Cis-diamminedichloroplatinum (cisplatin) is a commonly used anticancer drug that is effective for the treatment of various malignant solid tumors. It is currently understood that cisplatin covalently binds with DNA to form adducts that activate signal transduction pathways involved in DNA-damage recognition and repair, cell cycle arrest, and programmed cell death/apoptosis. The clinical use of cisplatin is associated with a dose-limiting renal tubular dysfunction typically occurring in up to 40% of patients despite the introduction of preventative measures, such as i.v. hydration therapy and prolongation of infusion duration. Cisplatin is known to abundantly accumulate in the human renal cortex against a concentration gradient, and it can competitively inhibit active uptake of the cation tetraethylammonium in mouse kidney slices and basolateral membrane vesicles of the renal cortex of rats. Furthermore, cisplatin has been shown to inhibit the renal clearance of organic ions from the basolateral site in isolated perfused rat kidney. These observations led to the recognition that an organic cation transporter (OCT) was probably regulating the cellular uptake of cisplatin into renal proximal tubular cells. In recent years, this transporter has been identified as OCT2, encoded by the gene SLC22A2, which is expressed at high levels in the basolateral membrane of renal tubular epithelial cells. Indeed, mice with a deficiency of the murine ortholog transporters Oct1 and Oct2 are protected from experiencing severe tubular damage following exposure to cisplatin, and similarly, patients carrying a variant of the OCT2 gene associated with reduced function are protected from cisplatin-induced nephrotoxicity. Moreover, concurrent administration of cisplatin with the OCT2 inhibitor cimetidine was demonstrated to result in at least partial protection against renal toxicity.

Although OCT2 has been linked to cisplatin-induced nephrotoxicity, it remains unclear whether this transporter contributes to the antitumor properties of cisplatin. In the current study, we evaluated the extent to which the transport of cisplatin by OCT2 is an important source of interindividual variability in efficacy and systemic disposition of cisplatin-based chemotherapy, which is a marker of antitumor efficacy in patients with head and neck cancer. We used in vitro and in vivo experimental approaches involving cell lines with variable expression levels of OCT2, a murine tumor model, and a cohort of patients with head and neck cancer receiving cisplatin with or without cimetidine in a randomized crossover fashion.

RESULTS
Effect of cimetidine on cisplatin uptake and cytotoxicity in vitro and in vivo
Previous studies reported that, among the National Cancer Institute's NCI-60 panel of cell lines, expression of the OCT2 gene
SLC22A2 was the highest in the ovarian cancer cell lines SKOV-3 and IGROV-1. A direct comparison indicates that the expression of SLC22A2 in IGROV-1 cells is equivalent to that observed in SKOV-3 cells (Figure 1a). Because SKOV-3 cells, unlike IGROV-1 cells, are p53 deficient and thereby experience increased intrinsic resistance to cisplatin, we focused on IGROV-1 cells to assess the influence of OCT2 inhibition on cisplatin-induced cytotoxicity. Despite the detectable expression levels of SLC22A2, coinubcation with cimetidine did not result in altered cisplatin uptake in IGROV-1 cells as compared with cells exposed to cisplatin alone (P = 0.86; Figure 1b). Moreover, coinubcation of cimetidine with cisplatin had no effect on cell growth inhibition as compared with IGROV-1 cells exposed to cisplatin alone (Figure 1c).

We next sought to assess whether coadministration of cimetidine with cisplatin would alter treatment efficacy in vivo using immunodecient nu/nu mice. Phenotypic characterization of these mice indicated that the cumulative urinary excretion of cisplatin was only ~25% of the dose, which is considerably lower than that observed in Friend Virus B-Type (FVB) mice used previously in toxicity studies (see Supplementary Figure S1a online). In line with this observation, we found that the nu/nu mice are relatively resistant to cisplatin-induced nephrotoxicity as compared with FVB mice, as determined by both histological examination of the kidney and by the toxicity markers blood urea nitrogen and serum creatinine (see Supplementary Figure S1b-d online). These findings suggest that the effects of cimetidine on the antitumor eficacy of cisplatin can be properly assessed in nu/nu mice without considering injurious effects on the kidney that may affect morbidity and mortality.

Administration of either cimetidine alone, cisplatin alone, or a combination of cisplatin and cimetidine to female nu/nu mice xenografted with luciferase-positive IGROV-1 cells had no effect on overall body weight, whereas mice that received saline alone experienced a signicant increase in weight (P = 0.04), presumably due to a progressively increasing tumor size (Figure 2a). The IGROV-1 tumor volume, measured on the basis of luciferase intensity, was dramatically decreased in mice that received cisplatin or the combination of cisplatin and cimetidine, as compared with controls, and did not signicantly differ from each other (P = 0.39, Figure 2b,c). Mice that had received cimetidine alone had no changes in tumor volume as compared with control mice (P = 0.09).

To further understand why cimetidine does not alter the uptake of cisplatin or cisplatin-induced cytotoxicity in cells expressing SLC22A2, we assessed localization of the OCT2 protein in these cells by immunofluorescence. We found that OCT2 was not detected at the plasma membrane of IGROV-1 or SKOV-3 cells (Figure 3a,b), whereas HEK293 cells engineered to overexpress OCT2, used as a positive control, had an abundance of OCT2 localized to this region (Figure 3c). OCT2 was also absent from the plasma membrane of HEK293 cells transfected with a vector control (Figure 3d).

**Effects of cimetidine coadministration on cisplatin pharmacokinetics**

A total of 19 patients diagnosed with head and neck cancer were recruited to the study, but samples from one patient had undetectable levels of cimetidine, and data from this patient were excluded from subsequent analyses. Baseline demographic data from the remaining 18 patients are summarized in Table 1. Of these patients, 10 received coadministration of cimetidine with cisplatin during cycle 1 and cisplatin alone during cycle 2 (arm A), whereas 8 patients received the reverse sequence (arm B). Two of 18 patients (both in arm A) were found to be heterozygous for the SLC22A2 808G > T variant (rs316019). The pharmacokinetic parameter estimates were not signicantly altered when data from these two patients were excluded, and therefore data from all patients were included irrespective of SLC22A2 genotype.

Two patients had received proton pump inhibitors, which have been previously identified as OCT2 inhibitors; however, pharmacokinetic parameter estimates were not signicantly altered when data from these two patients were excluded (see Supplementary Figure S2 online), and therefore data reported in this study included all patients. The areas under the curve of cimetidine in patients enrolled in Arms A and B were 29.0 ± 2.40 µg·h/ml and 24.5 ± 2.14 µg·h/ml, respectively, which is in line with previous

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**Figure 1** Regulation of platinum accumulation and sensitivity by OCT2. (a) Expression of SLC22A2 (organic cation transporter 2 (OCT2)) normalized to expression of GAPDH, in IGROV-1 and SKOV-3 tumor cells. (b) Cellular accumulation of platinum in IGROV-1 and HEK293 cells transfected with OCT2 or a vector control following a 30-min incubation with cisplatin (500 µmol/l) in the absence or presence of cimetidine (1 mmol/l). (c) Survival of IGROV-1 cells exposed to various concentrations of cisplatin and normalized to untreated controls in the presence or absence of cimetidine (100 µmol/l). Error bars represent standard error of the mean (n = 6). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
The concentrations of unbound cimetidine were consistently above the observed levels of unbound cisplatin at all time points in both Arms A and B (Figure 4a,b), with an average area under the curve ratio of unbound cimetidine to unbound cisplatin of 5.55 ± 0.50. Because a cimetidine-to-cisplatin concentration ratio of 2:1 was previously found to reduce OCT2-mediated uptake of cisplatin by fourfold,19 these clinical findings support the selection of our cimetidine dosing regimen.

In arm A, the area under the curve of unbound cisplatin was unaffected by concurrent use of cimetidine (4.47 ± 0.21 vs. 4.47 ± 0.20 µg·h/ml; P = 0.80), and similar results were obtained in arm B (4.26 ± 0.24 vs. 4.27 ± 0.40 µg·h/ml; P = 0.99; Figure 5a,b; Table 2). Likewise, the clearance of unbound cisplatin was unaffected by cimetidine, independent of the treatment sequence (Figure 5c,d; Table 2). Combining results from the two treatment arms generated similar results (Table 2). In addition, urinary excretion of platinum was unaffected by cimetidine (Table 2), which is consistent with previous findings that showed that patients with the reduced-function SLC22A2 808G > T variant do not have altered urinary excretion.28

**Discussion**

The current study addressed the concern regarding whether coadministration of cisplatin with the OCT2 inhibitor cimetidine would alter the efficacy and pharmacokinetic properties of cisplatin. Using in vitro models, we showed that cimetidine had no effect on the cytotoxicity of cisplatin or tumoral uptake. Moreover, coadministration of cimetidine with cisplatin in mice bearing IGROV-1 xenografts demonstrated no significant change in efficacy as compared with mice receiving cisplatin alone. Finally, we report that patients with head and neck cancer who received a combination of cisplatin and cimetidine showed no alterations in cisplatin pharmacokinetics as compared with when the same patients received cisplatin alone.

The incentive for our study was based on a growing body of literature that suggests that concurrent use of cimetidine can be protective against cisplatin-induced nephrotoxicity. The mechanism by which cimetidine affects cisplatin-induced nephrotoxicity is believed to be competitive inhibition of the renal tubular transporter OCT2, which restricts the accumulation of cisplatin in the kidney and subsequent downstream events resulting in apoptosis. This supposition is consistent with ex vivo preclinical findings indicating that cimetidine can alter cisplatin uptake in mouse kidney slices9 as well as in vivo observations in rodents.
and patients with cancer.\textsuperscript{20,29} Although the above studies reveal that cimetidine can be of great benefit in ameliorating cisplatin-induced nephrotoxicity, evidence to show whether its use would alter treatment efficacy is limited.

Currently available expression data demonstrate that OCT2 is either absent or detectable only at low levels in tumors, suggesting that it would be highly unlikely that this transporter regulates uptake of cisplatin into tumor cells.\textsuperscript{14,23} Our current study is in agreement with this notion demonstrating that even in cells that are among the highest expressers of OCT2, cimetidine does not appear to alter tumor cell uptake or efficacy either \textit{in vitro} or \textit{in vivo}. These observations are consistent with other recently reported studies involving cell lines with high expression of OCT2 either in culture or in

| Table 1 Patient demographics |
|-----------------------------|
| Patient characteristic      | Total | Arm A | Arm B |
| Patients, n                 | 18    | 10    | 8     |
| Sex                         |       |       |       |
| Male                        | 15 (83.3%) | 8 (80%) | 7 (87.5%) |
| Female                      | 3 (16.7%) | 2 (20%) | 1 (12.5%) |
| Age (years)                 |       |       |       |
| Mean (range)                | 57.5 (43–71) | 59.1 (43–71) | 55.5 (46–70) |
| Race                        |       |       |       |
| Caucasian                   | 16 (88.9%) | 10 (100%) | 6 (75.0%) |
| Asian                       | 2 (11.1%) | 0 (0%) | 2 (25.0%) |
| SLC22A2 808G > T genotype   | 2 (11.1%) | 2 (20%) | 0 (0%) |
| Tumor type                  |       |       |       |
| Head and neck               | 18 (100%) | 10 (100%) | 8 (100%) |
| BSA (m\textsuperscript{2})  |       |       |       |
| Mean (range)                | 1.95 (1.55–2.26) | 1.98 (1.55–2.26) | 1.90 (1.66–2.10) |
| Accumulative cisplatin dose (mg) |   |   |   |
| Mean (range)                | 387.5 (310–452) | 395.9 (310–452) | 377.0 (332–420) |
| Serum creatinine (µmol/l)   |       |       |       |
| Mean (range)                | 72.7 (45–101) | 72.2 (47–99) | 73.4 (45–101) |
| Blood urea nitrogen (mmol/l)|       |       |       |
| Mean (range)                | 5.0 (2.3–7.2) | 5.2 (2.3–7.2) | 4.8 (2.3–6.0) |
| Estimated GFR (ml/min/1.73 m\textsuperscript{2}) |   |   |   |
| Mean (range)                | 105.7 (71.3–141.6) | 110.1 (87.9–141.6) | 100.7 (71.3–124.0) |
| Hemoglobin (mmol/l)         |       |       |       |
| Mean (range)                | 8.49 (6.0–9.7) | 8.66 (7.9–9.7) | 8.3 (6.0–9.7) |
| Thrombocytes (x10\textsuperscript{9}/l) |   |   |   |
| Mean (range)                | 260.5 (171–405) | 236.8 (176–405) | 290.1 (171–378) |
| Leukocytes (x10\textsuperscript{9}/l) |   |   |   |
| Mean (range)                | 8.26 (4.6–12.4) | 8.4 (6.0–12.2) | 8.1 (4.6–12.4) |
| Bilirubin (µmol/l)          |       |       |       |
| Mean (range)                | 6.3 (3.0–13.0) | 6.2 (3.0–13.0) | 6.5 (4.0–12.0) |
| Alkaline phosphatase (U/l)  |       |       |       |
| Mean (range)                | 75.5 (40–105) | 75.9 (40.0–97.0) | 75 (63–105) |
| γ-Glutamyl transpeptidase (U/l) |   |   |   |
| Mean (range)                | 39.8 (9–110) | 39.2 (9.0–110.0) | 40.6 (20–75) |
|Alanine aminotransferase (U/l) |   |   |   |
| Mean (range)                | 25.7 (11–43) | 26.4 (14–28) | 24.9 (11–43) |
| Glutamate pyruvate transaminase (U/l) |   |   |   |
| Mean (range)                | 28.3 (7.0–88.0) | 32.7 (9.0–88.0) | 22.7 (7.0–46.0) |

BSA, body surface area; GFR, glomerular filtration rate.
Moreover, we provide data to show that, although tumor cells may express high levels of OCT2, localization and function of this transporter are not necessarily factors in the uptake of cisplatin into tumor cells. The process may instead rely on other transporters, such as the organic anion–transporting polypeptide OATP1B3 or the copper transporter 1, although the latter’s involvement in cisplatin transport has recently been questioned. In addition to the preclinical observations, patient data obtained throughout our study indicate that inhibition of OCT2 function with cimetidine has no impact on plasma levels or systemic clearance of cisplatin, which is consistent with results found in mice with a deficiency of both Oct1 and Oct2.

Although our study supports further clinical exploration of cimetidine as a modulator of cisplatin toxicity, it should be pointed out that the introduction of cimetidine to cisplatin-containing chemotherapy regimens in routine practice could remain problematic. Indeed, although loss of OCT2 function in vivo substantially diminishes cisplatin-induced nephrotoxicity, the degree of protection that is offered remains only partial, suggesting that other pathways unaffected by cimetidine may contribute to the overall side-effect profile. Furthermore, it should be kept in mind that (i) the action of cimetidine on OCT2 is via a competitive inhibitory mechanism, (ii) patients with a genetic predisposition to poor cimetidine absorption and/or rapid elimination may experience inadequate renoprotection, and (iii) cimetidine can inhibit the metabolism of other chemotherapeutics, such as paclitaxel, that are sometimes given in combination with cisplatin. Adding further to the complexity, the inhibition constant (Ki) values of cimetidine for multidrug and toxin extrusion protein (MATE)1 and MATE2-K, two solute carriers expressed on the luminal membrane of renal tubular cells linked with the terminal elimination of platinum chemotherapeutic drugs, are 1.1 and 7.3 µmol/l, respectively, which is comparable to the Ki for OCT2. Therefore, cimetidine is likely to inhibit the function of MATE1 and MATE2-K at relatively low doses and may lead to nonoptimal efflux of cisplatin out of tubular cells.

In conclusion, our results indicate that use of the OCT2 inhibitor cimetidine does not sacrifice the antitumor effects of xenograft models. Moreover, we provide data to show that, although tumor cells may express high levels of OCT2, localization and function of this transporter are not necessarily factors in the uptake of cisplatin into tumor cells. The process may instead rely on other transporters, such as the organic anion–transporting polypeptide OATP1B3 or the copper transporter 1, although the latter’s involvement in cisplatin transport has recently been questioned. In addition to the preclinical observations, patient data obtained throughout our study indicate that inhibition of OCT2 function with cimetidine has no impact on plasma levels or systemic clearance of cisplatin, which is consistent with results found in mice with a deficiency of both Oct1 and Oct2.

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cisplatin, irrespective of tumoral OCT2 expression, and that cimeti- dine has no influence of the disposition of cisplatin. These results support the future exploration of treatment strategies aimed at ameliorating cisplatin-induced nephrotoxicity using OCT2 inhibitors that can offer improved protection and selectivity as compared with cimidine.

METHODS

**In vitro and in vivo antitumor efficacy.** Expression levels of SLC22A2 in IGROV-1 and SKOV-3 cells were assessed as previously described and normalized to the expression of the gene for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Cellular cisplatin uptake was measured by determining total platinum levels following incubation with cisplatin (500 µmol/l) for 30 min in the presence or absence of cimidine (1 mmol/l), as previously described. The cell growth inhibitory potential of cisplatin in IGROV-1 cells in the presence or absence of 100 µmol/l cimidine was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay following continuous exposure for 72 h.

Efficacy studies were carried out in female immunodeficient CD-1 nude mice (Charles River, Wilmington, MA) injected with 3 × 10⁶ Luc²⁻YFP¹-transduced IGROV-1 cells, which were transduced with a lentivector (obtained from the Vector Core Facility at St Jude Children’s Research Hospital, Memphis, TN) containing the firefly luciferase and yellow fluorescent protein genes (CL20SF2–Luc²aYFP). Yellow fluorescent protein–positive cells were sorted by flow cytometry and expanded in culture to evaluate cellular localization of OCT2 expression. Localization of OCT2 was assessed in IGROV-1 and SKOV-3 cells, as well as in HEK293 cells transfected with human OCT2 or a control vector. A total of 250,000 cells were plated in six-well plates with sterile glass coverslips and incubated under standard conditions overnight. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton-X, and incubated in phosphate-buffered saline with 4% fetal bovine serum, after which a rabbit anti-human OCT2 antibody (Sigma-Aldrich, St Louis, MO) at 1:40 dilution was added and left overnight at 4 °C. Specificity of this antibody has been demonstrated by the Human Protein Atlas (http://www.proteinatlas.org/ENSG00000112499/subcellular). Samples were then incubated with a goat anti-rabbit Alexa Fluor 555 secondary antibody (1:500 dilution; Invitrogen, Carlsbad, CA) and stained with Alexa Fluor 488 phalloidin (1:100 dilution; Invitrogen). Coverslips were mounted on glass microscope slides with Prolong Gold antifade reagent with 4',6-diamidino-2-phenylindole (Invitrogen) for observation by confocal microscopy (LSM 510 Meta; Carl Zeiss) with a ×63 objective lens.

**Patient selection.** All patients had a histologically or cytologically confirmed diagnosis of irresectable and/or metastatic head and neck cancer, which at the time was not treated with cisplatin. Eligibility criteria included the following: (i) availability to receive treatment with high-dose cisplatin (once every 3 weeks, 100 mg/m²), without any other systemic anticancer treatment; (ii) at least 18 years of age; (iii) World Health Organization performance status of 0 or 1; (iv) adequate hematological functions (absolute neutrophil count >1.5 × 10⁹/l and platelets >100 × 10⁹/l); (v) adequate renal function (serum creatinine <1.25 × upper limit of institutional normal (ULN)); (vi) hepatic functions (bilirubin <1.25 × ULN; alanine aminotransferase <2.5 × ULN); and (vii) oncologic and co-medications for cytochrome P450 3A (CYP3A) and/or the ATP-binding cassette drug transporters P-glycoprotein (ABCBI)/breast cancer resistance protein (ABCG2), dietary supplements, or other inhibiting compounds that prohibited the understanding and providing of informed consent; (viii) using cimetidine within 4 weeks prior to study entry; (ix) any history of a psychiatric disorder (i) pregnant or lactating; (ii) experiencing serious illness or a medically unstable condition requiring treatment; (iii) having symptomatic metastases of the central nervous system or a history of a psychiatric disorder that prohibited the understanding and providing of informed consent; (iv) currently undergoing cisplatin therapy; (v) previously diagnosed with chronic kidney disease; (vi) undergoing major surgery within 4 weeks of the start of treatment; (vii) chronic users of inhibiting and/or inducing medications for cytochrome P450 3A (CYP3A) and/or the ATP-binding cassette drug transporters P-glycoprotein (ABCBI)/breast cancer resistance protein (ABCG2), dietary supplements, or other inhibiting compounds of putative relevance to cimidine (http://medicine.iupui.edu/flockhart/table.htm); (viii) unwilling to change medication; or (ix) using cimetidine within 4 weeks prior to study entry.

**Pretherapy evaluations.** Urine samples were collected within 24 hours of the start of treatment to establish baseline kidney function, and blood samples were collected to allow subsequent genotyping of patients. A complete blood count to assess white blood cell differentiation, and serum biochemistry, including levels of sodium, potassium, calcium, phosphorus, creatinine, creatine kinase, transaminases, γ-glutamyltransferase, alkaline phosphatase, total and conjugated bilirubin, total protein, α1-acid glycoprotein, albumin, glucose, blood urea nitrogen, and urea, were performed within 2 weeks before therapy. All patients were genotyped for the SLC22A2 808G > T variant, as previously described. Baseline creatinine clearance was determined by the Cockcroft–Gault equation:

\[
\text{CrCl} = \frac{140 \times \text{height} \times \text{weight}}{72 \times \text{serum creatinine} \times (1 - 0.013 \times \text{age})}
\]

where CrCl is the creatinine clearance, height is in meters, weight is in kilograms, serum creatinine is in milligrams per deciliter, and age is in years. The AUC of unbound cisplatin in patients who received cotreatment with cimetidine in the (a) first cycle only (arm A) or (b) second cycle only (arm B). The overall mean clearance of unbound cisplatin in patients who received cotreatment with cimetidine in the (c) first cycle only (arm A) or (d) second cycle only (arm B). The difference in pharmacokinetics of cisplatin between the period without and the period with concomitant use of cimetidine was evaluated using a paired Student’s t-test for comparison of the mean absolute difference between the two periods. AUC, area under the curve.

**Figure 5** The AUC of unbound cisplatin in patients who received cotreatment with cimetidine in the (a) first cycle only (arm A) or (b) second cycle only (arm B). The overall mean clearance of unbound cisplatin in patients who received cotreatment with cimetidine in the (c) first cycle only (arm A) or (d) second cycle only (arm B). The difference in pharmacokinetics of cisplatin between the period without and the period with concomitant use of cimetidine was evaluated using a paired Student’s t-test for comparison of the mean absolute difference between the two periods. AUC, area under the curve.
formulas, and estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula, which considers serum creatinine, blood urea nitrogen, and albumin levels.

**Study design and treatment.** Patients were deemed evaluable when treated once every 3 weeks with cisplatin at an i.v. dose of 100 mg/m² given as a 3-hour infusion for at least 2 two cycles of treatment, and when a complete pharmacokinetic sampling and toxicity assessment had been performed. Enrollment of patients continued until at least 18 patients had evaluable pharmacokinetic data for both cimetidine and cisplatin. Under these conditions, the probability is 91% that the study will detect a difference in the pharmacokinetics of unbound cisplatin between treatment groups at a two-sided 5.0% significance level, if the true difference between the treatments is 0.300 units. This is based on the assumption that the within-patient SD of the response variable is 0.200.41 No reductions or alterations of cisplatin dose were permitted in this study; therefore, if patients experienced severe nephrotoxicity following the first cycle, they were immediately removed from the study. As a result, the randomized crossover study design of this trial did not allow for accurate assessment of the renoprotective effects of cimetidine, which would instead require a randomized, parallel, two-arm, placebo-controlled, and adequately powered study to definitively address its protective effects.

Participants were randomized as follows: approximately half of the patients received concomitant administration of cimetidine with cisplatin on the initial day of treatment and no cimetidine on the second day of treatment (arm A), while the remaining patients received cisplatin alone on the initial day of treatment and concomitant administration of cimetidine with cisplatin on the second day of treatment (arm B). Cimetidine was given at a dose of 800 mg p.o. 30 min before the initiation of cisplatin infusion and another 800 mg p.o. 1.5 h after the start of infusion in order to achieve an unbound concentration at least three times that of cisplatin, which would be the level necessary to achieve renoprotection.16 Patients also received aprepitant (125 mg), granisetron (1 mg), and dexamethasone (10 mg) on the day of cisplatin infusion and continued to receive aprepitant (80 mg a day) on days 2 and 3 after infusion and dexamethasone (6 mg/day) on days 2–4. Some patients also received proton pump inhibitors, and most received pain-killing medication (acetaminophen and morphine). These agents were found not to interfere with the function of OCT2 in preclinical studies at clinically achievable concentrations (see Supplementary Figure S3 online).19 No other anticancer chemotherapy or use of biological response modifiers, hormone therapy, or immunotherapy was permitted during the study period.

**Clinical pharmacokinetic studies.** The pharmacokinetics of unbound total cisplatin was assessed from the first day of hospitalization to the following day in each treatment cycle. Blood samples were collected from the peripheral vein immediately before cisplatin administration and then at 1, 2, 3, 3.5, 4, 5, 6, and 24 h following the start of the infusion. Samples were processed to obtain plasma, which was stored frozen until analysis. Cisplatin concentrations were measured by a validated atomic absorption method, as previously described.14 The same samples were also used for the determination of cimetidine concentrations using liquid chromatography–tandem mass spectrometry. In brief, quantitation of cimetidine was carried out with a Waters ACQUITY separation system (Milford, MA) and TQD triple-quadrupole system (Beverly, MA). Separation was achieved on a Waters ACQUITY BEHC18 column (1.7 µm, 100 x x 2.1 mm) using a column heater operating at 40 °C with a Waters ACQUITY in-line filter. The autosampler temperature was maintained at 15 ± 5 °C, and the gradient mobile phase was composed of acetonitrile and 10 mM mmol/l ammonium bicarbonate in water. The flow rate was 0.6 ml/min, and the separation was completed within 3.5 min. The instrument was equipped with an electrospray interface and was controlled using Masslynx 4.1 software (Micromass, Manchester, UK). The analysis was performed in multiple reaction monitoring mode: m/z 253.08 > 158.95 for cimetidine, and cimetidine_d3 (m/z 256.02 > 162.02) was used as the internal standard. The tandem mass spectrometry conditions were as follows: capillary voltage: 0.7 kV, source temperature: 150 °C, desolvation temperature: 450 °C, cone gas flow: 10 l/h, and desolvation gas flow: 900 l/h. Calibration curves of cimetidine were created by plotting the peak area ratios of analyte to the internal standard against the analyte concentrations in the spiked plasma. The within- and between-run precisions for cimetidine were always less than 5.0%, and the mean measured concentrations (accuracy) were always within ±5.3% of the nominal value. All pharmacokinetic parameters were calculated using WinNonlin 6.2 (Pharsight, St Louis, MO).

**Statistical considerations.** All data are presented as mean values with standard error. Group differences as a function of cell type or treatment arm were evaluated using a t-test. Two-tailed P values less than 0.05 were considered statistically significant. All statistical calculations were performed using NCSS version 2004 (Number Cruncher Statistical System, Kaysville, UT).

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at http://www.nature.com/cpt

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**AUTHOR CONTRIBUTIONS**
J.A.S. and A.S. wrote the manuscript; J.A.S., L.L., R.H.M., and A.S. designed the research; J.A.S., L.v.D., S.H., L.v.G., P.d.B., L.L., A.A.G., and R.H.M. performed the research; P.d.B. and L.L. contributed new reagents/analytical tools; and J.A.S., L.L., R.H.M., and A.S. analyzed the data.

**CONFLICT OF INTEREST**
The authors declared no conflict of interests. None of the funding bodies had a role in the study design, data interpretation, or preparation of the manuscript. Associate Editor Alex Sparreboom was not involved in the review or decision process for this article.

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**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**
✓ Inhibitors of OCT2 can ameliorate cisplatin-induced nephrotoxicity.

**WHAT QUESTION DID THIS STUDY ADDRESS?**
✓ Whether renoprotective strategies involving the OCT2 inhibitor cimetidine influence the antitumor properties and systemic disposition of cisplatin.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**
✓ Use of cimetidine does not influence the pharmacokinetics or antitumor effects of cisplatin, irrespective of tumoral OCT2 expression.

**HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS**
✓ This study provides support to future exploration of treatment strategies aimed at ameliorating cisplatin-induced nephrotoxicity using OCT2 inhibitors.
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