Evaluation of the Drug–Drug Interaction Potential of Acalabrutinib and Its Active Metabolite, ACP-5862, Using a Physiologically-Based Pharmacokinetic Modeling Approach

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Acalabrutinib, a selective, covalent Bruton tyrosine kinase inhibitor, is a CYP3A substrate and weak CYP3A/CYP2C8 inhibitor. A physiologically-based pharmacokinetic (PBPK) model was developed for acalabrutinib and its active metabolite ACP-5862 to predict potential drug–drug interactions (DDIs). The model indicated acalabrutinib would not perpetrate a CYP2C8 or CYP3A DDI with the sensitive CYP substrates rosiglitazone or midazolam, respectively. The model reasonably predicted clinically observed acalabrutinib DDI with the CYP3A perpetrators itraconazole (4.80-fold vs. 5.21-fold observed) and rifampicin (0.21-fold vs. 0.23-fold observed). An increase of two to threefold acalabrutinib area under the curve was predicted for coadministration with moderate CYP3A inhibitors. When both the parent drug and active metabolite (total active components) were considered, the magnitude of the CYP3A DDI was much less significant. PBPK dosing recommendations for DDIs should consider the magnitude of the parent drug excursion, relative to safe parent drug exposures, along with the excursion of total active components to best enable safe and adequate pharmacodynamic coverage.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?✔ Acalabrutinib, a Bruton tyrosine kinase inhibitor, received accelerated approval by the US Food and Drug Administration for the treatment of adult patients with mantle cell lymphoma who have received at least one prior therapy.

WHAT QUESTION DID THIS STUDY ADDRESS?✔ Physiologically-based pharmacokinetic (PBPK) modeling predicted the effect of CYP3A modulators on the pharmacokinetics of acalabrutinib and its active metabolite ACP-5862. Acalabrutinib as victim or perpetrator of CYP enzymes was assessed to guide its appropriate dosing in clinical practice.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?✔ PBPK modeling recommended a dose of 100 mg once a day and 200 mg twice a day for use with a moderate CYP3A inhibitor or a strong CYP3A4 inducer, respectively. PBPK analysis shows that acalabrutinib has no clinically relevant effects on sensitive CYP3A4 or CYP2C8 substrates. CYP3A4 modulator effects on acalabrutinib pharmacokinetics may be less significant when a total active component is considered.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?✔ PBPK models of drug interactions that incorporate the impact of exposure to active metabolites, when verified with suitable clinical trial results, may give more relevant estimates of drug–drug interaction magnitude and therefore afford dosing recommendations that ensure both safety and adequate pharmacodynamic coverage.
patients with mantle cell lymphoma who have received at least one prior therapy.\textsuperscript{9,10} Acalabrutinib is currently under development for other hematological malignancies, including CLL and diffuse large B-cell lymphoma.

Acalabrutinib and ibrutinib have similar biological profiles in primary CLL cells; however, acalabrutinib appears to have fewer off-target effects in healthy B lymphocytes than ibrutinib.\textsuperscript{11-13} In a clinical study of patients with relapsed CLL, the overall response rate with acalabrutinib was 95% after a median follow-up of 14.3 months (range 0.5–20).\textsuperscript{5} In a clinical study of patients with relapsed/refractory mantle cell lymphoma, the investigator-Assessed overall response rate was 81% (95% confidence interval, 73–87%) after a median follow-up of 15.2 months (range 0.3–23.7).\textsuperscript{14} Acalabrutinib exhibited a favorable safety profile in these studies.

Acalabrutinib is rapidly absorbed with a short oral half-life of about 1.57 hours in healthy subjects, with an absolute oral bioavailability of 25%.\textsuperscript{15} The pharmacokinetics (PK) of acalabrutinib were generally linear in the 75–250 mg range in patients, and no accumulation of acalabrutinib was observed after multiple doses.\textsuperscript{16} During clinical studies, ACP-5862 was identified as the major, and pharmacologically active, metabolite of acalabrutinib in plasma. ACP-5862 has \textsim 50% potency for BTK inactivation relative to parent acalabrutinib and has a similar kinase selectivity profile.\textsuperscript{9} These data indicate that ACP-5862 may also contribute to efficacy and safety after acalabrutinib administration.

Based on \textit{in vitro} studies, acalabrutinib is a substrate of P-glycoprotein (P-gp) and is predominantly metabolized by CYP3A enzymes.\textsuperscript{9} Further metabolism of ACP-5862 is also mainly mediated by CYP3A. Following a single oral dose of 100 mg acalabrutinib, the half-life of ACP-5862 was 6.9 hours and the mean exposure was approximately twofold to threefold higher than that of acalabrutinib.\textsuperscript{9} In clinical drug–drug interaction (DDI) studies with itraconazole and rifampicin, itraconazole increased acalabrutinib area under the curve (AUC) by approximately fivefold and rifampicin reduced acalabrutinib AUC to \textsim 20% (about fivefold).\textsuperscript{17} Acalabrutinib and ACP-5862 were also shown to be weak time-dependent \textit{in vitro} inactivators of CYP3A4 and CYP2C8, respectively.\textsuperscript{9}

A physiologically-based PK (PBPK) modeling approach was used to evaluate the DDI potential of acalabrutinib and its active metabolite, ACP-5862, after oral 100 mg twice a day (b.i.d.) administration with CYP3A inhibitors and inducers. Clinical DDI studies with a strong CYP3A inhibitor (steady-state itraconazole) or a strong inducer (steady-state rifampicin) were used to develop and verify the PBPK model. The derived PBPK model was used to evaluate the impact of potential DDIs and to define potential acalabrutinib dose adjustments. Because of the weak time-dependent inactivation of CYP3A and CYP2C8, the model was also used prospectively to predict the impact of a steady-state clinical 100 mg b.i.d. dose of acalabrutinib on exposure to the sensitive CYP3A substrate midazolam and the sensitive CYP2C8 substrate rosiglitazone.

**METHODS**

The acalabrutinib and ACP-5862 PBPK model was developed in two steps. The PBPK model for acalabrutinib alone was developed and verified using several different sets of clinical study data, including itraconazole and rifampicin DDI studies (Figure 1). After the active metabolite ACP-5862 was identified during clinical development and quantified in multiple clinical studies, the model was extended to include the metabolite. In the second stage, the acalabrutinib compound file was also updated with newly available physicochemical experimental data (logP, pKₐ, and blood-to-plasma partition ratio). The combined parent/metabolite model was then verified using data from two additional clinical studies (Figure 1). Finally, the combined PBPK model was applied to predict the potential impact of coadministration with other CYP3A inhibitors and inducers on the PK of acalabrutinib and ACP-5862 in healthy subjects. The overall modeling strategy is shown in Figure 1. The potential interactions between multiple oral doses of acalabrutinib and a single oral dose of midazolam or rosiglitazone were also investigated to assess the potential for acalabrutinib and ACP-5862 to perpetrate a drug interaction by inhibition of CYP3A4 or CYP2C8, respectively.

**Clinical studies**

ACE-HV-001 was a phase I, dose-escalation study in healthy subjects in which a single dose of 25, 50, 75, and 100 mg of acalabrutinib from cohorts 3, 4, 5, and 6, respectively, were used for model development and verification. Data from cohort 7 (N = 16), which studied a single oral dose of 50 mg acalabrutinib administered in the absence of itraconazole and on the last day of 6 consecutive days of dosing with itraconazole (200 mg b.i.d.), were used to calibrate the model for the effect of a strong CYP3A inhibitor.\textsuperscript{17} ACE-HV-004 was a phase I, drug-interaction study in healthy subjects in which part 3 evaluated a 100 mg dose of acalabrutinib administered in the absence of rifampicin and again on the last day of 9 consecutive days of dosing with rifampicin (600 mg once a day (q.d.); N = 24),\textsuperscript{17} which was used to verify CYP3A contribution to the overall acalabrutinib metabolism. Both studies were completed before ACP-5862 was identified, so ACP-5862 concentrations were not analyzed.

ACE-HV-005 was a phase I study to evaluate the effects of single therapeutic (100 mg) and supratherapeutic (400 mg) oral doses of acalabrutinib on the QTc interval in healthy subjects (N = 40). ACE-HV-113 was a phase I study to measure intrasubject variability in PK and determine gastric pH effects on acalabrutinib PK in healthy subjects (N = 13) after a single 100 mg dose was administered on two different occasions.\textsuperscript{17} ACE-HV-009 was an absolute bioavailability, PK, excretion, and metabolism study of \textsuperscript{14}C-actalabrutinib in healthy subjects (N = 8) in which the active metabolite was first identified and quantified.\textsuperscript{15} When a synthetic standard and validated assay was available, ACP-5862 concentrations were measured in 18 subjects in the ACE-HV-005 study and in all subjects in the ACE-HV-113 study. Details on these clinical studies can be found in Table 1.

These studies were conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice. The final protocol and informed consent form were approved by the institutional review boards at the respective study sites. Informed consent was obtained from all volunteers before any study procedures were conducted.
PBPK Model of Acalabrutinib and Its Metabolite
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Model development

Parent model. A combination of physicochemical parameters, parameters from in vitro absorption and metabolism studies, and concentration-time data from clinical studies (Table 2) were used to build a physiologically based model for acalabrutinib and ACP-5862 in Simcyp, version 14 (Certara, Sheffield, UK). A minimal PBPK model with a single adjusting compartment (SAC), which considers metabolism in both the liver and intestine and lumps other tissues together, was incorporated in Simcyp for acalabrutinib. The SAC is a nonphysiological compartment that permits the adjustment of the drug concentration profiles in the systemic compartment and represents a lump of all tissues, excluding the liver and the portal vein. The concentration-time profiles of six individuals from ACE-HV-001 cohort 6 who received 100 mg acalabrutinib were used to estimate the rate of drug in and out of the SAC and the volume of the compartment using the parameter estimation module within Simcyp. A range of human intestinal effective permeability ($P_{\text{eff,man}}$) values were investigated using the clinical results from ACE-HV-001 cohort 4 and cohort 6 (Figure S1), and a $P_{\text{eff,man}}$

Table 1 Summary of acalabrutinib clinical studies

| Study                  | Dose (mg) | N  | Age (year) | % Female | Acalabrutinib | ACP-5862 |
|------------------------|-----------|----|------------|----------|---------------|----------|
| ACE-HV-001 cohort 3    | 25        | 6  | 30–59      | 83       | Yes           | No       |
| ACE-HV-001 cohort 4    | 50        | 6  | 27–59      | 50       | Yes           | No       |
| ACE-HV-001 cohort 5    | 75        | 12 | 26–56      | 50       | Yes           | No       |
| ACE-HV-001 cohort 6    | 100       | 6  | 24–56      | 0        | Yes           | No       |
| ACE-HV-001 cohort 7    | 50        | 16 | 19–57      | 19       | Yes           | No       |
| ACE-HV-004 part 3      | 100       | 24 | 18–58      | 33       | Yes           | No       |
| ACE-HV-113             | 100       | 12 | 18–58      | 33       | Yes           | Yes      |
| ACE-HV-005             | 100, 400  | 18 | 19–63      | 40       | Yes           | Yes      |
| ACE-HV-009             | 100       | 14 | 18–65      | 36       | Yes, semiquantitative |

The last two columns indicate if acalabrutinib and/or ACP-5862 were measured in the specific study.
value of $4.0 \times 10^{-4}$ cm/second was most consistent with the observed acalabrutinib peak plasma concentration ($C_{\text{max}}$) values and was therefore applied in the model. The volume of distribution at steady state was predicted using logP, pKa, and blood-to-plasma partition ratio values by the Rodgers and Rowland method. Fraction unbound in enterocyte was assumed to be the same as unbound fraction in plasma (Table 2).

In vitro incubation of 20 $\mu$M $^{14}$C-acalabrutinib (for qualitative metabolite profiling) with human hepatocytes (two male donors, CellzDirect-Invitrogen, Austin, TX) indicated relative contributions of glutathione and CYP-mediated metabolic pathways of 21% and 79%, respectively, and the CYP3A enzyme was the main enzyme (~ 90%) involved in the formation of CYP metabolites (data on file). Eventually, the percentage of acalabrutinib metabolized by CYP3A4 was assigned as 0.82 based on itraconazole interaction study ACE- HV- 001, cohort 7. The renal clearance of 1.33 L/hour for acalabrutinib observed in clinical study ACE- HV-009 was applied directly. The intrinsic metabolic clearance of acalabrutinib was estimated from the in vivo total clearance of 169 L/hour (ACE-HV-001) with the retrograde method. The total intrinsic metabolic clearance was then separated into CYP3A4 and undefined components based on the percentage of acalabrutinib metabolized by CYP3A4. The intrinsic clearance value of 9.63 $\mu$L/minutes/pmol was assigned for CYP3A4 with
289.5 μL/minutes/mg for additional human liver microsome clearance for the elimination of acalabrutinib (Table 2).

In vitro studies using MDCKII-MDR1 cells (Netherlands Cancer Institute, Amsterdam, The Netherlands) indicated that acalabrutinib is a substrate of P-gp with efflux ratio (42 at 1 μM; data on file). In the absence of detailed information on the specificity and affinity of acalabrutinib for transport, it was not possible to include the active transport process in the model. However, the sensitivity of the final parent model to the impact of intestinal efflux was investigated using the advanced dissolution, absorption and metabolism model within Simcyp in conjunction with a range of in vitro intestinal P-gp maximum rate of transporter mediated efflux and Michaelis-Menten constant (K_m) values.

Combined parent and metabolite model. The combined parent metabolite model was developed as an extension of the parent-alone model (Table 2). The logP, pKa, and blood-to-plasma partition ratio of acalabrutinib in the parent-alone model were updated with experimental values. The volume of distribution at steady state of 0.36 L/kg for ACP-5862 was model were updated with experimental values. The volume of distribution at steady state of 0.36 L/kg for ACP-5862 was predicted using the Rodgers and Rowland method. A SAC was also applied for the metabolite, and the concentration-time profile from the ACE-HV-113 period 2 oral dose (100 mg acalabrutinib) were used to optimize the volume of SAC and the rate of drug in and out of the SAC for ACP-5862.

The metabolism of acalabrutinib to ACP-5862 was characterized in recombinant CYP enzymes, and CYP3A4 was identified as the sole enzyme responsible for the conversion of acalabrutinib to ACP-5862. The reported maximum rate of metabolism formation (V_max) of 4.13 pmol/minutes/pmol and K_m of 2.78 μM in CYP3A4 were incorporated in the model to describe the conversion of acalabrutinib to ACP-5862. Therefore, the elimination of acalabrutinib via CYP3A4 (9.63 μL/minutes/pmol) was further divided into two clearance pathways: one to the active metabolite ACP-5862 (V_max of 4.13 pmol/minutes/pmol and K_m of 2.78 μM) and the other to the rest of the metabolites (8.14 μL/minutes/pmol). The in vitro studies also demonstrated that the CYP3A enzyme was responsible for the further metabolism of ACP-5862, and a clearance value of 23.6 μL/minutes/mg protein in human liver microsomes was assigned to CYP3A4 for the elimination of ACP-5862. A renal clearance of 0.3 L/hour for ACP-5862 reported in ACE-HV-009 was applied in the model. Such assignment suggested that the ACP-5862-related metabolites contributed about 12% of total acalabrutinib elimination, which is in close agreement with the 10% of total dose observed in human mass balance study.15

The competitive and time-dependent inhibition parameters against the CYP2C8, CYP2C9, CYP2C19, and CYP3A enzymes obtained from in vitro studies were applied for both acalabrutinib and ACP-5862 (Table 2). Acalabrutinib did not cause significant induction of the metabolism of the marker substrates of CYP1A2, CYP2B6, or CYP3A4 at any concentration evaluated.

Model verification
The parent-alone model was verified with the clinical study ACE-HV-001 cohort 3 and cohort 5, in which a single dose of 25 and 75 mg of acalabrutinib was given, as well as ACE-HV-004 part 3, in which acalabrutinib in the absence and presence of multiple doses of rifampicin was evaluated. The combined parent and metabolite model was verified again with the DDI studies with itraconazole and rifampicin. The model was also verified with studies ACE-HV-005 and ACE-HV-113 period 1, in which both acalabrutinib and its active metabolite were measured (Table S1). Default compound files for the CYP3A4 substrate midazolam and the CYP2C8 substrate rosiglitazone were applied directly. Simulations of the DDIs between itraconazole and midazolam or gemfibrozil and rosiglitazone were conducted to verify these two compound files.

Default Simcyp parameter values for creating a virtual North European Caucasian or virtual Healthy Volunteer population (population, physiological parameters including liver volume and blood flows, enzyme abundances) were applied in the current analysis. All simulations were run with virtual subjects matched as closely as possible with respect to age and sex to those in the corresponding actual clinical studies and according to the same study design as summarized in Table 1. The detailed simulation condition for each study can be found in Table S1.

Model application
Various clinical DDI scenarios were simulated using the final model of acalabrutinib and ACP-5862 to evaluate the likely impact of the coadministration of multiple doses of CYP3A4 inhibitors and inducers on the kinetics (C_max and AUC) of both acalabrutinib and ACP-5862 in healthy subjects. The AUC ratios of acalabrutinib and ACP-5862 in the absence and presence of a CYP3A inhibitor or inducer were calculated. The total active component (acalabrutinib plus ACP-5862) in the absence and presence of a CYP3A inhibitor or inducer were also determined as sum of the AUC of acalabrutinib and ACP-5862. In addition, ACP-5862 exposure was further adjusted for its potency for BTK inactivation relative to the parent acalabrutinib.

Because acalabrutinib and ACP-5862 exhibited as time-dependent inhibitors of CYP3A4 and CYP2C8, respectively, from in vitro studies (Table 2), the potential interactions between multiple 100 mg b.i.d. oral doses of acalabrutinib and a single oral dose of midazolam or rosiglitazone were investigated to assess the potential for acalabrutinib and ACP-5862 to inhibit CYP3A4 or CYP2C8. Compound files were used as provided by the Simcyp software for all CYP3A inhibitors (itraconazole, clarithromycin, fluconazole, fluvoxamine, diltiazem, erythromycin), inducers (rifampicin, efavirenz, carbamazepine) and substrate (midazolam) and CYP2C8 substrate (rosiglitazone). The detailed simulation condition for each interaction study design is provided in Table S1.

RESULTS
The initial simulations of the PK of acalabrutinib alone (ACE-HV-001 cohorts 4 and 6) and in the presence of itraconazole (ACE-HV-001 cohort 7) were used to optimize the parameter inputs for the parent-alone model (Figure 1). Simulated profiles for the acalabrutinib and itraconazole interactions were then compared with observed data (Figure S2). The model was further verified with two additional clinical studies.
The combined model was verified by comparing the predicted PK changes in acalabrutinib and ACP-5862 after 100 mg b.i.d. was coadministered with moderate or weak CYP3A4 inhibitors, such as diltiazem and fluconazole (Table S4). Similarly, the ratios of total exposure by considering the relative BTK potency between acalabrutinib and ACP-5862 in the absence and presence of CYP inhibitors/inducers has also been presented in Table S4, which are similar as ratios of total exposure without considering the relative BTK potency. The simulated DDIs are summarized as a forest plot in Figure 4.

When 100 mg b.i.d. acalabrutinib was coadministered with a multiple-dose regimen of the strong CYP3A inducer rifampin (600 mg q.d. for 9 days), even though parent-drug AUC was decreased by fivefold to 17% of control exposure, the predicted geometric mean total AUC of the two active components (acalabrutinib plus ACP-5862) decreased by only twofold (46% of acalabrutinib alone). At an acalabrutinib dose of 200 mg b.i.d., the simulation indicated exposure to acalabrutinib plus metabolite would be 92% of the exposure to acalabrutinib 100 mg b.i.d. without rifampin (Table S4 and Figure 4). Similarly, in the presence of the moderate CYP3A inducer efavirenz, the combined AUC of acalabrutinib and ACP-5862 decreased by 1.3-fold (75% of acalabrutinib alone).

The combined model was also applied to predict the impact of multiple 100 mg b.i.d. doses of acalabrutinib as a perpetrator on the sensitive substrates of CYP3A and CYP2C8. The predicted geometric mean midazolam AUC and Cmax ratios with coadministered itraconazole and rifampin (Table S3) also demonstrate that the assigned parameters were appropriate to predict changes in the acalabrutinib and ACP-5862 plasma concentration-time profiles during potential drug interactions.

After the verification of the acalabrutinib and ACP-5862 PBPK model, it was applied to predict untested clinical outcomes for DDIs with other CYP3A inhibitors or inducers. The moderate CYP3A inhibitors fluconazole, diltiazem, and erythromycin were predicted to increase steady-state acalabrutinib AUC by 2.44-fold, 2.28-fold, and 2.76-fold, respectively. The weak CYP3A inhibitor fluvoxamine was predicted to increase steady-state acalabrutinib AUC by 37%.

The model also predicted that the steady-state acalabrutinib AUC would decrease by 61% in the presence of CYP3A inducer efavirenz or carbamazepine (Table S4).

When acalabrutinib was coadministered at 100 mg b.i.d. at the end of a multiple-dose itraconazole regimen (200 mg b.i.d. for 6 days), the predicted acalabrutinib geometric mean AUC was about 4.59-fold higher relative to acalabrutinib given alone. However, the ACP-5862 AUC was significantly reduced in the presence of itraconazole, 0.15-fold when compared with acalabrutinib given alone (Table S4). Therefore, the total active component (acalabrutinib plus ACP-5862) in the presence of itraconazole was within 6% of the total active component when acalabrutinib was given in the absence of itraconazole, indicating that even with a fivefold increase in parent AUC, there was little net change in the total AUC of the two active components (parent and metabolite) in the presence of a strong CYP3A4 inhibitor. Simulations showed similar outcomes when acalabrutinib 100 mg b.i.d. was coadministered with moderate or weak CYP3A4 inhibitors, such as diltiazem and fluconazole (Table S4). Similarly, the ratios of total exposure by considering the relative BTK potency between acalabrutinib and ACP-5862 in the absence and presence of CYP inhibitors/inducers has also been presented in Table S4, which are similar as ratios of total exposure without considering the relative BTK potency. The simulated DDIs are summarized as a forest plot in Figure 4.

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DDI clinical data and then applied to prospective predictions of acalabrutinib-drug combinations for which no clinical data were available.

The PK of acalabrutinib was linear in the therapeutic range of 75–250 mg, although a slightly higher than proportional exposure was observed at the 400 mg dose. *In vitro* studies indicated that acalabrutinib is a substrate of P-gp with an efflux ratio around half that of the established P-gp substrate digoxin, and acalabrutinib is not a P-gp inhibitor. Sensitivity analysis indicated a slight change in acalabrutinib exposure only when extreme high affinity was applied (Figure S3). In addition, the unchanged acalabrutinib accounted for only 1.2% of the excreted dose in the human mass-balance study, suggesting a negligible P-gp contribution to the acalabrutinib PK. Considering the linear PK for acalabrutinib at a therapeutic dose of 100 mg, the

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**Figure 2** Plasma concentrations of acalabrutinib and ACP-5862 after single oral dosing of 100 mg acalabrutinib in healthy volunteers for study ACE-HV-113 and ACE-HV-005. Simulated values are displayed as lines, including means (solid lines) and 5% and 95% percentiles (dashed lines). Observed data from clinical studies are displayed as solid dots. Inserts show semilog plots of individual observations. hr, hour.
The efflux process is unlikely to be of clinical significance for acalabrutinib absorption. The *in vitro* study indicated that acalabrutinib was a weak inhibitor of CYP3A, CYP2C8, and CYP2C9 and a time-dependent inhibitor of CYP3A with a $K_I$ value (the concentration at half of the maximal inactivation rate) of 10.1 μM. However, the competitive and time-dependent inhibition has minimal clinical impact, and even though acalabrutinib is mainly metabolized by CYP3A, time-dependent accumulation of acalabrutinib was not observed. In addition, the model also predicted that steady-state exposure to acalabrutinib and ACP-5862 is almost the same, with or without consideration of the time-dependent inhibition.

The elimination of acalabrutinib was estimated using *in vivo* oral clearance observed in a phase I clinical study, and
the fraction metabolized by CYP3A4 was determined using the itraconazole interaction study. Total CYP3A metabolism was further divided into two portions: one to its active metabolite ACP-5862, which was determined by in vitro study using recombinant CYP3A4 enzyme, and another to the rest of the metabolism of acalabrutinib. The current model also applied CYP3A4 as the only enzyme involved in the further metabolism of ACP-5862 based on in vitro experiments. Sensitivity analysis of a CYP3A4 fraction for the elimination of ACP-5862 was conducted. With a lower CYP3A4 fraction, the AUC of ACP-5862 did increase in the presence of multiple doses of rifampicin, but the overall increase was not significant (data not shown). Application of these two sets of in vitro parameters to the formation and elimination of ACP-5862 reasonably capture the ACP-5862 PK profile in both 100 mg and 400 mg dose groups, indicating that this in vitro to in vivo extrapolation works well for ACP-5862.

One of the limitations of the available data is the lack of confirmation of the metabolite exposure in CYP3A inhibition and induction studies that are needed to confirm the

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**Figure 4** Summary of all drug–drug interaction predictions. Ratio of geometric means in drug–drug interaction simulations. All simulations were performed for 100 mg twice a day of acalabrutinib in the absence and presence of different CYP3A inhibitor/inducer. *, shows the ratio of 200 acalabrutinib plus rifampicin twice a day vs. 100 acalabrutinib alone twice a day. The dots and numbers nearby are the geometric mean of ratios in each scenario, and the horizontal segments are the 90% confidence intervals of the geometric mean. Green indicates the observed values in itraconazole and rifampicin clinical drug–drug interaction studies (ACE-HV-001 cohort 7 and ACE-HV-004 part 3), which was a single dose of acalabrutinib with/without itraconazole or rifampicin. Blue indicates the values calculated for parent only. Red indicates the values calculated for metabolite only. The values in black are the ratios calculated considering the total component of parent and metabolite (P+M). Gray area is the 80–125% criteria for bioequivalence.
predictions for the metabolite exposures are appropriate. However, the PBPK model predicted PK of both parent and metabolite under no DDI conditions at the clinically relevant dose of 100 mg (Table S3). The final PBPK model also adequately predicted an acalabrutinib exposure increase and decrease with a strong CYP3A inhibitor and inducer, respectively (Table S4). Therefore, the model was appropriate to use with confidence to predict the magnitude of DDIs for acalabrutinib and its metabolite as victims of a CYP3A-mediated DDI. PBPK simulations with acalabrutinib and moderate CYP3A inhibitors (erythromycin, fluconazole, diltiazem) showed that coadministration increased acalabrutinib $C_{\text{max}}$ and AUC increased by twofold to almost threefold (Table S5), resulting in a recommendation for a dose reduction to 100 mg q.d. when acalabrutinib is coadministered with moderate CYP3A inhibitors, per the US package insert. However, the total active component (acalabrutinib plus ACP-5862) exposure had negligible change in the presence of moderate CYP3A inhibitors because of the ACP-5862 exposure reduction in the presence of these inhibitors. Currently, clinical information is being compiled to confirm such predictions, which may provide more precise dosing recommendations in the presence of strong or moderate CYP3A inhibitors. Based on in vitro data and PBPK modeling, no interaction with sensitive CYP2C8 or CYP3A data and PBPK in vitro simulations showed that acalabrutinib is unlikely to perpetrate a drug interaction on sensitive CYP2C8 or CYP3A substrates.

In conclusion, the PBPK model adequately captured the PK profiles of acalabrutinib and its active metabolite ACP-5862 as well as parent, metabolite, and total parent and metabolite (total active component) exposure in drug interactions with CYP3A inhibitors and inducers. The model indicated that there was little change in exposure to total active component in the presence of strong or moderate CYP3A inhibitors and only a 50% decrease in exposure to total active components in the presence of a strong CYP3A inducer. The PBPK model guided package insert dose change recommendations for CYP3A inhibitors and inducers and also showed that acalabrutinib is unlikely to perpetrate a drug interaction on sensitive CYP2C8 or CYP3A substrates.

Supporting Information. Supplementary information accompanies this paper on the CPT: Pharmacometrics & Systems Pharmacology website (www.psp-journal.com).

Supplementary Figures and Tables
Table S1. Trial design for each simulation.
Table S2. The predicted and observed geometric mean AUC and $C_{\text{max}}$ with coefficient of variation (%CV) of acalabrutinib and ACP-5862.
Table S3. The predicted and observed geometric mean acalabrutinib $AUC_{\text{last}}$ and geometric mean ratio in the absence and presence of itraconazole and rifampin.
Table S4. The predicted geometric mean ratio of acalabrutinib, ACP-5862, and total active component (acalabrutinib plus ACP-5862) in the absence and presence of different CYP3A inhibitors and inducers.
Figure S1. Evaluation of the impact of $P_{\text{eff,man}}$ on the $C_{\text{max}}$ of acalabrutinib.
Figure S2. Mean simulated and observed plasma concentrations of acalabrutinib and ACP-5862 as victims of a strong CYP3A inhibitor.

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