traditionally affecting middle-aged women, recent data show rising cases among premenopausal females, making it the third most prevalent carcinoma in women (Koppikar et al., 2023).

Despite the common use of CA125 test for follow-up, its limitations in screening restrict its utility. Other biomarker tests suffer from a lack of specificity. Treatment is further complicated by multidrug resistance to platinum-based drugs. Cancer, which often presents late, contributes to high mortality with median survival under 3.5 years, and remission typically occurs within 1 to 1.5 years post-chemotherapy (Garzon et al., 2020).

To address these challenges, this study introduces a simulation-based teaching model for biomedical science students, centered on the therapeutic potential of Pien Tze Huang (PZH), a traditional Chinese medicine with anti-inflammatory and anti-carcinogenic effects (Huang et al., 2016). PZH has demonstrated efficacy in colorectal (Gou et al., 2023), breast (Yan et al., 2023), lung (Chen et al., 2022), and bone cancers (Zhang et al., 2013). This research investigates its effects on endometrioid ovarian carcinoma using A2780s (cisplatin-sensitive) and A2780cp (cisplatin-resistant) cell lines. The study aims to: (1) assess time- and dose-dependent effects of PZH on cell viability and migration; (2) compare responses between sensitive and resistant cell lines. It utilizes cell culture, processing and immunohistochemistry test using PD-L1 antibody, quantified using Tumor Proportion Score (TPS) and staining intensity.

This simulation-based model offers students hands-on experience in cancer pharmacology, cell culture handling and immunohistochemistry, without ethical concerns of using real human tissue. It integrates medicine with laboratory techniques for image analysis, fostering critical thinking and technical skills in a re-producible, professional setting. The study contributes to PD-L1 research as a biomarker and immunotherapy target, encouraging interdisciplinary exploration and innovation in biomedical education.

Literature Review

Understanding ovarian cancer requires not only studying tumor biology but also building strong training models for students and researchers. The way cancer cells are grown in the lab can change how they respond to drugs. For example, A2780 ovarian cancer cells show different multidrug-resistance (MDR) behaviors when grown in flat 2D dishes compared to 3D cultures. This means that the learning environment must teach students how culture conditions can affect drug testing and interpretation (Koshkin et al., 2021). Since ovarian cancer often comes back after chemotherapy, training models that highlight resistance mechanisms are important for preparing learners to think critically about treatment challenges (Garzon et al., 2020).

Traditional Chinese Medicine (TCM), especially PZH, provides another teaching opportunity. PZH has been shown to influence immune signaling in sepsis models through bile acid-mediated pathways (Li et al., 2024). In cancer studies, PZH and related compounds can trigger cell death and reduce tumor growth (Chen et al., 2022; Gou et al., 2023; Yan et al., 2023; Zhang et al., 2013). These findings can be turned into training exercises where students practice dose-

time viability assays, migration studies, and immunohistochemistry to see how TCM compounds affect cancer cells (Xu et al., 2025). However, learners must also understand how routine lab steps, like trypsinization, can change cell proteins and influence results if not carefully standardized (Huang et al., 2010).

Checkpoint biology is another area where training is essential. PD-L1 testing is widely used to guide immunotherapy. Studies show that antibodies like Dako 22C3 can be used across different staining platforms if protocols are adjusted, which is a valuable lesson in assay reliability (Ilie et al., 2017). Teaching students how to measure PD-L1 consistently prepares them for real-world applications, especially as new therapies such as bispecific CAR-T cells targeting PD-1 and C-Met are developed (Yang et al., 2025).

Together, these studies support a simulation-based training framework. By combining lessons on MDR, TCM compounds like PZH, standardized lab techniques, and checkpoint assays, learners can practice connecting biology, pharmacology, and immunology. This approach not only improves reproducibility but also helps future biomedical scientists understand how research decisions impact cancer treatment outcomes (Gou et al., 2023; Yan et al., 2023).

Methodology

Cell Lines

This study used cisplatin-sensitive A2780s and cisplatin-resistant A2780cp human ovarian cancer cell lines to evaluate the therapeutic effects of PZH on endometrioid ovarian carcinoma. A2780s, derived from untreated patients, models drug sensitivity, while A2780cp, developed via prolonged cisplatin exposure, models resistance through enhanced drug efflux. The well-characterized lines allow students to study drug responses mechanisms safely without human tissues. Using these models supports simulation-based biomedical education by enabling hands-on experience in cancer pharmacology and biomedical research, fostering professional skills and critical analysis.

Culture Medium

Complete medium was prepared using Dulbecco's Modified Eagle Medium (DMEM; Cat. 11965-084) supplemented with 10% fetal bovine serum (FBS; Cat. A56707-01) and 1% antibiotic-antimycotic (Cat. 15240062). DMEM provides essential nutrients, vitamins, salts, and high glucose, supporting the growth of mammalian cells, including the ovarian cancer lines used. Sodium bicarbonate buffers pH to physiological levels (7.0–7.4) under 5–10% CO₂, with phenol red serving as a pH indicator; yellowing signals acidity or CO₂ shifts, prompting medium changes. FBS enriches the medium with growth factors and proteins, with formulation following reference standard (Koshkin et al., 2021).

Cell Culture

A2780s and A2780cp ovarian cancer cell lines were thawed at 37°C using a water bath, then cultured in flasks under standard conditions (37°C, 5% CO₂). Cells were passaged at 70–80% confluence based on doubling times: A2780s (2–5 days) and A2780cp (5–7 days), reflecting slower growth due to cisplatin resistance (Xu et al., 2025). PBS (Cat. 10010023) was used to rinse cells before adding 0.25% trypsin-EDTA (Cat. 25200-056) for detachment. Over-trypsinization was avoided to preserve surface proteins like PD-L1 (Huang et al., 2010). Cells

were centrifuged (200g, 5 min), resuspended, and subcultured with even distribution. Morphologically, A2780s showed epithelial traits; A2780cp appeared round.

PZH Preparation

PZH stock solution was prepared at 20 mg/ml by dissolving 300 mg of powder in 15 ml PBS (pH 7.4) and sonicated for 30 minutes at 70 Hz to ensure complete dissolution (Li et al., 2024). To preserve stability and prevent freeze-thaw damage, stocks were stored at 4°C in the dark or used promptly. Using PBS at physiological pH protects active components and prevents osmotic stress, simulating oral ingestion. Before application, the solution was warmed to 37°C, centrifuged, and diluted with pre-warmed medium for cell treatment. This method instructs students in precise, reproducible drug preparation essential for cancer research. The microscopic images of cell morphology were obtained from Figure 1 and 2.

Figure 1

Microscopic Photo of A2780s Cell Morphology

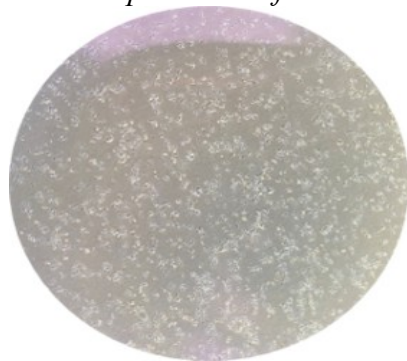
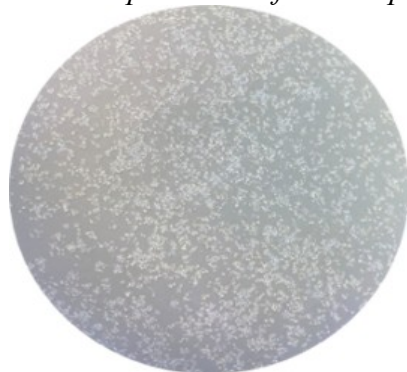


Figure 2

Microscopic Photo of A2780cp Cell Morphology



Colony Formation Assay

The colony formation assay evaluated cancer cell growth by measuring colony development in vitro. For this, 2,000 cells were evenly seeded per well in 6-well plates with 1.5 ml complete medium, using manual counting verified by an auto-mated cell counter (LUNA II) to ensure accuracy and viability. After 24 hours, cell adhesion was confirmed before applying PZH in six concentrations (0 to 1500 µg/ml) diluted in 500 µl warmed medium, added without removing existing medium. Half maximal inhibitory concentrations, the concentrations of PZH that reduce the number of cell colonies by 50% (IC50) in A2780s and A2780cp cell lines were determined. This method teaches students the precise cell handling and dose-response testing

critical for cancer research. Arrangement of the 6-well plate for colony formation assay was shown in Table 1.

Table 1

Arrangement of the 6-Well Plate for Colony Formation Assay

Well 1	Well 2	Well 3
PZH 0 $\mu\text{g/ml}$ (control) 0 μl PZH with 500 μl of complete medium	PZH 300 $\mu\text{g/ml}$ 30 μl PZH with 470 μl of complete medium	PZH 600 $\mu\text{g/ml}$ 60 μl PZH with 440 μl of complete medium
Well 4	Well 5	Well 6
PZH 900 $\mu\text{g/ml}$ 90 μl PZH with 410 μl of complete medium	PZH 1200 $\mu\text{g/ml}$ 120 μl PZH with 380 μl of complete medium	PZH 1500 $\mu\text{g/ml}$ 150 μl PZH with 350 μl of complete medium

Cell Block Preparation, Tissue Processing and Sectioning

To prepare cell blocks for staining, ovarian cancer cells (A2780s and A2780cp) were treated with PZH at control (0 $\mu\text{g/ml}$) and IC50 doses (1200 $\mu\text{g/ml}$ for A2780s, 900 $\mu\text{g/ml}$ for A2780cp) at 70% confluence. After 24 hours, cells were fixed with 10% buffered formalin for 30 minutes, scraped, and centrifuged. HistoGel (3%) was liquefied, cooled to 37°C, and mixed with the pellet to form a firm gel block, avoiding overheating to preserve protein integrity. The gel block was trimmed, wrapped, and processed using the Eprelia processor, ensuring deep tissue penetration for uniform fixation. After wax infiltration, blocks were embedded in molds with care to avoid sample carryover. Sections were cut at 4 μm , floated in a 50°C water bath, and mounted on poly-L-lysine-coated slides to pre-vent detachment during IHC staining (Agilent Technologies, 2021). Control and IC50 samples were placed on the same slide (Figure 3).

Figure 3

Tissue Sections on Positively Charged Slides



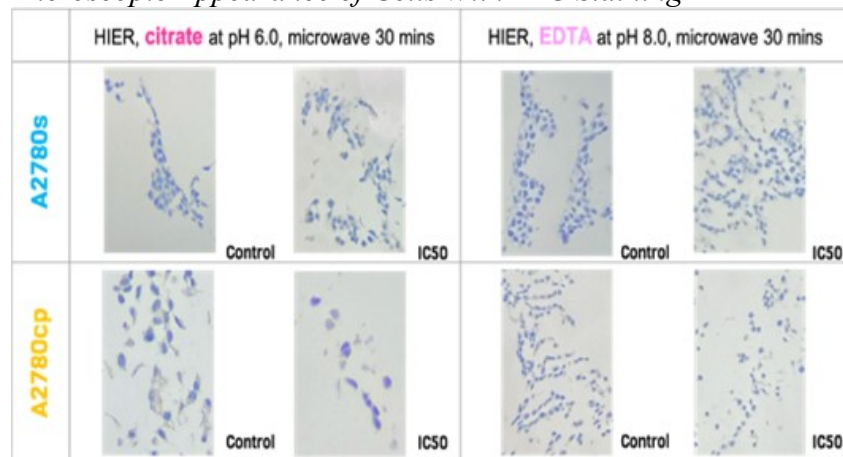
H&E Staining and IHC Staining of PD-L1

Before PD-L1 IHC staining, H&E stain was performed to confirm specimen quality and presence of over 100 viable tumor cells. Slides were dewaxed, rehydrated, and stained with Harris hematoxylin and eosin to visualize cell structures and ensure suitability for IHC. Enzyme-based retrieval was avoided to protect PD-L1 epitopes. IHC staining followed Leica's Bond Polymer Refine protocol (DS9800), using Dako's PD-L1 Clone 22C3 antibody (M3653) at 1:50 dilution. Detection involved HRP-linked polymer and DAB substrate, followed by

hematoxylin counterstaining which showed in Figure 4. Tonsil tissue served as a positive control (Agilent Technologies, 2021). PD-L1 was expressed in control A2780s and A2780cp blocks, but reduced in A2780cp treated with PZH at IC50, suggesting downregulation of PD-L1 (Yang et al., 2025).

Figure 4

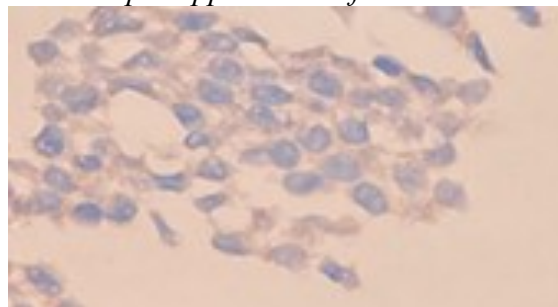
Microscopic Appearance of Cells With IHC Staining



Results

This study used colony formation assays to determine IC50 of PZH in cisplatin-sensitive A2780s and cisplatin-resistant A2780cp ovarian cancer cell lines. Results showed lower IC50 values at 48 hours than at 24 hours for both lines, indicating a time-dependent effect. A2780cp had lower IC50 values than A2780s, suggesting greater sensitivity to PZH despite its drug-resistant nature. Statistical analysis using F-tests and type II t-tests confirmed significant differences in IC50 between 24 and 48 hours for both cell lines ($p < 0.05$), and between A2780s and A2780cp at 24 hours. H&E staining was used to confirm sample adequacy before PD-L1 IHC staining was performed on a total of 12 cases. Due to extensive cell death at 48 hours, IHC was only performed on 24-hour samples. This decision taught students to critically assess specimen quality and interpret viability-linked biomarkers like PD-L1. The workflow emphasized hands-on learning in experimental design, statistical analysis, and histological validation, the core competencies in biomedical science education.

To validate PD-L1 immunohistochemistry (IHC), tonsil tissue was used as an internal positive control, following the Dako 22c3 interpretation guide (Agilent Technologies, 2021). Known for PD-L1 expression in germinal centers, tonsil samples confirmed reagent and system reliability. Staining intensity was graded from 1+ to 4+, with our control showing a strong 4+ dark brown membrane signal. This step taught students the importance of internal controls to ensure reproducibility and accuracy in IHC protocols. After confirming cell block quality via H&E staining, IHC was performed on A2780s and A2780cp cell blocks using the optimized protocol. PD-L1 expression was observed in both control and IC50-treated samples at 24 hours, suggesting baseline expression in ovarian cancer cells (Figure 5). This aligned with previous findings [12], where PD-L1 was detected at medium levels in A2780s via flow cytometry.

Figure 5*Microscopic Appearance of Cells With PD-L1 Expression*

Tumor Proportion Score (TPS) was calculated using the Dako formula: $TPS (\%) = (\text{PD-L1-stained viable tumor cells} / \text{total viable tumor cells}) \times 100\%$ Only membrane-stained, viable cells were counted, excluding cytoplasmic and necrotic cells. All samples exceeded the minimum 100-cell threshold, ensuring valid scoring. A2780s consistently showed 1+ intensity and 100% TPS across trials. A2780cp also showed 100% TPS, but with reduced staining intensity post-PZH treatment (from 3+/2+ to 1+), indicating a possible downregulation of PD-L1.

Students learned to assess staining quality, quantify biomarker expression, and interpret semi-quantitative data. The experiment also confirmed antibody-platform compatibility, confirming that Dako 22c3 could be used with the Leica BOND system under specific conditions: 1:50 antibody dilution, overnight incubation at 4°C, citrate buffer (pH 6.0), and extended incubation steps. This reinforced the importance of protocol optimization and adaptability in translational research.

Discussion

This study revealed that PZH had greater efficacy at 48 hours than at 24 hours, with significantly lower IC₅₀ values in both cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) ovarian cancer cell lines. Students learned that prolonged drug exposure enhances therapeutic impact, possibly due to cumulative stress, reactive oxygen species (ROS) buildup, and activation of apoptotic pathways like caspase 2 and 3. These findings highlight the importance of time-dependent drug action and cell cycle coverage in cancer pharmacology.

A2780cp showed greater sensitivity to PZH than A2780s, introducing students to the concept of collateral sensitivity, where drug-resistant cells become vulnerable to unrelated compounds. This was linked to altered metabolism, slower proliferation, and stress adaptation. Students also explored how glycosylation differences and signaling pathway modulation (e.g., PI3K/Akt, NF-κB) may influence drug uptake and resistance reversal.

The study emphasized hands-on learning in experimental design, statistical analysis, and staining interpretation, encouraging students to connect traditional medicine with modern cancer biology and explore mechanisms behind drug efficacy and resistance. Students explored how PZH may influence PD-L1 expression and cancer cell death through molecular pathways. Ginsenosides in PZH, such as Rg3 and Rb1, were shown to suppress cytokines like IL-6 and IL-8 via NF-κB and MAPK/p38 inhibition, promoting apoptosis. A2780cp cells exhibited lower p38 levels than A2780s, possibly linked to drug resistance. This introduced learners to the role of signaling pathways in immune evasion and cancer progression.

Minimal changes in PD-L1 expression observed via IHC led to discussions on microRNA regulation, especially miR-424, and its impact on PD-L1 levels. Students learned how cytokines like IFN- γ activate JAK/STAT signaling, influencing PD-L1 expression and immune response. To validate these findings, students proposed molecular assays such as qPCR and Western blotting. They are suggested to further practice RNA extraction, cDNA synthesis, and protein quantification, reinforcing skills in experimental design, biomarker analysis, and pathway investigation. This hands-on approach deepened their understanding of how traditional compounds like PZH interact with modern cancer biology.

Conclusion

This study showed that PZH reduced cell growth in both A2780s and A2780cp ovarian cancer cell lines in a time- and dose-dependent manner. The colony formation assay revealed lower IC50 values at 48 hours compared to 24 hours, highlighting the importance of prolonged drug exposure. Interestingly, the cisplatin-resistant A2780cp cells were more sensitive to PZH than the cisplatin-sensitive A2780s, introducing students to the concept of collateral sensitivity. PD-L1 immunohistochemistry confirmed baseline expression in both cell lines, with reduced staining intensity in A2780cp after PZH treatment, suggesting possible downregulation.

Implications

The findings suggest that PZH may have therapeutic potential in overcoming drug resistance in ovarian cancer, while also serving as a valuable teaching tool. Students learned how traditional medicine can be studied with modern biomedical techniques, gaining skills in cell culture, drug preparation, statistical analysis, and immunohistochemistry. This hands-on training reinforced critical thinking about drug efficacy, biomarker expression, and the complexity of cancer pharmacology.

Limitations

The study was limited by focusing on only two cell lines. PD-L1 changes were minimal and assessed only at 24 hours due to extensive cell death at 48 hours, which restricted deeper analysis of time-dependent biomarker regulation.

Recommendations

Future student-led experiments should expand to additional ovarian cancer cell lines and include molecular techniques to validate PD-L1 regulation and pathway involvement. Longer treatment durations and combination studies with other drugs could help clarify the mechanisms of collateral sensitivity. Incorporating assays for apoptosis, ROS generation, and signaling pathways (e.g., PI3K/Akt, NF- κ B, JAK/STAT) would provide a more complete picture. From an educational perspective, integrating these advanced methods into training will further strengthen biomedical research skills and prepare students for translational cancer studies.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The author declares that the AI-assisted writing software of Microsoft Copilot, was used in proofreading and refining the language used in the manuscript. The usage was limited to

correcting grammatical and spelling errors and rephrasing statements for accuracy and clarity. The author further declares that, apart from that, no other AI or AI-assisted technologies have been used to generate content in writing the manuscript. The ideas, design, procedures, findings, analyses, and discussion are originally written and derived from careful and systematic conduct of the research.

References

- Chen, Q., Hong, Y., Weng, S., Guo, P., Li, B., Zhang, Y., Yu, C., Wang, S., & Mo, P. (2022). Traditional Chinese medicine Pien-Tze-huang inhibits colorectal cancer growth and immune evasion by reducing β -catenin transcriptional activity and PD-L1 expression. *Frontiers in Pharmacology*, *13*, 1–10.
- Garzon, S., Laganà, A. S., Casarin, J., Raffaelli, R., Cromi, A., Franchi, M., Barra, F., Alkatout, I., Ferrero, S., & Ghezzi, F. (2020). Secondary and tertiary ovarian cancer recurrence: What is the best management? *Gland Surgery*, *9*(4), 1118–1129.
- Gou, H., Su, H., Liu, D., Wong, C. C., Shang, H., Fang, Y., Zeng, X., Chen, H., Li, Y., Huang, Z., Fan, M., Wei, C., Wang, X., Zhang, X., Li, X., & Yu, J. (2023). Traditional medicine Pien Tze Huang suppresses colorectal tumorigenesis through restoring gut microbiota and metabolites. *Gastroenterology*, *165*(6), 1404–1419.
- Huang, H.-L., Hsing, H.-W., Lai, T.-C., Chen, Y.-W., Lee, T.-R., Chan, H.-T., Lyu, P.-C., Wu, C.-L., Lu, Y.-C., Lin, S.-T., Lin, C.-W., Lin, C.-H., Chang, H.-T., Chou, H.-C., & Chan, H.-L. (2010). Trypsin-induced proteome alteration during cell subculture in mammalian cells. *Journal of Biomedical Science*, *17*(1). <https://doi.org/10.1186/1423-0127-17-36>
- Huang, M., Xu, W., Zhang, Y., Liu, J., Zhang, X., Lin, J., Chen, J., Li, Y., Wang, L., & Zhao, Q. (2016). Identification and quantification of the anti-inflammatory constituents in Pien-Tze-huang by liquid chromatography combined with quadrupole time-of-flight and triple quadrupole mass spectrometry. *Journal of Chromatography B*, *1027*, 27–39.
- Ilie, M., Long-Mira, E., Bence, C., Butori, C., Lassalle, S., Bouhleb, L., & Hofman, P. (2017). Comparative study of the PD-L1 expression and 22C3 antibody staining using Dako and Leica platforms in NSCLC. *Annals of Oncology*, *28*(Suppl 2), ii76–ii77.
- Koppikar, S., Oaknin, A., Babu, K. G., Lorusso, D., Gupta, S., Wu, L. Y., Colombo, N., Kim, J. W., Takeuchi, S., Ochi, H., Lim, M. C., Lee, J., Park, S. Y., & Bhatla, N. (2023). Pan-Asian adapted ESMO clinical practice guidelines for the diagnosis, treatment and follow-up of patients with endometrial cancer. *ESMO Open*, *8*(1), 100774.
- Koshkin, V., De Oliveira, M., Peng, C., Ailles, L., Liu, G., Covens, A., Bowering, V., & Karasinska, J. (2021). Multi-drug-resistance efflux in cisplatin-naive and cisplatin-exposed A2780 ovarian cancer cells responds differently to cell culture dimensionality. *Molecular and Clinical Oncology*, *15*(2). <https://doi.org/10.3892/mco.2021.2323>
- Li, B., Zhang, Y., Liu, X., Zhang, Z., Zhuang, S., Zhong, X., Chen, L., Wang, H., Zhou, Y., Fang, Y., & Xu, J. (2024). Traditional Chinese medicine Pien-Tze-huang ameliorates LPS-induced sepsis through bile acid-mediated activation of TGR5-STAT3-A20 signaling. *Journal of Pharmaceutical Analysis*, *14*(4), 100915.
- Ovarian Cancer Research Alliance. (n.d.). High-grade serous ovarian cancer. *Ovarian Cancer Research Alliance*. <https://ocrahope.org>

- Xu, Z., Jiang, Y., Shan, T., Hu, L., Wu, M., Ji, H., Li, L., Yi, Y., Wang, H., & Wang, L. (2025). Study on the effects and mechanism of Corilagin on A2780 cell apoptosis. *Current Issues in Molecular Biology*, 47(2), 105.
- Yan, X., Liu, Y., Li, C., Mao, X., Xu, T., Hu, Z., Chen, Y., Wang, J., Zhou, F., Zhang, L., & Sun, R. (2023). Pien-Tze-huang prevents hepatocellular carcinoma by inducing ferroptosis via inhibiting SLC7A11-GSH-GPX4 axis. *Cancer Cell International*, 23(1), 1–14.
- Yang, H., Zhang, Y., Li, Y., Peng, S., An, R., Du, N., Cao, J., Chu, F., & Min, J. (2025). Bispecific C-Met/PD-1 CAR-T cells have enhanced therapeutic effects on solid tumor. *Technology in Cancer Research & Treatment*, 24. <https://doi.org/10.1177/15330338251336850>
- Zhang, Y., Wang, Q., Niu, S., Liu, J., & Zhang, L. (2013). Pien Tze Huang induces apoptosis in multidrug-resistant U2OS/ADM cells via downregulation of bcl-2, survivin and P-gp and upregulation of Bax. *Oncology Reports*, 31(2), 763–770.