Evaluation of Ipatasertib Interactions with Itraconazole and Coproporphyrin I and III in a Single Drug Interaction Study in Healthy Subjects

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ABSTRACT

Ipatasertib is a pan-AKT inhibitor in development for the treatment of cancer. Ipatasertib was metabolized by CYP3A4 to its major metabolite, M1 (G-037720), and was a P-gp substrate and OATP1B1/1B3 inhibitor in vitro. A phase I drug-drug interaction (DDI) study (n = 15) was conducted in healthy subjects to evaluate the effect of itraconazole (200-mg solution QD, 4 days), a strong CYP3A4 and P-gp inhibitor, on pharmacokinetics of ipatasertib (100-mg single dose). Itraconazole increased the Cmax and AUCl0–∞ of ipatasertib by 2.3- and 5.5-fold, respectively, increased the half-life by 53%, and delayed the tmax by 1 hour. The Cmax and AUCl0–72h of its metabolite M1 (G-037720) reduced by 91% and 68%, respectively. This study confirmed that CYP3A4 plays a major role in ipatasertib clearance. Furthermore, the interaction of ipatasertib with coproporphyrin (CP) I and CPII, the two endogenous substrates of OATP1B1/1B3, was evaluated in this study. CPI and CPII plasma levels were unchanged in the presence of ipatasertib, both at exposures of 100 mg and at higher exposures in combination with itraconazole. This indicated no in vivo inhibition of OATP1B1/1B3 by ipatasertib. Additionally, it was shown that CPI and CPII were not P-gp substrates in vitro, and ipatasertib had no effect on CPI and CPII concentrations in vivo. The latter is an important finding because it will simplify interpretation of future DDI studies using CPI/CPII as OATP1B1/1B3 biomarkers.

SIGNIFICANCE STATEMENT

This drug-drug interaction study in healthy volunteers demonstrated that CYP3A4 plays a major role in ipatasertib clearance, and that ipatasertib is not an organic anion transporting polypeptide 1B1/1B3 inhibitor. Furthermore, it was demonstrated that itraconazole, an inhibitor of CYP3A4 and several transporters, did not affect CPI/CPII levels in vivo. This increases the understanding and application of these endogenous substrates as well as itraconazole in complex drug interaction studies.

Introduction

Ipatasertib (GDC-0068) is a novel, potent, highly selective small-molecule inhibitor of the three isoforms of serine/threonine kinase Akt (Akt1, Akt2, and Akt3) (Blake et al., 2012; Lin et al., 2013; Yan et al., 2013) or protein kinase B. It is being developed to be used in combination with chemotherapy, hormonal agents, or targeted agents for the treatment of breast and prostate cancers (Kim et al., 2017; de Bono et al., 2019).

In vitro assessments of drug metabolizing enzyme- and transporter-mediated pharmacokinetic drug-drug interactions (DDI) were conducted with ipatasertib, as patients with breast and prostate cancers may often require concomitant medications. Here, we present both the in vitro metabolism and transport studies as well as the clinical DDI study conducted to assess the clinical impact. In vitro, ipatasertib is primarily metabolized by cytochrome P450 (P450) 3A4. An N-dealkylated metabolite, M1 (G-037720), which circulates at concentrations of approximately 40% of the parent drug at steady state, is formed via CYP3A4 (Supplemental Fig. 1). Ipatasertib is also a substrate of the efflux transporter P-glycoprotein.
I T Z o n C P I a n d C P I I I exposure was investigated in vivo. CPI and CPIII disposition. Additionally, the in vivo effect of performed with CPI and CPIII to rule out any role of P-gp in inhibitor, an in vitro P-gp vesicular transport experiment was performed at three concentrations of ipatasertib (0.2, 2, and 60 μM), representing a range bracketing expected maximal plasma concentration of ~1.56 μM. The highest concentration represented the maximum tolerated concentration in the assay. Briefly, assay buffer containing ipatasertib in the presence and absence of PSC833 (P-gp inhibitor) (Solvay Biotechnology, Szeged, Hungary) or Ko134 (BCRP inhibitor) was added to the appropriate apical or basolateral chamber and incubated at 37°C. Digoxin or prazosin (Sigma-Aldrich, St Louis, MO) efflux ratio was determined as a positive control for MDR1 or BCRP function, with and without PSC833. Ipatasertib or control samples were taken from the receiver chambers at 0 and 120 minutes to determine the amount that permeated through the monolayer. Ipatasertib samples were analyzed by LC-MS/MS, whereas [3H]digoxin and [3H]prazosin (Perkin Elmer, Waltham, MA) were analyzed by liquid scintillation counting.

In Vitro Inhibitory Assay of Ipatasertib on Uptake Activity of OATP1B1/3

The effect of ipatasertib on OATP1B1 and OATP1B3-mediated transport was determined using intracellular accumulation of the probe substrates [3H]estradiol-17β-glucuronide (E217βG) and [3H]leucocysteokinine-4 (CKK-4), respectively, in HEK293K cells overexpressing either OATP1B1 or OATP1B3 in the presence of ipatasertib as previously described (Sane et al., 2020).

Cell Culture. HEK293 cells were transfected with vectors containing cDNA for OATP1B1, OATP1B3, or vectors only by Solvo Biotechnology, Inc (Szeged, Hungary). HEK293 cells overexpressing OATP1B1, OATP1B3, and empty vector were cultured in Dulbecco’s modified Eagle’s medium supplemented with fetal bovine serum (8.9%), antibiotic/antimycotic (0.89%), and l-glutamate (1.79 mM) in a humidified culture chamber (37 ± 1°C, 95 ± 5% relative humidity, and 5 ± 1% CO2). The medium was replaced every 2 to 3 days, and the cells were passaged when they became confluent.

OATP Inhibition Assay. Ipatasertib, E17βG (Sigma-Aldrich, St Louis, MO), and CCK-8 (Sigma-Aldrich, St Louis, MO) were prepared in DMSO spiked into incubation medium at 0.1% DMSO. The cells were plated onto standard 96-well tissue culture plates in cell culture medium 1 to 3 days prior to the experiment. OATP1B1 and OATP1B3-expressing and control cells were incubated with butyric acid for 24 hours prior to the experiment to inhibit suppression of the transport. Incubations of HEK293 cells were carried out in incubation medium Henseleit-Krebs (HK) buffer. Prior to the experiment, the cells were rinsed once with 1 mL of incubation medium. Cells were then preincubated with 0.3 μM of incubation medium containing ipatasertib or solvent control. After 15 minutes, the medium was replaced with incubation medium containing 50 nM of E17βG (OATP1B1) or CCK-8 (OATP1B3) and ipatasertib or solvent control. After 3

Materials and Methods

In Vitro CYP3A4 and P-gp Substrate Assay for Ipatasertib

CYP3A4 Substrate Assay. To identify the P450 isoforms responsible for the metabolism of ipatasertib, pooled human liver microsomes (Corning, Tewksbury, MA) were incubated with ipatasertib in the presence or absence of chemical inhibitors of specific P450 isoforms. Selective P450 inhibitors (furafylline for CYP1A2, tranylcypromine for CYP26A1, ticlopidine for CYP2B6, quercetin for CYP2C8, sulfaphenazole for CYP2C9, ticlopidine for CYP2C19, quinidine for CYP2D6, ketoconazole for CYP3A4/5) or P450 inactivators (troleandomycin and 1-aminobenzotriazole) were preincubated with human liver microsomes and NADPH for 5 or 15 minutes at 37°C with NADPH (1 mM). Reactions were initiated with the addition of ipatasertib. Samples were collected from each HLM reaction at the 0- and 60-minute timepoints, centrifuged, and the supernatant was analyzed for ipatasertib and M1 (G-037720) by liquid chromatography–mass spectrometry (LC-MS/MS).

Recombinant Cytochrome P450 P450. Various rCYP isoforms (rCYP1A2, rCYP2A6, rCYP2B6, rCYP2C5, rCYP2C9, rCYP2C19, rCYP2D6, rCYP2E1, rCYP3A4, and rCYP3A5) were used as a qualitative assessment for the formation of the metabolites. rCYP (40 pmol/mL) and NADPH were preincubated for 5 minutes at 37°C. The reaction was initiated with the addition of ipatasertib (1 μM) and quenched after 60 minutes. Samples were centrifuged, and the supernatant was analyzed by LC-MS/MS. Formation of M1 (G-037720) was monitored after incubations of ipatasertib with rCYP isoforms.

P-gp and BCRP Substrate Assay. To assess whether ipatasertib was a substrate of P-gp and BCRP, Madin-Darby canine kidney (MDCK) II transfected with human MDR1 gene (MDCKII-MDR1) or human BCRP gene (MDCKII-BCRP) were used. Monolayer assay was performed at three concentrations of ipatasertib (0.2, 2, and 60 μM, representing a range bracketing expected maximal plasma concentration of ~1.56 μM). The highest concentration represented the maximum tolerated concentration in the assay. Briefly, assay buffer containing ipatasertib in the presence and absence of PSC833 (P-gp inhibitor) (Solvay Biotechnology, Szeged, Hungary) or Ko134 (BCRP inhibitor) was added to the appropriate apical or basolateral chamber and incubated at 37°C. Digoxin or prazosin (Sigma-Aldrich, St Louis, MO) efflux ratio was determined as a positive control for MDR1 or BCRP function, with and without PSC833. Ipatasertib or control samples were taken from the receiver chambers at 0 and 120 minutes to determine the amount that permeated through the monolayer. Ipatasertib samples were analyzed by LC-MS/MS, whereas [3H]digoxin and [3H]prazosin (Perkin Elmer, Waltham, MA) were analyzed by liquid scintillation counting.

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Ipatasertib Interactions with Itraconazole and CPI and CPIII

(CPI and CPIII) P-gp Vesicular Transport Experiment

CPI and CPIII were tested at 2.5 and 10 nM using the Solvo PRE-DIZEV MDR1 Vesicular Kit in accordance with the protocol with minor modifications (Solvo Biotechnology, Szeged, Hungary). Briefly, CPI, CPIII, and N-methyl-quinidine (NMQ) (positive control, Herediti-Szabo et al., 2013) were added to the plate containing vesicles and allowed to equilibrate for 15 minutes at 37 °C. The reaction was initiated by adding the ATP and AMP solutions. The plate was gently mixed on a plate shaker and placed in a 37 °C incubator. At 5, 10, and 20 minutes, 200 μL of ice-cold wash buffer was added to vesicle samples to quench the reaction. All solution was transferred to a 96-well plate, diluted with scintillation fluid, and analyzed on a MicroBeta2 liquid scintillation counter (Perkin Elmer, Waltham, MA). The amount of protein in each incubation was determined by bicinchoninic acid analysis.

Clinical DDI Study

A phase 1, single-center, open-label, two-period, fixed-sequence, DDI study in healthy subjects was conducted to evaluate the effect of ITZ on the pharmacokinetics (PK) of ipatasertib and its primary metabolite M1 (G-037720). The protocol, informed consent forms, and other study related evaluations were performed at screening, at specific times during the study, and at follow-up or study completion and/or at early termination.

Fig. 1. Schematic of study design.
chromatography-tandem mass spectrometry with multiple reaction monitoring and electrospray ionization in the positive mode by Covance Laboratories, Inc (Madison WI), (Liu et al., 2018). The LC-MS/MS analysis was carried out with an ACE C18 Column (3 μm, 50 × 3 mm) on a Shimadzu High Performance Liquid Chromatography system coupled with a Sciex API 5000 mass spectrometer. Chromatographic separation was performed under gradient conditions with a mobile phase composed of 10 mM ammonium formate (mobile phase A) and methanol (mobile phase B). Ipatasertib, M1 (G-037720), ITZ, 1-hydroxyitraconazole, and CPI/CPIII were monitored by m/z 460.2 → 389.2; 416.2→217.2; 712.4→392.3; 721.4→208.3; and 655.3→596.3, respectively. Assay employed deuterated internal standards for ipatasertib, M1, ITZ, and 1-hydroxy itraconazole or stable labeled internal standards for coproporphyrin. The LLOQ for ipatasertib and M1 was 0.463 ng/mL, LLOQ for ITZ and 1-hydroxy itraconazole was 5 ng/mL, LLOQ for CPI was 50 ng/mL, and LLOQ for CPIII was 20 ng/mL.

The following PK parameters were derived from plasma concentrations of ipatasertib and M1 (G-037720) using the noncompartmental methods: Cmax, tmax, AUC0-t, AUC0-∞, t1/2, CL/F (ipatasertib only), Vz/F (ipatasertib only), metabolite ratio based on metabolite and parent Cmax and AUC. For CPI and CPIII analysis, Cmax, tmax, and AUC0-t was determined. PK calculations were performed using commercial software Phoenix WinNonlin (Certara Inc., version 6.4 or higher).

For the primary analysis of DDI with ITZ, the effect of ITZ on ipatasertib and M1 (G-037720) PK was assessed. Natural log-transformed Cmax, AUC0-t, and AUC0-∞ values for ipatasertib were compared between period 1 (reference; ipatasertib alone) and period 2 (test; ipatasertib and M1 (G-037720)). For CPI and CPIII analysis, Cmax, tmax, and AUC0-t was determined. PK calculations were performed using commercial software Phoenix WinNonlin (Certara Inc., version 6.4 or higher).

In Vitro Metabolism of Ipatasertib to M1 (G-037720)

HLM in the presence of selective chemical inhibitors and human rCYP isoforms were used to identify the major human P450 isoforms responsible for the metabolism of ipatasertib and formation of M1 (G-037720). Metabolism of ipatasertib in HLM was inhibited by CYP3A selective inhibitor, ketoconazole, as well as troloxandmycin by >94%, but not other CYP450 inhibitors. These selective CYP3A inhibitors inhibited the formation of M1 (G-037720), suggesting CYP3A was the major contributor to the metabolism of ipatasertib. Incubation of ipatasertib with several recombinant P450 isoforms (rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, rCYP3A4, rCYP3A5) confirmed that CYP3A4 was the primary P450 responsible for the formation of M1 (G-037720). M1 itself had very low turnover in vitro.

Reaction phenotyping results are reported in Supplemental Table 1 in the supplemental file.

In Vitro BCRP Substrate Assay

Ipatasertib incubated at 0.2, 2, and 60 μM in MDCKII-BCRP monolayers resulted in efflux ratios of 1.41, 6.14, and 12.64, respectively, which reduced to 1.20, 1.78, and 1.73 in the presence of the P-gp inhibitor, PSC833. These results suggested that ipatasertib was a substrate of P-gp. Results are shown in Supplemental Fig. 2 in the supplemental file.
was administered with ITZ, with Cmax and AUC both showing marked decrease in presence of ITZ (Fig. 4; Table 3). Arithmetic mean $t_{1/2}$ of M1 (G-037720) were 36.0 hours and 47.6 hours when ipatasertib was coadministered with ITZ versus ipatasertib alone, respectively. Statistical analysis to evaluate the effect of ITZ on the PK of M1 (G-037720) yielded geometric least-square (LS) means for AUC0-72 and Cmax of approximately 68% and 91%, respectively (Table 2). Coadministration of ipatasertib with ITZ decreased the geometric mean metabolic ratios (MR$_{Cmax}$, MR$_{AUC0-\infty}$, and MR$_{AUC0-72}$) by approximately 25-, 12-, and 15-fold, respectively (Table 3).

After single-dose oral administration of ipatasertib 100 mg alone, M1 (G-037720) detectable plasma concentrations appeared within 30 minutes for most subjects (Fig. 4; Table 3). Whereas, after oral dosing of ipatasertib 100 mg coadministered with ITZ 200 mg, first detectable concentrations of M1 (G-037720) were not observed for most subjects until approximately 2 hours postdose. Median $t_{max}$ appeared delayed by about 6 hours when ipatasertib was coadministered with ITZ compared with ipatasertib alone (Table 3).

**Pharmacokinetic Results for Itraconazole and Metabolite 1-Hydroxyitraconazole.** Plasma concentrations of ITZ and its primary metabolite (1-hydroxyitraconazole) were measured predose and postdose on day 18 (4th consecutive day of ITZ dosing) over a 24-hour period. After administration of ITZ, geometric mean (geometric CV%) Cmax and median (min, max) $t_{max}$ for ITZ were 1640 ng/mL (17.5%) and 3.00 (2.00, 4.03) hours, respectively. In general, geometric mean ITZ trough (predose) concentrations on days 18 and 19 were similar (313 and 383 ng/mL, respectively). For 1-hydroxyitraconazole, geometric mean (geometric CV%) Cmax and median (min, max) $t_{max}$ were 1280 ng/mL (15.6%) and 4.00 (3.00, 5.00) hours, respectively.

**CPI and CPIII Plasma Levels.** Ipatasertib, at the exposures achieved in this study with or without ITZ, did not cause any changes in CPI and CPIII plasma levels (Fig. 5; Tables 4 and 5). ITZ administration also did not alter the CPI and CPIII plasma levels (Fig. 5; Table 4).

**Summary of Adverse Events.** Overall, both ipatasertib and ITZ were well tolerated when given alone or in combination to the healthy male and female subjects in this study. All
Itraconazole also appeared to impact the \( t_{\text{max}} \) of ipatasertib. The first detectable concentration and median \( t_{\text{max}} \) of ipatasertib was slightly delayed in the presence of itraconazole. This observation did not appear to be an artifact of sampling times or an anticipated effect of itraconazole-mediated CYP3A and P-gp inhibition. Given that the range of \( t_{\text{max}} \) overlapped between the two treatment groups, it is likely that this observation was attributed to the high variability of ipatasertib PK.

Also, for M1 (G-037720), the \( t_{\text{1/2}} \) values should be interpreted with caution, as there were a limited number of quantifiable concentrations available to include in the estimation of the terminal rate constant. The apparent change in V/F in presence of itraconazole is likely due to reduced first pass effect, and therefore, a change in oral bioavailability (F).

Given the magnitude of the observed DDI, the coadministration of other strong CYP3A4 inhibitors such as posaconazole, clarithromycin, and grapefruit juice are being avoided in clinical studies with ipatasertib or used with a reduced dose of ipatasertib ([https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers](https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers)). In this study, a 100-mg single dose of ipatasertib was administered, which is lower than the intended therapeutic dose of 400 mg. At 400 mg, the magnitude of DDI is expected to be somewhat lower than that observed at 100 mg, as the competition for CYP3A4 is typically concentration-dependent. Physiologically based PK modeling is ongoing to predict the DDI at 200- and 400-mg dose of ipatasertib to provide guidance for use with weak and moderate inhibitors of CYP3A4.

The PK of itraconazole and its primary metabolite, 1-hydroxyitraconazole, were also assessed in this study to ensure sufficient inhibitor concentrations were achieved. The \( C_{\text{max}} \) and \( t_{\text{max}} \) of itraconazole were comparable to that previously observed after administration of 200 mg itraconazole oral solution under fasted conditions for 15 days at 1963 ng/mL and 2.5 hours, respectively (Sporanox oral solution United States Prescribing Information). The \( C_{\text{max}} \) of 1-hydroxyitraconazole observed on day 18 in this study was slightly lower than that previously reported; however, the geometric mean concentrations of itraconazole and 1-hydroxyitraconazole were well above the reported IC\(_{50}\) values [approximately 29 nM (20.5 ng/mL) and 37 nM (26.7 ng/mL), respectively], indicating they provided sufficient inhibition of CYP3A4 in this study (Isoherranen et al., 2004).

Initial single or multiple ascending dose studies may often be desired to evaluate the effect of a drug on endogenous biomarkers. However, ipatasertib is in late stages of clinical development and phase 3 trials, with its commercial approval currently pending. Additional clinical studies with ipatasertib or used with a reduced dose of ipatasertib (i.e., 100-mg single dose of ipatasertib) are ongoing to provide guidance for use with strong CYP3A4 inhibitors such as posaconazole, clarithromycin, and grapefruit juice in clinical studies with ipatasertib or used with a reduced dose of ipatasertib ([https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers](https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers)). In this study, a 100-mg single dose of ipatasertib was administered, which is lower than the intended therapeutic dose of 400 mg. At 400 mg, the magnitude of DDI is expected to be somewhat lower than that observed at 100 mg, as the competition for CYP3A4 is typically concentration-dependent. Physiologically based PK modeling is ongoing to predict the DDI at 200- and 400-mg dose of ipatasertib to provide guidance for use with weak and moderate inhibitors of CYP3A4.

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Initial single or multiple ascending dose studies may often be desired to evaluate the effect of a drug on endogenous biomarkers. However, ipatasertib is in late stages of clinical development and phase 3 trials, with its commercial approval currently pending. Additional clinical studies with ipatasertib or used with a reduced dose of ipatasertib (i.e., 100-mg single dose of ipatasertib) are ongoing to provide guidance for use with strong CYP3A4 inhibitors such as posaconazole, clarithromycin, and grapefruit juice in clinical studies with ipatasertib or used with a reduced dose of ipatasertib ([https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers](https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers)). In this study, a 100-mg single dose of ipatasertib was administered, which is lower than the intended therapeutic dose of 400 mg. At 400 mg, the magnitude of DDI is expected to be somewhat lower than that observed at 100 mg, as the competition for CYP3A4 is typically concentration-dependent. Physiologically based PK modeling is ongoing to predict the DDI at 200- and 400-mg dose of ipatasertib to provide guidance for use with weak and moderate inhibitors of CYP3A4.
development with several patient studies ongoing in combination with other agents (Kim et al., 2017; de Bono et al., 2019). This study in healthy subjects was considered suitable to evaluate the in vivo effect of ipatasertib on OATP1B1/1B3, since ipatasertib was given as a single agent. In typical oncology clinical studies, patients are taking other comedications that can confound DDI study results. Therefore, it was important to have a study population with minimal confounder effects, as there was expected to be minimal or no effect of ipatasertib inhibition on OATP1B1/1B3 in vivo. Any confounding effects due to comedications could have made the results inconclusive. Based on in vitro predictions using the methods described in the FDA DDI guidance, at the clinically intended dose of 400 mg, and the observed IC50 values, the predicted AUC ratios (AUC in presence of inhibitor/AUC in absence of inhibitor), using the estimated maximum unbound liver inlet concentration (I_inlet, unbound) of ipatasertib and default values for fraction absorbed (Fa), intestinal availability (Fg) and absorption rate constant (Ka) (as worst case scenario), were calculated to be 1.25 and 1.19 for OATP1B1 and OATP1B3 inhibition, respectively. These are slightly higher than the FDA cutoff of 1.1 (FDA DDI guidance). However, when using a realistic Ka value estimated from population PK analysis of ipatasertib (data on file) to calculate the maximum unbound liver inlet concentration, these ratios were 1.08 and 1.06, respectively, for OATP1B1 and OATP1B3, less than the FDA cutoff of 1.1. Therefore, this CYP3A4 DDI study was leveraged to measure the changes in CPI and CPIII levels before and after administration of ipatasertib. As expected, after 100 mg ipatasertib administration, no change was observed in CPI and CPIII exposures, which were surrogates for OATP1B1/1B3 activity.

In this present clinical DDI study, subjects received 100 mg ipatasertib, which was lower than intended clinical dose of 400 mg (Kim et al., 2017; de Bono et al., 2019). This dose was selected for the study to minimize unnecessary drug exposure in healthy subjects, especially in anticipation of a DDI. Consequently, the exposure of ipatasertib in presence of itraconazole was comparable to the exposure observed after administration of 400 mg ipatasertib in patients. Therefore, CPI and CPIII were also evaluated in presence of both ipatasertib and itraconazole to assess the impact of ipatasertib exposures expected at intended therapeutic dose. There was no change in CPI and CPIII plasma levels even at higher ipatasertib exposures, indicating ipatasertib did not inhibit OATP1B1/1B3 in vivo at the clinically relevant exposures. After administration of ipatasertib alone, itraconazole alone, or coadministration of ipatasertib with itraconazole, CPI and CPIII were generally similar to the respective values at baseline (maximum decreases of ≤11% from baseline). Given that itraconazole also did not impact CPI and CPIII exposure, the conclusion that ipatasertib did not impact CPI and CPIII levels when coadministered with itraconazole was not confounded. Overall, OATP1B1/1B3 substrates can be coadministered with ipatasertib without a risk of DDI even at clinically relevant concentrations of ipatasertib.

To facilitate interpretation of CPI and CPIII data, an uptake experiment was prospectively conducted which demonstrated that neither CPI nor CPIII were substrates of P-gp. Itraconazole, a P-gp inhibitor, did not alter CPI and CPIII levels in our study, and this is consistent with an observed lack of P-gp involvement in CPI and CPIII transport (Fig. 3). This

### Table 3
Summary of PK parameters of M1 (G-037720) after single oral dose of ipatasertib 100 mg alone or coadministered with itraconazole 200 mg

<table>
<thead>
<tr>
<th>M1 (G-037720) PK Parameters</th>
<th>Ipatasertib (100 mg)</th>
<th>Ipatasertib (100 mg) + Itraconazole (200 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 15</td>
</tr>
<tr>
<td>AUC0-72 (ng*h/mL)</td>
<td>233 (35.7)*</td>
<td>110 (57.7)*</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>179 (34.7)</td>
<td>58.0 (38.5)*</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>2.03 (1.02, 3.15)</td>
<td>8.00 (2.00, 12.00)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>47.6 (14.0)</td>
<td>36.1 (12.7)*</td>
</tr>
<tr>
<td>MR_{Cmax}</td>
<td>0.452 (50.2)</td>
<td>0.0178 (41.0)</td>
</tr>
<tr>
<td>MR_{AUC}</td>
<td>0.787 (19.8)</td>
<td>0.0646 (33.9)*</td>
</tr>
<tr>
<td>MR_{AUC0-72}</td>
<td>0.728 (20.1)</td>
<td>0.0484 (23.7)*</td>
</tr>
</tbody>
</table>

* N=14.
+ N=4.

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Fig. 5. Arithmetic mean (+S.D.) concentration-time profile of CPI (A) and CPIII (B) at baseline and after oral administration of ipatasertib 100 mg alone, itraconazole 200 mg alone, or coadministration of ipatasertib and itraconazole in healthy subjects (N = 15).
observation was in line with previously reported in vitro data; CPI and CPIII have been reported as being substrates of OATP1B1/3 and the multidrug resistance-associated protein (MRP) 2 and MRP3. CPI and CPIII were not substrates of other uptake or efflux transporters including BCRP, the bile salt export pump, P-gp, the multidrug and toxin extrusion proteins 1/2, MRP4, the oAT1/3, the organic cation transporter 1/2, and the sodium taurocholate cotransporting polypeptide (Kunze et al., 2018).

The observation that CPI and CPIII levels were not altered in a clinical setting by the presence of itraconazole, which is an inhibitor of CYP3A4, P-gp, and BCRP, adds to the scientific knowledge in the field of transporter-mediated DDI. Itraconazole has been shown to increase the concentration of OATP1B1/3B3 substrates such as rosuvastatin and pravastatin in previous studies (Vaidyanathan et al., 2016). However, the lack of effect on CPI and CPIII in our study suggests inhibition of other transporters such as P-gp and/or BCRP by itraconazole and/or 1-hydroxylitraconazole may be playing a role in the DDI studies with statins. If a new molecule is an inhibitor of CYP3A4 and multiple transporters such as P-gp, BCRP, and OATP1B1/3B3, CPI and CPIII offer an appropriate option to evaluate an exclusive effect on OATP1B1/3B3 substrates such as atorvastatin, rosuvastatin, or simvastatin, which are substrates of other enzymes or transporters. This study demonstrates, in a clinical setting, that CPI and CPIII exposures are not affected by itraconazole, a CYP3A4, P-gp, and BCRP inhibitor. These results make interpretation of itraconazole DDI studies simpler, when the victim molecules are substrates of multiple transporters, such as fexofenadine (Shimizu et al., 2006). This type of study design also offers the opportunity to evaluate CPI and CPIII at a higher concentration of the inhibitor drug, which may be needed for studying complex DDI or DDI in special populations, scenarios in which higher drug exposures may occur. Itraconazole can boost perpetrator concentrations which may aid CPI and CPIII evaluation at a higher inhibitor concentration without any interference.

Overall, using this approach of evaluating two DDIs in one study has provided evidence that ipatasertib is a substrate of CYP3A4 in vivo and therefore a victim of DDI with strong CYP3A4 inhibitors, and that ipatasertib does not inhibit OATP1B1/3B3 in vivo. As polypharmacy is not uncommon in patients with cancer, this study has provided critical information to ensure safe use of ipatasertib in patients and provides DDI risk mitigation strategies for ongoing clinical studies and future clinical use of ipatasertib. The study also offers new insights by demonstrating lack of CPI/CPIII interaction with itraconazole.

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Authorship Contributions
Participated in research design: Sane, Liederer, Malhi, Musib.
Conducted experiments: Sane, Cho, Hanover, Plise, Wong.
Contributed new reagents or analytic tools: Sane, Plise.
Performed data analysis: Malhi, Liederer, Wong.
Wrote or contributed to the writing of the manuscript: Sane, Cheung, Liederer, Malhi, Plise.

References

Itraconazole

TABLE 5
Statistical analysis of the effect of ipatasertib on PK parameters of Coproporphyrins I and III

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parameter</th>
<th>GMR (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coproporphyrin I</td>
<td>AUC₀₋₂₄ (pg/h/mL)</td>
<td>97.4 (94.0, 100.8)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (pg/mL)</td>
<td>96.7 (88.4, 105.9)</td>
</tr>
<tr>
<td>Coproporphyrin III</td>
<td>AUC₀₋₂₄ (pg/h/mL)</td>
<td>95.9 (89.6, 102.8)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (pg/mL)</td>
<td>95.8 (86.4, 106.2)</td>
</tr>
</tbody>
</table>


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