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Oral AZD5438 is a clinically translatable otoprotectant against cisplatin-induced hearing loss

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ABSTRACT

Cisplatin-based chemotherapy causes hearing loss in 40–60 % of all patients, yet effective preventative options remain limited. Building on prior animal studies, we demonstrate that oral administration of AZD5438, a potent and selective CDK2 inhibitor, provides dose-dependent protection against hearing loss in a clinically relevant multi-dose cisplatin mouse model. Protective doses (4.7 and 9.4 mg/kg b.i.d.) fall within the human-equivalent maximum tolerated dose range established in AstraZeneca trials, and exhibit plasma pharmacokinetics comparable to those in humans. Importantly, AZD5438 at 9.4 mg/kg b.i.d. does not reduce cisplatin's anti-tumor efficacy in a testicular cancer xenograft model, consistent with *in vitro* findings. These results support AZD5438 as a promising candidate for clinical trials to prevent cisplatin-induced hearing loss while preserving cancer treatment efficacy.

Introduction

According to the World Health Organization, approximately 430 million people live with varying degrees of hearing loss [1]. Cisplatin, or *cis*-diamminedichloroplatinum (II), is a platinum-based chemotherapeutic agent widely used to treat various solid tumors, including testicular, ovarian, head and neck, lung and breast cancers [2]. While cisplatin is highly effective for cancer treatment, one of its major side effects is hearing loss or ototoxicity, resulting in 40-60 % of both pediatric and adult patients [3–6]. In the inner ear, cisplatin damages multiple cell types, particularly the sensory hair cells of the cochlea and neurons, which are responsible for detecting sound vibrations, converting them into electrical signals, and transmitting the signals to the CNS [7]. Cisplatin predominantly causes loss of outer hair cells (OHCs) in the high-frequency regions of the cochlea through mechanisms such as oxidative stress and reactive oxygen species (ROS) production, mitochondrial apoptotic signaling, inflammation, and DNA damage [8].

Cisplatin-induced hearing loss is associated with significant long-term consequences, including social isolation, anxiety, depression, and cognitive decline, such as dementia [9–11]. Therefore, there is a critical need for therapies that can prevent or mitigate this debilitating side effect and improve patients' quality of life. Currently, sodium thiosulfate (STS), marketed as Pedmark®, is the only FDA-approved treatment for cisplatin-induced hearing loss [12–14]. STS, an antioxidant, is approved exclusively for pediatric patients aged one month to under 18 years receiving cisplatin therapy for non-metastatic solid tumors. Beyond STS, a number of investigational agents–including antioxidants, apoptosis inhibitors, and statins–have been explored as potential otoprotectants, though with varying levels of success [15–19]. Given that approximately 500,000 cancer patients in the United States receive cisplatin and other platinum-based chemotherapy annually, the prevention and treatment of cisplatin-induced ototoxicity represents a large and urgent unmet medical need.

In 2018, Teitz et al. performed a high-throughput screen (HTS) of a bioactive compound library containing 4,385 unique molecules in HEI-OC1 cells, an immortalized mouse cochlear cell line, to identify agents that protect against cisplatin-induced cell death [20]. Unexpectedly, three of the top 10 protective compounds were found to target CDK2, a canonical cell cycle-dependent kinase. Building on this finding, Hazlitt et al. conducted a second HTS using a focused library of 187 CDK2 inhibitors in HEI-OC1 cells for protection against cisplatin-induced cell death, followed by testing 9 of these compounds in cochlear explants [21]. Among them, AZD5438 emerged as one of the most potent candidates, exhibiting an EC_{50} of 5 nM in cochlear explants.

AZD5438, originally developed by AstraZeneca, is a potent, orally bioavailable CDK inhibitor with an *in vitro* IC_{50} of 6 nM against cyclin E/

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CDK2, 16 nM against cyclin B1/CDK1, 45 nM against cyclin A/CDK2, and 450 nM against cyclin D1/CDK4 [22,23]. It demonstrated acceptable plasma pharmacokinetics, including a rapid Tmax (0.5–3 h) and a relatively short $t_{1/2}$ (1–3 h) following a single oral dose. In preclinical and early-phase clinical studies, AZD5438 was shown to inhibit tumor growth through suppression of downstream targets such as phosphorylated Rb (pRb) [23,24]. However, clinical trials in cancer patients revealed poor tolerability at high doses upon prolonged treatment durations, leading to early termination of those trials [22]. Based on these observations, we hypothesized that systemic delivery of AZD5438 at lower doses with shorter treatment regimens–distinct from the protocols used in cancer studies–could safely and effectively mitigate cisplatin-induced ototoxicity. This repurposing strategy has the potential to reduce systemic toxicity while preserving the otoprotective benefits of AZD5438 in patients undergoing cisplatin chemotherapy.

Hazlitt et al. first evaluated AZD5438 in an acute cisplatin ototoxicity model using FVB mice treated with a single intraperitoneal dose of cisplatin (30 mg/kg), which resulted in auditory threshold elevations of 5–20 dB at 8, 16, and 32 kHz [21]. Transtympanic administration of AZD5438 (50 μM) one hour before and 24 h after cisplatin significantly attenuated cisplatin-induced hearing loss (CIHL), as evidenced by reduced auditory brainstem response (ABR) thresholds at 32 kHz. Histological analysis further showed that locally delivered AZD5438 protected against outer hair cell (OHC) loss in the 32 kHz region. Ingersoll et al. subsequently demonstrated that AZD5438 protects against cisplatin-induced hair cell loss *in vivo* using zebrafish neuromasts, with efficacy observed at a concentration of 100 nM [25]. In the same study, oral administration of AZD5438 at 75 mg/kg once daily for three days via gavage significantly protected against CIHL at 32 kHz in the same single-dose cisplatin mouse model. Similar otoprotective effects were observed when AZD5438 (75 mg/kg) was administered orally in a noise-induced hearing loss (NIHL) model, with improved auditory thresholds at 8 and 16 kHz [25]. These findings suggest that AZD5438's mechanism of protection is unlikely to involve direct cisplatin inactivation but rather inhibition of shared cell death pathways triggered by different cochlear insults [15]. Furthermore, co-treatment with AZD5438 and dabrafenib (a BRAF inhibitor) provided enhanced protection in the NIHL model, with significant auditory preservation at 8, 16, and 32 kHz [25]. Together, these studies demonstrate that AZD5438 is an effective otoprotective agent against both cisplatin- and noise-induced hearing loss.

However, the tolerability and efficacy of previously tested AZD5438 doses in the context of human clinical use remain unclear. Specifically, it is unknown whether these preclinical doses fall within the human-equivalent tolerated dosing range—i.e., below the maximum tolerated dose (MTD) established in oncology trials. Moreover, it is critical to establish that clinically relevant, otoprotective doses do not interfere with cisplatin's anti-tumor efficacy. Demonstrating both tolerability and lack of negative interaction with cisplatin's therapeutic effect is essential for clinical translation.

Single high-dose cisplatin regimens have been widely used as an initial step for evaluating otoprotective compounds in murine models as they are relatively short and give a fast reading on the potential protective effects of a drug. But the single-dose cisplatin protocols often result in high mortality. In contrast, cisplatin is typically administered to patients as multiple low-dose infusions over several days, with recovery periods spanning weeks to minimize systemic toxicity. Therefore, it is essential to evaluate AZD5438 in an animal model that more accurately reflects clinical cisplatin treatment regimens. To address this need, Fernandez et al. developed an optimized multi-cycle cisplatin administration protocol using CBA/CaJ mice–an approach that more closely mimics the dosing schedules used in human oncology patients [26]. This clinically relevant model has been widely adopted due to its ability to induce stable and significant hearing loss across multiple frequencies, while minimizing mortality and reducing interindividual variability [27–32]. Notably, Fernandez et al. and Ingersoll et al. demonstrated that even four months after completing the multi-cycle cisplatin regimen, mice exhibited persistent hearing loss and remained viable, further validating the robustness and translational relevance of this model [26,30].

In this study, we evaluated the otoprotective effects of oral AZD5438 across multiple doses in the clinically relevant multi-cycle cisplatin CBA/CaJ mouse model. We further characterized the plasma pharmacokinetic profiles of AZD5438 in mice and compared them to those observed in humans. Finally, we assessed whether AZD5438 interferes with the anti-cancer efficacy of cisplatin both *in vitro* in multiple cancer cell lines and *in vivo* in a xenograft mouse model. Our results demonstrate that oral AZD5438 is effective at doses within the human-equivalent maximum tolerated dose (MTD) range in the multi-cycle cisplatin mouse model, and does not compromise cisplatin's anti-tumor activity both *in vitro* and *in vivo*. Together, these findings support a novel strategy to prevent cisplatin-induced ototoxicity and highlight AZD5438's potential as a clinically translatable therapeutic to preserve hearing and improve quality of life in cancer patients receiving cisplatin chemotherapy.

Material and methods

Animal experiments

Multi-cycle cisplatin ototoxicity mouse model

A multi-cycle cisplatin-induced ototoxicity mouse model was employed in CBA/CaJ mice (Strain #:000654, Jackson Laboratory [JAX], Bar Harbor, ME) with minor modifications from previously published methods [26]. A total of 112 mice, aged 7–8 weeks at the start of the 42-day experimental protocol, were used. The animals were given 1 week to acclimate to the Animal Resource Facilities (ARF) at the Scintillon Institute (San Diego, CA, USA). All animals were housed in standard cages with *ad libitum* access to food and water in accordance with the NIH NIDCD/NINDS guidelines and the Institutional Animal Care and Use Committee-approved protocol (2024-YH-001). At the beginning of the study, the average body weight was 20.4 ± 0.9 g for females (range: 18.1–21.8 g) and 25.7 ± 1.5 g for males (range: 23.1–29.1 g). Animals were randomly assigned to all experimental groups, with approximately equal numbers of males and females in each group.

For cisplatin-induced ototoxicity, mice received 3 cycles of once-daily cisplatin (#479306, MilliporeSigma, St. Louis, MO) at the dosage of 3 mg/kg via intraperitoneal (IP) injection. Each treatment cycle consisted of four consecutive days of cisplatin administration followed by a 10-day recovery period (Fig. 2A). Cisplatin solution was freshly prepared immediately before injection at a concentration of 1.8 mg/mL in sterile saline pre-warmed to 37°C, using pipette mixing. Control animals received an equivalent volume of sterile saline, adjusted for body weight, at the same frequency as the cisplatin-treated animals. Mouse overall condition and body weight were monitored throughout the study to assess the general health status. To minimize dehydration after cisplatin administration, a subcutaneous injection of sterile saline (500 μL/animal) was performed twice daily. Additionally, cages were partially placed on heating pads to prevent hypothermia and supplemental food (Nutra-Gel diets; Product # NGB-1 and S4798, Bio-Serv) was provided for nutritional support throughout the experimental protocol.

For otoprotectant treatment, AZD5438 (# HY-10012) was obtained from MedChemExpress (MCE, Monmouth Junction, NJ, USA), dissolved in a vehicle composed of 10 % DMSO (#D2650, MilliporeSigma), 5 % Tween-80 (#HY-Y1891, MCE), 40 % PEG-300 (#HY-Y0873, MCE), and 45 % saline solution, and administered to mice via oral gavage (OG). Increasing doses (1.2, 4.7, 9.4, 18.8 and 37.5 mg/kg) were given twice daily (b.i.d) with a 6-h interval between administrations for six consecutive days (Day 1–6) at the beginning of each cisplatin cycle. On the day of the cisplatin administration (Day 2–5), AZD5438 was given to

mice one hour prior to cisplatin (Fig. 2A). The treatment timing was based on plasma pharmacokinetic data of AZD5438 in CBA mice (Fig. 1) together with our previous *in vivo* efficacy data on AZD5438 [21,25].

Mouse xenograft model

NOD.Cg-Prkdcscid/J (SCID) mice were purchased from JAX and housed in the animal facility of K2 Biolabs (Houston, TX). Experiments were conducted on 12-wk-old male SCID mice in full accordance with the K2 Biolabs IACUC protocol (2023-005). All human tumor xenografts were established by subcutaneously injecting 100 µL of NCCIT cells (1×10^7 cells mixed 1:1 with Matrigel). NCCIT, a mediastinal mixed germ cell human testicular carcinoma cell line, was obtained from American Type Culture Collection (ATCC, Manassas, VA, # CRL-2073), and propagated in RPMI 1640/10 % FBS (#11875093/ #26140079, Thermo Fisher Scientific, Waltham, MA) before the engraftment. The day before tumor engraftment, mice were numbered and initial body weights were recorded. After tumor engraftment, mice were measured up to three times per week with calipers and tumor volumes were calculated as described previously [33].

For tumor killing, NCCIT tumor-bearing SCID mice were administered with cisplatin when tumors reached a mean size of approximately 100 mm^3. Cisplatin was administered using the same formulation and dosage as in the cisplatin ototoxicity model, but at a reduced frequency: on the first and second days of each 7-day treatment cycle (Fig. 3A).

For AZD5438 treatment, NCCIT tumor-bearing SCID mice received AZD5438 twice daily via oral gavage at 9.4 mg/kg on days 1, 2, and 3 of each 7-day treatment cycle. AZD5438 preparation and administration followed the same protocol as used in the cisplatin ototoxicity model. The total treatment period consisted of three cycles (21 days). Animals were randomized into the following four treatment groups ($n = 10$): (i) saline/vehicle (Vehicle group), (ii) saline/AZD5438 (AZD5438 group), (iii) cisplatin/vehicle (Cisplatin group), or (iv) cisplatin/AZD5438 (Cisplatin + AZD5438 group). Mice were continuously monitored for three weeks post-treatment for tumor volume and body weight changes.

Pharmacokinetics

LC-MS/MS analysis of AZD5438 was performed by BioQual Solutions (San Diego, CA). Briefly, AZD5438 was extracted from plasma by protein precipitation extraction using 40 volumes of acetonitrile and then centrifuged at 13,000 g for 10 mins. After centrifugation, 100 µL of the supernatant was transferred to an injection plate which contained 100 µL of mobile phase A, making a 1:1 ratio of water and acetonitrile. The standards were prepared by spiking AZD5438 ranging from a final concentration of 1 ng/ml to 1000 ng/ml. AZD5438 was analyzed by liquid chromatography/tandem mass spectrometry (LC/MS–MS).

The LC/MS–MS system consisted of Sciex 5500 Triple Quad mass spectrometer. Mass spectrometer was attached to a UHPLC system, which consisted of CTC PAL autosampler, LC-20AD pumps, a Shimadzu CBM20A controller, a DGU-20A 5R degasser, and a Shimadzu CTO20AC column oven. Chromatographic separation was carried out using Kinetex C18 3 u, 2.1 × 50 mm column at a temperature of 40°C. The elution of AZD5438 and internal standard was carried out by a gradient method using solvents (A) Water with 0.1 % Formic Acid and (B) Acetonitrile with 0.1 % Formic Acid with a flow rate of 0.5 mL/min. Elusion started with 10 % solvent B, which was increased to 100 % over a period of 1.0 min and constant till 2 min after which it was returned to initial mobile phase conditions. The total elution time was 2 min and the total run time was 4 min. For every run, 5 µL of sample was injected into the column. Analyst software was used for data acquisition and data analysis.

Auditory testing

For all auditory assessments, animals were anesthetized via intraperitoneal injection of a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture. To maintain proper thermoregulation during the entire recording session, mice were placed on heating pads that maintained their body temperature at 37± 0. 2 °C. Ophthalmic ointment was applied to the eyes to prevent corneal desiccation during the procedure.

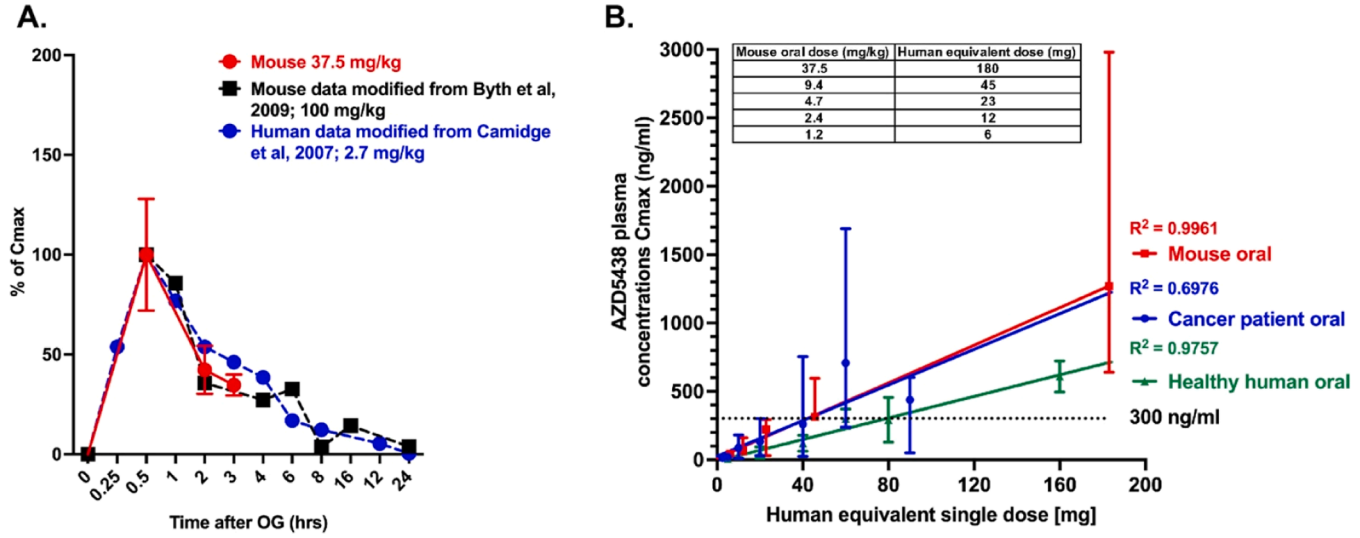

Fig. 1. Dose-dependent plasma pharmacokinetics of single oral AZD5438 dosing in mice. A) Plasma pharmacokinetics of single oral AZD5438 were sampled at 0, 0.5, 2, and 3 hrs post oral administration in CBA/CaJ and SCID mice (red line). Plasma pharmacokinetics of single oral AZD5438 administration were plotted from previously published data in mouse (black dotted line) and human (blue dotted line). Plasma AZD5438 levels were determined by LC-MS. Results were normalized to the Cmax of each experiment and presented as a percentage of Cmax. Means +/- SEM are plotted. **B)** Plasma concentrations of AZD5438 were sampled at 0.5 hrs after oral administration with various doses in CBA/CaJ mice, which were extrapolated to human oral doses (table inset). Each data point represents the median plasma concentration, with error bars indicating the range of values. A linear regression analysis was performed on the median plasma concentrations versus the administered doses. The red solid line represents the linear regression fit, with a coefficient of determination (R2) of 0.9989. Plasma concentrations (medians and ranges) of AZD5438 from two previous clinical studies with single dosing were plotted and subjected to linear regression for curve fitting (blue and green lines).

ABR and wave I amplitude measurements

ABRs were recorded from the left ear only, as previously described with minor modifications [20]. Briefly, following anesthesia, ABR waveforms were acquired in a sound-attenuated chamber (Industrial Acoustics Company) using a closed-field configuration. Saline was applied to the electrode sites, and subdermal needle electrodes were inserted at the vertex (active), behind the left pinna (reference), and at the base of the tail (ground). The response signals were amplified using a low-impedance digital biological amplifier (Medusa RA4L system; Tucker-Davis Technologies [TDT]; Alachua, FL, USA) with a 20 dB gain. Three stimulus frequencies (8, 16, and 32 kHz) were tested. For each frequency, the sound pressure level (SPL) was decreased in 5 dB steps from 90 to 0 dB to determine the auditory threshold, defined as the lowest SPL eliciting a response above background noise. Each ABR waveform was averaged over 500 tone bursts. Signals were bandpass filtered between 300 Hz and 3 kHz. Baseline ABR recordings were performed within 1 week prior to any treatment (when mice were 7–8 weeks old), and final recordings were conducted within two weeks after the 42-day experimental protocol concluded (mice aged 13–14 weeks). Thresholds were independently determined by 2 to 3 experimenters per mouse. ABR wave 1 amplitudes were measured as the difference between the positive peak and the following negative trough. Data analysis was performed using BioSigRZ software.

DPOAE measurements

Distortion product otoacoustic emissions (DPOAEs) were measured from the left ear only, as previously described with slight modifications [30]. Following anesthesia, DPOAEs were recorded in a sound attenuated chamber (Industrial Acoustics Company) using the RZ6 Multi-I/O processor (TDT). To ensure clear access to the tympanic membrane, a DPM1 microphone system (TDT) was inserted into the ear canal. Four stimulus frequencies (10, 14, and 29 kHz) were tested using an f2/f1 ratio of 1.2. At each frequency, the stimulus intensity was decreased in 5 dB steps from 75 to 0 dB SPL to determine the DPOAE threshold, defined as the lowest SPL producing an emission detectable above background noise. Changes in DPOAE thresholds were calculated by subtracting baseline values from those obtained at the end of the experimental protocol. Thresholds were independently determined by 2 to 3 experimenters per mouse. Data analysis was performed using BioSigRZ software.

Tissue preparation and immunohistochemistry

Following auditory testing at the end of the 42-day protocol, mice were euthanized via CO_2 asphyxiation followed by decapitation. The temporal bones were immediately harvested and rinsed with PBS until all visible blood was removed. Cochleae were then fixed in 4 % paraformaldehyde (PFA) at 4 °C for six days. Fixed tissues were washed three times with PBS and decalcified in 150 mM EDTA (pH 8.0) for 72 to 96 h at 4 °C. Once decalcified, each cochlea was micro-dissected and incubated in a blocking solution consisting of 0.1 % Triton X-100 (Bio-Rad) in PBS (PBST) with 10 % normal horse serum, with gentle rocking. Samples were then incubated overnight at 4 °C with the anti-myosin-VIIa primary antibody (rabbit anti-myosin VIIa; 1:300; Proteus Biosciences #26-6790) diluted in the same blocking solution. After four 10-min washes in PBST at 4 °C with rocking, samples were incubated overnight at 4 °C with secondary antibody conjugated to Alexa Fluor 568 (anti-rabbit; 1:300; Invitrogen #A10042). Following four additional 10-min washes in PBST at 4 °C with rocking, each cochlea was then dissected into three segments (apical, middle, and basal turns) and mounted using ProLong™ Gold Antifade mounting media (Invitrogen).

Imaging and data acquisition

To count IHCs and OHCs, images were acquired at room temperature using a Zeiss Axio Imager M2 microscope or a Leica Stellaris 5 Confocal Microscope with a 20X objective for hair cell imaging. Images acquired using the Zeiss system were processed with ZEN Blue Edition software, while those acquired using the Leica system were processed with Leica Application Suite software. IHC and OHC counts were expressed as the number of cells per 160 μm segment of the organ of Corti. All counts were performed manually at regions corresponding to 8, 16, and 32 kHz along the cochlear spiral. To map cochlear positions to their respective frequency regions, a cochlear frequency map was generated using the Measure Line plug-in in ImageJ software. Minor linear adjustments to brightness and contrast were applied uniformly across images, and final figures were assembled using Adobe Photoshop and Illustrator software.

Assessment of cell viability

For JKT1 and SEM-1 cell lines, cell viability was assessed through a colorimetric MTT assay as previously described [34]. Briefly, cells were plated in 24-well plates to achieve 60–70 % confluence. Cultures were then treated with various concentrations of AZD5438 and/or cisplatin in growth medium for 24 h. A 10 % (v/v) stock solution of MTT ((3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; Thermo Fisher Scientific) was added, and plates were incubated at 37°C for 20 min. Following incubation, the culture medium was removed and DMSO was added to dissolve the formazan crystals. Absorbance was then read at 490 nm. For NCCIT, cell confluence was evaluated using IncuCyte® Live-Cell Analysis System (Sartorius, Ann Arbor, MI) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were conducted using Prism software (GraphPad Software, version 10.3.1, San Diego, CA). Comparisons of multiple groups were conducted using either ordinary one-way analysis of variance (ANOVA), followed by either Tukey's post hoc multiple comparisons test, Dunnett's multiple comparisons test, or two-way ANOVA to detect the cisplatin ototoxicity, as well as the main effect of AZD5438 treatment across multiple groups. A significance threshold was set at $p < 0.05$.

Results

Dose-dependent plasma pharmacokinetics of single oral AZD5438 dosing in mice

To better characterize the pharmacokinetics of orally administered AZD5438 in mice, we first measured plasma drug concentrations at multiple time points after a single oral dosing at 37.5 mg/kg (Fig. 1A). At 0.5 hrs post-dosing, the plasma concentration reached a peak (Cmax) at 1467 +/- 916 ng/ml, and gradually reduced to 621 +/- 305 ng/ml at 2 hrs and 510 +/- 135 ng/ml at 3 hrs. We observed that the Cmax at 0.5 hr post-dosing was variable and the variance was larger than those at 2 and 3 hrs post-dosing. A comparison with published results from AstraZeneca showed consistency in Tmax and T1/2 with their mouse and human data following single AZD5438 dosing (Fig. 1A) [22–24]. These agreements not only validate our experimental procedures and measurements but also indicate that Cmax is a good parameter to assess human and mouse exposure of oral AZD5438 dosing.

Next, we examined Cmax (at 0.5 hrs) post-various oral doses of AZD5438 in mice. We chose the initial single oral dose at 37.5 mg/kg as used in previous mouse otoprotection studies [25], and then gradually reduced it to 9.4, 4.7, 2.4, and 1.2 mg/kg in mice. As expected, plasma concentrations of the drug increased progressively with dose, from 50 ng/ml at 1.2 mg/kg to 1467 ng/ml at 37.5 mg/kg (Fig. 1B). Moreover, the linearity curve ($R^2 = 0.9992$) demonstrates an almost perfect correlation between average plasma concentrations and the administered doses of AZD5438, indicating dose-proportional pharmacokinetics and exposure within the tested range. To compare these Cmax values with

those from human clinical trials, we converted mouse doses to human equivalent doses. This conversion utilized a factor of 12.3 (accounting for species differences in body surface areas, metabolism rates, and other factors) and assumed an average human body weight of 60 kg (Fig. 1B; [35]). Interestingly, our mouse Cmax follows similar linear relationships to human dose-dependent Cmax in both healthy individuals and cancer patients, despite significant variations of Cmax range in both human datasets [22,24]. These comparisons further suggest that dose responses of AZD5438 exposure in mice can be reasonably extrapolated to those in humans.

Dose-dependent protection against cisplatin-induced hearing loss by oral AZD5438 in mice

After confirming the plasma pharmacokinetics of oral AZD5438 in mice, we next sought to evaluate its protective potential against cisplatin-induced hearing loss in a multi-cycle, low cisplatin dose treatment model using CBA/CaJ mice [26]. In this model, CBA/CaJ mice received cisplatin at 3 mg/kg via intraperitoneal injection once daily for four consecutive days followed by 10 days of recovery in each of the three treatment cycles (Fig. 2A). In each cycle, various oral doses of AZD5438 were administered twice daily (b.i.d), starting one day prior to cisplatin treatment and continuing throughout the dosing period as well as one day afterward (a total of 6 continuous days of AZD5438 treatment). This clinically relevant murine model mimics the therapeutic regimen currently used in cisplatin-treated patients [36,37].

Considering the choices of AZD5438 doses in mice, we compared the two clinical phase I studies of healthy individuals and cancer patients with regard to the tolerable doses by AstraZeneca. The healthy male volunteers had a maximum tolerated dose (MTD) of 80 mg single dose [24], while the cancer patients exhibited non-tolerated doses (NTDs) of $>$ 90 mg four times for one day per week and 40 mg four times daily for at least 14 and 28 consecutive days without determining the MTDs [22]. Based on these two datasets of MTDs/NTDs, we conjectured that MTD is 80 mg single daily dose in healthy individuals and 20-90 mg four times daily in cancer patients. Furthermore, based on the ranges of single dose exposures in healthy individuals and cancer patients (Fig. 1B), we estimated that Cmax tolerated by cancer patients is ideally below ~300 ng/ml, and therefore the human equivalent mouse doses should be ideally $\leq$9.4 mg/kg (Fig. 1B black dotted horizontal line). Given the short half-life of AZD5438, we also assumed that Cmax at 9.4 mg/kg b.i.d. is similar to a single dose of 9.4 mg/kg in mice. We therefore chose five doses (1.2, 4.7, 9.4, 18.8, and 37.5 mg/kg b.i.d.) of oral AZD5438 plus a vehicle negative control for otoprotection experiments in mice, while the two higher doses (18.8 and 37.5 mg/kg b.i.d.) were to confirm AZD5438 otoprotection as positive controls in the new multi-cycle cisplatin CBA/CaJ mouse model.

Auditory tests, including auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE), were performed on all animals at the beginning and end of the 42-day protocol (Fig. 2A). The representative ABR traces from several treatment groups are shown (**Fig. S1**). Body weights were recorded for each mouse. A total of four cohorts of ~30 mice each were conducted over the course of a year and each cohort consisted of multiple mice treated with vehicle only and cisplatin alone as controls with minimum variations among four cohorts. Each treatment group had approximately equal numbers of males and females. Survival rates were calculated for the entire course. Mice were sacrificed after completing the entire protocol for cochlear morphological analysis of hair cell counts using myosin 7a immunostaining.

Only a few mice mostly in the cisplatin + 37.5 mg/kg AZD5438 group died during the entire experiments and the body weight losses (both means and SEM) were similar to those of previous reports by other investigators (Fig. 2B, C; [26,30,32]). The ABR threshold shifts at 8, 16, and 32 kHz in the cisplatin group were 25-40 dB and those in the vehicle only treated group were close to zero (Fig. 2D red and black lines, respectively). These threshold shifts (both means and SEM) are consistent with other reports using similar mouse models [26–28,30,32], demonstrating the reproducibility and reliability of cisplatin ototoxicity using this model as well as the accuracy of our measurements. The groups with AZD5438 alone did not show any ABR threshold shifts, indicating that AZD5438 at these doses are not ototoxic (Fig. 2D). Interestingly, only three groups with 4.7, 9.4, and 18.8 mg/kg AZD5438 + cisplatin significantly reduced the cisplatin-induced ABR threshold shifts, while the 1.2 and 37.5 mg/kg AZD5438 + cisplatin groups did not

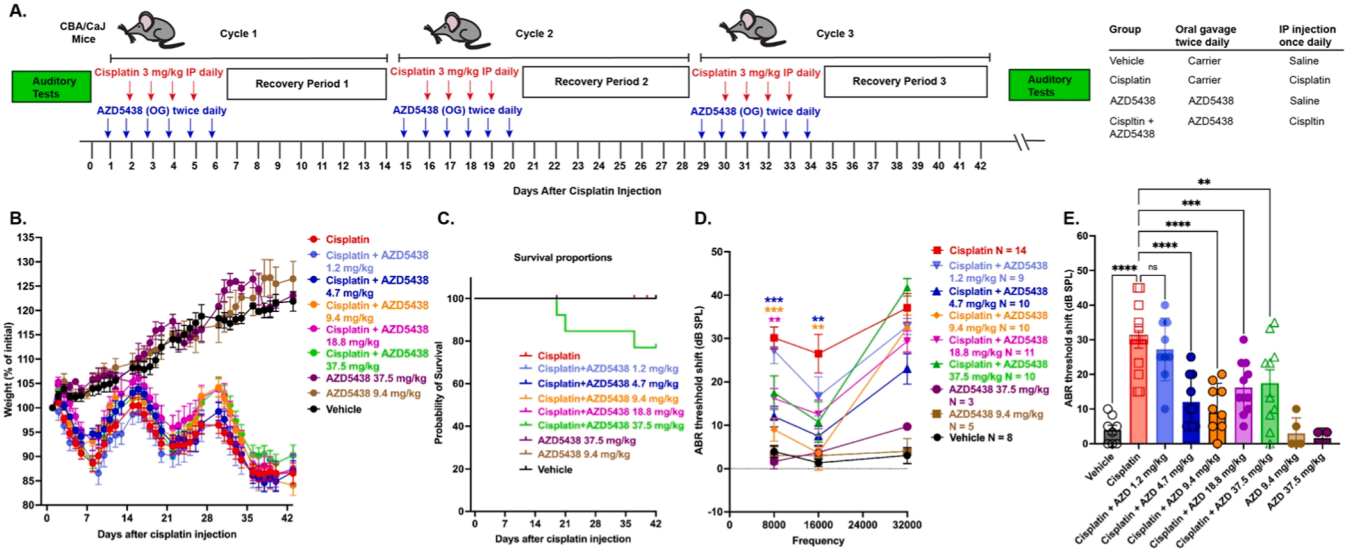

Fig. 2. Dose dependent protection against cisplatin-induced hearing loss by oral AZD5438 in mice.
A) Experimental design of the multi-cycle, cisplatin and AZD5438 treatment model. The treatment of carrier, saline, cisplatin or AZD5438 for each group is indicated in the right table panel. **B, C)** Mouse body weight and survival were indicated across the 3 cycles of cisplatin administration protocol. Means ± SEM, n = 9 to 14 mice for cisplatin groups. **D, E)** Hearing thresholds were assessed by ABR before (baseline) and after the treatment. Hearing loss is reported as threshold shifts (the difference between baseline and end point ABR thresholds) (D). Threshold shifts at 8 kHz were demonstrated in a bar graph for multiple comparison (E). Statistical analysis was performed using two-way ANOVA (D) and one-way ANOVA (E) with Dunnett's multiple comparisons test. ns, not significant. **, ***, and **** represent $p < 0.01$, 0.001, and 0.0001, respectively. Means +/- SEM are plotted in all panels.

(Fig. 2D) when two-way ANOVA and Dunnett's multiple comparisons test were used. Notably, the frequencies at which AZD5438 significantly ameliorated cisplatin-induced ABR threshold shifts are 8 and 16 kHz, and not 32 kHz. When the ABR threshold shifts at 8 kHz were analyzed by comparing each co-treatment group to the cisplatin only group using one-way ANOVA and Dunnett's multiple comparisons test, we found significant protection against cisplatin-induced ABR threshold shifts, at the 4.7, 9.4, 18.8 and 37.5 mg/kg groups (Fig. 2E).

We further measured the wave 1 amplitudes at three frequencies tested and found that vehicle and cisplatin groups exhibited significant differences in the input and output functions of the 90 dB level at 32 kHz but not at 8 and 16 kHz using one-way ANOVA and post-hoc Tukey's test (**Fig. S2**). Such differences are consistent with other reports [32], further demonstrating accuracy of our measurements. However, no significant protection was observed in any combination groups when compared to the cisplatin only group (**Fig. S2**). Similarly, the wave 1 latency did not reveal significant improvements in combination groups vs the cisplatin only group of all levels at 8 and 16 kHz (**Fig. S3**).

To further examine functions of outer hair cells, we analyzed DPOAE thresholds and amplitudes. Similarly, significant differences were detected between cisplatin only group and vehicle group at all tested frequencies for thresholds and at 10, 14 and 29 kHz for amplitudes, but no significant protection was observed between any combination groups and the cisplatin only group, although there were trends of improvements by AZD5438 (**Fig. S4**). Input and output analysis of DPOAE amplitudes revealed significant differences between cisplatin only group and vehicle group at 29 kHz but not at 10 and 14 kHz frequencies, consistent with a previous report [32]. However, no significant protection was observed between any combination groups and the cisplatin only group (**Fig. S5**).

When we analyzed the hair cell counts by using myosin 7a immunostaining, we found that none of the groups caused any inner hair cell loss, but the cisplatin alone group did show significant outer hair cell loss at the 32 kHz region, whereas AZD5438 co-treated groups did not significantly reduce cisplatin-induced outer hair cell loss at 32 kHz (**Fig. S6**).

No interference of AZD5438 with cisplatin anti-cancer activity in vitro and in mice

Because of previous findings that sodium thiosulfate (STS) chemically chelates cisplatin thus complicating the clinical outcomes of cisplatin ototoxicity trials [38], it remains possible that AZD5438 also inactivates cisplatin thus compromising cisplatin's tumor-killing efficacy. We thus first tested the interactions *in vitro* between AZD5438 and cisplatin in multiple cancer cell lines with testicular cancer origins: NCCIT, JKT1 and SEM-1 cells. For NCCIT cells, we confirmed that AZD5438 and cisplatin caused cell death and combination groups caused significantly higher cell death compared to either drug alone over 40 h *in vitro* (**Fig. S7A**). Dose-dependent analysis revealed that AZD5438 is a potent anti-tumor agent with IC_{50} of 1.38 μM, more potent than cisplatin with IC_{50} of 9 μM (**Fig. S7B**), consistent with previous reports [23]. When co-treated with 5 μM cisplatin, AZD5438 significantly shifted IC_{50} to 0.28 μM (**Fig. S7B** green line), demonstrating that AZD5438 synergistically enhances (rather than inactivates) cisplatin's tumor killing ability *in vitro*. The NCCIT cell line harbors a p53 mutation, which is different from most clinical testicular tumors when initially diagnosed [39]. Once the testicular tumors are treated with chemotherapy they can acquire p53 mutations. Therefore, we further tested anti-cancer activity of cisplatin in JKT1 and SEM-1 cells. Of these two cell lines, JKT1 is known to have functional p53, whereas the p53 status is unclear in SEM-1 [40]. When JKT1 and SEM-1 cells were treated with various doses (~0.02-100 μM) of AZD5438 together with cisplatin, no substantial interferences were observed in tumor cell loss compared to cisplatin treatment only (**Fig. S8**). In addition, Ingersoll *et* al., 2020 and Pushpan et al., 2023 reported similar results that AZD5438 does not interfere with cisplatin's tumor killing in nine cancer cell lines of neuroblastoma, lung, and testicular cancer origins (IMR-32, SH-SY5Y, SKN-AS, SHP-77, H115, A549, Kelly, JKT1, NCCIT) *in vitro,* consistent with our results here [25,34].

We next tested whether AZD5438 interferes with cisplatin's antitumor activity *in vivo*. Given that the human equivalent efficacious and tolerated dose of AZD5438 in mice was 9.4 mg/kg or lower, we therefore chose the 9.4 mg/kg dose for this test. SCID immunodeficient mice were engrafted with NCCIT cells and, when the tumor volume reached to ~100 mm^3, mice were then treated with multiple doses of cisplatin (3 mg/kg) at similar frequency as in the CBA/CaJ mouse model and as in cancer patient cisplatin treatment. The frequency of cisplatin treatment is reduced to two days per week to accommodate the SCID mouse's more vulnerable immune status and ensure their survival while the 9.4 mg/kg b.i.d. AZD5438 was given via oral gavage only for 3 days per week (Fig.3A).

The vast majority of mice survived but several deaths occurred in the combination group as well as the AZD5438 only group (Fig. 3B). Over the entire course of the experiment, the cisplatin + AZD5438 group did have a trend of body weight loss more than the cisplatin only group, although insignificantly (Fig. 3C). Importantly, while the tumor volume increased at similar rates in the vehicle only and the AZD5438 group, the cisplatin + AZD5438 group completely obliterated the tumor volumes as efficiently as the cisplatin only group and no significant differences were observed between the two groups (Fig. 3D). These results indicate that ≤9.4 mg/kg b.i.d. oral AZD5438 treatment in mice does not interfere with cisplatin anti-tumor efficacy *in vivo*.

Discussion

Hearing loss is a major global health issue, affecting >10 % of the world's population, yet it remains a significant unmet medical need. Recent advances such as the successful *OTOF* gene therapy for congenital deafness [41,42], and the FDA approval of sodium thiosulfate (STS) as the first otoprotective agent for pediatric patients with non-metastatic solid tumors receiving cisplatin [12,13], represent important progress. However, the need for safer and more broadly effective therapeutics for the prevention and treatment of hearing loss remains urgent. High-throughput screening efforts across diverse platforms–including cell-based assays, in silico modeling, cochlear explants, zebrafish, and murine models–have led to the identification of numerous candidate otoprotective compounds with activity against cisplatin-, aminoglycoside-, and noise-induced auditory damage [20,21,25,27,28,30,32,43–47]. Despite this progress, translating these findings into clinically safe and efficacious therapies remains a major challenge. Here, we demonstrate that oral AZD5438, a potent and selective CDK2 inhibitor, is efficacious within a defined dose range in a clinically relevant multi-cycle cisplatin mouse model. This dose range corresponds to a human-equivalent tolerated exposure window based on prior clinical trials in both healthy individuals and cancer patients. Furthermore, we show that oral AZD5438 at these doses does not impair cisplatin's anti-tumor efficacy in a testicular cancer xenograft model, consistent with *in vitro* results of multiple additional cancer cell lines. Together, these results provide compelling preclinical evidence to support clinical trials evaluating AZD5438 as a repurposed therapeutic for preventing cisplatin-induced hearing loss.

AZD5438 was initially discovered as a potent CDK2 inhibitor, with an *in vitro* IC_{50} of approximately 5 nM, and was subsequently developed as an oral anticancer agent in several preclinical and clinical studies by AstraZeneca [22–24,48]. In both animal and human studies, oral AZD5438 displayed favorable plasma pharmacokinetic (PK) properties, including a short half-life ($t_{1/2}$ of 1–3 h), a Tmax of 0.5–3 h, and dose-dependent linear Cmax values ranging from 5 to 1,000 ng/mL. Pharmacodynamic (PD) assessments showed that downstream targets of CDK2, such as phosphorylated Rb, were modulated within 1–3 h of administration, correlating with effective plasma concentrations [22].

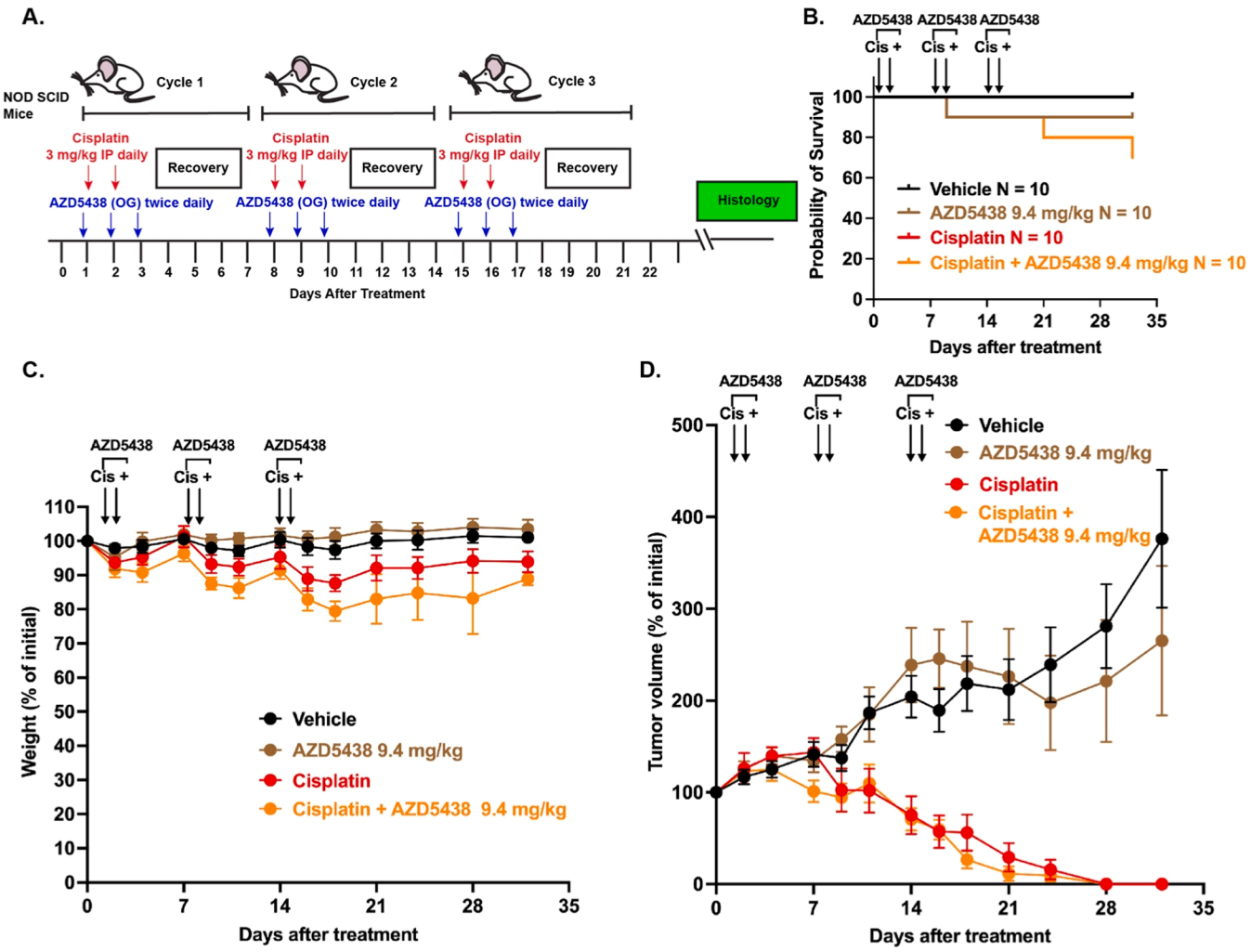

Fig. 3. No interference of AZD5438 with cisplatin anti-cancer activity in mice.
A) Experimental design of the multi-cycle cisplatin treatment with the NCCIT xenograft mouse model. **B, C)** Mouse survival and body weight were indicated across the 3 cycles of cisplatin and AZD5438 administration protocol. $n = 10$ mice per experimental group. **D)** Tumors were measured up to three times per week with calipers to calculate tumor volumes. The data were then plotted as normalized tumor volume (to the initial volume at the onset of the treatment) for each group versus time. Statistical analysis was performed using two-way ANOVA with Tukey's multiple comparisons. Means +/- SEM are plotted in all panels.

In clinical dose-escalation studies, the maximum tolerated dose (MTD) of oral AZD5438 was established at 80 mg single dose in healthy male volunteers. However, based on non-tolerated doses observed in cancer patients, the estimated MTD was lower, ranging from 20 to 90 mg QID (4×/day). This estimation is based on the immediate lower doses of the clinical dose-escalation scheme, which documented non-tolerated doses at 40 mg with long-term daily treatment and at > 90 mg QID with one-day-per-week treatment schedule [22,24]. Importantly, the plasma Cmax at these doses fell within the same linear range observed in animal models, supporting the translatability of preclinical dosing. In our study, we demonstrated that oral AZD5438 administered at 4.7 and 9.4 mg/kg in mice conferred protection against cisplatin-induced hearing loss (Fig. 2). These doses fall within the human-equivalent MTD range and produce similar dose-dependent Cmax values to those seen in human trials (Fig. 1B), supporting their tolerability and relevance for clinical translation. Previous clinical trial data showed that two separate cancer patient cohorts experienced adverse events leading to early trial termination when receiving continuous, long-term daily AZD5438 at 40 mg QID. Adverse events in the continuous dosing of AZD5438 were gastrointestinal in origin, including profound anorexia and fatigue in some patients, accompanied by nausea and vomiting. However, a third cohort, which received AZD5438 only one day weekly well tolerated doses as high as 90 mg QID. These findings suggest that shorter treatment durations–such as 3–5 days during each cycle of cisplatin infusion–may permit higher MTDs and improved tolerability, expanding the potential therapeutic window in otoprotective applications. Additionally, the patient populations in AstraZeneca's oncology trials had an average age of ~60 years and were primarily composed of individuals with advanced-stage solid tumors, factors that likely contributed to increased toxicity. In contrast, younger cancer populations, such as testicular cancer patients (average onset ~32 years), may tolerate AZD5438 more similarly to the healthy male volunteers (average age 35.4 years) in whom higher tolerability was reported [24].

Despite its overall promise, AZD5438 exhibited variable oral absorption and exposure (Cmax and AUC) in both humans and mice, for which there is no clear explanation [22]. In our mouse studies, similar interindividual variability was observed in plasma Cmax following oral gavage (Fig. 1). New oral formulations with more consistent and improved exposure of AZD5438 may help in future studies. Alternatively, our preliminary unpublished data show that intraperitoneal (IP) administration of AZD5438 reduces this PK variability, suggesting that alternative delivery routes—such as IV or IM—may enhance consistency and efficacy in future clinical applications. Furthermore, our prior work demonstrated that local delivery of AZD5438 via transtympanic injection in mice also protected against cisplatin-induced hearing loss [21]. These results, together with the potential for improved tolerability via

non-oral routes, suggest that AZD5438 remains a viable candidate for both systemic and local delivery in otoprotection. Finally, given that multiple other CDK2 inhibitors have shown similar protective effects against cochlear injury [20,21], there is strong potential for next-generation CDK2 inhibitors with improved pharmacologic and clinical properties to be repurposed as cisplatin otoprotectants [49].

Sodium thiosulfate (STS) has been approved by the FDA for the prevention of cisplatin-induced ototoxicity; however, this approval carries significant restrictions, as it excludes adults and patients with metastatic tumors. Furthermore, STS is known to chemically chelate cisplatin. Because of this antagonism, a strict requirement is in place for delayed administration–typically six hours after cisplatin infusion–to achieve a balance between otoprotection and anti-tumor efficacy. These findings underscore the necessity of demonstrating that candidate otoprotective agents do not interfere with the anti-cancer activity of cisplatin in preclinical models before advancing to clinical trials. In the present study, we show that AZD5438 does not compromise cisplatin's anti-tumor effects in a testicular cancer xenograft mouse model *in vivo*. While xenograft models require immunocompromised hosts and thus impose limitations on the use of multi-cycle cisplatin regimens that more closely reflect clinical practice, we were able to test AZD5438 at 9.4 mg/kg b.i.d.–a dose equivalent to the human MTD. The absence of interference at this dose strongly supports the translational potential of AZD5438 as an otoprotective agent against CIHL within a clinically relevant and tolerated dosing range (4.7–9.4 mg/kg b.i.d), potentially serving as the basis for dose and schedule selection in futures human studies for adults and children. Complementary *in vitro* studies using multiple human testicular, lung and neuroblastoma cancer cell lines also confirmed that AZD5438 does not reduce cisplatin-induced tumor cell killing (**Fig. S3A** and [25,34]). These results extend our *in vivo* findings and further reinforce the safety of co-administering AZD5438 with cisplatin across a broad spectrum of cancer types. Although no loss of cochlear hair cells or changes in CtBP2-positive ribbon synapse counts were observed in cisplatin-treated SCID mice compared to vehicle-treated controls, future studies are warranted to evaluate auditory function in these immunocompromised models, which may exhibit differential sensitivity to cisplatin ototoxicity. Cisplatin chemotherapy is known to produce multiple systemic side effects, including nephrotoxicity, neurotoxicity, and myelosuppression, in addition to ototoxicity. Therefore, it will be important to assess potential pharmacodynamic interactions between AZD5438 and cisplatin in other vulnerable organ systems. Encouragingly, recent data showed that AZD5438 also protected against cisplatin-induced nephrotoxicity in mice [34], and co-treated animals in our study showed no significant adverse effects based on body weight or behavior (Figs. 2 and 3). Given that the AZD5438 doses used fall within the human-equivalent MTD range, the likelihood of significant drug-drug interactions appears low. Nonetheless, careful monitoring of kidney, nervous system, and hematologic parameters in future clinical trials will be prudent, particularly as such monitoring is already standard practice in cisplatin-based chemotherapy regimens. Taken together, our results support the continued evaluation of AZD5438 as a promising otoprotective agent with a favorable tolerability profile and no detectable interference with cisplatin's anti-tumor activity.

It has been proposed that the multi-cycle, low-dose cisplatin model serve as the standard preclinical model for evaluating cisplatin-induced ototoxicity prior to clinical translation [25–28,30,32,50]. Although the single high-dose cisplatin protocol gives useful assessment of the potential of a drug to protect against cisplatin-induced hearing loss, it harbors the limitation of high mortality rate of the mice. In contrast, the multi-cycle, low-dose cisplatin mouse models achieve near 100 % survival, induce robust and persistent hearing loss up to 164 days post-treatment, and provide greater reproducibility and clinical relevance [25–28,30,50]. Such models have been validated by multiple independent groups using a variety of candidate otoprotective compounds. Notably, atorvastatin demonstrated consistent auditory protection (5–8 dB) in both this CBA/CaJ model and a retrospective human study, reinforcing the predictive power of this mouse model for human clinical outcomes [18,19]. Furthermore, the model enables direct comparison of different otoprotectants—via local or systemic routes—under standardized conditions, which is essential for ranking and optimizing therapeutic candidates. There is interest in studying larger animal models (e.g., guinea pigs, mini-pigs) for otoprotection. However, in the case of AZD5438, we show that plasma pharmacokinetics (Tmax, T1/2, Cmax, AUC) are comparable between mice and humans across equivalent linear dose ranges (Fig. 1), suggesting that murine data are translationally informative. While species-specific differences in blood-labyrinth-barrier permeability cannot be ruled out, AZD5438 demonstrated similar otoprotective effects across multiple mouse strains (C57BL/6, CBA/CaJ, 129S and FVB), implying comparable inner ear exposure. Although larger animal models may provide additional safety and pharmacokinetics data, human trials remain essential to fully validate AZD5438's clinical potential.

Mechanistically, our previous work demonstrated that AZD5438 mediates otoprotection through CDK2 inhibition, as confirmed using CDK2 knockout mice [20,21]. Interestingly, both AZD5438-treated and CDK2 knockout mice showed frequency-specific protection, primarily at 8 and 16 kHz, suggesting that drug distribution or cellular susceptibility varies along the cochlear tonotopic axis. Disparities in drug delivery between apical and basal cochlear turns likely contribute to reduced protection at higher frequencies (e.g., 32 kHz), as supported by cochlear explant and transtympanic delivery studies [20,21]. Further investigation into inner hair cell synaptopathy, such as CtBP2-positive ribbon synapse counts, may help clarify AZD5438's cellular targets—particularly in inner hair cells (IHCs) and spiral ganglion neurons. In addition, previous findings that AZD5438 synergizes with dabrafenib (an FDA-approved BRAF inhibitor) suggest that both compounds may act on complementary stress response or apoptotic signaling pathways in the inner ear [25].

In summary, we demonstrate that oral AZD5438 confers otoprotection in a clinically relevant, multi-cycle cisplatin mouse model at effective and tolerated doses (4.7–9.4 mg/kg), without interfering with cisplatin's anti-tumor activity in a testicular cancer xenograft model. These findings support further clinical development of AZD5438 and provide a compelling rationale for dose-acceleration studies in Phase I/II clinical trials targeting cisplatin-induced hearing loss in cancer patients.

CRediT authorship contribution statement

Yunlong Huang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xintian Zhang:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Enrick Vesanes:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Tal Teitz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Jian Zuo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

All authors are employees of Ting Therapeutics Inc. J.Z. and T.T. hold equity shares in Ting Therapeutics Inc., which has an option to obtain an exclusive license for the issued patent US11,446,308B2 related to CDK2 inhibitors' otoprotection from St. Jude Children's Research Hospital. All other authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2025.101250.

References

[1] WHO, Deafness and hearing loss. Key Facts on Deafness and Hearing Loss, 2025.
[2] S. Dasari, P.B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, Eur. J. Pharmacol. 740 (2014) 364–378.
[3] S.A. Funt, A. Knezevic, K. Wilson, M. Bromberg, A. Budnick, K.L. O'Connor, D. J. McHugh, E. Larsen, D.F. Bajorin, R.J. Motzer, et al., Ototoxicity associated with high-dose carboplatin for patients with previously treated germ cell tumors, Cancer 129 (2023) 3952–3961.
[4] D.R. Feldman, J. Sheinfeld, D.F. Bajorin, P. Fischer, S. Turkula, N. Ishill, S. Patil, M. Bains, L.M. Reich, G.J. Bosl, et al., TI-CE high-dose chemotherapy for patients with previously treated germ cell tumors: results and prognostic factor analysis, J. Clin. Oncol. 28 (2010) 1706–1713.
[5] O. Rose, T. Croonenberg, S. Clemens, T. Hinteregger, S. Eppacher, P. Huber-Cantonati, M. Garcia-Miralles, R. Liuni, S. Dossena, Cisplatin-induced hearing loss, oxidative stress, and antioxidants as a therapeutic strategy-A State-of-the-art review, Antioxidants 13 (2024).
[6] D.R. Freyer, P.R. Brock, K.W. Chang, L.L. Dupuis, S. Epelman, K. Knight, D. Mills, R. Phillips, E. Potter, D. Risby, et al., Prevention of cisplatin-induced ototoxicity in children and adolescents with cancer: a clinical practice guideline, Lancet Child Adolesc. Health 4 (2020) 141–150.
[7] E.A. Elmorsy, S. Saber, R.S. Hamad, M.A. Abdel-Reheim, A.F. El-Kott, M. A. AlShehri, K. Morsy, S.A. Salama, M.E. Youssef, Advances in understanding cisplatin-induced toxicity: molecular mechanisms and protective strategies, Eur. J. Pharm. Sci. 203 (2024) 106939.
[8] A. Callejo, L. Sedo-Cabezon, I.D. Juan, J. Llorens, Cisplatin-induced ototoxicity: effects, mechanisms and protection strategies, Toxics 3 (2015) 268–293.
[9] X. Huo, T.M. Reyes, C.J. Heijnen, A. Kavelaars, Cisplatin treatment induces attention deficits and impairs synaptic integrity in the prefrontal cortex in mice, Sci. Rep. 8 (2018) 17400.
[10] A.H. Alhowail, Cisplatin induces hippocampal neurotoxicity and cognitive impairment in rats through neuroinflammation, oxidative stress, and overexpression of glutamatergic receptors mRNA, Front. Pharmacol. 16 (2025) 1592511.
[11] A. Chattaraj, M.P. Syed, C.A. Low, T.K. Owonikoko, Cisplatin-induced ototoxicity: a concise review of the burden, prevention, and interception strategies, JCO Oncol. Pract. 19 (2023) 278–283.
[12] E. Orgel, K.R. Knight, D. Villaluna, M. Krailo, A.J. Esbenshade, L. Sung, D. R. Freyer, Reevaluation of sodium thiosulfate otoprotection using the consensus International Society of Paediatric Oncology Ototoxicity Scale: a report from the Children's Oncology Group study ACCL0431, Pediatr. Blood Cancer (2023) e30550.
[13] P.R. Brock, R. Maibach, M. Childs, K. Rajput, D. Roebuck, M.J. Sullivan, V. Laithier, M. Ronghe, P. Dall'Igna, E. Hiyama, et al., Sodium thiosulfate for protection from cisplatin-induced hearing loss, N. Engl. J. Med. 378 (2018) 2376–2385.
[14] D.R. Freyer, A.L. Frazier, L. Sung, Sodium thiosulfate and cisplatin-induced hearing loss, N. Engl. J. Med. 379 (2018) 1180–1181.
[15] A. Orasan, M.C. Negru, A.I. Morgovan, R.C. Fleser, D. Sandu, A.M. Sitaru, A. C. Motofelea, N.C. Balica, Strategies to mitigate cisplatin-induced ototoxicity: a literature review of protective agents, mechanisms, and clinical gaps, Audiol. Res. 15 (2025).
[16] L. Carles, A. Gibaja, V. Scheper, J.C. Alvarado, C. Almodovar, T. Lenarz, J.M. Juiz, Efficacy and mechanisms of antioxidant compounds and combinations thereof against cisplatin-induced hearing loss in a rat model, Antioxidants 13 (2024).
[17] X. Tan, Y. Zhou, A. Agarwal, M. Lim, Y. Xu, Y. Zhu, J. O'Brien, E. Tran, J. Zheng, D. Gius, et al., Systemic application of honokiol prevents cisplatin ototoxicity without compromising its antitumor effect, Am. J. Cancer Res. 10 (2020) 4416–4434.
[18] K. Fernandez, K.K. Spielbauer, A. Rusheen, L. Wang, T.G. Baker, S. Eyles, L. L. Cunningham, Lovastatin protects against cisplatin-induced hearing loss in mice, Hear. Res. 389 (2020) 107905.
[19] K.A. Fernandez, P. Allen, M. Campbell, B. Page, T. Townes, C.M. Li, H. Cheng, J. Garrett, M. Mulquin, A. Clements, et al., Atorvastatin is associated with reduced cisplatin-induced hearing loss, J. Clin. Invest. 131 (2021).
[20] T. Teitz, J. Fang, A.N. Goktug, J.D. Bonga, S. Diao, R.A. Hazlitt, L. Iconaru, M. Morfouace, D. Currier, Y. Zhou, et al., CDK2 inhibitors as candidate therapeutics for cisplatin- and noise-induced hearing loss, J. Exp. Med. 215 (2018) 1187–1203.
[21] R.A. Hazlitt, T. Teitz, J.D. Bonga, J. Fang, S. Diao, L. Iconaru, L. Yang, A.N. Goktug, D.G. Currier, T. Chen, et al., Development of second-generation CDK2 inhibitors for the prevention of cisplatin-induced hearing loss, J. Med. Chem. 61 (2018) 7700–7709.
[22] D.S. Boss, G.K. Schwartz, M.R. Middleton, D.D. Amakye, H. Swaisland, R. S. Midgley, M. Ranson, S. Danson, H. Calvert, R. Plummer, et al., Safety, tolerability, pharmacokinetics and pharmacodynamics of the oral cyclin-dependent kinase inhibitor AZD5438 when administered at intermittent and continuous dosing schedules in patients with advanced solid tumours, Ann. Oncol. 21 (2010) 884–894.
[23] K.F. Byth, A. Thomas, G. Hughes, C. Forder, A. McGregor, C. Geh, S. Oakes, C. Green, M. Walker, N. Newcombe, et al., AZD5438, a potent oral inhibitor of cyclin-dependent kinases 1, 2, and 9, leads to pharmacodynamic changes and potent antitumor effects in human tumor xenografts, Mol. Cancer Ther. 8 (2009) 1856–1866.
[24] D.R. Camidge, D. Smethurst, J. Growcott, N.C. Barrass, J.R. Foster, S. Febbraro, H. Swaisland, A. Hughes, A first-in-man phase I tolerability and pharmacokinetic study of the cyclin-dependent kinase-inhibitor AZD5438 in healthy male volunteers, Cancer Chemother. Pharmacol. 60 (2007) 391–398.
[25] M.A. Ingersoll, E.A. Malloy, L.E. Caster, E.M. Holland, Z. Xu, M. Zallocchi, D. Currier, H. Liu, D.Z.Z. He, J. Min, et al., BRAF inhibition protects against hearing loss in mice, Sci. Adv. 6 (2020).
[26] K. Fernandez, T. Wafa, T.S. Fitzgerald, L.L. Cunningham, An optimized, clinically relevant mouse model of cisplatin-induced ototoxicity, Hear Res. 375 (2019) 66–74.
[27] E.J. Sailor-Longsworth, R.D. Lutze, M.A. Ingersoll, R.G. Kelmann, K. Ly, D. Currier, T. Chen, J. Zuo, T. Teitz, Oseltamivir (Tamiflu), a commonly prescribed antiviral drug, mitigates hearing loss in mice, Clin. Transl. Med. 14 (2024) e1803.
[28] R.D. Lutze, M.A. Ingersoll, R.G. Kelmann, T. Teitz, Trametinib, a MEK1/2 inhibitor, protects mice from cisplatin- and noise-induced hearing loss, Pharmaceuticals 17 (2024).
[29] V. Manickam, M. Zallocchi, Paving the way for better ototoxicity assessments in cisplatin therapy using more reliable animal models, Front. Cell Neurosci. 19 (2025) 1552051.
[30] M.A. Ingersoll, R.D. Lutze, C.K. Pushpan, R.G. Kelmann, H. Liu, M.T. May, W. J. Hunter, D.Z. He, T. Teitz, Dabrafenib protects from cisplatin-induced hearing loss in a clinically relevant mouse model, JCI Insight 8 (2023).
[31] B.K. Gersten, T.S. Fitzgerald, K.A. Fernandez, L.L. Cunningham, Ototoxicity and platinum uptake following cyclic administration of platinum-based chemotherapeutic agents, J. Assoc. Res. Otolaryngol. 21 (2020) 303–321.
[32] C.Y.W. Sung, N. Hayase, P.S.T. Yuen, J. Lee, K. Fernandez, X. Hu, H. Cheng, R. A. Star, M.E. Warchol, L.L. Cunningham, Macrophage depletion protects against cisplatin-induced ototoxicity and nephrotoxicity, Sci. Adv. 10 (2024) eadk9878.
[33] M.M. Tomayko, C.P. Reynolds, Determination of subcutaneous tumor size in athymic (nude) mice, Cancer Chemother. Pharmacol. 24 (1989) 148–154.
[34] C.K. Pushpan, D.F. Kresock, M.A. Ingersoll, R.D. Lutze, D.L. Keirns, W.J. Hunter, K. Bashir, T. Teitz, Repurposing AZD5438 and Dabrafenib for cisplatin-induced AKI, J. Am. Soc. Nephrol. 35 (2024) 22–40.
[35] A.B. Nair, S. Jacob, A simple practice guide for dose conversion between animals and human, J. Basic Clin. Pharm. 7 (2016) 27–31.
[36] P. Rajkumar, B.S. Mathew, S. Das, R. Isaiah, S. John, R. Prabha, D.H. Fleming, Cisplatin concentrations in long and short duration infusion: implications for the optimal time of radiation delivery, J. Clin. Diagn. Res. 10 (2016) XC01–XC04.
[37] P. Szturz, K. Wouters, N. Kiyota, M. Tahara, K. Prabhash, V. Noronha, A. Castro, L. Licitra, D. Adelstein, J.B. Vermorken, Weekly low-dose versus three-Weekly high-dose cisplatin for concurrent chemoradiation in locoregionally advanced non-nasopharyngeal head and Neck cancer: a systematic review and meta-analysis of aggregate data, Oncologist 22 (2017) 1056–1066.
[38] S. Dhillon, Sodium thiosulfate: pediatric first approval, Paediatr. Drugs 25 (2023) 239–244.
[39] H. Burger, K. Nooter, A.W. Boersma, C.J. Kortland, A.P. van den Berg, G. Stoter, Expression of p53, p21/WAF/CIP, bcl-2, bax, bcl-x, and bak in radiation-induced apoptosis in testicular germ cell tumor lines, Int. J. Radiat. Oncol. Biol. Phys. 41 (1998) 415–424.
[40] D.A. Tweddle, A.J. Malcolm, M. Cole, A.D. Pearson, J. Lunec, p53 cellular localization and function in neuroblastoma: evidence for defective G(1) arrest despite WAF1 induction in MYCN-amplified cells, Am. J. Pathol. 158 (2001) 2067–2077.
[41] J. Lv, H. Wang, X. Cheng, Y. Chen, D. Wang, L. Zhang, Q. Cao, H. Tang, S. Hu, K. Gao, et al., AAV1-hOTOF gene therapy for autosomal recessive deafness 9: a single-arm trial, Lancet 403 (2024) 2317–2325.
[42] H. Wang, Y. Chen, J. Lv, X. Cheng, Q. Cao, D. Wang, L. Zhang, B. Zhu, M. Shen, C. Xu, et al., Bilateral gene therapy in children with autosomal recessive deafness 9: single-arm trial results, Nat. Med. 30 (2024) 1898–1904.
[43] H.C. Ou, F. Santos, D.W. Raible, J.A. Simon, E.W. Rubel, Drug screening for hearing loss: using the zebrafish lateral line to screen for drugs that prevent and cause hearing loss, Drug Discov. Today 15 (2010) 265–271.
[44] A. Kurabi, K. Pak, E.J. Lee, A.F. Ryan, Combinatorial protection of cochlear hair cells: not too little but not too much, Front. Cell Neurosci. 18 (2024) 1458720.

[45] S. Vijayakumar, J.A. DiGuiseppi, P.J. Dabestani, W.G. Ryan, R.V. Quevedo, Y. Li, J. Diers, S. Tu, J. Fleegel, C. Nguyen, et al., In silico transcriptome screens identify epidermal growth factor receptor inhibitors as therapeutics for noise-induced hearing loss, Sci. Adv. 10 (2024) eadk2299.
[46] M.A. Ingersoll, R.D. Lutze, R.G. Kelmann, D.F. Kresock, J.D. Marsh, R.V. Quevedo, J. Zuo, T. Teitz, KSR1 Knockout mouse model demonstrates MAPK Pathway's key role in cisplatin- and noise-induced hearing loss, J. Neurosci. 44 (2024).
[47] C.L. Kennedy, B. Shuster, R. Amanipour, B. Milon, P. Patel, R. Elkon, R. Hertzano, Metformin protects against noise-induced hearing loss in male mice, Otol. Neurotol. 44 (2023) 956–963.
[48] D.R. Camidge, M. Pemberton, J. Growcott, D. Amakye, D. Wilson, H. Swaisland, C. Forder, R. Wilkinson, K. Byth, A. Hughes, A phase I pharmacodynamic study of the effects of the cyclin-dependent kinase-inhibitor AZD5438 on cell cycle markers within the buccal mucosa, plucked scalp hairs and peripheral blood mononucleocytes of healthy male volunteers, Cancer Chemother. Pharmacol. 60 (2007) 479–488.
[49] S. Hati, M. Zallocchi, R. Hazlitt, Y. Li, S. Vijayakumar, J. Min, Z. Rankovic, S. Lovas, J. Zuo, AZD5438-PROTAC: a selective CDK2 degrader that protects against cisplatin- and noise-induced hearing loss, Eur. J. Med. Chem. 226 (2021) 113849.
[50] Y. Chen, C. Cheng, A. Li, D. Ma, S. Li, H. Wang, S. Gao, D. Liu, P. Song, C. Yu, et al., Pinoresinol diglucoside attenuates nuclear receptor coactivator 4-mediated ferritinophagy associated with cisplatin-induced hearing loss, Adv. Sci. 12 (2025) e2408777.