

Original Article

Potential benefits of combined treatment with Hsp90 inhibitor AUY922 and cisplatin for overcoming drug resistance in nasopharyngeal carcinoma

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Received November 30, 2024; Accepted January 16, 2025; Epub February 15, 2025; Published February 28, 2025

Abstract: Nasopharyngeal carcinoma (NPC) initially responds well to platinum-based therapy but often develops resistance. Combining therapies may offer a viable approach to address this resistance. Heat shock protein 90 (Hsp90) has shown promising anticancer activity in various cancer types. This study aimed to investigate the efficacy of an Hsp90 inhibitor, luminespib (AUY922), and evaluate the synergistic effect of combining AUY922 with cisplatin on two cisplatin-resistant human NPC cell lines. The response of cisplatin-resistant NPC cells to AUY922 and/or cisplatin was assessed through proliferation assay, cell cycle analysis, Annexin V apoptosis detection, Western blot analysis, *in vivo* investigation, and histological analysis. Our results indicated that AUY922/cisplatin combination significantly inhibited the proliferation of both non-resistant and resistant NPC cells. Moreover, Annexin V analysis indicated apoptosis when AUY922 was administered alone or in combination with cisplatin. Consistently, Western blot analysis revealed increased cleavage of PARP. Most importantly, the combination treatment demonstrated enhanced tumor growth inhibition in nude mice xenograft models, without notable adverse effects. These findings highlight the antiproliferative effects and anticancer activity of the AUY922/cisplatin combination in cisplatin-resistant NPC cells. The combination treatment of AUY922 and cisplatin holds promise as a strategy to overcome drug resistance in NPC patients.

Keywords: AUY922, cisplatin resistance, combination treatment, heat shock protein (Hsp90), Hsp90 inhibitor, nasopharyngeal carcinoma (NPC)

Introduction

Nasopharyngeal carcinoma (NPC) exhibits a unique epidemiological pattern, with a high prevalence in specific geographic regions, particularly Southeast Asia. The disease demonstrates a male predominance and is associated with specific ethnic groups [1]. NPC is characterized by its invasive and metastatic nature, as well as its strong link to Epstein-Barr virus [2]. Several treatment challenges are posed by NPC, including late diagnosis, local and regional invasion, distant metastasis, radiation-induced toxicities, treatment resistance, and the absence of targeted therapies. In fact, resistance to cisplatin therapy commonly arises in cases of recurrent NPC. Overcoming cisplatin resistance is crucial for improving treatment outcomes, enhancing treatment response rates, and expanding the range of available therapeutic options [3]. It is imperative to understand the underlying

mechanisms of resistance and develop innovative approaches, such as targeted therapy and combination therapy, to overcome cisplatin resistance and enhance the treatment efficacy in NPC [4].

Heat shock protein 90 (Hsp90) is a molecular chaperone protein that plays a significant role in the folding, stabilization, and activation of various client proteins. It is intricately involved in cancer development and progression by stabilizing and activating client proteins implicated in oncogenic signaling pathways. Its participation in protein folding, maturation, and stress response mechanisms positions it as a critical player in cancer cell survival and adaptation [5]. Targeting Hsp90 has emerged as a potential therapeutic strategy to disrupt oncogenic signaling and overcome drug resistance in cancer treatment. While targeting Hsp90 as an anti-cancer approach has shown promise, challeng-

es persist in developing an Hsp90 inhibitor with optimal efficacy, selectivity, and tolerability [6]. Additionally, ongoing research focuses on patient selection based on biomarkers that predict Hsp90 dependence and sensitivity to inhibitors. Nevertheless, the rationale for targeting Hsp90 lies in its central role in maintaining the stability and activity of client proteins critical for cancer cell survival and oncogenic signaling [7].

The Hsp90 inhibitor luminespib (AUY922) has demonstrated potent anticancer activity in various cancer types, such as lymphoma [8, 9], lung cancer [10-13], breast cancer [14, 15], hepatocellular carcinoma (HCC) [16], pancreatic cancer [17], multiple myeloma [18], ovarian cancer [19], prostate cancer [20], and glioblastoma [21]. It has exhibited efficacy in inhibiting tumor growth, inducing apoptosis, and suppressing metastasis. AUY922 demonstrates potential in overcoming resistance to various targeted therapies by effectively targeting and destabilizing client proteins that contribute to resistance mechanisms. Inhibition of Hsp90 by AUY922 sensitizes immune-refractory tumors, allowing for improved response to adoptive T cell transfer and PD-1 blockade. Additionally, it revitalizes the immune cycle of tumor-reactive T cells, enhancing their ability to recognize and eliminate tumor cells [22]. Phase I/II trials of AUY922 have focused on specific cancer types, including lung cancer [23-26], breast cancer [27], and gastrointestinal stromal tumors [28]. These studies have evaluated AUY922 as a monotherapy or in combination with other agents. Encouraging clinical activities have been observed, including disease stabilization and partial response. However, the efficacy of AUY922 in NPC remains largely unexplored. This study aimed to investigate the preclinical anticancer efficacy of AUY922 and the potential synergistic effect of combining AUY922 with cisplatin in cisplatin-resistant NPC cells.

Materials and methods

Cell lines and cell culture

Two human NPC cells (Hone 1 and HK1) were utilized in this study. The Hone1 cell line was received as a gift from Prof. SW Tsao of the University of Hong Kong (Hong Kong SAR, China), while the HK1 cell line was established by our laboratory [29]. The cisplatin-resistant cell lines were developed through prolonged continuous exposure to increasing concentra-

tions of cisplatin. These resulting cisplatin-resistant cell lines (Hone1-R and HK1-R) and their corresponding mock control cell lines (Hone1-M and HK1-M) were used for both *in vitro* and *in vivo* experiments. The various cell lines were cultured in RPMI 1640 medium (ThermoFisher Scientific Inc., Waltham, USA) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% sodium pyruvate in a 37°C humidified incubator with 5% CO₂.

Cell proliferation assay

Cancer cells were seeded at the desired cell densities in each well of 96-well plates and incubated overnight in a 37°C humidified incubator with 5% CO₂. These cancer cells were then treated with various concentrations of AUY922 or cisplatin for a duration of 72 h, divided into three distinct time intervals (24 h, 48 h, and 72 h). MTS working solution was added to each well and incubated at 37°C for 2 h. The absorbance was measured at 490 nm using a microplate reader, and the percentage of growth inhibition was calculated using the formula: $(1 - \text{treatment absorbance/control absorbance}) \times 100\%$. The inhibitory concentration (IC)₅₀ values were determined by performing non-linear regression analysis using Graphpad Prism v7.0.

Drug combination analysis

The optimal drug combination was identified by analyzing the synergy score and combination index (CI) values.

Calculation of synergy score using the SynergyFinder web application: The combination of two drugs, AUY922 and cisplatin, was investigated using the MTS assay with various drug combinations. Cancer cells were seeded at the desired cell density and incubated overnight in a 37°C humidified incubator with 5% CO₂. Freshly prepared drug combinations were added to the corresponding wells after removing the liquid. After 72 h, MTS working solution was added into each well and incubated at 37°C for 2 h. The absorbance at 490 nm was measured using a multiplate reader, and the growth inhibition was calculated as $(1 - \text{absorbance at drug combination/absorbance of control}) \times 100\%$. The data were organized in matrix form and input into Microsoft Excel. The matrix was then uploaded to the SynergyFinder v2.0 server (<https://synergyfinder.fimm.fi/synergy/>) to calculate the synergy score. A synergy score > 1 indicates synergy, with a higher value indicating

a greater level of synergy in the drug combination.

Determination of CI using the CompuSyn software: In addition to SynergyFinder, the CompuSyn v1.0 program (ComboSyn Inc., Paramus, USA) was used to calculate the CI [30]. Data obtained from analysis results were input into the CompuSyn program to determine the CI. A CI value < 1 indicates synergy, whereas a CI value > 1 suggests an antagonistic effect. The smaller the CI value, the greater the observed synergy in the drug combination.

Cell cycle analysis

A cell suspension containing 2×10^6 cells was seeded per well in a 6-well plate. After 24 h of acclimatization, selected cancer cells were treated with either the absence or presence of AUY922 at $\frac{1}{2} IC_{50}$ and IC_{50} concentrations determined in the MTS assay, as well as a combination of 0.01 μM AUY922 + 2 μM cisplatin determined by the SynergyFinder and CompuSyn program. Following a 72 h incubation in a 37°C humidified incubator with 5% CO₂, the cells were harvested and fixed overnight at -20°C using 70% ice-cold ethanol. The fixed cells were then washed twice with phosphate buffer solution (PBS) at pH 7.4 and centrifuged at 500×g for 8 min to remove the ethanol. Next, the cells were permeabilized using 1% bovine serum albumin and incubated in the dark overnight at 4°C with a propidium iodide (PI) staining mixture at pH 8.0. The stained cells were filtered through 40 μm nylon-net filter (Millipore, Burlington, USA) prior to analysis. Finally, the stained cells were analyzed using a BD FACSAria Fusion flow cytometer (Becton, Dickinson and Company, Franklin Lakes, USA), and the cell cycle distribution was determined using BD FACSDiva v8.0.

Apoptosis assay

Annexin V-FITC staining was used to distinguish apoptotic cells from necrotic cells in the presence of AUY922 and cisplatin in various cisplatin-resistant and mock cancer cell lines. The Annexin V-FITC staining kit (Biovision, Milpitas, USA) was employed in conjunction with a confocal microscope. A cell aliquot containing an initial cell number of 1.25×10^3 cells was cultured in an SPL 8-well chamber-slide. This cell aliquot was pre-incubated for 24 h in a 37°C humidified incubator with 5% CO₂. After the 24 h pre-incubation period, the cells were treated with

PBS (as a control), 0.01 μM AUY922, 2 μM cisplatin, and a combination of both drugs. After incubation for 48 h, the medium was removed and the slide was rinsed with PBS. Each chamber of the slide was then added Annexin V-FITC labeling solution and incubated at room temperature in the dark for 5 min. After labeling, the slides were directly analyzed using a confocal microscope (Leica TCS SPE). An excitation wavelength of 488 nm and an emission wavelength of 515 nm were used to evaluate Annexin V-fluorescein, while an excitation wavelength of 488 nm and an emission wavelength of 561 nm were used for PI.

Western blot analysis

The Hone1-M/R cancer cells were subjected to different treatments, including 1% dimethyl sulfoxide (as a control), 0.01 μM AUY922, 2 μM cisplatin, and a combination of both drugs. For protein analysis, a sodium dodecyl-sulfate polyacrylamide gel electrophoresis was prepared, protein extracts from the cancer cells were loaded onto the gel for electrophoresis. After electrophoresis, the proteins were transferred onto a polyvinylidene difluoride membrane using the Western blot technique. Specific antibodies were used to target the proteins on the membrane and their visualization was achieved using a chemiluminescent imaging system. The protein bands were normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Tumor xenograft

4 groups (5 per group) of nude mice were used to examine the combined effects of drugs *in vivo*. A total of 1×10^6 Hone1-M and Hone1-R cancer cells were subcutaneously injected into the left and right flanks of each nude mouse, respectively. The tumors were allowed to grow until they reached a size of 100 mm³. Once the tumors reached the desired size, different treatments (saline control, 0.093 $\mu g/20$ g AUY922, 12.04 $\mu g/20$ g cisplatin, and a combination of both drugs) were administered intraperitoneally three times a week for 31 d. The tumor size (calculated as long length \times short length²/2) and body weight of the mice were monitored twice a week. The percentage of tumor growth was calculated as (tumor size at day x - tumor size at day 0)/tumor size on day 0 \times 100%. At the end of the study, the mice were euthanized. The tumors were excised, weighed, divided into multiple sections, and then fixed in formalin for

AUY922 combined with cisplatin to overcome resistance in NPC

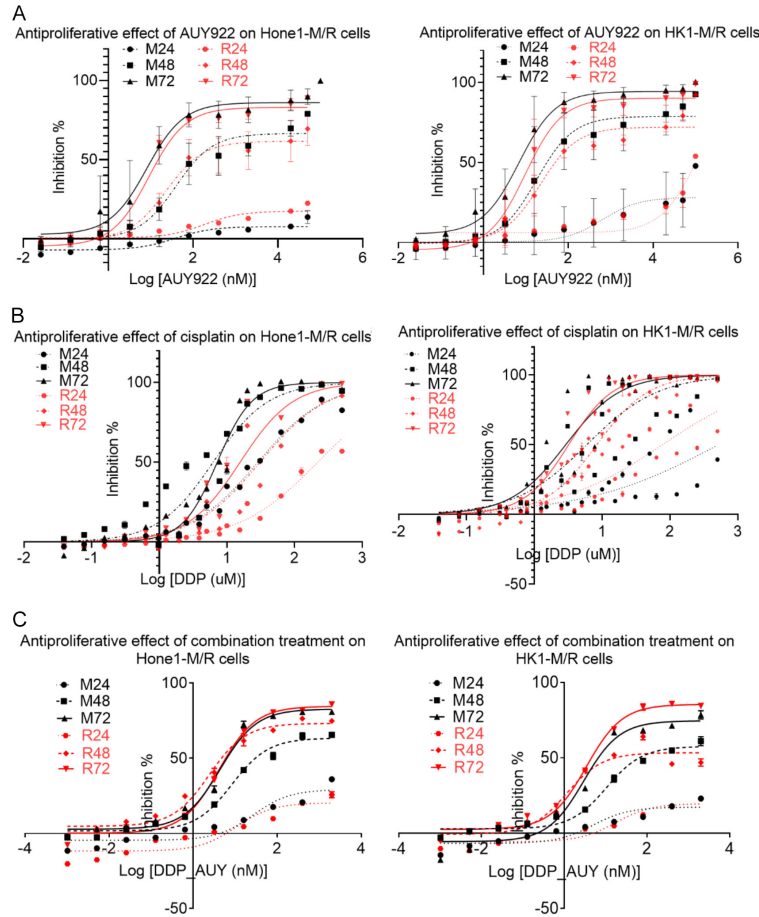


Figure 1. Antiproliferative effects of AUY922 and cisplatin on nasopharyngeal carcinoma cells. The Hone1-M/R and HK1-M/R cell lines were treated with (A) AUY922, (B) cisplatin, or (C) their combination at various concentrations for 24 h, 48 h, and 72 h. The results are presented as the mean \pm standard deviation of triplicate determinations from three independent experiments. M, mock; R, resistant; DDP, cisplatin; AUY, AUY922.

Table 1. Inhibitory concentration (IC_{50}) values of AUY922 and/or cisplatin on nasopharyngeal carcinoma cells proliferation

Cell line	IC_{50} value [Log μ M]		
	AUY922	Cisplatin	Combination
Hone1-Mock	0.056	7.190	0.004
Hone1-Resistant	0.049	15.650	0.004
HK1-Mock	0.006	2.903	0.003
HK1-Resistant	0.007	3.204	0.003

embedding. Additionally, 5 organs (heart, liver, spleen, lung, and kidney) were dissected and weighed.

Histological staining

The tumor tissues were fixed in 4% paraformaldehyde, followed by paraffin embedding and

sectioning. The resulting sections were then deparaffinized and subjected to hematoxylin and eosin staining before being sealed. Subsequently, the tumor tissues were examined under a microscope to observe any morphological and structural alterations.

Statistical analysis

All experiments were randomized and repeated at least three times. Data analysis was performed using Microsoft Excel and GraphPad Prism Software version 7.0 (GraphPad Software, La Jolla, USA). All data is expressed as the mean \pm standard error of the mean (SEM) unless otherwise specified. Student's two-tailed t-tests were performed to calculate *p*-values. A *P*-value < 0.05 was considered statistically significant.

Results

Treatment with AUY922 and/or cisplatin yielded antiproliferative effects in cisplatin-resistant human NPC cells

To evaluate the antiproliferative effects of AUY922 and/or cisplatin, we performed a comparative analysis on cisplatin-resistant human NPC cells (Hone1-R and HK1-R), along with their respective mock control cell lines (Hone1-M and HK1-M). The inhibition rates of AUY922, cisplatin, or their combination were evaluated after 24 h, 48 h, and 72 h of treatment.

Both AUY922 and cisplatin, either alone or in combination, substantially inhibited the *in vitro* growth of all the four cell lines in a dose- and time-dependent manner (Figure 1). The IC_{50} [Log μ M] values obtained from the 72-h treatment of AUY922 and/or cisplatin are presented in Table 1. Our findings indicate that the combi-

AUY922 combined with cisplatin to overcome resistance in NPC

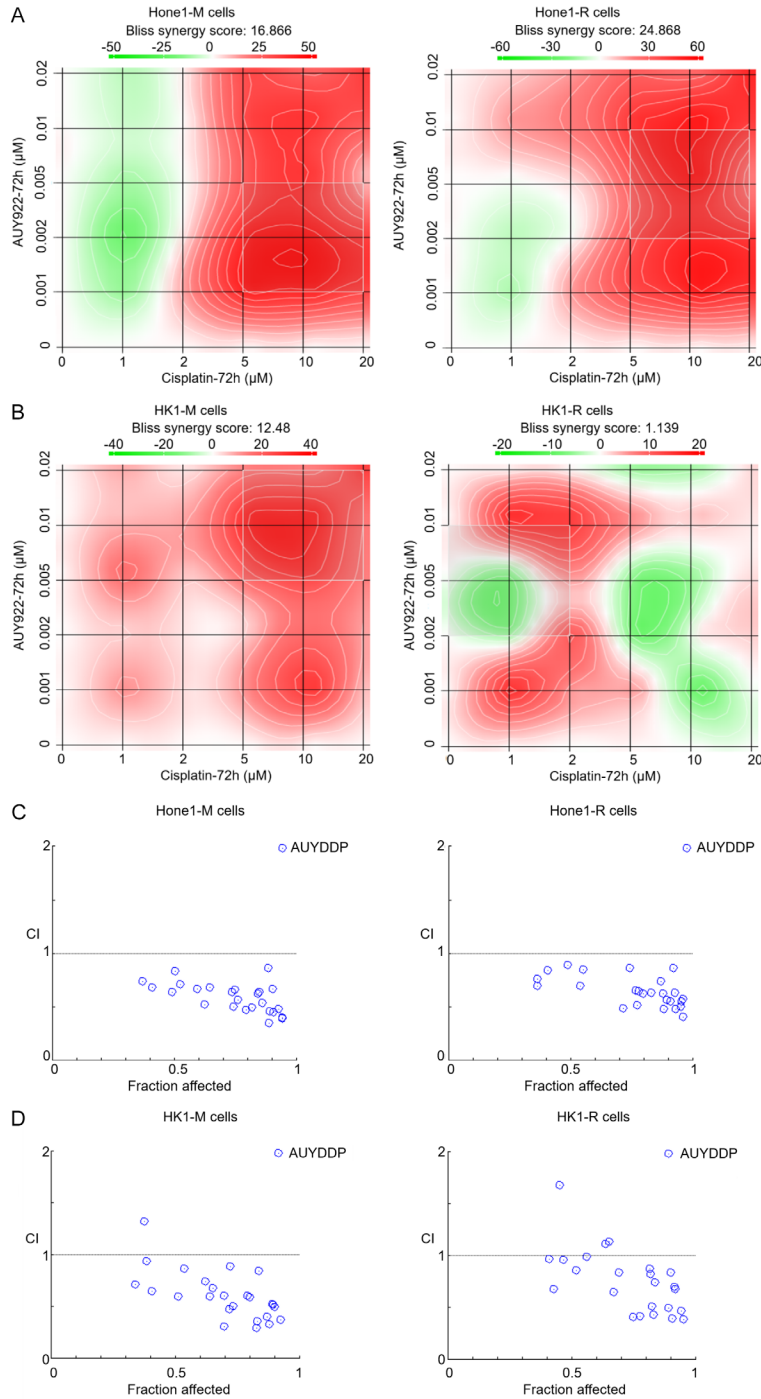


Figure 2. Comparative analysis of synergy scores and combination index resulting from a 72 h exposure to various concentrations of AUY922 and cisplatin in combination. The findings are visually represented through a heatmap, illustrating the synergy scores observed in both (A) Hone1-M/R and (B) HK1-M/R nasopharyngeal carcinoma cell lines for each drug combination. Scatter plots are provided, depicting the CI values in (C) Hone1-M/R and (D) HK1-M/R cell lines for each drug combination. M, mock; R, resistant; CI, combination index; AUYDDP, AUY922 + cisplatin.

nation treatment yielded superior IC_{50} values compared to the individual drug treatments,

suggesting an enhanced anti-proliferative efficacy of both drugs when used in combination.

AUY922 and cisplatin exhibited synergistic inhibitory effects on the growth of human NPC cells

To assess the efficacy of the combined treatment comprising AUY922 and cisplatin on human NPC cell lines, Hone1-M/R and HK1-M/R cells were exposed to different concentrations of both drugs in combination for 72 h. Synergy-Finder analysis was performed to evaluate the interaction between AUY922 and cisplatin in Hone1-M/R (**Figure 2A**) and HK1-M/R (**Figure 2B**) cells. The synergistic effect of the combination was determined by calculating the CI using the CompuSyn program in Hone1-M/R (**Figure 2C**) and HK1-M/R (**Figure 2D**) cells. Our findings revealed the most favorable drug combination (0.01 μ M AUY922 + 2 μ M cisplatin) based on the analysis of synergy score and CI values. This indicates that the combination treatment of AUY922 and cisplatin exhibits synergistic inhibitory effects on the growth of human NPC cells, potentially enhancing the overall therapeutic outcome.

Cell cycle progression of NPC cells and cisplatin-resistant NPC cells following combined treatment of AUY922 and cisplatin

Flow cytometric analysis was conducted to examine the effects of AUY922 alone and its combination with cisplatin on the cell cycle progression of human NPC M/R cells over a period of 72 h. The data demonstrated that AUY922 treatment

AUY922 combined with cisplatin to overcome resistance in NPC

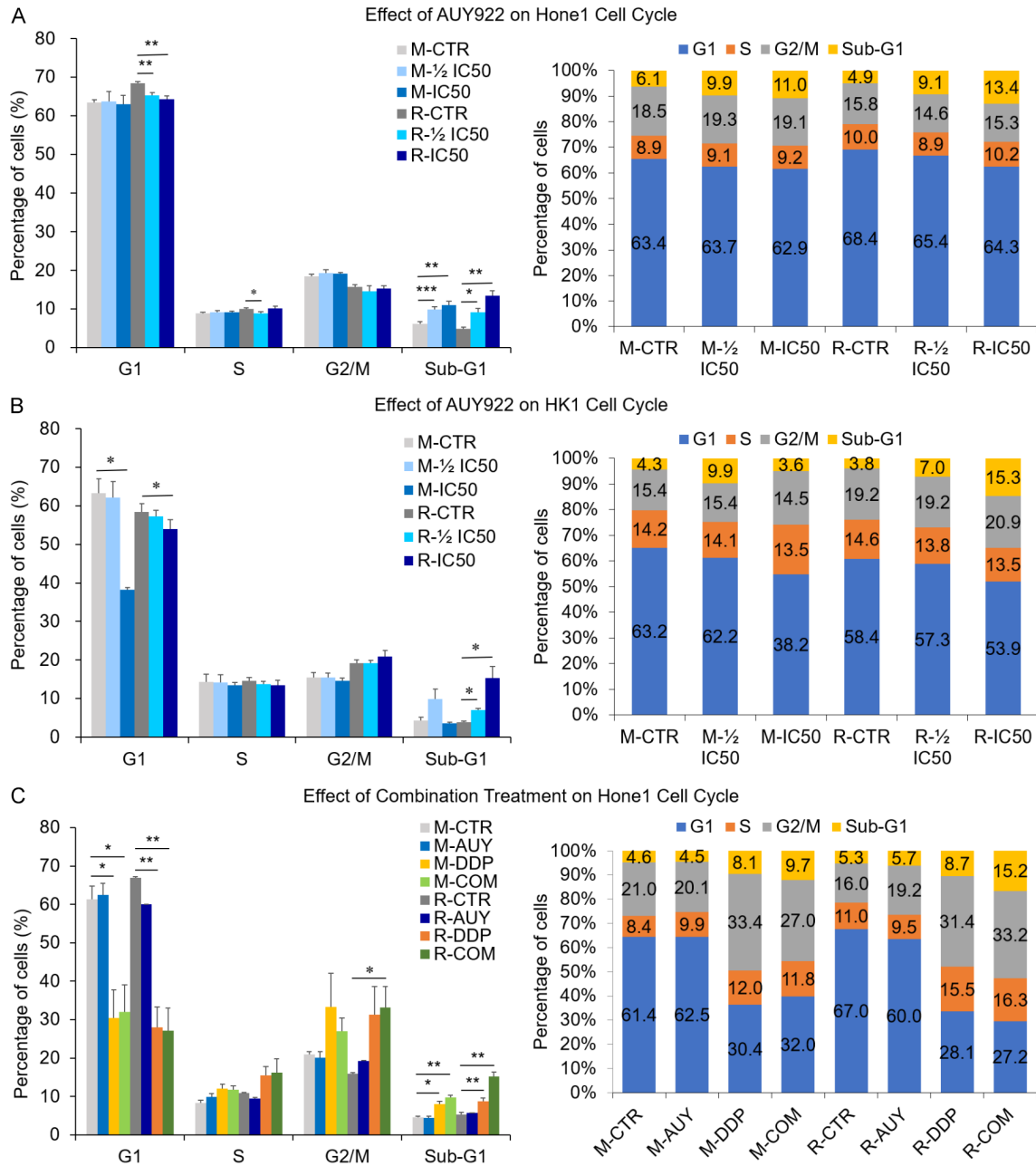


Figure 3. Cell cycle alterations of nasopharyngeal carcinoma cells after 72 h treatment of AUY922 alone and its combination with cisplatin. The effect of AUY922 treatment ($\frac{1}{2}$ IC₅₀ or IC₅₀ concentrations) on cell cycle progression was examined for (A) Hone1-M/R and (B) HK1-M/R cancer cells. (C) The impact of treatment with 0.01 μ M AUY922 alone, 2 μ M cisplatin alone, or the combination of both drugs on the cell cycle progression of cisplatin-resistant cancer cells (Hone1-R) and their mock control cancer cells (Hone1-M). M, mock; R, resistant; CTR, control; AUY, AUY922; DDP, cisplatin; COM, combination treatment. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

led to a notable cell death in the cell cycle compared to the untreated control groups in both Hone1-M/R cells (Figure 3A) and HK1-M/R cells (Figure 3B). Furthermore, the combined treatment of AUY922 and cisplatin resulted in a distinct sub-G1 peak observed in the flow

cytometry analysis of Hone1-M/R cells as well (Figure 3C). These findings suggest that both AUY922 alone and its combination with cisplatin elicit an apoptotic response, leading to cell cycle arrest and impeding the uncontrolled growth of NPC cells.

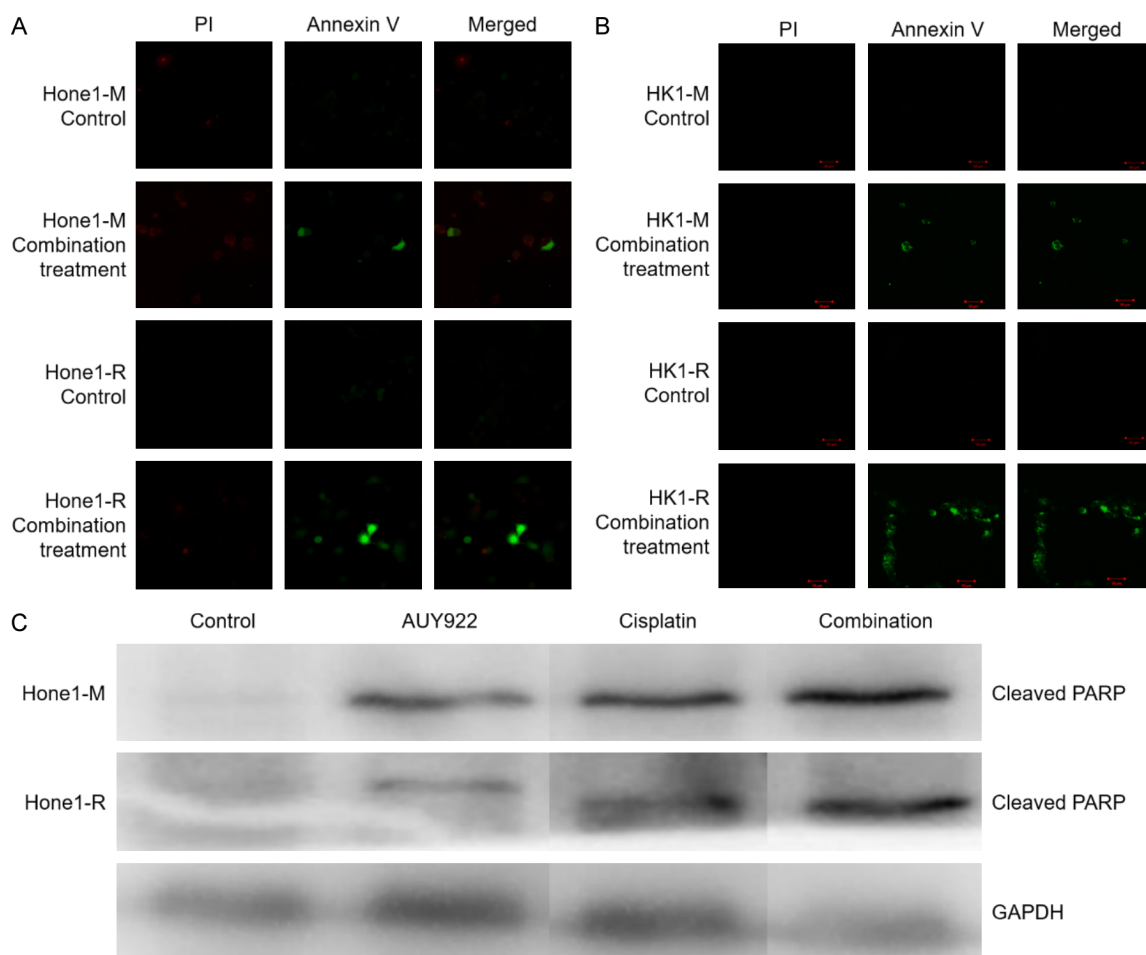


Figure 4. Induction of apoptosis by the combination of AUY922 and cisplatin. Fluorescence microscopy analysis was performed on (A) Hone1-M/R cancer cells and (B) HK1-M/R cancer cells after treatment with a combination of 0.01 μ M AUY922 + 2 μ M cisplatin. The Annexin V-FITC staining assay was used to analyze these cells following treatment. (C) Western blot analysis of PARP was conducted on Hone1-M/R nasopharyngeal carcinoma cells after treatment with AUY922 alone, cisplatin alone, or the combination of both drugs. M, mock; R, resistant; PI, propidium iodide; PARP, poly (ADP-ribose) polymerase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

AUY922/cisplatin combination treatment induced apoptosis

To determine whether the reduction in cell proliferation induced by AUY922/cisplatin is associated with cell apoptosis, cancer cells were treated with AUY922 and cisplatin alone or in combination. The cells were then analyzed via confocal microscopy with Annexin V/PI staining. Our results showed that the AUY922/cisplatin combination treatment induced a pro-apoptotic response in Hone1-M/R cells (**Figure 4A**) and HK1-M/R cells (**Figure 4B**) compared to the control. Moreover, apoptosis was also evident from Western blot analysis in Hone1-M/R cells compared to the control. A substantial increase was observed in cleaved poly (ADP-ribose) poly-

merase (PARP) after co-treatment with AUY922 and cisplatin, indicating an increased level of apoptosis. The increase in cleaved PARP was greater than that with either drug alone (**Figure 4C**). These findings suggest that the combination treatment of AUY922 and cisplatin induces apoptosis, contributing to the suppression of NPC cell growth.

Combination of AUY922 and cisplatin caused tumor regression in xenograft models

To assess the therapeutic efficacy of AUY922 and cisplatin combination treatment *in vivo*, we established Hone1-M/R subcutaneous tumor xenograft models with nude mice. When the tumor size reached 100 mm³, twenty tumor-

AUY922 combined with cisplatin to overcome resistance in NPC

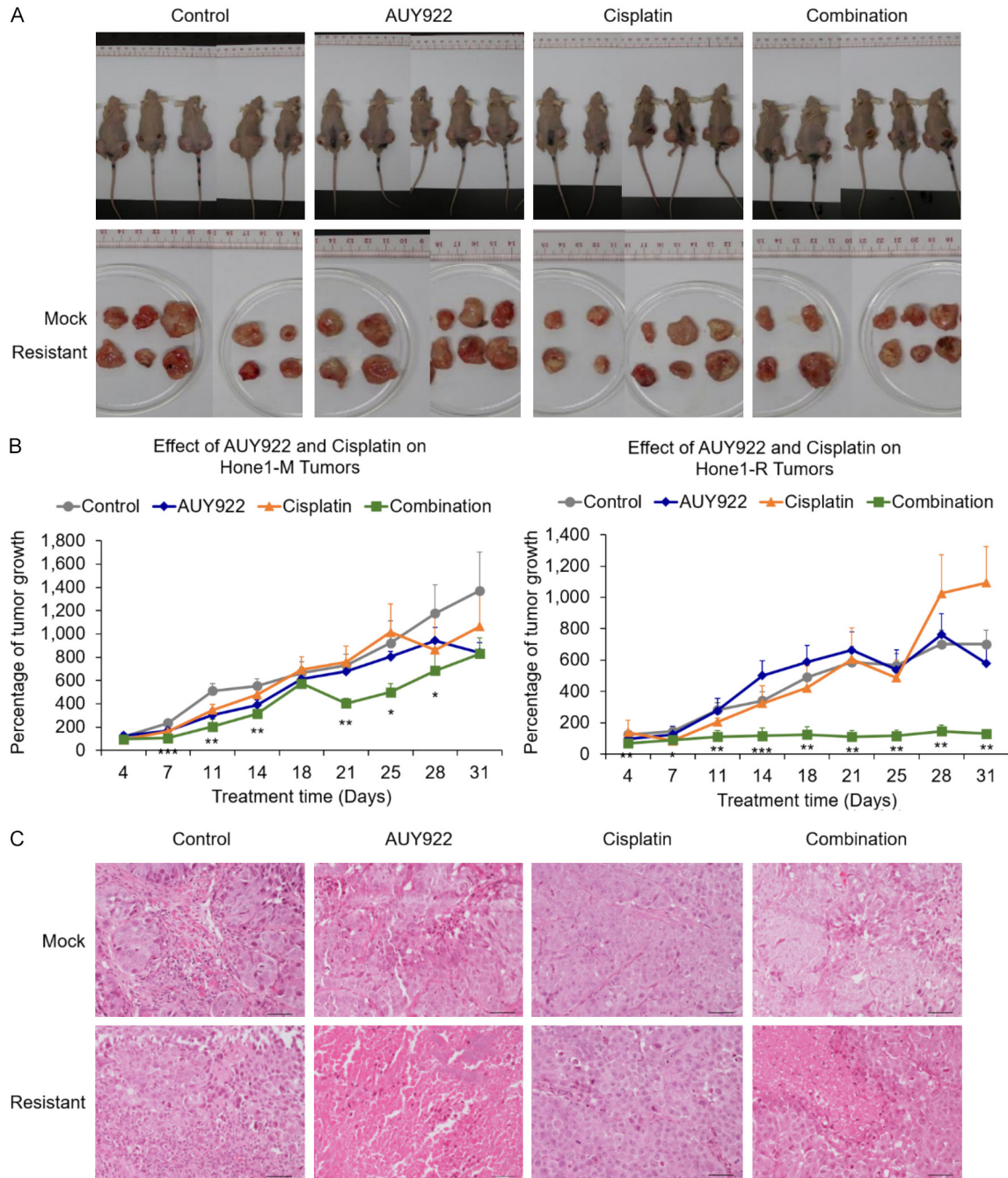


Figure 5. Inhibitory effect of combination treatment on xenografts. A. The general condition and tumor growth of Hone1-M/R tumor-bearing mice on the 31st day are presented ($N = 20$). The accompanying photographs depict the excised tumors from mice subjected to various treatment groups. B. The percentage of tumor growth was recorded during treatments in xenografts bearing Hone1-M and Hone1-R tumors. The inhibitory effects of the combination group were compared to those of the control group. M, mock; R, resistant. $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$. C. The histological appearance of tumor tissues from different treatment groups was observed through hematoxylin and eosin staining (magnification, $\times 20$; scale bar, $100 \mu\text{m}$).

bearing mice were randomly assigned to four groups and were administered saline control, AUY922, cisplatin, or the combination treatment three times per week for 31 d (Figure 5A).

On the 7th and 4th days, treatment with AUY922/cisplatin combination significantly inhibited the percentage of tumor growth in Hone1-M and Hone1-R xenografts, respectively (Figure

5B). The administration of the AUY922/cisplatin combination did not have a significant impact on the body weight of the mice, indicating that the dose used was well-tolerated by the animals (Supplementary Figure 1A). In addition, no significant impact was shown in the weight of 5 organs (heart, liver, lung, spleen, and kidney) treated with AUY922/cisplatin combination (Supplementary Figure 1B-F). Furthermore, histological staining showed no substantial morphological and structural alteration in the tumor tissue from the animals treated with AUY922/cisplatin combination (Figure 5C). These results demonstrate that the combination treatment of AUY922 and cisplatin leads to tumor regression in xenograft models of NPC, supporting its potential as an effective therapeutic strategy *in vivo*.

Discussion

AUY922, a specific Hsp90 inhibitor, has undergone a number of preclinical studies to explore its potential in cancer treatment. For example, a previous study revealed that AUY922 could effectively inhibit the function of EGFR exon 20 insertion mutations in advanced lung cancer cells [12]. AUY922 also downregulated IGF-1R β protein in pancreatic cancer cells, leading to growth inhibition and apoptosis [17]. Furthermore, it was reported that AUY922 induced apoptosis and autophagy in glioblastoma cells, leading to cell death and reducing the expression of EGFR, PDGFRA, CDK4, and NF1 [21].

In a resistance study against mantle cell lymphoma, AUY922 demonstrated its efficacy on overcoming resistance to ibrutinib *in vitro* and *in vivo* [8]. AUY922 also effectively inhibited HCC cell growth *in vitro* and *in vivo*. Its treatment induced molecular changes on β -catenin cleavage and increased expression of p53, Mcl-1, or NUPR1 to suppress resistance development [16]. Moreover, the administration of AUY922 could restrict metastatic and drug-resistant phenotypes in non-small cell lung cancer (NSCLC) cells even at low doses [13].

Several clinical trials have been conducted to assess the safety and efficacy of AUY922 in cancer treatment. For instance, a Phase I/II study was performed to determine the maximum tolerated dose, safety profile, and preliminary efficacy in EGFR-mutant lung cancer patients who progressed on erlotinib [23]. Addi-

tionally, there were several Phase II trials evaluating the tolerability and efficacy of AUY922 in different cancer types, including refractory gastrointestinal stromal tumors [28], previously treated and molecularly defined patients with advanced NSCLC [24], and NSCLC patients with EGFR exon 20 insertions [25].

Given the complex nature of cancer, AUY922 has been investigated in combination with various other therapies in both preclinical and clinical settings. A preclinical study combining AUY922 with the estrogen receptor antagonist Fulvestrant in breast cancer showed that AUY922 countered the feedback reactivation effect induced by Fulvestrant by targeting multiple proteins associated with ErbB receptors, PI3K/AKT, and ERK pathways [14]. An investigation on the synergistic effects of AUY922 with a PI3K inhibitor omipalisib in KRAS-mutant NSCLC found that AUY922 effectively suppressed the PI3K/AKT/mTOR and RAF/MEK/ERK signaling pathways, enhancing the cells' sensitivity to omipalisib [10]. The combination of AUY922 with a BCL-2 inhibitor in BCL-2-expressing small cell lung cancer also synergistically induced apoptosis and suppressed resistance to the BCL-2 inhibitor by downregulating AKT and ERK [11].

In clinical studies, a Phase I trial assessed the safety and tolerability of combining AUY922 with pemetrexed in previously treated metastatic non-squamous NSCLC patients [26]. Another Phase IB/II trial evaluated the combination of AUY922 with trastuzumab in patients with locally advanced or metastatic HER2-positive breast cancer who had prior chemotherapy and anti-HER2 therapy [27]. Both studies reported well-tolerated combinations, with some patients achieving stable disease or partial response. Combining AUY922 with chemotherapy agent or targeted therapy appears to be a viable option for cancer treatment.

NPC is known for its aggressive nature and resistance to conventional treatments [31]. Therefore, identifying novel strategies to improve the efficacy of existing treatments is of great importance. An earlier study has demonstrated that the interaction of a curcumin analogue (FM807) with the N-terminus of Hsp90 disrupts Hsp90/client complexes. This disruption leads to the degradation of the Hsp90 client protein EGFR and the inhibition of down-

stream signaling pathways, including Raf/MEK/ERK and PI3K/AKT [32]. Researchers has also indicated that miR-302c-5p modulates the AKT pathway by controlling HSP90AA1 expression. This regulation alters the resistance of NPC cells to cisplatin and influences the characteristics of tumor stem cells [33]. Additionally, a previous study has uncovered that a natural chalcone (flavokawain C) hampers glucose metabolism and tumor angiogenesis in NPC by targeting the HSP90B1/EGFR/PI3K/Akt/mTOR signaling axis [34]. A recent study has shown that p53-R280T may undergo misfolding, forming aggregates with the assistance of Hsp90. Consequently, this misfolding impairs the ability of sequestered p53 to initiate the transcription of downstream target genes in NPC [35]. These studies collectively underscore that NPC-specific pathways and molecular characteristics represent promising targets for Hsp90 inhibition.

Both AUY922 and cisplatin have shown anti-cancer activities in various types of cancer [36], but their combined effects in NPC treatment had not been well investigated. Therefore, studying the combination may fill this knowledge gap and provide insights into the potential benefits of this specific drug combination in NPC treatment. By studying the combination of AUY922 and cisplatin, we aimed to explore a potential treatment approach that could overcome the limitations of monotherapy and potentially provide a more comprehensive and targeted treatment for NPC patients. We revealed that the combination of these two drugs resulted in synergistic effects, which had several beneficial outcomes in the context of NPC treatment.

One outcome of the combination treatment involving AUY922 and cisplatin was the effective inhibition of NPC cell growth. We observed that the simultaneous administration of both drugs resulted in a stronger suppression of NPC cell growth compared to using either drug alone. This finding is significant because inhibiting the growth of cancer cells is a fundamental objective in cancer treatment. By effectively inhibiting cancer cell growth, it becomes possible to prevent tumor progression and potentially improve patient outcomes [37].

In addition to inhibiting cell growth, the combination treatment also induced cell cycle arrest

in NPC cells. The cell cycle is a tightly regulated process that controls cell division. Dysregulation of the cell cycle is a hallmark of cancer, leading to uncontrolled cell division and tumor growth [38]. By halting the progression of NPC cells at various stages of the cell cycle, the combination of AUY922 and cisplatin effectively prevents their uncontrolled growth and division. This mechanism further contributes to the anti-cancer activity of the combination therapy and enhances its effectiveness in combating NPC.

Another outcome found in this study was the promotion of apoptosis in NPC cells by the combination treatment. Apoptosis is a programmed cell death process that eliminates damaged or abnormal cells. It is a fundamental mechanism of maintaining tissue homeostasis and preventing the growth of cancer cells. Apoptosis induction is a desirable outcome in cancer treatment, as it helps to target and eliminate cancer cells specifically, without harming healthy cells [39]. The ability of the combination therapy to induce apoptosis in NPC cells suggests that it can effectively eliminate cancer cells and reduce tumor burden.

It has been reported that the IRF2/CENP-N/AKT signaling axis stimulates proliferation, cell cycling, and resistance to apoptosis in NPC cells by elevating aerobic glycolysis [40]. A previous study revealed that the downregulation of the ERK1/2 and Akt signaling pathways is associated with drug-induced apoptosis [41]. PTEN also plays a crucial role in inhibiting cell proliferation, regulating migration and invasion, promoting autophagy and apoptosis, and influencing radiotherapy resistance in NPC [42]. Treatment with AUY922 resulted in the upregulation of HSP70 and concomitant depletion of HSP90 client proteins [43]. Given that HER2 is a client protein of the molecular chaperone Hsp90, the targeting of Hsp90 by AUY922 has proven beneficial in HER2-positive cancer cases [44]. On the other hand, a high level of NIX protein enhances the antioxidant capacity of tumor cells and diminishes the apoptosis induced by AUY922 [45]. Combining AUY922 with a histone deacetylase inhibitor significantly amplifies the potency of cisplatin in cancer cells by inducing apoptosis and affecting the expression of apoptosis-related genes and proteins [46]. An earlier study found that AUY922 diminishes the mRNA and protein expressions

of EGFR, PDGFRA, CDK4, and NF1, suggesting that these molecules may act as biomarkers for assessing AUY922 treatment efficacy [47].

In the present study, we demonstrated that the combination treatment had the potential to inhibit the development of drug resistance. Drug resistance is a significant challenge in cancer treatment, as cancer cells can acquire the ability to survive and proliferate despite the presence of therapeutic agents. This can lead to treatment failure and disease progression [48]. The synergistic effects of AUY922 and cisplatin revealed in this study may help overcome or delay the development of drug resistance. This is a crucial advantage, as it can prolong the effectiveness of the treatment and improve long-term outcomes for NPC patients.

The results of our study highlight the potential benefits of combining AUY922 with cisplatin in the treatment of NPC. The synergistic effects revealed in the combination therapy resulted in enhanced anti-cancer activity, including inhibition of cell growth, induction of cell cycle arrest and apoptosis, and prevention of drug resistance. These outcomes suggest that this combination therapy holds promise as a valuable therapeutic approach for improving patient outcomes in NPC treatment, particularly in the case of cisplatin resistance. By elucidating the *in vitro* and *in vivo* efficacy of AUY922 and its combination treatment, we aim to establish a foundation for further preclinical and clinical investigations. This study may lead to the development of novel therapeutic strategies for cisplatin-resistant NPC patients and potentially improve their treatment outcomes and overall survival rates. However, further researches and clinical trials are necessary to validate these findings and determine the optimal dosage and treatment regimens for this combination therapy in NPC patients.

Conclusion

Our findings demonstrate the anticancer activities of AUY922/cisplatin combination *in vitro* and *in vivo*. These results suggest that AUY922 may be a valuable therapeutic option for NPC, either as a standalone agent or in combination with cisplatin. Most importantly, our study provides preclinical evidence supporting the efficacy of AUY922/cisplatin combination in overcoming drug resistance in NPC treatment. The

discovery of the anticancer activity of AUY922 in NPC holds promise in advancing our understanding of novel therapeutic options for chemo-resistant NPC. Further studies are warranted to translate these findings into clinical application.

Disclosure of conflict of interest

None.

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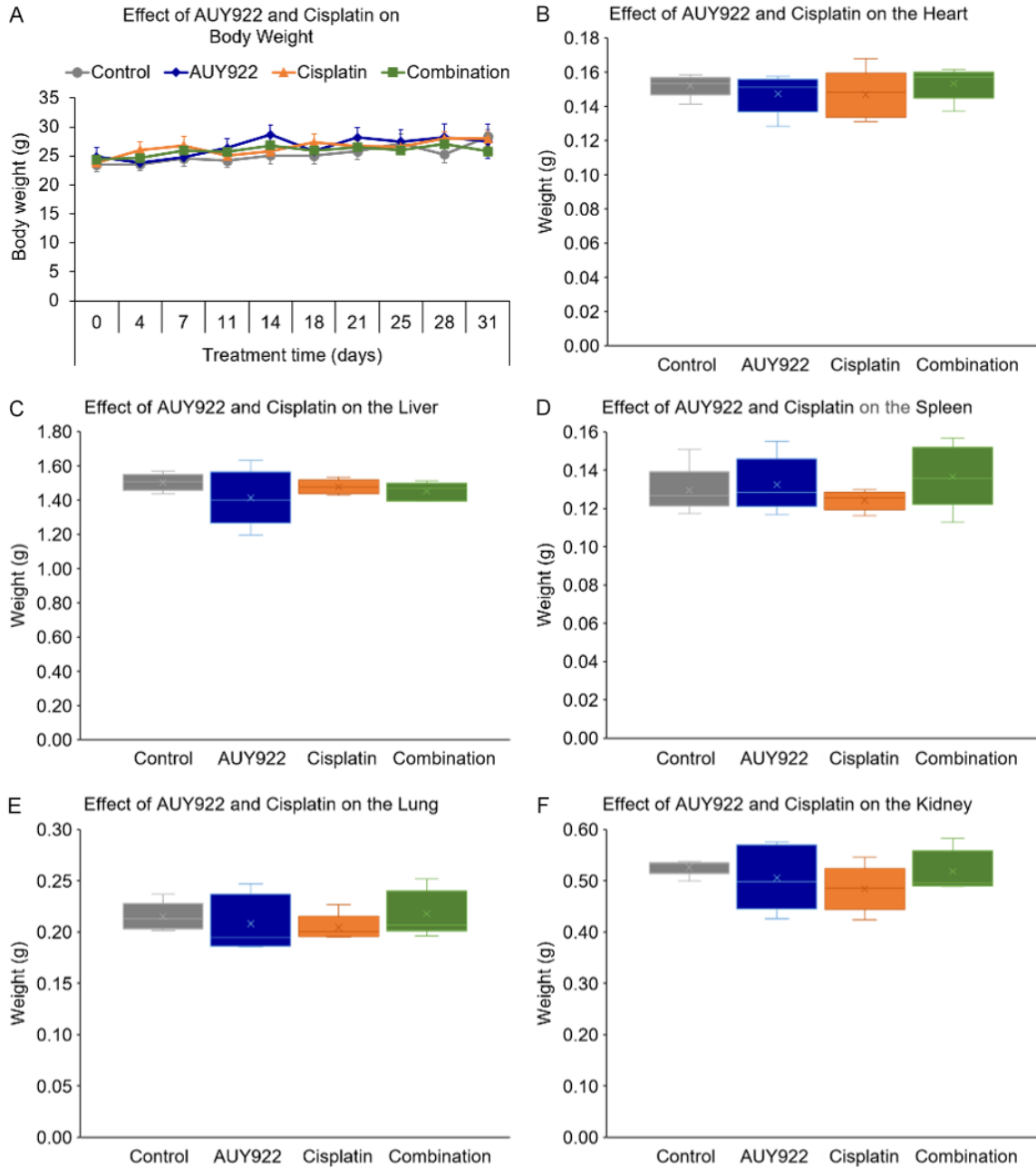
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AUY922 combined with cisplatin to overcome resistance in NPC



Supplementary Figure 1. Effect of AUY922 and cisplatin on xenografts bearing Hone1 tumors ($N = 20$). A. Body weights (g) were monitored during treatments in mice bearing Hone1 tumors. The body weights of the treatment groups were compared to those of the control group. The administration of the AUY922/cisplatin combination did not significantly affect the body weight of the mice, indicating that the animals tolerated the dosage well. B-F. At the end of the experiment, the five organs (heart, liver, spleen, lung, and kidney) were excised and weighed. The weights of the five organs of the treatment groups were compared to those of the control group. No significant difference was observed in the weights of these five organs when treated with AUY922/cisplatin or their combination.