2 SYNOPSIS

Title of study: Absolute Bioavailability, Pharmacokinetics, Excretion, and Metabolism of [^{14}C]ACP-196 (Acalabrutinib) in Healthy Subjects

Study drug: ACP-196 (acalabrutinib)

Sponsor: Acerta Pharma, BV, PPD 5349 AB Oss, The Netherlands

Investigator: PPD

Study site: Covance Clinical Research Unit, Inc., PPD Madison, WI 53704, USA

Publications: None

Period of study:	**Phase of development:**
03 March 2016 (date of first informed consent) to 13 April 2016 (date of final poststudy observation)	Clinical Phase 1

Objectives:

The primary objectives of this study were:

- To determine the absolute bioavailability of acalabrutinib and intravenous (IV) pharmacokinetics (PK) of [^{14}C]ACP-196
- To determine route, rate, and extent of excretion of total ^{14}C related to [^{14}C]ACP-196 after oral administration

The secondary objective of this study was:

- To evaluate the safety and tolerability of acalabrutinib

The exploratory objectives of this study were:

- To characterize metabolites of acalabrutinib after oral administration of [^{14}C]ACP-196
- To evaluate the effect of select genetic polymorphisms of 3 drug metabolism and transport genes on acalabrutinib disposition
- To quantify ^{14}C related to [^{14}C]ACP-196 in blood, plasma, and select blood cell fractions after oral administration of [^{14}C]ACP-196

Methodology:

This was a single-center, open-label, nonrandomized, single oral dose study conducted in 2 cohorts to assess the absolute bioavailability, PK, excretion, and metabolism of [^{14}C]ACP-196. Cohort 1 received a single oral dose of acalabrutinib, with an IV microtracer dose of [^{14}C]ACP-196 (<10 µg; ≤1 µCi) administered as a 2-minute IV push timed to finish at 1 hour after the oral dose. Cohort 2 received a single oral 100-mg dose of acalabrutinib containing a microtracer (<10 µg; ≤1 µCi [^{14}C]ACP-196) dose.

Number of subjects (planned and analyzed):

It was planned to study a total of 14 subjects: Cohort 1 having 8 subjects, and Cohort 2 having 6 subjects. Fourteen subjects were enrolled in the study and dosed. Twelve subjects completed the study. Two subjects discontinued the study and were not replaced. Data for all 14 subjects entered into the study were included in the PK and safety analyses.

Diagnosis and main criteria for inclusion:

Healthy male and female subjects aged between 18 and 65 years, inclusive, and with a body mass index between 18.5 and 29.9 kg/m^2, inclusive, were enrolled according to the inclusion and exclusion criteria listed in the protocol.

Test product, dose, mode of administration, and lot number:

Cohort 1

For Cohort 1, on the morning of Day 1, after an overnight fast of at least 8 hours, all subjects received a single 100-mg oral capsule dose of acalabrutinib (lot number CCI), followed 58 minutes post-oral-dose by a single IV microtracer solution dose (<10 µg; ≤1 µCi) of [C]ACP-196 (actual concentration 1.64 µg/mL; 193.87 nCi/mL [approximately 0.194 µCi/mL]; lot number CCI) as an approximately 5-mL IV push over 2 minutes.

Cohort 2

For Cohort 2, on the morning of Day 1, after an overnight fast of at least 8 hours, all subjects received a single 100-mg oral dose of acalabrutinib, administered as 100 mL of a 1.0-mg/mL oral solution containing a microtracer dose (<10 µg; ≤1 µCi) of [^{14}C]ACP-196 (actual concentration 9.22 nCi/mL [approximately 0.00922 µCi/mL]; lot number CCI).

Duration of treatment:
A single oral capsule dose of acalabrutinib and single IV dose of [^{14}C]ACP-196 were administered to each subject in Cohort 1 on Day 1, and single oral solution of acalabrutinib and [^{14}C]ACP-196 was administered to each subject in Cohort 2 on Day 1. Subjects resided at the Clinical Research Unit from Day -1 to Day 5 (Cohort 1) or Day 8 (Cohort 2).

Criteria for evaluation:
Pharmacokinetics:
For Cohort 1, blood samples were collected for plasma PK analysis of acalabrutinib for oral PK parameters and [^{14}C]ACP-196 plasma concentrations for IV PK parameters at specified timepoints, and urine samples were collected for PK analysis of [^{14}C]ACP-196 (IV microtracer) and for total acalabrutinib (oral unlabeled dose) during specified time intervals.
For Cohort 2, blood samples were collected for quantification of ^{14}C in whole blood and plasma, analysis of plasma acalabrutinib concentrations, and metabolite profiling in plasma at specified timepoints; urine samples were collected for PK analysis of total ^{14}C concentrations, total acalabrutinib, and metabolite profiling and identification (ID) during specified time intervals; and fecal samples for analysis of total ^{14}C concentrations and metabolite profiling and ID were collected during specified time intervals. Additional blood samples were collected at specified timepoints for peripheral blood mononuclear cell isolation for determination of total ^{14}C.

Cohort 1:
Parameters for Acalabrutinib and [^{14}C]ACP-196 in Plasma: area under the concentration-time curve (AUC) from Hour 0 to the last quantifiable concentration (AUC_{0-t}); AUC from Hour 0 to Hour 12 (AUC_{0-12h}); AUC from Hour 0 to Hour 72 (AUC_{0-72h}); AUC extrapolated to infinity ($AUC_{0-\infty}$); percentage extrapolated from the last quantifiable concentration to infinity ($\%AUC_{extrap}$); maximum observed postdose concentration (C_{max}); time to maximum observed postdose concentration (t_{max}); apparent terminal elimination half-life ($t_{1/2}$); apparent terminal elimination rate constant (λ_z); apparent total clearance (CL/F); total clearance (CL); apparent volume of distribution (V_z/F); volume of distribution during the terminal phase (V_z); volume of distribution at steady state (V_{ss}); absolute bioavailability (F); and mean residence time (MRT).
Parameters for Acalabrutinib and Total [^{14}C]ACP-196 in Urine: amount of drug excreted in urine over each sampling interval (A_{eu}); cumulative amount of drug excreted in urine over the entire sample collection period (Cumulative A_{eu}) renal clearance (CL_R); percent excreted over each sampling interval (% f_{eu}); and cumulative percent excreted in urine over the entire sample collection period (Cumulative % f_{eu}).

Cohort 2:
Parameters for Total ^{14}C Radioactivity in Whole Blood and for Acalabrutinib and Total ^{14}C Radioactivity in Plasma: AUC_{0-t}; AUC_{0-12h}; AUC from Hour 0 to Hour 168 (AUC_{0-168h}); $AUC_{0-\infty}$; $\%AUC_{extrap}$; C_{max}; t_{max}; $t_{1/2}$; λ_z; CL/F; V_z/F; ratio of whole blood to plasma $AUC_{0-\infty}$ for total radioactivity (B:P $AUC_{0-\infty}$ ratio); and ratio of plasma $AUC_{0-\infty}$ of acalabrutinib to plasma $AUC_{0-\infty}$ of total ^{14}C radioactivity (plasma acalabrutinib: ^{14}C radioactivity $AUC_{0-\infty}$ ratio).
Parameters for Acalabrutinib and Total ^{14}C Radioactivity in Urine: A_{eu}; Cumulative A_{eu}; CL_R; % f_{eu}; and Cumulative % f_{eu}.
Pharmacokinetic Parameters for Total ^{14}C Radioactivity in Feces: amount of drug excreted in feces over each sampling interval (A_{ef}); cumulative amount of drug excreted in feces over the entire sample collection period (Cumulative A_{ef}); percent excreted over each sampling interval (% f_{ef}); and cumulative percent excreted in feces over the entire sample collection period (Cumulative % f_{ef}).

Safety:
Adverse events (AEs), vital signs, 12-lead electrocardiograms (ECGs), clinical laboratory evaluations, and physical examinations.

Statistical methods:
Safety and PK parameters were listed and summarized using standard descriptive statistics, as appropriate.

Summary - Conclusions:
Pharmacokinetic results:
The absolute bioavailability of acalabrutinib, assessed using a 100-mg oral dose, was 25.3%. In addition, the terminal half-lives were consistent for the oral and IV routes of administration, with $t_{1/2}$ values of 1.57 and 1.78 hours, respectively, in Cohort 1. Following oral administration of a single 100-mg dose, the CL/F and V_z/F values for acalabrutinib in plasma were 163 L/h and 344 L, respectively. After IV administration of a single microtracer dose (<10 µg; ≤1 µCi) of [^{14}C]ACP-196 at 58 minutes post-oral-dose, CL was 39.4 L/h, V_z was 98.0 L, and V_{ss} was 34.2 L for [^{14}C]ACP-196 in plasma. The mean V_{ss} (34.2 L ± 40.2%) was slightly less than the standard total body water of approximately 40 L for a 70 kg person.

Fecal excretion was the primary route of elimination following a single 100-mg oral dose of acalabrutinib containing a microtracer dose (<10 µg; ≤1 µCi) of [^{14}C]ACP-196 in Cohort 2. Radioactivity was recovered in urine and feces through 168 hours postdose (the last collection interval), with >80% of the administered total radioactivity recovered in the first 96 hours postdose. Mean recoveries of total radioactivity in urine and feces were 12.0% and 83.5%, respectively, for an overall recovery of approximately 96% of the administered dose.

Total ^{14}C radioactivity in whole blood accounted for approximately 86% of the total radioactivity in plasma based on mean AUC_{0-12} and for approximately 184% of the total radioactivity in plasma based on mean AUC_{0-168}. While the total radioactivity levels observed in whole blood and plasma were very similar during the initial absorption and elimination phases, a clear trend of increased blood-to-plasma ratio over time was observed for total radioactivity during the terminal elimination phase. This result may be partially attributed to covalent binding of acalabrutinib to Bruton tyrosine kinase, which is predominantly expressed in B cells, a component of whole blood.

Following a single 100-mg oral dose of acalabrutinib containing a microtracer dose (<10 µg; ≤1 µCi) of [^{14}C]ACP-196 in Cohort 2, the total radioactivity initially declined rapidly in plasma and whole blood, similar to parent acalabrutinib in plasma, which has a mean plasma $t_{1/2}$ value of 1.47 hours. The total ^{14}C radioactivity continues to be measurable at later timepoints and has a mean terminal $t_{1/2}$ value of 46.5 hours. The mean acalabrutinib (ACP-196) to ^{14}C radioactivity ratio was 0.0635 for $AUC_{0-\infty}$, indicating that acalabrutinib only contributed a small proportion of total ^{14}C radioactivity exposure with the majority of total ^{14}C radioactivity arising from metabolites. Furthermore, the mean acalabrutinib plasma concentration-time profile was lower than the mean total plasma radioactivity-time profile, suggesting that metabolites contributed to the circulating total radioactivity in plasma. Comparison of the plasma and whole blood total radioactivity data and plasma acalabrutinib data also indicate that a large percentage of exposure was due to metabolites.

Visual inspection of the PK parameters indicated there were no notable gender differences.
Safety results:
The incidence of treatment-emergent AEs (TEAEs) was minor. All TEAEs, irrespective of relatedness by the Investigator, were mild (Grade 1) in severity and resolved by the end of the study. All drug-related TEAEs occurred in Cohort 1, possibly due to differences between the cohorts in procedure, formulation, and/or individual sensitivity (direct correlations cannot be verified). There were no serious AEs and no subjects discontinued the study due to a TEAE. Clinical safety assessments, including AEs, clinical laboratory evaluations, vital sign measurements, and 12-lead ECGs, were unremarkable. Single oral doses of 100 mg acalabrutinib were therefore safe and well tolerated when administered to healthy male and female subjects in this study.
Conclusions:
- After a single 100-mg oral dose of acalabrutinib followed 58 minutes later by a single IV microtracer solution dose (<10 µg; ≤1 µCi) of [^{14}C]ACP-196, the absolute bioavailability of acalabrutinib was 25.3% (range 20.7% to 31.3%);
- For the IV dose, after reaching C_{max}, plasma [^{14}C]ACP-196 declined at approximately the same rate as the orally-derived acalabrutinib concentrations with $t_{1/2}$ values of 1.78 hours for [^{14}C]ACP-196 and 1.57 hours for acalabrutinib;
- Mean CL/F and V_z/F values for acalabrutinib in plasma were 163 L/h and 344 L, respectively, following oral administration of 100 mg acalabrutinib. Mean CL and V_z values were 39.4 L/h and

98.0 L, respectively, following the IV microtracer dose;

- Following a single 100-mg oral dose of acalabrutinib containing a microtracer dose (<10 µg; ≤1 µCi) of [^{14}C]ACP-196, the majority of total ^{14}C radioactivity was eliminated in feces with mean recoveries of total ^{14}C radioactivity in feces and urine of 83.5% and 12.0%, respectively, and mean overall recovery of 95.7%;
- Levels of exposure for acalabrutinib in plasma were lower than total ^{14}C radioactivity in plasma (with a mean acalabrutinib [ACP-196] to total ^{14}C radioactivity ratio of 0.0635 for $AUC_{0-\infty}$), indicating that acalabrutinib contributed only a small proportion of total ^{14}C radioactivity exposure with the majority of total ^{14}C radioactivity arising from metabolites;
- While the total radioactivity levels observed in whole blood and plasma were very similar during the initial absorption and elimination phases, a clear trend of increased blood-to-plasma ratio over time was observed for total radioactivity during the terminal elimination phase. Total radioactivity in whole blood accounted for approximately 86% and 184% of the total radioactivity in plasma based on mean AUC_{0-12} and AUC_{0-168}, respectively;
- Single oral doses of 100 mg acalabrutinib were safe and well tolerated when administered to healthy male and female subjects in this study;
- All drug-related TEAEs were experienced by subjects in Cohort 1, possibly due to differences between the cohorts in procedure, formulation, and/or individual sensitivity (direct correlations cannot be verified);
- No clinically significant changes or findings were noted from AEs, clinical laboratory evaluations, vital sign measurements, or 12-lead ECGs for this study.

Date of report: 07 October 2016