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Cisplatin is an effective chemotherapy agent for a wide variety of solid tumors, but its use is dose-limited by serious side effects including acute kidney injury (AKI) and hearing loss. There are no FDA-approved drugs to treat both side effects. Recently, two anti-cancer oral drugs, AZD5438 and dabrafenib, were identified as protective against cisplatin-induced hearing loss in mice. We hypothesize that similar cell stress
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Conclusion
Cisplatin-induced damage to the inner ear and kidneys share similar cellular beneficial responses to AZD5438 and dabrafenib highlighting the potential therapeutic use of these agents to treat both cisplatin-mediated kidney damage and hearing loss.

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To combat both untoward effects of nephrotoxicity and ototoxicity in cisplatin-treated patients, two potential therapeutic oral anti-cancer drugs AZD5438 and dabrafenib, a Phase-2 clinical trial protein kinase CDK2 inhibitor and an FDA-approved drug BRAF inhibitor respectively, were tested in an established mouse acute kidney injury (AKI) model. Both drugs have previously been shown to protect significantly against cisplatin-induced hearing loss in mice. Each drug ameliorated cisplatin-induced increases in the serum biomarkers BUN, creatinine, and NGAL. Drugs also improved renal histopathology and inflammation, mitigated cell death by pyroptosis and necroptosis, and significantly enhanced overall survival of cisplatin-treated mice.

Study Group

No
**Clinical Trials Registration:** My study was a clinical trial.

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T.T. is a co-founder of Ting Therapeutics, LLC which has patents for drugs AZD5438 and dabrafenib for protection from hearing loss. T.T. and K.B. are inventors on a provisional patent application filed for the use of AZD5438 and dabrafenib in cisplatin-induced AKI by Creighton University. The other authors declare they have no competing interests.
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Repurposing AZD5438 and Dabrafenib for Cisplatin-Induced Acute Kidney Injury

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Running title: AZD5438 and dabrafenib reduce cisplatin-induced AKI

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Abstract

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Cisplatin is an effective chemotherapy agent for a wide variety of solid tumors, but its use is dose-limited by serious side effects including acute kidney injury (AKI) and hearing loss. There are no FDA-approved drugs to treat both side effects. Recently, two anti-cancer oral drugs, AZD5438 and dabrafenib, were identified as protective against cisplatin-induced hearing loss in mice. We hypothesize that similar cell stress and death pathways are activated in kidney and inner ear cells when exposed to cisplatin, and tested whether these drugs alleviate cisplatin-induced AKI.

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The HK-2 cell line and adult FVB mice were utilized to measure the protection from cisplatin-induced cell death and AKI by these drugs. Serum markers of kidney injury, BUN, creatinine, and NGAL, as well as histology of kidneys were analyzed. Levels of markers of kidney cell death including necroptosis and pyroptosis, pERK, and PCNA, were also examined by western blotting and immunofluorescence. Additionally, CDK2 KO mice were utilized to confirm AZD5438 protective effect is through CDK2 inhibition.

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**Conclusion**

Cisplatin-induced damage to the inner ear and kidneys share similar cellular
beneficial responses to AZD5438 and dabrafenib highlighting the potential therapeutic
use of these agents to treat both cisplatin-mediated kidney damage and hearing loss.
Introduction

Ten percent of all cancer patients world-wide receive cisplatin as part of their solid-tumor chemotherapy regimen, but unfortunately, it is particularly toxic to certain tissues in the body in which the drug accumulates, including the kidney and the inner ear.\(^1\)-\(^7\) Cisplatin concentrates in the kidney during glomerular filtration and tubular secretion, which results in a fivefold increase in cisplatin concentration compared to serum levels, causing severe damage to kidney proximal tubular cells.\(^8\)-\(^{10}\) Cisplatin buildup in the inner ear causes hair cell death, and damage to the supporting cells, neuron fibers, and stria vascularis cells.\(^7\)\(^{,11,12}\) Despite its potent antitumor effect, the clinical use of cisplatin is dose-limited by nephrotoxicity which is commonly manifested as acute kidney injury (AKI) or chronic renal dysfunction.\(^13\)-\(^{17}\) The renal function impairment can be progressive, and eventually lead to chronic kidney disease.\(^18\) Cisplatin can also cause renal tubular dysfunction, salt wasting, hypomagnesemia, and anemia.\(^19\) These side effects are observed in roughly one-third of patients despite intensive prophylactic measures such as hydration, diuresis, magnesium supplementation, and amifostine treatment.\(^1\),\(^4\),\(^16\) Moreover, the incidence and severity of renal failure increases with subsequent courses and can eventually become irreversible.\(^8\),\(^20\) As a result, discontinuing therapy with cisplatin is generally indicated in those who develop evidence of progressive renal impairment.\(^21\) More than half of patients also suffer from irreversible hearing loss after cisplatin treatment.\(^22\),\(^23\) To date, no drugs have been approved by the Food and Drug Administration (FDA) for protection from cisplatin-induced kidney damage and hearing loss.\(^24\)-\(^{26}\)
In this study, we reasoned that drugs which protect from ototoxicity may also alleviate renal toxicity. Cisplatin has been shown in both tissue types to cause DNA damage, mitochondrial injury, production of reactive oxygen species (ROS), triggering of inflammatory responses, and cell death signaling pathways including pyroptosis and necroptosis.\textsuperscript{27,28} The kidney and inner ear cells share similar transport proteins such as ATP6V1B1, and ATP6V0A4, that can contribute to relatable pharmacological activity of drugs in the two organs.\textsuperscript{24-26,29,30} Herbal Chinese medicine points to similarities between drugs that work in the kidney and ear tissues.\textsuperscript{31-33} Interestingly, congenital abnormalities like Alport syndrome and branchio-oto-renal (BOR) syndrome share renal and hearing defects.\textsuperscript{18,31}

Protein kinases are particularly promising therapeutic targets as they regulate critical cellular functions, and many kinase inhibitors have been approved by the FDA for effective cancer therapy.\textsuperscript{34,35} Recently, we reported that two protein kinase inhibitors and anti-cancer drugs, a clinical phase-2 drug CDK2 kinase inhibitor AZD5438,\textsuperscript{22,36-39} and an FDA-approved drug dabrafenib\textsuperscript{40-42} protect the post-mitotic inner ear cells against cisplatin-induced ototoxicity in mice by oral delivery. Both drugs were identified as top hits in inner ear cell line-based high-throughput screens for protection from cisplatin-induced cell death, and were shown to not interfere with cisplatin killing efficacy in lung and neuroblastoma cancer cell lines.\textsuperscript{22,24} Here, we evaluated the two drugs activity against cisplatin-induced kidney injury \textit{in vitro}, in HK-2 cells, and \textit{in vivo} in adult mice. The anti-cancer activity of the drugs with cisplatin was confirmed in a neuroblastoma cell line and in two testicular cancer cell lines, tumor types in which cisplatin is the standard of care. We studied the mechanism of action of AZD5438 in the
kidney by analyzing CDK2-null mice, and by complementing WT and KO CDK2 mice with the drug. Dabrafenib was shown to inhibit, in vivo, the phosphorylation of the downstream target ERK1/2. Our experimental results identify the two drugs as promising therapeutic candidates for prevention of both cisplatin-induced kidney injury and hearing loss.

METHODS

Animals

FVB/NJ mice (Strain # 001800) were purchased from The Jackson Laboratory and bred in Creighton University animal facility. The germline CDK2 KO mice were derived as described in Teitz et al., 2018 by crossing CDK2-floxed/floxed mice with EIIA-Cre on C57BL/6/129 mixed backgrounds, from which offspring possessing the CDK2 deletion and lacking Cre were intercrossed to obtain homozygous CDK2 germline KO mice. Heterozygous mixed-background CDK2 KO mice were bred in Creighton University animal facility for 5 generations to the FVB/NJ background. All animal experiments were approved by the Institutional Animal Care and Use Committee of Creighton University.

Cell lines

Human immortalized proximal tubular HK-2 cells (CRL-2190) were purchased from the American Type Culture Collection (ATCC) and were maintained as per ATCC specifications in Keratinocyte serum-free medium, (Invitrogen, Gibco, Catalog Number
17005-042) and supplemented with additives 0.05 mg/ml bovine pituitary extract and 5 ng/ml EGF human recombinant epidermal growth factor, to promote growth, purchased from Invitrogen (Gibco). The cell line was grown at 37°C and 5% CO₂ and passaged using 0.05% (w/v) trypsin- 0.53 mM EDTA 1× (25300-054, Gibco). Testicular cell lines (JKT-1 & NCCIT) and neuroblastoma cell line (Kelly) were grown in RPMI 1640 (A1049101) medium with the ATCC® modification (Gibco) with a final concentration of 10% fetal bovine serum (FBS) and 200µL of 0.04 mg/mL ampicillin.

Drugs

AZD5438 (HY-10012) and Dabrafenib (HY-14660A) were obtained from MedChem Express.

Cell Titer-Glo viability assays

Human immortalized proximal tubular HK-2 cells were plated in 96-well plates in 6 replicates and incubated overnight at 37°C in 5% CO₂. After 24 hours, 9,600 cells were pretreated with AZD5438 or dabrafenib, and one-hour later, cells were treated with 5 µM cisplatin plus drugs AZD5438 or dabrafenib for 48 hours. CellTiter-Glo Luminescent Assay (Promega, G7571) was employed to determine the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells. For controls, media alone, cisplatin alone and drug alone treated cells were tested. Cell viability was calculated as percent survival compared to media alone treated cells.

For tumor cell lines viability experiments, two testicular cell lines (JKT-1 & NCCIT) and a neuroblastoma cell line (Kelly) were utilized. 9600 cells per well were
plated in 6-replicates in 96 well plates and allowed to attach overnight at 37°C in 5% CO2. The following day, the tumor cell lines were pretreated with 3 µM of dabrafenib or AZD5438 alone for 1 hour. The cells were then treated with 10 µM cisplatin and incubated for 48 hours. Cell viability was then measured using the Cell Titer-Glo (Promega) assay. Medium alone-, cisplatin alone-, and drug alone were used as controls and the percent viability was calculated as the viability compared to the medium alone treated cells. Drug plus cisplatin treated cells were compared to cisplatin alone treated cells to determine whether dabrafenib or AZD5438 interfered with cisplatin’s tumor killing ability.

Mouse model for cisplatin-induced acute kidney injury

10 mg cisplatin (479306; Sigma-Aldrich) powder was dissolved in 10 ml sterile saline (0.9% NaCl) at 37°C for 40-60 min. Cisplatin at doses (15, 20, 25 and 30 mg/kg) was administered by intraperitoneal (IP) injection to 8-10 weeks old FVB/NJ mice, females, and males in equal ratio. To decrease the dehydration during cisplatin treatment, mice were injected subcutaneously with sterile saline 1 ml one day before cisplatin injection, and twice daily throughout the experimental period. The cisplatin-treated mice were placed on a heating pad, and fresh water-mushed food was given daily. Body weights were monitored daily. Blood samples were collected by cardiac puncture on day 3 or 21 after cisplatin treatment for quantifying kidney injury markers in serum. Kidney tissues were harvested for histopathology, and western-blot analysis.
Administration of drugs by oral gavage

AZD5438 and dabrafenib mesylate were dissolved in a solution containing 10% DMSO (BP231-100, Fisher), 5% Tween 80 (P1754, Sigma-Aldrich), 40% PEG-E-300 (91462, Sigma-Aldrich), and 45% saline (0.9% NaCl) as described previously. The mice were pretreated with AZD5438 (35 mg/kg) or dabrafenib (12 mg/kg) by oral gavage 45 minutes before injecting with cisplatin. The mice were given AZD5438 by oral gavage twice daily for 3 consecutive days. Dabrafenib was administered three times daily, 6 hours apart, for 3 consecutive days. Mice were sacrificed after 72 hours (day 3). Blood was collected by cardiac puncture. Kidneys were harvested and transferred immediately to 10% neutral buffered formalin for histological studies, and tissue was stored at -80°C for western blotting. Different doses of AZD5438 (30, 35, 40 mg/kg) were tested for its protective effects. 12 mg/kg body weight dose of dabrafenib was used.

For 14-days mouse survival studies, cisplatin (22 mg/kg) was IP-injected and AZD5438 and dabrafenib was given orally twice daily for three consecutive days. For longer-term studies of 21-days, mice were injected with 30 mg/kg of cisplatin and given 12 mg/kg of dabrafenib twice daily for three consecutive days by oral gavage.

Kidney injury markers BUN, NGAL and Creatinine to determine renal function

BUN serum levels were analyzed with Blood Urea Nitrogen (BUN) colorimetric detection kit (EIABUN, Thermo Fisher Scientific). NGAL serum levels was analyzed by ELISA using Invitrogen Lipocalin-2 mouse ELISA KIT (EMLCN2). Creatinine serum
levels were analyzed by Colorimetric Assay Kit (Cayman Chemical, #700460). Control proteins provided in the kits were used to graph a dose-response curve.

**Kidney histological examination**

Mouse kidney tissues were fixed in 10% neutral buffered formalin solution for 24 hours, embedded in paraffin, sectioned (3 µm) and stained with Hematoxylin and Eosin (H&E) or Periodic Acid-Schiff (PAS). Three sections from each mouse were observed under a microscope (Nikon Eclipse Ci) for histological examination. Semiquantitative pathological scoring system was used as described in references. The grading system used scores 0, 1, 2, and 3 which indicates the percentage of damage in each section, and were examined by an experienced pathologist in a double-blinded manner. Grade 0- no visible injury and normal kidney morphology; Grade 1- mild tubular dilation, presence of casts, condensed nuclei, partial loss of brush borders in the membranes in <1/3 tubules; Grade 2- clear dilation of many tubules, loss of brush border membranes, loss of nuclei and presence of casts in <2/3 of tubules; Grade 3- severe dilation of most tubules and complete loss of brush border membranes and loss of nuclei in > 2/3 of tubules.

**TUNEL assay**

Cell death in the mouse kidney sections was performed by TUNEL Assay Kit (Abcam, ab66110) according to manufacturer instruction. Briefly, harvested mouse kidneys were fixed in 10% formaldehyde for 24 hours and then embedded in paraffin. Three-micron thick sections were then sliced, deparaffinized, rehydrated using ethanol gradient (100%, 95%, 85%, 70%, 50%) and transferred to 0.85% NaCl solution for 5 min,
and Phosphate Buffered Saline (PBS) for 5 min. After washing with PBS, 20 µg/mL Proteinase K is added, and fixed with 4% formaldehyde. The sections were labeled with Br-dUTP for 60 min at room temperature, and treated with anti-BrdU-Red antibody for half an hour. Following coating with an anti-fading solution (Fluoromount-G™, Thermo Fisher Scientific), the sections were covered with a glass cover slip, and sealed. The images are scanned by Olympus BX61VS; Olympus America, Center Valley, PA fluorescent microscope, and the stained area is quantified using NIH software ImageJ.\textsuperscript{45} Each experimental group had 3-5 mice. Three kidney sections were analyzed from each mouse, and five images from each kidney section (covering more than 80% of the kidney total area) were quantified for mean fluorescence intensity (MFI) using ImageJ software. The nuclei number was analyzed from each image using “Analyze Particle” mode. The ratio of MFI to the number of nuclei was used for each image for statistical analysis.

**PCNA and pERK immunohistochemistry**

Tissue sections were analyzed for the expression of PCNA and pERK1/2 by immunostaining using a standard protocol.\textsuperscript{46} Antigen retrieval was done using HIER (Heat Induced Epitope Retrieval) buffer at 95°C for 20 min. The sections were blocked in PBS containing 0.25% Triton-X and 10% Fetal bovine serum (FBS) for 30 min at room temperature. Primary antibodies anti-PCNA (MA5-11358) or anti-pERK1/2 (9101), both purchased from Cell Signaling Technology, were used at a dilution of 1:500 in 10% FBS in PBS solution for overnight incubation. After washing with PBS, the slides were incubated for 1 h with respective secondary antibodies anti-mouse Alexa Fluor 647 (A31571) or anti-rabbit Alexa Fluor 488 (A11034), purchased from Invitrogen, at
dilution of 1:500. Kidney sections were then counterstained with the nuclei marker, 4’,6-diamidino-2-phenylindole (DAPI) for 10 min, and covered with glass coverslip using anti-fading solution and sealed. Slides were imaged using a fluorescent microscope (Olympus BX51; Olympus America, Center Valley, PA). Each experimental group had 6 mice, and three kidney sections were analyzed from each experimental mouse. Five square areas from each kidney section, covering more than 80% of the total kidney area, were imaged, and used for quantification of mean fluorescence intensity (MFI) using ImageJ software. The nuclei number was analyzed from each image using “Analyze Particle” mode. The ratio of MFI to the number of nuclei was used for each image for statistical analysis.

**Western blotting**

HK-2 cells and kidney tissue protein lysates were prepared in cell lysis buffer (9803, Cell Signaling Technology), containing protease (complete ULTRA Tablet 05892791001) and phosphatase (Phos STOP 04 906 837 001) inhibitors (Roche). The lysates were centrifuged for 10 min at 15,000g at 4°C, and the supernatants were collected. Protein concentrations in supernatants were determined with the BCA protein kit (23235, Thermo Fisher Scientific). Forty micrograms of total cell lysates for HK-2 cells or 60 micrograms of kidney lysates were loaded on 10% Tris-Glycine-polyacrylamide gel electrophoresis gels. The following antibodies were used for immunoblotting: anti-BRAF (14814), anti-pBRAF (Ser445, 2696), anti-ERK1/2 (4695), anti-pERK1/2 (Thr202/Tyr204, 9101), anti-MEK1/2 (9122), anti-pMEK1/2 (Ser217/221, 41G9), anti-RIP3 (95702), anti-RIP1 (3493), p-MLKL (3733), GSDME Cleaved (38821), GSDME (AB215191), CASPASE-3 Cleaved (14220) were obtained from Cell Signaling.
Technology, anti-β-actin (C4; SC-47778), was purchased from Santa Cruz, and anti-α Tubulin (T9026, Millipore) and total-MLKL (MAB C604) was purchased from Millipore. The antibodies were used at dilutions ranging from 1:500 to 1:1000. Anti-mouse (P0447) and anti-rabbit (P0448) secondary antibodies were purchased from Dako Laboratories and diluted 1:5,000. Images were quantified and band intensities were recorded as a ratio to loading control. Blot intensities were quantified using NIH Image J software. As positive controls in western blots, Phospho-MLKL Positive Control, product PC-PMLKL, FabGennix International Inc, and Caspase-3 Control, Cell Extracts Cell Signaling Technology #9663, were used.

**Statistical Analysis**

Results are expressed as mean ± SEM using Graph Pad Software, *P<0.05, **P< 0.01, ***P<0.001, compared by one-way or two-way ANOVA with Bonferroni post hoc test to analyze data, and unpaired Student T- test if only two conditions were compared.

**RESULTS**

**AZD5438 and dabrafenib protected human kidney HK-2 proximal tubular cells from cisplatin-induced cell death**

To determine IC₅₀ for cell death with cisplatin in the human HK-2 proximal tubular cell line, cells were treated with increasing concentrations of cisplatin for 48 hours, and cell viability was determined by CellTiter-Glo assay. Treatment with cisplatin showed a dose-dependent increase in cell death with an IC₅₀ of 5 µM (Figure 1A), consistent with
previous results published for this cell line. Dose-response of AZD5438 or dabrafenib alone showed no cytotoxicity (Figure 1B and C). HK-2 cells were then pretreated with different concentrations of AZD5438 or dabrafenib for one hour, and co-treated with 5 µM cisplatin and either drug for 48 hours. Results showed that AZD5438 and dabrafenib pretreatment protected HK-2 cells from cell death with an IC$_{50}$ of 0.026 µM and 0.77 µM respectively (Figure 1B and C).

AZD5438 and dabrafenib do not interfere with or enhance cisplatin tumor-killing efficacy in tumor cells

It is essential to test that AZD5438 and dabrafenib co-treatments with cisplatin for reducing AKI damage would not interfere with cisplatin killing efficacy of the treated tumors. Previously in our hearing-focused studies, we have shown that AZD5438 and dabrafenib do not interfere with tumor killing efficacy of cisplatin in three neuroblastoma and three lung cancer cell lines. Here, we broadened our tests to include an additional neuroblastoma genetic cell line Kelly, and two testicular cancer cell lines- seminoma JKT-1, and pluripotent embryonal carcinoma cell line, NCCIT. Neuroblastoma and testicular tumors are routinely treated with cisplatin as part of standard medical care. To evaluate interference with or enhancement of cell survival with drug treatments, cells were treated with the drugs alone or in combination with cisplatin for 48 hours (Figure 1D). Cell viability was measured by CellTiter-Glo assay in which 100% cell viability was the medium alone treated cells. The results indicated no interference with cisplatin killing activity in the three tumor cell lines tested (Figure 1D).
Oral administration of AZD5438 or dabrafenib protected from acute cisplatin-induced renal injury in mice, measured by reduction in levels of injury and histopathology biomarkers on day 3

To generate an AKI *in vivo* model, we employed an established protocol in FVB/NJ strain mice. Renal injury was determined on day 3 after cisplatin treatment by the serum levels of blood urea nitrogen (BUN) and creatinine, markers for kidney damage, and renal neutrophil gelatinase-associated lipocalin (NGAL), an early kidney injury marker.

To test the cisplatin-induced nephrotoxicity in our mouse model, and to evaluate how it mimics the human ailment, escalating doses of cisplatin (15, 20, 25, 30 mg/kg body-weight) were given by intraperitoneal injection (IP) to 8-10 weeks FVB/NJ old mice, and serum levels of BUN, NGAL and histopathology markers were measured 72 hours after cisplatin treatment (Supplementary Figure S1).

The histological scores of the cisplatin-treated kidneys were determined in a double-blind manner, by an experienced pathologist, employing a grading system described previously. H&E staining and Periodic acid-Schiff (PAS) staining of kidney sections of mice treated with 25 mg/kg or 30 mg/kg cisplatin showed extensive tissue damage characterized by the presence of cellular casts, loss of brush border, presence of dying cells, and inflammation 72 hours after cisplatin treatment, while lower doses of cisplatin exhibited less damage (Supplementary Figure S1C and D). The recorded histological grades in mice treated with escalating cisplatin doses correlated well with the measured BUN and NGAL levels (Supplementary Figure S1A and B).
Employing the mouse AKI model, 35 mg/kg dose of AZD5438 twice daily (Figure 2A), and 12 mg/kg dabrafenib three times daily (Figure 2D), were tested against 25 mg/kg cisplatin for their nephroprotective effects. The doses/regimen of the AZD5438 and dabrafenib drugs were chosen based on the lowest effective in vivo doses determined in our previous studies to be non-toxic to the inner ear cells and to confer protection against cisplatin-induced hearing loss in mice.24 The drug doses were confirmed in this study to be non-toxic to kidney cells by themselves or with cisplatin co-treatment in mice (Figure 2, Supplementary Figure S2).

In the mice, serum kidney injury markers were highly elevated on day 3 with cisplatin treatment, as determined by colorimetric detection of BUN and creatinine, and ELISA measurements of NGAL. Average BUN values were 108 ± 6 mg/dl, creatinine values were 4.3 ± 0.2 mg/ml and NGAL values were 96 ± 6 ng/ml, consistent with a previous publication employing this mouse model.43 In comparison, mice given oral gavage of carrier alone had BUN values of 34 ± 5 mg/dl, creatinine values of 0.2 ± 0.03 and NGAL of 7 ± 2 ng/ml (Figure 2). Importantly, AZD5438 cotreatment resulted in 2.1-fold decrease in BUN values to 51 ± 7 mg/dl, 4.3-fold decrease to 1.0 ± 0.1 mg/dl creatinine and 1.6-fold decrease in NGAL to 59 ± 8 ng/ml (Figure 2B). For dabrafenib cotreatment, BUN decreased 1.8-fold, to 59 ± 8 mg/dl, creatinine decreased 2.1 fold to 2.0 ± 0.2 mg/dl, and NGAL decreased 1.4-fold to 65.3 ± 4.0 ng/ml (Figure 2E). Scoring of H&E and PAS-stained kidney sections indicated reduced values from 3 ± 0 grade kidney damage in cisplatin alone treated mice, to 0.5 ± 0.2 with AZD5438 co-treatment (Figure 2B and C), and 0.8 ± 0.2 with dabrafenib co-treatment (Figure 2E and F).
AZD5438 inhibited cell proliferation measured by PCNA levels and cell death measured by TUNEL in cisplatin co-treated kidneys on day 3

Previous reports of cisplatin treatment in mice measured elevated levels of proliferating cell nuclear antigen (PCNA) occurring in post-mitotic kidney cells undergoing AKI.\textsuperscript{43,54} Therefore, we evaluated PCNA levels after AZD5438 co-treatment knowing the drug is a CDK2 kinase inhibitor that blocks proliferation and cell cycle progression that occurs with PCNA upregulation.\textsuperscript{49} PCNA immunofluorescent staining of kidney sections of cisplatin co-treated mice with AZD5438 indeed showed a 2.2-fold decreased expression of PCNA protein on day 3 compared to cisplatin alone treated mice (Figure 3A and B).

To determine if the mechanism of protection of AZD5438 against cisplatin-induced AKI is through reduction of cell death \textit{in vivo}, an immunofluorescence Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay to detect double-strand DNA breaks generated during cell death was performed on kidney sections of the different mouse experimental groups. As previously reported,\textsuperscript{55} cisplatin-treated mouse kidneys showed a high presence of DNA breaks per cell (0.06 ± 0.005 MFI/cell), in both proximal convoluted tubule and glomeruli, compared to the carrier alone mouse group with 0.016 ± 0.003 MFI/cell. The renal tubular cells and glomeruli of the AZD5438-treated mice had a 3.0-fold lower number of double-strand DNA breaks (0.02 ± 0.004 MFI/cell) compared to the cisplatin-treated mouse group, and close to the average number of DNA breaks of the carrier only mouse group (Figure 3C and D). Overall, both PCNA protein levels and cell death were significantly reduced in AZD5438 co-treated mice.
Dabrafenib inhibited cisplatin-induced ERK phosphorylation and cell death measured by TUNEL assay in kidneys

Mice cotreated with dabrafenib and cisplatin had a reduction in pERK levels compared to cisplatin alone, showing 2.6-fold reduction of pERK with dabrafenib cotreatment compared to cisplatin alone treated mice (Figure 3E and F). TUNEL assay performed on mouse kidney sections showed increased cell death in cisplatin alone treated mice (average 0.07±0.007 MFI/cell) on day 3 of treatments, and significant protection from cell death (average 0.03±0.008 MFI/cell) with dabrafenib co-treatment (Figure 3G and F). In summary, dabrafenib co-treatment decreased ERK phosphorylation of the kidney tissues at 72 hours after treatment and decreased total cell death at 72 hours in vivo.

Understanding the cellular mechanisms through which cisplatin induces nephrotoxic effects is important for identifying compounds for treatment. For dabrafenib, a specific BRAF inhibitor, we tested first if the canonical Mitogen-Activated Protein Kinase (MAPK) cellular pathway (schematically illustrated in Figure 4A) is upregulated with cisplatin treatment by following pBRAF (S445), pMEK (S217/221), and pERK (T202/Y204) protein expression, the activated forms of the kinases, in kidney HK-2 cells treated with cisplatin. Five µM cisplatin treatment of HK-2 (IC_{50} for death of HK-2 cells by cisplatin, determined in Figure 1A) was found to increase pMEK and pERK expression after 5 hours of cisplatin treatment by western blotting (Figure 4B). For testing in vivo, mice were IP injected with cisplatin and pERK1/2 protein was quantified in total kidney protein lysates at timepoints of 0, 1, 2, 5, and 8 hours after cisplatin treatment and compared to cisplatin and dabrafenib cotreatment (Figure 4C and D). An
additional experiment tested pERK protein levels at 72 hours post-cisplatin treatment in individual mice, with or without oral treatment of dabrafenib, 12 mg/kg three times a day for three days (Figure 4E and F). The levels of pERK protein after cisplatin treatment were found to be 6.3-fold upregulated at the 5 h, and 14.5-fold at 72 h timepoints compared to carrier alone mice. At both timepoints, the average pERK protein levels were reduced 3.1 and 2.7–fold respectively, with dabrafenib cotreatment (Figures 4D and F, Supplementary Figure S3-S5,S8-9). The western blot results at 72 h were consistent with the immunofluorescence data (Figure 3E and F), showing 2.6-fold reduction of pERK with dabrafenib co-treatment compared to cisplatin alone treated mice.

**Deletion of CDK2 attenuates cisplatin-induced AKI and complementation of the CDK2 knockout mouse with AZD5438 administration does not confer additional protection**

To confirm that the mechanism through which AZD5438 offers protection is inhibition of CDK2 and no other off-targets, a germline CDK2 knockout (KO) mouse model in which the CDK2 gene is deleted in all tissues was used.\(^{22}\) Importantly, the CDK2 KO mouse was backcrossed for five generations to the FVB/NJ background to compare to the genetic background the AKI model was tested. CDK2 KO mice were viable and had normal kidney structure. CDK2 WT and KO mice littermates were subjected to cisplatin IP injection (25 mg/kg) and on day 3, their blood and tissue samples were analyzed. As shown in Figure 5F, BUN levels in CDK2 WT (93.2 ± 2.5 mg/dl) mice were significantly higher compared to CDK2 KO (67.1 ± 3.7 mg/dl) mice. Histology results showed that the kidneys of CDK2 KO mice exhibited less tissue
damage with a histology grade of 1.4 ± 0.8 compared to grade 2.8 ± 0.4 for WT mice (Figure 5A-C). In addition, CDK2 WT mice treated with cisplatin on day 3, had higher levels of cell death by TUNEL assay (0.060± 0.008 MFI/cell), compared to the CDK2 KO mice undergoing the same treatment (0.027 ± 0.009 MFI/cell) (Figure 5D and E). Significantly, administration of AZD5438 to the WT and KO CDK2 mice resulted in similar level of kidney protection, and no additional kidney protection was achieved when AZD5438 was given to the KO CDK2 mice (Figure 5F). Thus, CDK2 is a key target in cisplatin-induced kidney damage, and the protective effect of AZD5438 is primarily through inhibition of this cellular target.

**Dabrafenib inhibited kidney injury on Day 21 post-cisplatin treatment**

Dabrafenib 3-day treatment protected hearing loss in adult FVB/NJ mice after one dose of 30 mg/kg cisplatin that induces permanent hearing loss. Functional hearing was measured at 21-days post-cisplatin treatment by Auditory Brainstem Response (ABR) threshold shifts. In this study, we tested whether dabrafenib can also confer kidney protection at 21-days post-cisplatin treatment (Figure 6A). Tissue injury was still extensive in cisplatin alone injected mice on day-21 (Figure 6B-D), and dabrafenib co-treatment resolved the tissue damage as demonstrated by scoring H&E and PAS stained kidney sections (Figure 6B-D). Cisplatin-alone treated mice showed enhanced levels of pERK1/2 protein and TUNEL levels per cell on day-21 after treatment compared to carrier alone treated mice (Figure 6E-H). In contrast, dabrafenib co-treated mice showed 2.1-fold reduction of pERK protein levels, and 1.5-fold reduction of TUNEL signals per cell (Figure 6E-H). In all experimental groups on day 21, no fibrosis was detected in the kidneys by H&E and PAS staining, that would suggest
repair (Figure 6B-D), and BUN values were normal. In summary, dabrafenib exhibited protection of the kidney tissues by histology and reduced cell death manifested even after 21 days post cisplatin treatment.

**AZD5438 and dabrafenib inhibit cisplatin-induced necroptosis and pyroptosis**

The TUNEL assays (Figures 3, 5, and 6) showed down regulation of cell death in the kidney tissues after AZD5438 and dabrafenib co-treatment on day 3 after cisplatin treatment. To explore which cell death pathways were upregulated in the mouse kidney tissues, we ran western blots of total kidney protein lysates from treated mice and probed with known biomarkers of cell death mechanisms that occur in cisplatin-induced AKI. The markers tested included: for apoptosis detection- cleaved caspase-3, for necroptosis - RIPK1, RIPK3, total and p-MLKL proteins, and for pyroptosis detection - cleaved GSDME-N. Interestingly, the results show that RIPK-1, RIPK-3, total MLKL and GSDME-N cleaved proteins were upregulated in cisplatin alone treated mouse kidneys and upon co-treatments with AZD5438 or dabrafenib, the levels of these markers were significantly reduced (Figure 7A-G). AZD5438 co-treatment restored levels of GSDME-FL (full length), total MLKL, RIPK1 and RIPK3 to the levels of carrier alone treated mice, and inhibited cleavage of GSDME-FL to the cleaved form GSDME-N. Dabrafenib restored levels of total MLKL, inhibited cleavage of GSDME-FL, and significantly reduced RIPK1 and RIPK3 levels (Figure 7A-G and Supplemental Figure S6-7). No apoptosis was detected on day 3 by cisplatin treatment in this AKI model as measured by no change in the levels of cleaved caspase-3. The results strongly suggest that AZD5438 and dabrafenib inhibit necroptosis and pyroptosis cell death pathways.
occurring during AKI in the kidney tissues on day 3 after cisplatin treatment as illustrated in Figure 7H.

**AZD5438 and dabrafenib enhanced survival of mice treated with cisplatin**

FVB/NJ mice treated with 22 mg/kg cisplatin in our experiments had high frequency of death, 30-50% by day 4 after treatment. A survival study was performed to test if the co-treatment with AZD5438 or dabrafenib could reduce death of the mice. Mice were IP injected with cisplatin and treated orally with AZD5438 or dabrafenib for three consecutive days, twice daily, first treatment 45 minutes before the cisplatin injection, and their survival was monitored for 14 days. Eighty-eight percent of animals co-treated with AZD5438 or dabrafenib and cisplatin were alive on day 14, whereas the cisplatin alone treated mice had higher death rates, with only 37.5% mice surviving at day 14 (Figure 8A and B). These results show that co-treatment with AZD5438 or dabrafenib gives a significant survival advantage to cisplatin-treated mice.

**Testing combinations of AZD5438 and dabrafenib for treatment of cisplatin-induced AKI**

As both drugs, AZD5438 and dabrafenib, conferred protection from cisplatin-induced AKI, we sought to test whether their combination would allow administrating lower total doses of drugs and thus, reducing the overall toxicity of the treatments. Figure 8C-E shows the combinations tested in mice by measuring BUN and creatinine serum levels on day 3 of treatments. Consistent and significant protection was achieved with the lower dose combination of 12 mg/kg AZD5438 and 3 mg/kg dabrafenib given
together orally 3 times a day for 3 days. This drug combination reduced daily doses by 51% for AZD5438 (12x3/35x2 x100) and 25% for dabrafenib (3x3/12x3 x 100) and was still as effective at alleviating cisplatin-induced AKI in mice as the original higher doses of each drug alone (Figures 2 and 8C-E).

**Discussion**

Previous studies from our laboratory have shown that the CDK2 kinase inhibitor, AZD5438, and the BRAF kinase inhibitor, dabrafenib, protect against cisplatin-induced permanent hearing loss in mice, without interfering with the tumor killing efficacy of cisplatin. Here, the efficacy of these two drugs was tested for suppressing cisplatin-induced nephrotoxicity in human HK-2 kidney cells, and in an established mouse model for AKI. Activation of various cell death pathways triggered by cisplatin was described previously in kidney and inner ear cell lines, zebrafish, and mice. The adult kidney is a quiescent organ that lacks significant mitotic activity in normal conditions. The mitotic activity, cell proliferation, and cell death is triggered during injury to cells as a mechanism to maintain homeostasis and recovery from cell injury. Here, employing a battery of known kidney injury biomarkers – BUN, creatinine, NGAL, pERK, PCNA, TUNEL (cell death), necroptosis and pyroptosis biomarkers (RIPK1, RIPK3, total MLKL, p-MLKL, cleaved GSDM-E), and histopathology markers, we could show that both AZD5438 and dabrafenib protect from AKI in mice in doses that were also protective for cisplatin-induced hearing loss. Interestingly, cell death by apoptosis was not detected at day 3 after cisplatin treatment as measured by absence of caspase-3 cleavage, while necroptosis and pyroptosis were the major cell
death pathways detected by TUNEL and the above mentioned specific biomarkers. This is in agreement with recent literature that suggests apoptosis occurs at a later time point after necroptosis and pyroptosis in AKI.27,58

Importantly, for translational purposes, the doses of the drugs AZD5438 and dabrafenib tested in our studies were within the range or lower than the equivalent doses tested or approved for use in humans.36,39,59 For AZD5438, 35 mg/kg twice a day in mice, is within the range tested in phase-2 clinical trials in humans.36,38 For dabrafenib, 12 mg/kg dose in mice three times daily, is equivalent to 60% of the dose approved for treatment of melanoma patients (150 mg twice daily).40,42 For both drugs, three days of treatment in mice was sufficient for long-term protection of hearing and kidney functions, and significantly increased survival of the mice, probably as result of the increased health status of the kidney tissues. For dabrafenib, Bai et al. showed recently, that the BRAF kinase molecular target itself does not contribute to nephrotoxicity.60 Moreover, combination of the two drugs (12 mg/kg AZD5438 and 3 mg/kg dabrafenib, 3 times a day for 3 days) proved beneficial in increasing consistency and reducing the total dose of each of the drugs needed, 51% for AZD5438 and 25% for dabrafenib, for equivalent kidney protection levels as the higher doses tested initially in this study. Similarly, for protection from noise-induced hearing loss, we reported that combining the drugs AZD5438 and dabrafenib enhanced the protection effects and allowed administrating lower doses of the drugs to achieve full protection.24

In this study, phosphorylated ERK1/2 protein levels in the kidney were enhanced with cisplatin treatment at early time points starting at 5 h and sustained at 72 h. Dabrafenib co-treatment downregulated pERK protein expression as determined by
immunofluorescence and western blot analysis. Increase in phosphorylated ERK1/2 protein levels have been associated previously with cisplatin-induced cell death, and enhanced inflammation in mouse kidneys. In the inner ear, an increase in ERK1/2 phosphorylation was detected 1 hour after cisplatin treatment, which was also reduced with dabrafenib treatment.

Previous studies have demonstrated that inflammation and the immune response contributes to AKI following cisplatin. Studies suggest that activation of the MAPK pathway and CDK2 following tissue damage can influence the inflammatory and immune response. It is possible that dabrafenib and AZD5438, through inhibition of the BRAF and CDK2, are affecting the immune response which lowers the amount of kidney damage following cisplatin. This is a very intriguing downstream mechanism that will be further explored.

We show here that wild type adult CDK2 mice were more susceptible to cisplatin-induced kidney damage compared to CDK2 KO littermates. This is the first genetic proof that CDK2 directly has an in vivo role in mediating cisplatin-induced AKI. CDK2 critical role in cisplatin nephrotoxicity has been studied in detail previously by the Price group. Yu et al. from this research group, have shown that the molecular target E2F1 downstream of CDK2, and E2F1 KO mice are resistant to cisplatin-induced cell death, suggesting that the CDK2-E2F1 pathway is important for cisplatin-induced cell death and protection strategies.

The level of protection from cisplatin-induced cell death conferred by the CDK2 KO mice was similar to the one achieved by AZD5438 treatment, with about 50% reduction in BUN, histology score, and TUNEL levels compared to WT CDK2 mice.
Moreover, adding AZD5438 to the CDK2 KO mice did not confer additional cell death protection strongly suggesting that the protection mediated by AZD5438 is via inhibition of CDK2 and not by other cellular targets.

Previously, we reported that CDK2 KO adult mice are resistant to cisplatin-induced hearing loss compared to WT CDK2 mice, and could show that their P3 cochlear explants exhibited resistance to cisplatin-induced hair cell loss, and mitochondrial production of ROS.\textsuperscript{22} Altogether, this study and the previous ones confirm that CDK2 inhibitors such as AZD5438, are useful for shielding both the kidneys and the inner ear from cisplatin-induced damage.\textsuperscript{22,37}

In this study, two well-characterized anti-cancer drugs, dabrafenib, an FDA-approved drug, and AZD5438, a CDK2 inhibitor and clinical trial phase-2 drug, showed promising nephroprotection in mice when given orally. As novel drug development is a lengthy, high-cost, and high-risk process, drug repurposing is an increasingly efficient strategy for developing new therapeutics.\textsuperscript{70} CDK2 and BRAF inhibition proved beneficial for both kidney cells and inner ear cells for protection from cisplatin-induced cell death. Due to dabrafenib already being FDA-approved and well tolerated by patients, it is a more promising drug for the treatment of AKI compared to AZD-5438, which has been shown to be toxic at higher doses and in continuous treatment in humans.\textsuperscript{36} The drug AZD-5438 is still promising in combination treatment, because we show that it can be given at lower doses with a low dose of dabrafenib and significant protection from AKI is still observed. When these drugs are given at low doses together, it limits the total amount of drug given which can limit side effects. It would be exciting to test these drugs in clinical trials for amelioration of both cisplatin-induced AKI and hearing loss. In
addition, future studies can also address the possibility that these two drugs will have utility in other clinical settings associated with AKI such as Fanconi-like syndrome which can become a long-term sequela of cisplatin-induced kidney injury, aminoglycoside-induced AKI, ischemic injury, sepsis, and rhabdomyolysis.

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Disclosure Statement

T.T. is a co-founder of Ting Therapeutics, LLC which has patents for drugs AZD5438 and dabrafenib for protection from hearing loss. T.T. and K.B. are inventors on a provisional patent application filed for the use of AZD5438 and dabrafenib in cisplatin-induced AKI by Creighton University. The other authors declare they have no competing interests.

Supplementary Material

Supplemental Figure 1: Dose-dependent response of cisplatin treatments in the mouse AKI model.

Supplemental Figure 2: Dose-dependent response of AZD5438 treatments in the mouse AKI model.

Supplemental Figure 3: Protein levels of p-ERK in mouse kidney lysates at different timepoints after dabrafenib cotreatment with cisplatin.

Supplemental Figure 4: Raw western blot data from Figure 4B.

Supplemental Figure 5: Raw western blot data from Figure 4C and 4E.

Supplemental Figure 6: Raw western blot data from Figure 7.

Supplemental Figure 7: Raw western blot data from Figure 7.

Supplemental Figure 8: Raw western blot data from Supplemental Figure 3A.

Supplemental Figure 9: Raw western blot data from Supplemental Figure 3B

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Figure Legends

Figure 1: AZD5438 and dabrafenib protect the human proximal tubule cell line HK-2 from cisplatin-induced cell death while not interfering with cisplatin’s tumor killing efficacy. (A) HK-2 cells were treated with increasing concentrations of cisplatin for 48 hours. Cell-Titer Glo assay was performed, and dose-dependent increase in cell death of HK-2 cells was observed with an IC$_{50}$ of 5 µM. (B) HK-2 cells were pretreated with different doses of AZD5438, starting 1 hour before 5 µM cisplatin treatment for 48 hours, and IC$_{50}$ of 0.026 µM was determined for protection of cell viability. (C) HK-2 cells were pretreated with different doses of dabrafenib, starting 1 hour before 5 µM cisplatin treatment for 48 hours, and IC$_{50}$ of 0.77 µM was determined for protection of cell viability. (D) Three human cancer cell lines, neuroblastoma Kelly, testicular seminoma JKT-1 and pluripotent embryonal carcinoma cell line NCCIT, were studied. The tumor cell lines were initially pretreated with 3 µM of dabrafenib, AZD5438 or both drugs for 1 hour and then treated with 10 µM cisplatin and 3 µM AZD5438, dabrafenib, or both drugs and incubated for 48 hours. Cell viability was measured at 48 hours using the Cell Titer-Glo (Promega) assay.
Figure 2: AZD5438 and dabrafenib protects against cisplatin-induced kidney injury in adult FVB/NJ mice. (A) Experimental protocol of cisplatin IP injection, saline administration, and AZD5438 oral delivery. (B) Quantification of kidney function markers and overall kidney morphology. BUN, creatinine, and NGAL levels were measured for kidney function and histology scores were determined for overall kidney function. (C) Renal tissues were stained with H&E (hematoxylin and eosin stain) and PAS (Periodic Acid-Schiff stain). Kidney histological scores were obtained by a pathologist blinded to the experimental conditions using a semi-quantitative pathological scoring system described in Methods. (D) Experimental protocol of cisplatin injection, saline administration, and dabrafenib oral delivery. (E) Quantification of BUN, creatinine, and NGAL levels and kidney histology scores. (F) Renal tissues were stained with H&E and PAS stain, and scored by a pathologist blinded to the experimental conditions. In all bar graphs, dots represent number of mice (n= 6-17). Values expressed are mean ± SEM, *P < 0.05, ***P < 0.001 by one-way ANOVA with Bonferroni post hoc test. Scale bar: 50 µm.
Figure 3: PCNA levels and cell death are downregulated with AZD5438 while dabrafenib prevents pERK upregulation and cell death in mouse kidneys treated with cisplatin. Representative immunofluorescent images of (A) PCNA and (C) TUNEL staining in kidney sections of experimental mice treated with carrier, cisplatin, or cisplatin and AZD5438. Quantification of PCNA signals is shown in (B) and TUNEL positive cells in (D), DAPI staining was used to localize nuclei in kidney sections. Intensity of PCNA expression, and TUNEL positive cells were determined by ImageJ software. Mean fluorescence intensity (MFI) per nuclei was quantified for each specimen. Three sections were quantified for each mouse. The graph represents MFI per cell. Representative fluorescent images of (E) pERK1/2 and (F) graph showing quantification of pERK1/2. (G) TUNEL staining in kidney sections of experimental mice. (H) Graph shows the quantification of TUNEL positive cells expression. DAPI staining was used to localize nuclei. The intensity of TUNEL positive cells and pERK staining were acquired, and the MFI per nuclei was quantified from each specimen. In all bar graphs dots represent number of mice (n=6). Values expressed are mean ± SEM *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA with Bonferroni post hoc test. Scale bar: 50 µm.
Figure 4: Dabrafenib inhibits phosphorylation of ERK1/2 in co-treated mice. (A) Putative pathway for mitogen-activated protein kinases (MAPK) activated by cisplatin in kidney cells. (B) HK-2 cells were treated with cisplatin, and the expression of p-BRAF, p-MEK and pERK was quantified by western blot. Ratio of phosphorylated protein bands to β-actin bands were marked. (C) Representative western blot images for levels of pERK1/2 and ERK1/2 in kidney lysates from mice treated with cisplatin (25 mg/kg) or cisplatin and dabrafenib (12 mg/kg) at - 0,1,2,5,8 hours post treatments. (D) Quantification of 3 independent western blot experiments (the other two blots are shown in Supplementary Figure S3). Ratio of pERK1/2 levels to α-tubulin were plotted (E) Western blot images for levels of pERK1/2 and ERK1/2 in kidney lysates from mice treated with carrier, cisplatin (25 mg/kg) or cisplatin and dabrafenib (12 mg/kg) at 72 h. Dabrafenib (12 mg/kg body weight) was given by oral gavage, 3 times daily, 4 hours apart. (F) Quantification of western blot. Ratio of pERK1/2 levels to β-actin were plotted. In all bar graphs, dots represent number of mice (n=3). Values expressed are mean ± SEM, **P < 0.01, ***P < 0.001 by one-way ANOVA with Bonferroni post hoc test.
Figure 5: CDK2 KO mice are resistant to cisplatin-induced nephrotoxicity compared to their WT littermates. CDK2 WT and KO mice (FVB/NJ background) were injected with cisplatin 25 mg/kg body weight and analyzed 72 hours post cisplatin treatment. Significant difference in (a-c) histology, (d-e) TUNEL MFI per cell, and (f) BUN levels were observed. In all bar graphs, dots represent number of biologically independent samples (n= 6-10 mice). Values expressed are mean ± SEM, *P < 0.05, **P < 0.01, compared by Student’s t-test (c, e) and one-way ANOVA with Bonferroni post hoc test (f). Scale bar: 50 µm.
Figure 6: Dabrafenib protects against cisplatin-induced kidney injury on day 21.

Adult FVB mice injected with 30 mg/kg cisplatin and treated with 12 mg/kg dabrafenib, two times daily for 3 days, were sacrificed after 21 days, and examined for tissue morphology. (a) Experimental protocol for cisplatin injection and dabrafenib treatment. (b-d) shows H&E, PAS staining and histology score. Histology score was determined by an experienced pathologist blinded to the experimental conditions, using a semi-quantitative scoring system described in Supplementary Materials. Scores are expressed as mean ± SEM. (e-f) Dabrafenib treatment to cisplatin injected mice reduced levels of ERK1/2. Analysis of protein levels of pERK by immunofluorescence analysis. (e) The image shows pERK1/2 (green) and nuclear stain (blue) and (f) shows the quantification of protein pERK levels. (g) Dabrafenib treatment protects from cell death in cisplatin injected mice. Immunofluorescence for TUNEL assay, for cell death analysis and DAPI staining to localize nuclei and (h) quantification of TUNEL (MFI per cell) from each sample. In all bar graphs, dots represent number of biologically independent samples (n= 6 mice). The graph represents mean ± SEM, *P < 0.05, ***P < 0.001 compared by one-way ANOVA with Bonferroni post hoc test. Scale bar: 50 µm.
Figure 7. AZD5438 and dabrafenib inhibit cisplatin-induced pyroptosis and necroptosis. (a) Western blot images of kidney samples from carrier, cisplatin alone treatment, cisplatin +AZD5438, and cisplatin+dabrafenib treated mice. RIPK-1, RIPK-3, Gasdermin E (Full length and N terminal), Gasdermin-N, p-MLKL, Total-MLKL, caspase-3, and cleaved caspase-3 were studied. (b-h) Quantification of western blots ratio of protein levels to β-actin were plotted. In all bar graphs, dots represent number of mice (n=3). Values expressed are mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA with Bonferroni post hoc test. (i) Diagrammatic representation of cell death cellular pathways inhibited by AZD5438 or dabrafenib co-treatment with cisplatin in mouse kidneys.
Figure 8: Dabrafenib and AZD5438 enhanced survival rates in adult mice and the combination of both AZD5438 and dabrafenib protects from cisplatin-induced kidney damage at lower doses. FVB/NJ adult mice were treated with AZD5438 (25 mg/kg body weight, twice daily for three consecutive days), or dabrafenib (12 mg/kg body weight, two times daily for three consecutive days), followed by IP injection of cisplatin 22 mg/kg, and survival was determined for 14 days after cisplatin injection. Six out of 16 mice treated with cisplatin alone survived for the duration of experiment, whereas 8 out of 9 mice survived in (A) AZD5438 and (B) dabrafenib co-treated groups. (C) BUN levels of mice treated with high dose combinations of AZD5438 and dabrafenib (D-E) BUN levels of mice treated with low dose combination of 12 mg/kg body weight AZD5438 and 3 mg/kg body weight dabrafenib three times daily for 3 days showing protection. In all bar graphs, dots represent number of mice (n=6). Values expressed are mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA with Bonferroni post hoc test.
Repurposing AZD5438 and Dabrafenib for Cisplatin-Induced Acute Kidney Injury

METHODS

1. In vitro screening for nephroprotection of AZD5438 and dabrafenib using HK-2 cell lines
2. Cisplatin-induced acute kidney injury is mice treated with AZD5438 and dabrafenib

Parameters tested:
1. Serum biomarkers of kidney injury - BUN, Creatinine, iNOS, & histology
2. Protein expression of PCNA & p-ERK
3. Cell death
4. Percent survival of mice

OUTCOME

AZD5438 and dabrafenib ameliorate cisplatin-induced kidney injury

Cisplatin mediates acute kidney injury by activating CDK2 and ERK signaling

Drug treatments enhanced survival

Conclusion

Oral administration of AZD5438 or dabrafenib protects against cisplatin-induced acute kidney injury in mice and enhances their survival.

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