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ARTICLE

Evaluation of Drug–Drug Interactions of Rucaparib and CYP1A2, CYP2C9, CYP2C19, CYP3A, and P-gp Substrates in Patients With an Advanced Solid Tumor

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This phase I study (CO-338-044; NCT02740712), conducted in patients with advanced solid tumors, evaluated the effect of the poly(ADP-ribose) polymerase (PARP) inhibitor rucaparib on the pharmacokinetics (PK) of caffeine 200 mg, warfarin 10 mg, omeprazole 40 mg, and midazolam 2 mg (cytochrome P450 (CYP) 1A2, CYP2C9, CYP2C19, and CYP3A substrates; dosed as a cocktail) and digoxin 0.25 mg (P-glycoprotein (P-gp) substrate; dosed separately) without rucaparib and following oral rucaparib 600 mg b.i.d. Geometric mean (GM) ratios (90% confidence interval (CI)) of area under the concentration-time curve (AUC) from time zero to last quantifiable measurement with and without rucaparib were: caffeine, 2.26 (1.93–2.65); S-warfarin, 1.49 (1.40–1.58); omeprazole, 1.55 (1.32–1.83); midazolam, 1.39 (1.14–1.68); and digoxin, 1.20 (1.12–1.29). There was limited effect on peak concentration of the substrates (GM ratios, 0.99–1.13). At steady state, rucaparib 600 mg b.i.d. moderately inhibited CYP1A2, weakly inhibited CYP2C9, CYP2C19, and CYP3A, and marginally increased digoxin exposure.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Prior studies have described the single-dose and steady-state PK profiles of oral rucaparib, a PARP inhibitor; however, no studies have characterized DDIs for rucaparib in a clinical setting.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ What, if any, effect does oral rucaparib 600 mg b.i.d. have on the CYP enzymes CYP1A2, CYP2C9, CYP2C19, and CYP3A, and the P-gp transporter?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ At steady state, rucaparib 600 mg b.i.d. showed moderate inhibition of CYP1A2 and weak inhibition of CYP2C9, CYP2C19, and CYP3A as determined by fold increase in AUC and/or peak plasma concentration of probe drugs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These findings will provide clinicians with the ability to better manage administration of concomitant medications for patients who are receiving oral rucaparib 600 mg b.i.d.

Rucaparib (Clovis Oncology, Boulder, CO) is an oral poly(ADP-ribose) polymerase (PARP) inhibitor approved by the US Food and Drug Administration (FDA) as monotherapy for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy and for treatment of adult patients with deleterious *BRCA1* or *BRCA2* mutation (germline and/or somatic)-associated epithelial ovarian, fallopian tube, or primary peritoneal cancer who have been treated with two or more chemotherapies.[1–3]

The drug–drug interaction (DDI) potential of rucaparib as a cytochrome P450 (CYP) perpetrator was assessed *in vitro* (Clovis Oncology, data on file). In human liver microsomal studies, rucaparib inhibited CYP1A2 and CYP2C19, with inhibition constant (*K*i) values of 9.3 and 17.1 μM, respectively. Rucaparib showed mixed inhibition of CYP2C9 (competitive *K*i = 67 μM; uncompetitive *K*i = 31.5 μM), as well as reversible CYP3A inhibition (half maximal inhibitory concentration = 17.2 μM). In a CYP2D6 inhibition assay conducted in human liver microsomes, rucaparib showed no inhibition of CYP2D6 up to 25 μM. Additionally, rucaparib

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showed concentration-dependent CYP1A2 induction and CYP3A4 downregulation in human hepatocytes. Given the mean steady-state peak plasma concentration (C_{max}) of 6 μM following rucaparib 600 mg b.i.d., *in vivo* CYP interaction could not be ruled out. Based on results from an *in vitro* transporter interaction study using digoxin as a probe substrate across the monolayer of MDCKII cells transfected with MDR1, rucaparib is an inhibitor of P-glycoprotein (P-gp), with half maximal inhibitory concentration of 169 μM, suggesting potential P-gp inhibition in the gut.

In part 1 (dose escalation phase) of the phase I/II study CO-338-010 (study 10; NCT01482715), the single-dose and steady-state pharmacokinetic (PK) profiles of rucaparib administered orally q.d. (range = 40–500 mg) or b.i.d. (range = 240–840 mg) were evaluated.[4,5] The PK of rucaparib was linear, with time independence and dose proportionality across all dosages; steady state was achieved following 7 days of dosing.[4,5] Across the dosage levels evaluated, median time to C_{max} (T_{max}) ranged from 1.5–6 hours; the half-life ($t_{1/2}$) was ~17 hours.[4,5] A high-fat meal caused a 20% increase in C_{max} and 38% increase in area under the plasma concentration-time curve (AUC), but the effect was not clinically significant based on collective clinical efficacy and safety data.[2–4,6] Oral rucaparib 600 mg b.i.d. with or without food was selected as the recommended phase II dose.[4,5]

In this study (CO-338-044; NCT02740712), rucaparib was administered to patients with advanced solid tumors to characterize potential DDIs. For evaluation of CYP enzymes, four of the five probe drugs in the validated Cooperstown 5 + 1 cocktail[7] were used as substrates, including caffeine (CYP1A2), S-warfarin (CYP2C9), omeprazole (CYP2C19), and midazolam (CYP3A). Dextromethorphan, a CYP2D6 probe substrate used in the Cooperstown 5 + 1 cocktail, was not included in this study based on results of *in vitro* studies. To test potential interactions with a P-gp transporter, which is not part of the validated cocktail, digoxin was dosed separately (24 hours after the cocktail). A similar staggered digoxin dosing following administration of the Cooperstown cocktail has been previously reported.[8,9] The primary objective of this study was to determine the effect of rucaparib at the recommended clinical dose (600 mg b.i.d.) on the PK of the selected substrates of CYP1A2, CYP2C9, CYP2C19, and CYP3A (as part of a validated cocktail[7]) and P-gp (dosed separately) after a single oral dose of the substrates.

METHODS

Patients

Patients were eligible to enroll in the study if they were ≥ 18 years old, had a histologically or cytologically confirmed advanced solid tumor with evidence of measurable disease per Response Evaluation Criteria In Solid Tumors version 1.1,[10] and, in the opinion of the investigator, could potentially benefit from treatment with rucaparib. Patients must have had a body mass index 18.0–35.0 kg/m^2, Eastern Cooperative Oncology Group Performance Status of 0 or 1, and adequate organ function. All patients enrolled in this DDI study had adequate renal function (creatinine clearance ≥ 45 mL/min using Cockcroft Gault formula) and hepatic function (total bilirubin ≤ 1.5 × upper limit of normal, serum albumin ≥ 30 g/L, aspartate aminotransferase and alanine aminotransferase ≤ 3 × upper limit of normal). The cutoff values were selected to represent the real patient populations for the approved indications by the FDA and the European Medicines Agency. Patients were excluded if they had received chemotherapy, radiation, immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or experimental drugs within 14 days prior to day 1; had ongoing grade ≥ 1 adverse effects from such treatment per Common Terminology Criteria for Adverse Events (ongoing grade 2 nonhematologic toxicity related to most recent treatment regimen could be permitted with prior advanced approval from the study sponsor); or had prior treatment with any PARP inhibitor unless it was not the most recent treatment, and it had been discontinued > 3 months before the first planned dose of rucaparib. Patients should not be on treatment with any of the study probe drugs or strong/moderate perpetrators (including medicines and herbal products) of the tested CYP enzymes or P-gp. Ingestion of grapefruit, star fruit, Seville oranges, pomegranate, pomelo, or juices from these fruits was not allowed within 7 days prior to day 1 and for the duration of the study. Ingestion of alcohol or any products containing methylxanthine or caffeine (e.g., coffee, black and green tea, cocoa or chocolate, cola, and energetic beverages) was not allowed within 72 hours prior to day 1 and for the duration of the study. Use of nicotine-containing products was not allowed within 3 months prior to screening and for the duration of the study. Lifestyle restrictions included prohibition of strenuous activity, sunbathing, or contact sports from 4 days prior to entry in the study site until the end of the study; use of precautions against photosensitivity when going outside (e.g., applying sunscreen, covering exposed skin, and wearing a hat and sunglasses); and the practice of highly effective methods of contraception (e.g., progesterone-only injectable or implantable contraceptives, intrauterine device or system, bilateral tubal occlusion, vasectomy with documentation of absence of sperm in ejaculate, and/or true, complete abstinence) for patients of childbearing potential and their partners or postmenopausal women with amenorrhea < 1 year (unless postmenopausal status was confirmed by follicle-stimulating hormone test).

All patients provided written informed consent. The study was approved by the institutional review boards of each institution and was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonisation.

Study design

This was a phase I, open-label, sequential, DDI study in patients with advanced solid tumors. Patients were admitted and remained at the study sites from day –1 to day 3 and from day 11 to day 14 for dosing and serial PK blood collection (**Figure 1**). After discharge, the patients returned to the sites for outpatient visits on days 4, 5, 7, and 9. Patients received a single oral dose of a CYP substrate cocktail of caffeine 200 mg, S-warfarin 10 mg, omeprazole 40 mg, and midazolam 2 mg (substrates for CYP1A2, CYP2C9, CYP2C19, and CYP3A, respectively) on days 1 and 12, a single oral dose of digoxin 0.25 mg (P-gp substrate) on days

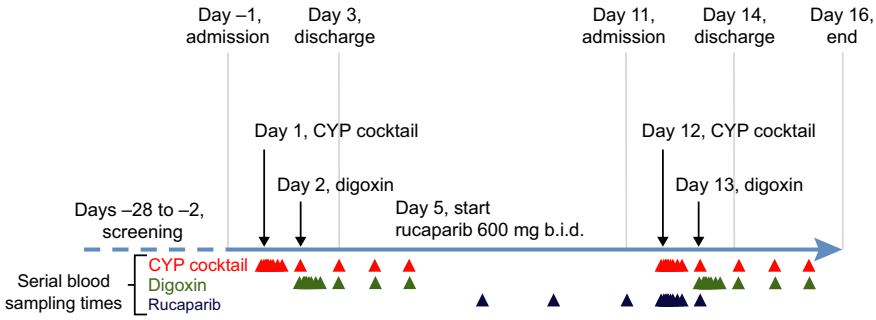

Figure 1 Study schema. CYP, cytochrome P450.

2 and 13, and oral rucaparib 600 mg b.i.d. from day 5 to day 16. Midazolam 2 mg was prepared in a solution of 5% dextrose in water and provided to patients orally. Vitamin K 10 mg (warfarin antagonist) was given on days 1 and 12 for bleeding prophylaxis; additional doses were given on days 2 and 13, if needed. The PK was monitored up to 96 hours for S-warfarin and 72 hours for the other substrates. Serial blood samples were obtained before dosing and at 0.25 (CYP cocktail only), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96 (S-warfarin only) hours after dose of the CYP cocktail or digoxin. For rucaparib PK, serial blood samples were obtained prior to dosing on days 7, 9, 11, and 12, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after dose on day 12. For predose (trough) samples, a 20-minute window before dosing was allowed; for postdose samples, a time window of ± 5 minutes for up to 2 hours, ± 10 minutes for 3–24 hours, and ± 2 hours for 48–96 hours was allowed.

Probe drugs were administered to patients in the morning after an overnight fast of ≥ 8 hours, and fasting continued for ≥ 2 hours after administration. Tablets were taken with ≥ 240 mL water. Water was restricted for 1 hour before and after administration.

Genotyping

Genotypes of CYP2C9 and CYP2C19 were determined using validated methods for all patients by Genelex (Seattle, WA). The genotyping included testing for CYP2C9 alleles *2–6, *8, *11, *13, and *15 and CYP2C19 alleles *2–10, *12, and *17. Absence of a positive test result for CYP2C9 and CYP2C19 for all variants were assigned as CYP2C9*1 and CYP2C19*1. If identified as poor metabolizers of CYP2C9 or CYP2C19, patients were excluded from the PK DDI data analysis for the respective probes.

PK variables

C_{max} and AUC from time zero to the last quantifiable measure ($AUC_{0\text{-}t}$), were the primary PK parameters analyzed for caffeine, S-warfarin, omeprazole, midazolam, and digoxin with and without rucaparib treatment. Other PK parameters analyzed for the probe drugs included AUC from time zero extrapolated to infinity ($AUC_{0\text{-}inf}$), $t_{1/2}$, and T_{max}. $AUC_{0\text{-}inf}$ was reported if the portion extrapolated from time of last quantifiable measurement to infinity did not exceed 20%. The PK parameters analyzed for rucaparib at steady state ($_{ss}$) include trough plasma concentration, $C_{max,ss}$, $T_{max,ss}$, and $AUC_{\tau,ss}$, where τ indicates the dosing interval (12 hours) at steady state. The PK parameters were calculated using noncompartmental analysis from the plasma concentration-time data using WinNonlin version 6.3 (Pharsight, Mountain View, CA).

Plasma sample analysis

Validated liquid chromatography-tandem mass spectrometry methods were used to determine the plasma concentrations of probe drugs (performed by Frontage Laboratories, Exton, PA) and rucaparib (performed by Q Squared Solutions BioSciences (formerly Advion Bioanalytical Laboratories), Ithaca, NY). Details are provided in the **Data S1**.

Safety

Safety and tolerability were assessed through adverse event monitoring, clinical laboratory tests, vital sign measurements, 12-lead electrocardiogram measurements, and physical examination. Adverse events were monitored throughout the study up to 28 days after the last dose of rucaparib and were classified in accordance with the Medical Dictionary for Drug Regulatory Activities classification system version 19.0[11] and graded for severity in accordance with the Common Terminology Criteria for Adverse Events version 4.03.[12]

Statistical analyses

Descriptive statistics for plasma concentrations and PK parameters were provided for each probe drug with and without rucaparib treatment, including number of patients, arithmetic mean, SD, percent coefficient of variation (%CV), SEM, minimum, median, maximum, and geometric mean (GM) with 90% CI. The primary PK parameters (C_{max} and $AUC_{0\text{-}t}$) and $AUC_{0\text{-}inf}$ were natural log-transformed before assessment with a linear mixed effects model. Treatment was used as fixed effect, and subject was used as a random effect. Point estimates for the means and point estimates and corresponding 90% CIs for the differences in means between the two conditions (with or without rucaparib) for each probe drug were obtained from the linear mixed effects model and then exponentiated to obtain GMs, GM ratios (GMRs), and respective 90% CIs on the original scale. The GMRs of AUC and C_{max} for probe drugs were calculated in the presence vs. absence of rucaparib. Individual and summary statistics of concentration-time data were collected for rucaparib. Descriptive statistics for

PK parameters were provided for rucaparib at steady state (day 12), including number of patients, arithmetic mean, SD, %CV, SEM, minimum, median, maximum, and GM (including 90% CI). All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patient demographics and study populations

Of the 17 patients enrolled, 17 (100%) were white and 13 (76.5%) were women. The median age was 55 years (range = 40–67 years), and the mean body mass index was 28.1 kg/m² (SD = 3.07). Patients had the following advanced solid tumor (carcinoma) types: ovarian (*n* = 8; 47.1%), colon (*n* = 4; 23.5%), nonsmall cell lung (*n* = 3; 17.6%), parotid gland (*n* = 1; 5.9%), and renal cell (*n* = 1; 5.9%). All patients had advanced metastatic disease and variable nodal disease (where assessable) at study entry.

All 17 patients were included in the PK and safety populations. One patient (5.9%) withdrew from the study on day 9 due to treatment-emergent adverse events (TEAEs) not considered related to rucaparib; therefore, the DDI analysis population included 16 patients (94.1%).

Genotyping

Based on the CYP2C9 genotyping results, two patients (11.8%) were determined to be poor metabolizers (CYP2C9*2/CYP2C9*3 and CYP2C9*3/CYP2C9*3), and the other patients were extensive metabolizers (CYP2C9*1/CYP2C9*1) or intermediate metabolizers (CYP2C9*1/CYP2C9*2 or CYP2C9*1/CYP2C9*3). The PK parameters were calculated for all patients, but the two CYP2C9 poor metabolizers were excluded from the DDI analysis for S-warfarin. Based on the CYP2C19 genotyping results, eight patients were rapid metabolizers (CYP2C19*1/CYP2C19*17), six patients were extensive metabolizers (CYP2C19*1/CYP2C19*1), and three patients were intermediate metabolizers (CYP2C19*1/CYP2C19*8 or CYP2C19*2/CYP2C19*17). No patients were excluded from the DDI analysis for omeprazole. No genotyping was done for CYP1A2, CYP3A4, or P-gp, and no patient was excluded from the DDI analyses for caffeine, midazolam, or digoxin.

PKs

Patients received oral rucaparib 600 mg b.i.d. from day 5 to day 16. The GM (%CV) $C_{max,ss}$ and $AUC_{\tau,ss}$ for rucaparib were 2,650 ng/mL or 8.19 μM (57%) and 25,800 h*ng/mL (57%) (**Table 1**), comparable to the exposures observed in other studies ($C_{max,ss}$ of 6 μM).[4]

The CYP cocktail (caffeine, S-warfarin, omeprazole, and midazolam) was dosed orally without rucaparib on day 1 and after the steady state of rucaparib was achieved on day 12. The GM concentration-time profiles of a single oral dose of each substrate in the CYP cocktail without and with rucaparib are shown in **Figure 2a–d**. Probe drug PK parameters (C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{1/2}$, and T_{max}) following a single dose of the CYP cocktail or digoxin with and without rucaparib are summarized in **Table 1**. Percent of extrapolated AUC for S-warfarin was > 20% in 15 of 16 patients and percent of extrapolated AUC for digoxin was > 20% in all 16 patients. Therefore, no summary statistics of $AUC_{0\text{-}inf}$ are reported for these two probe drugs, and no DDI analysis was conducted based on $AUC_{0\text{-}inf}$.

DDIs

To determine the inhibitory effect of rucaparib on CYP enzymes and P-gp, the GMRs of probe drugs with rucaparib at steady state to probe drugs alone were determined for each PK parameter (**Table 1**, **Figure 3**). The inhibition effect was categorized based on AUC increase of the probe drugs as weak (≥ 1.25-fold but < 2-fold), moderate (≥ 2-fold but < 5-fold), and strong (≥ 5-fold) per the FDA and European Medicines Agency guidance for drug interaction studies.[12,13] In this study, marginal inhibition was defined as an increase of the exposure (C_{max} and/or AUC) by > 1-fold but < 1.25-fold. Rucaparib had no apparent effect on the C_{max} of caffeine but moderately increased caffeine AUC from time 0 to 72 hours ($AUC_{0\text{-}72\ h}$) and $AUC_{0\text{-}inf}$. The C_{max} of S-warfarin was marginally increased, and the AUC from time 0 to 96 hours ($AUC_{0\text{-}96\ h}$) was weakly increased with rucaparib. Rucaparib marginally increased the C_{max} of omeprazole and weakly increased the $AUC_{0\text{-}72\ h}$ and $AUC_{0\text{-}inf}$. For midazolam, rucaparib marginally increased the C_{max} and weakly increased both $AUC_{0\text{-}72\ h}$ and $AUC_{0\text{-}inf}$. Rucaparib had no apparent effect on the C_{max} of digoxin and marginally increased the $AUC_{0\text{-}72\ h}$.

Safety data

In this study, 16 patients (16/17; 94.1%) experienced TEAEs. The most common TEAEs were nausea (9/17; 52.9%), dysgeusia (3/17; 17.6%), headache (3/17; 17.6%), urinary tract infection (3/17; 17.6%), and vomiting (3/17; 17.6%). The majority of TEAEs were grade 1 or 2; grade 3 coagulopathy, hematuria, and hydronephrosis were reported in one patient each (1/17; 5.9%). Individual laboratory parameters were generally within normal ranges. Additionally, there were four cases in which deviations from normal laboratory ranges were grade 3 or higher (two cases of low lymphocyte count (2/17; 11.8%), one case of elevated alanine aminotransferase (1/17; 5.9%), and one case of low serum sodium concentration (1/17; 5.9%)). One patient was withdrawn from the study on day 9 due to adverse events of large intestine perforation and large intestinal obstruction related to progression of the underlying adenocarcinoma of the colon.

DISCUSSION

In vitro data suggest that rucaparib could reversibly inhibit CYP1A2, CYP2C9, CYP2C19, CYP3A, and P-gp at clinical exposure levels in human liver microsomes (Clovis Oncology, data on file). Additionally, rucaparib induced CYP1A2 and downregulated CYP3A4 in cryopreserved human hepatocytes in a concentration-dependent manner (Clovis Oncology, data on file). The potential for complicated DDIs warranted *in vivo* assessment following repeated rucaparib administration at the recommended dosage (600 mg b.i.d.).

Cocktail DDI studies allow for an efficient assessment of the effect of a perpetrator drug on multiple DDI probe drugs. As there are no DDIs among the probe drugs in the

Table 1 Summary of plasma pharmacokinetics of probe drugs

PK parameters by probe drug	Patients, *n*[b]	Geometric mean (%CV)[a]		Ratio (90% CI)
		Without rucaparib	With rucaparib	
Caffeine				
C_{max} (ng/mL)	16	5,980 (30)	5,900 (16)	0.99 (0.90–1.08)
$AUC_{0–72\ h}$ (h*ng/mL)	16	57,500 (61)	130,000 (34)	2.26 (1.93–2.65)
$AUC_{0–inf}$ (h*ng/mL)	11	59,300 (76)	152,000 (31)	2.55 (2.12–3.08)
$t_{1/2}$ (h)	11	7.0 (78)	20.7 (25)	—
T_{max} (h)	16	0.5 (0.3, 2.0)	1.0 (0.5, 2.0)	—
S-warfarin[c,d,e]				
C_{max} (ng/mL)	14	721 (20)	759 (20)	1.05 (0.99–1.12)
$AUC_{0–96\ h}$ (h*ng/mL)	14	20,300 (26)	30,200 (29)	1.49 (1.40–1.58)
T_{max} (h)	14	1.0 (0.5, 3.0)	1.5 (0.5, 3.0)	—
Omeprazole				
C_{max} (ng/mL)	16	1,110 (71)	1,210 (54)	1.09 (0.93–1.27)
$AUC_{0–72\ h}$ (h*ng/mL)	16	2,910 (123)	4,510 (116)	1.55 (1.32–1.83)
$AUC_{0–inf}$ (h*ng/mL)	16	2,920 (123)	4,540 (116)	1.55 (1.32–1.83)
$t_{1/2}$ (h)	16	1.5 (91)	2.3 (91)	—
T_{max} (h)	16	2.0 (1.0, 3.0)	2.0 (2.0, 3.0)	—
Midazolam				
C_{max} (ng/mL)	16	19.4 (35)	22.0 (54)	1.13 (0.95–1.36)
$AUC_{0–72\ h}$ (h*ng/mL)	16	45.4 (65)	63.0 (69)	1.39 (1.14–1.68)
$AUC_{0–inf}$ (h*ng/mL)	16	48.0 (64)	66.5 (67)	1.38 (1.13–1.69)
$t_{1/2}$ (h)	16	6.8 (41)	7.8 (39)	—
T_{max} (h)	16	0.5 (0.3, 1.0)	0.5 (0.2, 2.0)	—
Digoxin[d,e]				
C_{max} (pg/mL)	16	1,940 (34)	1,860 (32)	0.96 (0.84–1.10)
$AUC_{0–72\ h}$ (h*pg/mL)	16	21,500 (20)	25,900 (27)	1.20 (1.12–1.29)
T_{max} (h)	16	1.0 (0.5, 3.0)	1.0 (0.5, 3.0)	—
Rucaparib				
$C_{max,ss}$ (ng/mL)	16	NA	2,650 (57)	—
$AUC_{\tau,ss}$ (h*ng/mL)	16	NA	25,800 (57)	—
$T_{max,ss}$ (h)	16	—	2.5 (0.5, 3.1)	—

%CV, percent coefficient of variation; AUC, area under the concentration-time curve; $AUC_{0\text{-}72\ h}$, AUC from time 0–72 hours; $AUC_{0\text{-}96\ h}$, AUC from time 0–96 hours; $AUC_{0\text{-}inf}$, AUC extrapolated from time 0 to infinity; $AUC_{\tau,ss}$, AUC over a dosing interval τ (12 hours) at steady state; CI, confidence interval; C_{max}, peak plasma concentration; $C_{max,ss}$, C_{max} during a dosing interval at steady state; CYP, cytochrome P450; DDI, drug–drug interaction; NA, not applicable; PK, pharmacokinetics; $t_{1/2}$, terminal half-life; T_{max}, time of maximum plasma concentration.

[a]For T_{max}, data are reported as median (minimum, maximum). [b]One patient (5.9%) withdrew from the study on day 9 due to treatment-emergent adverse events not considered related to rucaparib; therefore, DDI was only analyzed in 16 patients (94.1%). [c]Two patients (11.8%) were determined to be poor metabolizers of CYP2C9 per genotyping and were excluded from the DDI analysis for S-warfarin. [d]$AUC_{0\text{-}inf}$ is not reported because percent of extrapolated AUC was < 20% for ≤ 1 patient. [e]The $t_{1/2}$ is not reported due to uncertainty in the reliability of $t_{1/2}$ estimation.

cocktail used in this study,[7] observed *in vivo* interactions can be interpreted as the effect of the investigated drug on individual probe drugs and the corresponding enzymes. On the other hand, there has been limited success in drug-transporter cocktail DDI studies due to a lack of validated cocktails, as well as a lack of probe-transporter specificity.[14,15]

In this cocktail DDI study, the Cooperstown 5 + 1 cocktail[7] was modified to assess the effect of steady-state rucaparib on CYP1A2 (caffeine), CYP2C9 (S-warfarin), CYP2C19 (omeprazole), and CYP3A (midazolam). Dextromethorphan, a probe drug for CYP2D6 that is part of the validated cocktail, was not included in this study. Digoxin, a P-gp probe drug, is not a part of the validated cocktail, and was dosed 1 day after the cocktail. Similar staggered dosing schedules were used successfully in previously reported studies.[8,9]

Cocktail DDI studies for oncology drugs can be conducted in healthy subjects[8] or patients with cancer.[16,17] As rucaparib is a PARP inhibitor, and thus clastogenic,[18–21] this DDI study was conducted in patients with advanced solid tumors. Compared with studies in healthy subjects, a study in patients with advanced cancer could allow better DDI characterization as this target population may have altered CYP activity and PK of the drug under investigation.[17]

Among all the probe drugs, caffeine, omeprazole, and midazolam have relatively short $t_{1/2}$ values,[7] whereas $t_{1/2}$ values are longer for S-warfarin and digoxin. S-warfarin has a $t_{1/2}$ of ~21–43 hours.[22–24] Digoxin has a $t_{1/2}$ of ~36 hours (range = 24–48 hours),[25] which can be prolonged in patients

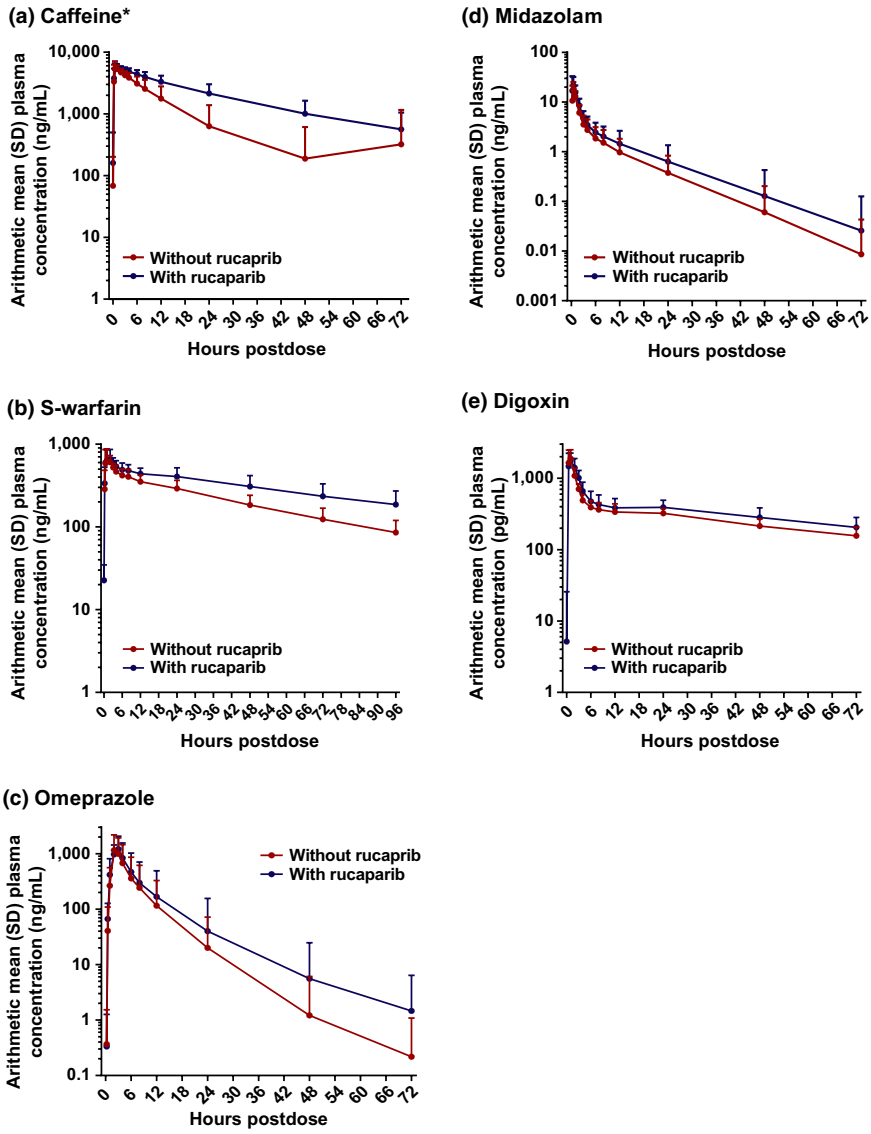

Figure 2 Arithmetic mean (SD) plasma concentration-time profiles for (**a**) caffeine, (**b**) S-warfarin, (**c**) omeprazole, (**d**) midazolam, and (**e**) digoxin administered with (blue line) and without (red line) rucaparib. *Following the caffeine dose on day 1, 9 of the 16 evaluable patients had caffeine concentrations lower than the quantification limit at 72 hours postdose. One subject had a higher than expected concentration at the same time point, presumably due to incidental caffeine intake, contributing to an apparent spike in the mean caffeine pharmacokinetic profile. The presumed incidental caffeine intake in this one patient had no impact on CYP1A2 DDI assessment based on C_{max} and $AUC_{0\text{-inf}}$. $AUC_{0\text{-inf}}$, area under the concentration-time curve extrapolated from time 0 to infinity; C_{max}, peak plasma concentration; DDI, drug–drug interaction.

with impaired renal function. The long $t_{1/2}$ values would mandate a long PK sampling period in order to capture the complete PK profiles (e.g., $5 \times t_{1/2}$). However, practical limitations had to be considered, and in this rucaparib DDI study, PK sampling periods of up to 96 and 72 hours were implemented for S-warfarin and digoxin, respectively. These were considered justifiable durations, as a study design with a PK sampling period of $5 \times t_{1/2}$ would have required the DDI assessment to be ≥ 27 days (vs. 16 days in this study). In the event of CYP2C9 or P-gp inhibition, the $t_{1/2}$ and the ideal PK sampling period would be even longer. Excessively long PK sampling could have negatively affected patient enrollment, delayed continuous rucaparib treatment (offered to patients who completed the DDI portion of the study), increased the risk of an unstable baseline, or confounded the DDI results in this single-sequence crossover DDI study. Published PK simulations indicate that the terminal $t_{1/2}$ and $AUC_{0\text{-inf}}$ can be reliably estimated with a PK sampling period of ~$2 \times t_{1/2}$, and clinical DDI studies with relatively short but adequate PK sampling periods for S-warfarin and digoxin have been reported. For example, the Cooperstown 5 + 1 cocktail was validated using a 96-hour PK sampling period for S-warfarin.[7] In a pazopanib DDI study, S-warfarin PK was monitored up to 96 hours.[17] In a cocktail DDI study of tipranavir and ritonavir, a 72-hour sampling period was applied for both S-warfarin and digoxin.[8]

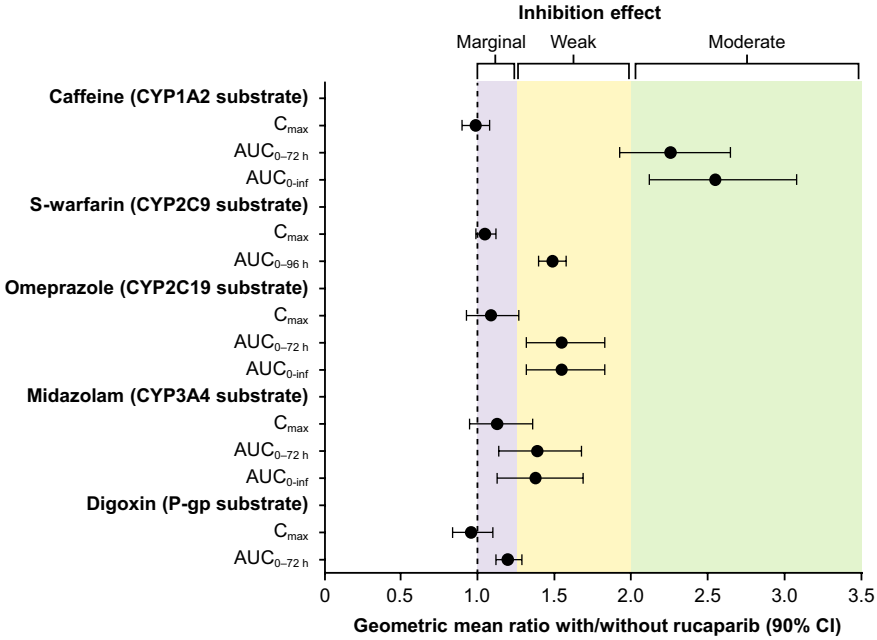

Figure 3 Effect of rucaparib on the pharmacokinetics of probe drugs. $AUC_{0\text{-}72\ h}$, area under the concentration-time curve from time 0–72 hours; $AUC_{0\text{-}96\ h}$, area under the concentration-time curve from time 0–96 hours; $AUC_{0\text{-}inf}$, area under the concentration-time curve extrapolated from time 0 to infinity; CI, confidence interval; CYP, cytochrome P450; C_{max}, peak plasma concentration; P-gp, P-glycoprotein.

Genotyping of CYP2C9 and CYP2C19 was conducted as part of this study. Two patients were poor metabolizers of CYP2C9 and were excluded from the S-warfarin DDI analysis. For these two patients, the day 12 to day 1 $AUC_{0\text{-}96\ h}$ ratios were 1.45 and 1.58, respectively, consistent with the GMR of 1.49 for the other 14 patients. The reason for the apparent CYP2C9 inhibition in the poor metabolizers is unknown; however, it fell within the expected intrasubject PK variability given the weak inhibition. Data from the two CYP2C9 poor metabolizers did not impact the conclusion that rucaparib is a weak inhibitor of CYP2C9. No other genotyping was conducted in this study because different genotypes are not believed to account for the majority of activity variability for CYP1A2,[7,17] CYP3A4,[7,26] or P-gp[8] in such studies.

Caffeine, S-warfarin, omeprazole, and midazolam are all sensitive *in vivo* index substrates (i.e., they are associated with a > fivefold increase in AUC when coadministered with a strong CYP inhibitor). A specific and sensitive index P-gp probe drug has not been identified in *in vivo* DDI studies.[12,13] Nevertheless, digoxin is a widely used P-gp probe substrate[27,28] and has a narrow therapeutic window; therefore, digoxin was used in this study as an *in vivo* P-gp probe drug.

In general, the observed rucaparib DDIs were limited in magnitude (**Table 1**, **Figure 3**). A limited effect of rucaparib on C_{max} was observed for all probe drugs, with mean GMR ≤ 1.13. This is clinically informative because CYP3A and P-gp have been reported to play a role in the intestinal absorption of midazolam and digoxin, respectively.[29,30] For caffeine, omeprazole, and midazolam, $AUC_{0\text{-}inf}$ GMRs were 2.55, 1.55, and 1.38, respectively, suggesting moderate (≥ 2-fold to < 5-fold) inhibition of CYP1A2 and weak (≥ 1.25-fold to < 2-fold) inhibition of CYP2C19 and CYP3A. For S-warfarin and digoxin, truncated AUCs were calculated. The GMR (90% CI) of S-warfarin $AUC_{0\text{-}96\ h}$ was 1.49 (1.40–1.58), and the GMR (90% CI) of digoxin $AUC_{0\text{-}72\ h}$ was 1.20 (1.12–1.29). These results suggest weak inhibition of CYP2C9 and marginal effect on digoxin PK.

The most common TEAE of any grade in this study was nausea, experienced by more than half of the patients. In this small study population, reported TEAEs were consistent with those observed in other studies of rucaparib.[3,4,6,31]

In summary, results from study CO-338-044 indicated that at steady state following 600 mg b.i.d. dosing, rucaparib moderately inhibited CYP1A2, weakly inhibited CYP2C9, CYP2C19, and CYP3A, and showed marginal effect on P-gp. Patients should be appropriately monitored, and dosage adjustments should be considered for CYP1A2, CYP3A, CYP2C9, and CYP2C19 substrates, particularly for medicines with a narrow therapeutic index, if clinically indicated.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Data S1. Supplementary methods.

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Conflict of Interest. J.J.X., J.I., J.B., and S.W. are employees of Clovis Oncology and may own stock or have stock options in that company. E.N. is a paid consultant for Clovis Oncology. R.K. and G.A. are employees of PRA Health Sciences. All other authors have nothing to disclose.

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1. Rubraca (rucaparib) tablets [prescribing information] (Clovis Oncology, Inc.: Boulder, CO, 2018).
2. Oza, A.M. *et al.* Antitumor activity and safety of the PARP inhibitor rucaparib in patients with high-grade ovarian carcinoma and a germline or somatic BRCA1 or BRCA2 mutation: Integrated analysis of data from study 10 and ARIEL2. *Gynecol. Oncol.* **147**, 267–275 (2017).
3. Coleman, R.L. *et al.* Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **390**, 1949–1961 (2017).
4. Kristeleit, R. *et al.* A phase I-II study of the oral PARP inhibitor rucaparib in patients with germline BRCA1/2-mutated ovarian carcinoma or other solid tumors. *Clin. Cancer Res.* **23**, 4095–4106 (2017).
5. Shapiro, G.I. *et al.* Pharmacokinetic study of rucaparib in patients with advanced solid tumors. *Clin. Pharmacol. Drug Dev.* Epub ahead of print (2018).
6. Swisher, E.M. *et al.* Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 part 1): An international, multicentre, open-label, phase 2 trial. *Lancet Oncol.* **18**, 75–87 (2017).
7. Chainuvati, S. *et al.* Combined phenotypic assessment of cytochrome p450 1A2, 2C9, 2C19, 2D6, and 3A, N-acetyltransferase-2, and xanthine oxidase activities with the "Cooperstown 5 + 1 cocktail". *Clin. Pharmacol. Ther.* **74**, 437–447 (2003).
8. Dumond, J.B. *et al.* A phenotype-genotype approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir. *Clin. Pharmacol. Ther.* **87**, 735–742 (2010).
9. Tachibana, M. *et al.* Evaluation of the pharmacokinetic drug interaction potential of tivantinib (ARQ 197) using cocktail probes in patients with advanced solid tumours. *Br. J. Clin. Pharmacol.* **84**, 112–121 (2018).
10. Eisenhauer, E.A. *et al.* New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur. J. Cancer* **45**, 228–247 (2009).
11. Brown, E.G., Wood, L. & Wood, S. The medical dictionary for regulatory activities (MedDRA). *Drug Saf.* **20**, 109–117 (1999).
12. European Medicines Agency. Guideline on the investigation of drug interactions. <https://www.ema.europa.eu/documents/scientific-guideline/guideline-investigation-drug-interactions_en.pdf> (2012). Accessed 30 September 2017.
13. U.S. Food and Drug Administration. Guidance for industry: Drug interaction studies — Study design, data analysis, implications for dosing, and labeling recommendations. <https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> (2012). Accessed 1 August 2017.
14. Ebner, T., Ishiguro, N. & Taub, M.E. The use of transporter probe drug cocktails for the assessment of transporter-based drug–drug interactions in a clinical setting—proposal of a four component transporter cocktail. *J. Pharm. Sci.* **104**, 3220–3228 (2015).
15. Zhang, L. & Sparreboom, A. Predicting transporter-mediated drug interactions: Commentary on: "Pharmacokinetic evaluation of a drug transporter cocktail consisting of digoxin, furosemide, metformin and rosuvastatin" and "Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A". *Clin. Pharmacol. Ther.* **101**, 447–449 (2017).
16. Gibbons, J.A. *et al.* Pharmacokinetic drug interaction studies with enzalutamide. *Clin. Pharmacokinet.* **54**, 1057–1069 (2015).
17. Goh, B.C. *et al.* An evaluation of the drug interaction potential of pazopanib, an oral vascular endothelial growth factor receptor tyrosine kinase inhibitor, using a modified Cooperstown 5 + 1 cocktail in patients with advanced solid tumors. *Clin. Pharmacol. Ther.* **88**, 652–659 (2010).
18. Polyak, K. & Garber, J. Targeting the missing links for cancer therapy. *Nat. Med.* **17**, 283–284 (2011).
19. Wahlberg, E. *et al.* Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors. *Nat. Biotechnol.* **30**, 283–288 (2012).
20. Thomas, H.D. *et al.* Preclinical selection of a novel poly(ADP-ribose) polymerase inhibitor for clinical trial. *Mol. Cancer Ther.* **6**, 945–956 (2007).
21. Jenner, Z.B., Sood, A.K. & Coleman, R.L. Evaluation of rucaparib and companion diagnostics in the PARP inhibitor landscape for recurrent ovarian cancer therapy. *Future Oncol.* **12**, 1439–1456 (2016).
22. Stockis, A., van Lier, J.J., Cawello, W., Kumke, T. & Eckhardt, K. Lack of effect of lacosamide on the pharmacokinetic and pharmacodynamic profiles of warfarin. *Epilepsia* **54**, 1161–1166 (2013).
23. Walker, G., Mandagere, A., Dufton, C. & Venitz, J. The pharmacokinetics and pharmacodynamics of warfarin in combination with ambrisentan in healthy volunteers. *Br. J. Clin. Pharmacol.* **67**, 527–534 (2009).
24. Coumadin (warfarin sodium) tablets [prescribing information] (Bristol-Myers Squibb Company: Princeton, NJ, 2017).
25. Bauer, L.A. Applied Clinical Pharmacokinetics. 2nd edn. New York, NY: McGraw-Hill Education. <https://accesspharmacy.mhmedical.com/content.aspx?bookid=510&sectionid=40843080> (2008). Accessed 16 May 2018.
26. Klein, K. & Zanger, U. Pharmacogenomics of cytochrome P450 3A4: Recent progress toward the "missing heritability" problem. *Front. Genet.* **4**, 12 (2013).
27. de Lannoy, I.A.M. & Silverman, M. The MDR1 gene product, P-glycoprotein, mediates the transport of the cardiac glycoside, digoxin. *Biochem. Biophys. Res. Commun.* **189**, 551–557 (1992).
28. Schinkel, A.H., Wagenaar, E., van Deemter, L., Mol, C.A. & Borst, P. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J. Clin. Invest.* **96**, 1698–1705 (1995).
29. Igel, S. *et al.* Increased absorption of digoxin from the human jejunum due to inhibition of intestinal transporter-mediated efflux. *Clin. Pharmacokinet.* **46**, 777–785 (2007).
30. Gorski, J.C., Jones, D.R., Haehner-Daniels, B.D., Hamman, M.A., O'Mara, E.M. & Hall, S.D. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. *Clin. Pharmacol. Ther.* **64**, 133–143 (1998).
31. Drew, Y. *et al.* Phase 2 multicentre trial investigating intermittent and continuous dosing schedules of the poly(ADP-ribose) polymerase inhibitor rucaparib in germline BRCA mutation carriers with advanced ovarian and breast cancer. *Br. J. Cancer* **114**, 723–730 (2016).