Absorption, Metabolism, Excretion, and the Contribution of Intestinal Metabolism to the Oral Disposition of [14C]Cobimetinib, a MEK Inhibitor, in Humans

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Abbreviations: AUC, area under the curve; CL, clearance, C_max, maximum concentration; F, bioavailability; F_a, fraction absorbed; F_g, fraction escaping gut wall elimination; F_h, fraction escaping hepatic elimination; HLM, human liver microsomes; PK, pharmacokinetics, SD, standard deviation; t_max, time to reach C_max.
Abstract

The pharmacokinetics, metabolism, and excretion of cobimetinib, a MEK inhibitor, were characterized in healthy male subjects (n=6) following a single 20 mg (200 μCi) oral dose. Unchanged cobimetinib and M16 (glycine conjugate of hydrolyzed cobimetinib) were the major circulating species, accounting for 20.5% and 18.3% of the drug-related material in plasma up to 48 h post-dose, respectively. Other circulating metabolites were minor, accounting for less than 10% of drug-related material in plasma. The total recovery of the administered radioactivity was 94.3% (±1.6%, standard deviation) with 76.5% (±2.3%) in feces and 17.8% (±2.5%) in urine. Metabolite profiling indicated that cobimetinib had been extensively metabolized with only 1.6% and 6.6% of the dose remaining as unchanged drug in urine and feces, respectively. In vitro phenotyping experiments indicated that CYP3A4 was predominantly responsible for metabolizing cobimetinib. From this study, we concluded that cobimetinib had been well absorbed (F_a = 0.88). Given this good absorption and the previously determined low hepatic clearance, the systemic exposures were lower than expected (F=0.28). We hypothesized that intestinal metabolism had strongly attenuated the oral bioavailability of cobimetinib. Supporting this hypothesis, F_g was estimated to be 0.37 based on F and F_a from this study and F_k from the absolute bioavailability study (F=F_a * F_k * F_g). PBPK modeling also showed that intestinal clearance had to be included to adequately describe the oral profile. These collective data suggested that cobimetinib was well-absorbed following oral administration and extensively metabolized with intestinal first-pass metabolism contributing in its disposition.
Introduction

Cobimetinib (GDC-0973/XL518, chemically identified as (S)-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)phenyl)(3-hydroxy-3-(piperidin-2-yl)azetidin-1-yl)methanone, Figure 1) is a novel therapeutic small molecule being developed by F. Hoffman-La Roche/Genentech. The molecule is a potent and highly selective inhibitor of MEK1/2, a kinase that activates ERK1/2 in the mitogen-activated protein kinase (MAPK) signaling cascade. The MAPK signaling cascade transduces multiple proliferative and differentiating signals within tumor cells and includes four major mammalian MAPK pathway modules: extracellular signal-regulated kinases (ERK) 1 and 2, c-Jun NH2-terminal kinase, p38 kinase, and ERK5 (Johnson and Lapadat, 2002; Roberts and Der, 2007). In the phase III coBRIM study, cobimetinib plus vemurafenib reduced the risk of disease worsening or death by half in patients with BRAF V600-mutated metastatic melanoma (hazard ratio = 0.51, 95% confidence interval = 0.39–0.68, P < 0.0001). The median progression-free survival was 9.9 months for cobimetinib plus vemurafenib compared to 6.2 months with vemurafenib alone (Larkin et al., 2014). The U.S. Food and Drug Administration (FDA) granted Priority Review for a New Drug Application for cobimetinib in combination with vemurafenib for the treatment of patients with BRAF V600 mutation–positive advanced melanoma in 2015.

Human radiolabeled studies are the accepted standard for providing a definitive understanding of the ADME properties of a drug since the radiolabel assures that all of the drug-related material can be accounted for. From the radiolabel study, in addition to obtaining PK for the drug, the identity and concentrations of circulating metabolites and the pathways of elimination (metabolism or excretion) are revealed (Beumer et al., 2006; Penner et al., 2009). Prior to the human mass balance study, the ADME properties of cobimetinib were characterized in the preclinical species with 14C-radiolabeled mass balance studies (Choo et al., 2012). In rats and dogs, the administered dose was well-absorbed with 70-80% of the radioactivity recovered in urine and bile. Metabolism was extensive with biliary excretion of metabolites as the primary pathway for elimination. The major metabolic pathways were oxidative though some
species differences in metabolism existed with rats primarily hydroxylating the aromatic core and dogs sequentially oxidizing the piperidine-azetidine moieties (manuscript in preparation).

The pharmacokinetics of cobimetinib following oral administration in patients with solid tumors have also been previously described (Musib et al., 2011). Cobimetinib exhibited dose-proportional kinetics (~3.5 to 100 mg) with coefficient of variability in exposure (AUC) ranging from 21-120%. It has a low apparent clearance with terminal half-life ($t_{1/2}$) of approximately 50 h supporting once-daily dosing. The pharmacokinetics of cobimetinib following oral and IV administration have also been characterized in healthy subjects and the absolute bioavailability of cobimetinib was determined to be 46.2% (Musib et al., 2013). The human mass balance study described here was conducted in healthy subjects and, therefore, the administered dose (20 mg) was lower than the clinical therapeutic dose for cancer patients (60 mg). Given its dose-proportional PK, extrapolation of data from the 20 mg dose group in healthy subjects was expected to adequately assess the fate of cobimetinib at its clinical therapeutic dose in patients. A caveat, however, is that differences in exposures between healthy subjects and cancer patients have been reported (Cheeti et al., 2013; Coutant et al., 2015).

Physiologically-based pharmacokinetic modeling (PBPK) is a powerful tool that allows for predictions and investigations; for example, drug-drug interactions (DDI), special populations, formulation changes, regional absorption, etc. (Agoram et al., 2001; Heikkinen et al., 2012; Huang and Rowland, 2012; Zhao et al., 2011). For cobimetinib, a base PBPK model was developed that described the IV PK profile. This model enabled simulations where the effects of food, permeability, particle size, and solubility on the absorption of cobimetinib were tested with sensitivity analysis and these data have been previously reported (Musib et al., 2013). The mass balance study in humans provided data which refined and verified the inputs for the cobimetinib PBPK model and, thereby, increased confidence in the model for simulating the effects of other clinical scenarios, such as DDI.
The objectives of the current study were to determine the routes of excretion, characterize the metabolites, and understand the factors affecting the biotransformation/disposition of cobimetinib. In addition, PBPK simulations were used to test the hypothesis that intestinal metabolism plays a role in the oral disposition of cobimetinib.

**Materials and Methods**

**Radiolabeled Drug and Reference Compounds.** Cobimetinib and [14C]cobimetinib (radiochemical purity >98%) were synthesized by F Hoffmann LaRoche, Ltd (Basel, Switzerland). For [14C]cobimetinib, the radiolabel was evenly distributed in the fluoro-iodoaniline ring (55 mCi/mmol specific activity, Figure 1). Synthetic standards for metabolites M12, M16, and M19 were synthesized at Genentech and Roche. 13C6-cobimetinib (used as an internal standard for bioanalysis) was synthesized at Ricerca Biosciences.

**Materials.** 1-Aminobenzotriazole (ABT) was purchased from Spectrum Chemical Corporation (Gardena, CA), CYP3cide was from Toronto Research Chemicals (Toronto, ON), and other chemical inhibitors (furafylline, tranylcypromine, ticlopidine, quercetin, sulfaphenazole, quinidine, ketoconazole, troleandomycin, fluconazole), reduced β-nicotinamide adenine dinucleotide phosphate tetrasodium salt (NADPH), uridine 5′-diphosphoglucuronic acid trisodium salt (UDPGA), and alamethicin were from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of analytic grade and were obtained from commercial sources. Human liver microsomes (HLM, pool of 150 donors, mixed sex), CYP Supersomes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, and 3A5), and UGT Supersomes (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17) were purchased from BD Biosciences (San Jose, CA). CYP3A5 genotyped single donor HLMs were from Xenotech (Lenexa, KS) and Corning Discovery Labware (Tewksbury, MA).

**Study Design.** This was a single-center, open-label, nonrandomized study with the oral administration of radiolabeled cobimetinib (20 mg with approximately 200 μCi of radioactivity) to six healthy male
subjects to determine the PK of the parent drug and to characterize metabolites in circulation and excreta.

The study was conducted at Covance Clinical Research Unit (Madison, WI). The study followed the guidelines of the World Medical Association Declaration of Helsinki in its revised edition, the current guidelines for Good Clinical Practice, and other applicable regulatory requirements. All subjects provided written informed consent.

**Subjects.** Volunteers that were eligible for inclusion in this study were male (18–55 years of age; body mass index, 18.5–29.9 kg/m²) in good health, as determined from a medical history, vital signs, 12-lead electrocardiogram, and clinical laboratory evaluations. Exclusion criteria included any clinically significant allergic disease or clinical manifestation of any significant metabolic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neurological, or psychiatric disorder, or receiving any other investigational drug within 5 half-lives or 30 days prior, whichever was longer, or a radiolabeled investigational drug within 6 months prior. Subjects with history of glaucoma or retinal vein occlusion, neurosensory retinal detachment, or predisposing factors to retinal vein occlusion were also excluded.

**Dose Preparation and Administration.** The final dose of cobimetinib was prepared by Covance Clinical Research Unit (Madison, WI) by dissolving 20 mg (200 µCi) of [14C]cobimetinib in Crystal Light® solution. The dosed radioactivity was not expected to represent a significant radiation exposure risk in man based on dosimetry from quantitative whole-body autoradiography using rats (Choo et al., 2012). Each subject was admitted on day -1 and on day 1, after at least a 10 h fast, received the single 20 mg dose as an oral solution followed by 240 ml of water to rinse the dosing container. Subjects remained ambulatory (seated or standing) for 1 h following dose administration. Subjects were restricted from caffeinated beverages, grapefruit juice, alcohol, and concomitant medications during the entire duration of the study. Subjects were confined to the clinic for a minimum of 13 days and a maximum of 28 days. Subjects were discharged when blood and plasma radioactivity levels were below the limit of quantitation in two consecutive samples; and ≥90% of the administered dose had been recovered or there was minimal excretion of radioactivity in urine and feces (<1% of dose) in consecutive collections.
Sample Collection. Blood samples for PK analysis of cobimetinib and metabolite profiling and identification were collected predose and at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 192, 264, 336, and 408 h post dose. Urine was collected predose and at 0 to 6 h, 6 to 12 h, 12 to 24 h; and then at 24-h intervals until the subject was discharged from the clinic. For each period, all urine produced by a subject was collected and stored in dark containers and refrigerated. At the end of the collection period, the bulk urine was mixed thoroughly, the total weight recorded, and aliquots transferred to polypropylene containers and stored frozen at approximately -70°C until analysis. Fecal samples were collected at predose and at 24-h intervals until the subject was discharged from the clinic. Each fecal collection was transferred to a tared container and homogenized with approximately 2 to 3 volumes of water and the weight of the fecal homogenate sample was recorded. For each collection interval, 10 g of each fecal homogenate sample was transferred to a separate container for measurement of total radioactivity by liquid scintillation counting (LSC) and the remaining bulk fecal homogenate samples were stored at -20°C or below until they were used for preparing pooled samples for biotransformation analyses.

Determination of Radioactivity and Cobimetinib Plasma Concentrations. Radioactivity and cobimetinib plasma concentrations were determined at Covance Laboratories Inc. (Madison, WI). The radioactivity in plasma and urine were determined by LSC, and in blood and feces were determined by combustion followed by LSC of the trapped $^{14}\text{C}O_2$. Cobimetinib was quantified in plasma using a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) method (Deng et al., 2014). The plasma samples were mixed with $^{13}\text{C}_6$-cobimetinib and then prepared using supported liquid extraction. The plasma extracts were concentrated under nitrogen, and reconstituted for analysis. The LC-MS/MS system consisted of a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC), LC-10AD pumps (Shimadzu, Columbia, MD), and an API4000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA). The samples were injected onto a Luna PFP (2×50 mm, 3 μm, Phenomenex, Torrance, CA) column and eluted with a gradient method that used mobile phases A (water with 0.1% formic acid) and B (acetonitrile (ACN) with 0.1% formic acid). The total run time was 3 min and the flow
rate was 0.5 ml/min. The ionization was conducted in the positive ion mode for cobimetinib and its internal standard, $^{13}$C$_6$-cobimetinib, using the selected reaction monitoring (SRM) transitions $m/z$ 532.1$\rightarrow$249.1 and 538.1$\rightarrow$255.1, respectively. The standard curve ranged from 0.200 to 100 ng/ml using 50 µl of plasma and the lower limit of quantification of cobimetinib was 0.200 ng/ml.

**Extraction of Metabolites from Biological Samples.** Plasma samples at predose, and 1, 2, 4, 8, 24, and 48 h post-dose were pooled by combining equal volumes of plasma from each subject and the subject pooled samples at these selected time points were profiled. Time-based pooling of plasma was not pursued since the recovery of radioactivity from plasma was different across time points and biased the metabolite profile to earlier time points. Plasma samples (2 ml) were extracted twice with two volumes of ACN. The extracts from each step were combined and concentrated under vacuum, then redissolved with water:methanol (2:1, v:v, ~0.2 ml for approximately 10x concentration of radioactivity) for radiometric analysis. Time pooled urine and feces samples were prepared for each subject by mixing an equal percentage of the excreted volume or weight of each collection. The pooled urine and feces samples represented >90% of the radioactivity excreted in that route (pooled urine were for periods of 0-144 to 0-192 h, and pooled feces were for periods of 0-96 to 0-312 h post-dose depending on the rate that radioactivity was excreted by the individual subject). Urine samples were concentrated under vacuum, the radioactivity components were re-dissolved with the addition of a small volume of ACN, and the supernatant was injected onto the LC column. Fecal homogenates were extracted twice with ACN and the supernatants were separated and pooled together. The combined supernatants were evaporated to dryness under vacuum and the residues were reconstituted with water:methanol (2:1, v:v) before injection onto the LC column.

**Metabolite Profiling.** Chromatographic separations were completed using an Accela LC system (Thermo Scientific, San Jose, CA) and CTC HTS PAL autosampler (LEAP Technologies) using a Luna C18(2) column (3 µm, 4.6×250 mm, Phenomenex) and mobile phases A (0.4% formic acid in water adjusted to pH 3.2 with ammonium hydroxide) and B (methanol). Gradient method A (total run-time 113 min) was used for urine and feces samples: initial conditions were 0% B for 3 min, increase to 15% B over 5 min,
increase to 30% over 15 min and maintain for 12 min, increase to 42% B over 45 min, increase to 70% over 7 min, and increased to 100% over 4 min. The column was flushed at 100% B for 5 min, then returned to 0% B over 2 min and re-equilibrated to these conditions for 15 min. Gradient method B (total run-time 128 min) was used for plasma samples to provide additional chromatographic separation of late-eluting metabolites. This gradient was the same as method A up to reaching 42% B, but then increased to 58% B over 22 min, to 100% B over 4 min, and then the column was flushed and re-equilibrated as in method A. For detection, the flow from the column was split 10:1 with the minor component directed to the mass spectrometer and the major component directed to a fraction collector for collection to DeepWell LumaPlate 96-well plates (Perkin Elmer, Waltham, MA) based on time (15 sec/fraction). Following fraction collection, the plates were dried under vacuum using a Savant (Thermo Scientific) with low or medium heat setting for up to 8 h. The radioactivity in each fraction was measured using a TopCount Scintillation and Luminescence Counter (Perkin Elmer) for 5 min. Radiochromatograms were reconstructed using Laura Evaluation software (LabLogic, Sheffield, UK) and all radiopeaks that were discernable over background (signal at least 3×) were integrated to determine the distribution of radioactivity in each sample.

**Metabolite Identification.** The proposed metabolites structures were determined from MS data that corresponded to the radiodetection of drug-related analytes. All radiopeaks in plasma and the radiopeaks that accounted for greater than 0.5% of the dose in urine or 1% of the dose in feces were targeted for identification. Mass spectra were obtained with an LTQ Orbitrap high-resolution mass spectrometer equipped with an electrospray ionization source from Thermo Scientific (San Jose, CA). The electrospray ion source voltage was 5.0 kV. The heated capillary temperature was 350ºC. The scan-event cycle consisted of a full-scan mass spectrum at a mass resolving power of 30,000 (at m/z 400) and the corresponding data-dependent MS/MS and MS^n scans were acquired at a resolving power of 7,500. Accurate mass measurements were performed using external calibration.
Pharmacokinetic Evaluation. PK parameters were calculated by noncompartmental methods (Gibaldi and Perrier, 1982) using WinNonlin (Pharsight, Mountain View, CA) version 5.1.1. AUC$_{0\rightarrow t}$ was calculated for hour 0 to the last measurable concentration (t), which was 264 h for total radioactivity in plasma and 408 h for cobimetinib in plasma. AUC$_{0\rightarrow \infty}$ was calculated as AUC$_{0\rightarrow t}$ plus the extrapolated area from the last measurable concentration to infinity. AUCs were calculated using the linear trapezoidal rule and $t_{1/2}$ was calculated from the apparent terminal elimination phase rate constant. Pharmacokinetic parameter estimates are presented as mean ± S.D. To estimate the plasma concentrations of metabolites, the percent contributions from radioprofiling were multiplied by the concentrations of total radioactivity (ng-eq per g) for the 1, 2, 4, 6, 24, and 48 h post-dose plasma samples. The plasma exposures for parent compound and metabolites (AUC$_{0\rightarrow 48\text{~h}}$) were calculated from the concentration-time data with the trapezoidal method using Excel 2003 (Microsoft, Redmond, WA).

In Vitro Metabolism. Cobimetinib was incubated with HLM (0.5 mg/ml) in potassium phosphate buffer (100 mM, pH 7.4) with the necessary cofactors. Incubations for P450-mediated clearances were supplemented with NADPH (1 mM). Incubations for UGT-mediated clearances were supplemented with MgCl$_2$ (10 mM) and UDPGA (5 mM) and the microsomes were activated with alamethicin (50 µg/mg microsomal protein, pre-incubated on ice for 5 min). For CYP phenotyping experiments, mechanism-based CYP inactivators (ABT, P450, 1 mM; furafylline, CYP1A2, 10 µM; or troleandomycin, CYP3A, 20 µM) were pre-incubated for 15 min with HLM that were supplemented with NADPH. Other inhibitors (tranylcypromine, CYP2A6, 1 µM; ticlopidine, CYP2B6/2C19, 10 µM; quercetin, CYP2C8/2C9, 10 µM; sulfaphenazole, CYP2C9, 10 µM; quinidine, CYP2D6, 1 µM; and fluconazole, UGT2B7, 2.5 mM) were added immediately before cobimetinib was added. The contribution of CYP3A5 to HLM clearance of cobimetinib was determined following similar experimental conditions as have been previously described (Tseng et al., 2014). For additional phenotyping experiments, cobimetinib was incubated with P450 Supersomes (40 pmol/ml) or UGT Supersomes (0.5 mg/ml). Reactions were at 1 µM (except for M15 generation experiments, when it was 20 µM) and conducted at 37°C. At 0 and 60 min, aliquots of the
reaction mixture were transferred to ACN containing internal standard. The samples were centrifuged at 2,000 × g for 10 min, and the supernatants were diluted with water and analyzed by LC-MS/MS. Cobimetinib was monitored with the SRM transition 532.1 → 249.1 and the percentage drug remaining in the reaction was determined by comparing to t=0 samples. For UGT experiments, M15 (glucuronide conjugate) formation was monitored by SRM with the neutral loss of 176 Da (708.1 → 532.1). For in vitro clearance estimates, the in vitro t₁/₂ was calculated as -0.693/k with k from the slope of the linear regression of log percentage substrate remaining vs incubation time. The in vitro t₁/₂ was scaled to hepatic clearance with the incubation conditions (concentration, time, and microsomal protein), microsomal content for liver taken to be 45 mg per g liver, and liver weight taken to be 20 g per kg body weight (Obach et al., 1997).

Estimating Oral Bioavailability (F), Fraction of Dose Absorbed (Fₐ), Fraction of Dose Escaping Gut Metabolism (Fₖ), and PBPK Modeling and Simulations. The hepatic clearance (assumed as CL_Total; as renal CL was negligible; 1.6% of dose unchanged in urine) of cobimetinib in healthy volunteers was taken from the IV dosing arm of the previously reported absolute bioavailability study (Musib et al., 2013). The mean dose normalized AUC from the current study was compared to that from the IV dosing study to estimate the oral bioavailability (F) for the current study. The fraction of the oral dose absorbed (Fₐ) was estimated as the sum of radioactivity recovered in urine and radioactivity in feces that was characterized as metabolites. This assumed that during gastrointestinal tract transit, unabsorbed drug did not degrade and metabolites did not revert to unchanged cobimetinib. The fraction of dose that escaped gut metabolism (Fₖ) was then calculated from the relationship of F=Fₐ*Fₖ*Fₕ with F and Fₐ from the current study and Fₕ from the IV dosing study.

The PBPKPlus™ module of GastroPlus™ simulation software (SimulationsPlus Inc, Lancaster, CA) was used to simulate the oral PK profile of cobimetinib from this ¹⁴C study using inputs as previously described (Musib et al., 2013). The enterocyte binding was set as 5.8% unbound (based on plasma protein binding). For all other parameters, default settings of GastroPlus™ were used.
Results

Demographic, Safety, and Tolerability Data. Six healthy male volunteer subjects (four Caucasian and two African-American) were enrolled. Five of the six enrolled subjects completed the study in accordance with the protocol. One subject withdrew 17 days after dosing, but was not replaced and was included in overall data analysis. This withdrawn subject received a full dose of cobimetinib on Day 1, had all plasma, whole blood, urine, and feces samples collected through 408 hours postdose, and the cumulative percent of radioactive dose recovered in urine and feces for this subject was >90%. The mean age, weight, and BMI for the subjects were 32 years (range: 21-47 years), 82.2 kg (range: 74.7-92.7 kg), and 25.5 kg/m² (range 20.5 to 27.6 kg/m²), respectively.

Overall, the oral dose of cobimetinib was well tolerated. There were no serious adverse events (AEs) and no subject was withdrawn from the study as a result of an AE. Five subjects (83.3%) experienced a total of 9 AEs, which were all Grade 1. All AEs occurred greater than 24 hours after dosing, were mild in severity, and were resolved by the end of the study. The majority (7 of 9 events) were considered drug-related by the Investigator. The most common AE was categorized as gastrointestinal disorders, which included change in bowel movement, hard feces, nausea, and diarrhea. To assist/improve their bowel movements, all of the subjects received concomitant single doses of docusate sodium (100 mg). There were no clinically important changes in clinical laboratory values, vital signs, physical examinations, electrocardiograms or visual disturbances.

Pharmacokinetics. The mean concentration-time profiles for plasma cobimetinib and total radioactivity are presented in Figure 2 with the PK parameter estimates summarized in Table 1. Cobimetinib and total radioactivity median $t_{\text{max}}$ were observed at 4.0 and 2.0 h, respectively. The mean $C_{\text{max}}$ for cobimetinib in plasma was 10.0 ng/ml, while the mean $C_{\text{max}}$ for total radioactivity in plasma was 68.2 ng eq/g. After reaching $C_{\text{max}}$, cobimetinib and total radioactivity concentrations in plasma declined with mean terminal $t_{1/2}$ values of 75.5 and 141 h, respectively. The mean $\text{AUC}_{0-\infty}$ for cobimetinib in plasma (495 ng·h/ml) was
approximately 10.7% of the mean total radioactivity $AUC_{0-\infty}$ in plasma (4621 ng eq·h/g). The mean blood/plasma ratio for total radioactivity was 0.805 and 0.979 for $C_{\text{max}}$ and $AUC_{0-\infty}$, respectively.

**Mass Balance/Excretion in Urine and Feces.** The cumulative recoveries of radioactivity from the six subjects are shown graphically in Figure 3. An average of 94.3%±1.6% of the administered 20 mg dose of $[^{14}\text{C}]$cobimetinib was recovered over the 408-hour study. The majority of the administered radioactivity was recovered in feces with a mean cumulative percentage of 76.5%±2.4% of the administered radioactivity. The radioactivity recovered in urine cumulatively accounted for 17.8%±2.5% of the administered radioactivity. The majority of the radioactivity (>80% of the dose) was recovered within the first 168 h post-dose.

**Metabolite Profiles in Plasma and Excreta.** Radioprofiles were determined for plasma samples at 1, 2, 4, 6, 24, and 48 h post-dose pooled across all six subjects. Representative profiles are shown in Figure 4 and the calculated concentrations of analytes determined for all plasma profiles are presented in Table 2. The recoveries of radioactivity from plasma by protein precipitation decreased as time increased post-dose. From the 1 h sample, 93% of the radioactivity recovered; whereas, from the 48 h sample, 56% of the radioactivity recovered. The most abundant analytes at time points up to 24 h were unchanged cobimetinib and M16 (glycine conjugate of hydrolyzed cobimetinib), which accounted for 20.5 and 18.3% of the exposures to total drug-related material up to 48 h, respectively. M15 (direct glucuronidation) accounted for 7.4% of the exposure up to 48 h. At 24 and 48 h post-dose, M16 and unchanged drug accounted for approximately equal percentages of total plasma radioactivity. Other minor circulating metabolites (accounting for less than 5% of the total circulating drug-related material) were oxidative products (M12, M18, M19, M21, and M40) and their glucuronide conjugates (M6, M20, M44, M45, M57, M59, and M60). Plasma samples from after 48 h post-dose did not have enough radioactivity in their extracts for profiling. Extracts for plasma from 24 through 192 h post-dose were analyzed by mass spectrometry using SRM for cobimetinib (532.1→249.1) and M16 (negative ion mode electrospray, 449.1→127.1), and M16 levels declined with a slope that was similar to cobimetinib (in-house data).
Urine and feces pools from each subject were analyzed for metabolite profiles. Representative metabolic profiles are shown in Figure 5 and the distributions of metabolites are presented in Table 3. The radioactivity was widely distributed with few major metabolites. Across subjects, the same profile of metabolites was observed with reasonable subject-to-subject variability in metabolite abundances (mean variability in abundances (CV) were 25% and 27% for the identified urinary and fecal metabolites, respectively). Unchanged cobimetinib in urine accounted for 1.6% of the administered dose. M15 was the major metabolite in urine, which accounted for 2.1% of the dose, and all other metabolites were trace level (<1% of the dose). In feces, unchanged cobimetinib accounted for 6.6% of the administered dose. The major metabolites in feces were M5 (dioxidation, +30 Da), M10 (mono-oxidation), M29 (tri-oxidation, +46 Da), M56 (tri-oxidation, +48 Da), and M62 (mono-oxidation of M40), which individually accounted for 5.2-10.3% of the administered dose. Six other minor metabolites (M21, M52, M53, M55, M28, and M40) individually accounted for 2.1-3.3% of the dose in feces, and the remaining radioactivity was accounted for by trace level metabolites (<2% of the dose). Assuming no degradation of unabsorbed drug and no metabolites reverted to unchanged cobimetinib during transit in the gastrointestinal tract, or enterohepatic recirculation (supported by observations in preclinical species), the fraction of the dose absorbed (Fa) following oral administration in healthy subjects was estimated to be 0.88 using the sum of radioactivity recovered in urine (17.8%) and radioactivity in feces that was characterized as metabolites (69.9%). It was noted that M15 (glucuronide conjugate) was absent in feces, presumably due to β-glucuronidase activities in the GI tract, so the estimated fraction absorbed was likely underestimated and represented the minimum value for Fa.

**Metabolite Identification.** Twenty-five metabolites were identified in the current study that were either in plasma samples or accounted for greater than 2% of the administered dose in urine and feces. The proposed structures of these metabolites were derived from elemental compositions calculated based on the accurate masses observed for their protonated molecular ions [MH]+ for all metabolites except M16, which was analyzed in negative ion mode, and structural elucidation of the product ions observed.
from collision-induced dissociation experiments. The human metabolites were compared to those reported in the preclinical species (manuscript to be submitted) based on their LC retention times, and MS/MS and MS<sup>n</sup> fragmentation patterns to confirm the same metabolite numbers were assigned to common metabolites. For M12, M16, and M19, synthetic standards were available to confirm the structures of the human metabolites. The proposed metabolic pathways for cobimetinib in humans are summarized in Figure 6 with the structural elucidation data for metabolites provided in Supplemental Table 2.

**In Vitro Metabolism of Cobimetinib.** Clearance (hepatic) values of 18.7, 2.9, and 17.8 ml/min/kg were observed for cobimetinib with HLM supplemented with NADPH, UDPGA, and both co-factors, respectively. In preliminary experiments, activation of microsomes with alamethicin did not impact the NADPH-dependent metabolism of cobimetinib and addition of 2% bovine serum albumin to activate UGT2B7 did not impact the UDPGA-dependent clearances of cobimetinib. The metabolism of cobimetinib by HLM was NADPH-dependent and fully inhibited by ABT (a broad-spectrum inactivator of P450), indicating that it was primarily P450-mediated. Ketoconazole and troleandomycin effectively inhibited the metabolism, which identified CYP3A as the major P450s involved in metabolizing cobimetinib (Figure 7A). This was consistent with the findings from experiments using individual expressed recombinant P450 Supersomes, where the greatest turnover was measured with rCYP3A4 and rCYP3A5 (Figure 7B). In the UGT reaction phenotyping study using Supersomes, M15 formation was mediated mainly by UGT2B7 and this was confirmed by inhibition by fluconazole (Figure 7C) (Uchaipichat et al., 2006). Clearance values were low with little difference between ketoconazole and CYP3cide inhibition with genotyped CYP3A5 HLM lots (CYP3A5*1/*1, *1/*3, or *3/*3), which suggested that cobimetinib was mostly or exclusively metabolized by CYP3A4 with minimal contribution by CYP3A5 (in-house data).

**Determining the Contribution of First-Pass Intestinal Metabolism (F<sub>p</sub>) to the Oral Bioavailability of Cobimetinib.** The oral bioavailability (F) for the current study was estimated to be 0.28 ± 0.10 from the ratio of the mean dose normalized AUC compared with that following IV dosing.
study (Musib et al., 2013). The relatively low intersubject variability (CV=28.2%, n=13) in the IV dosing study suggested that hepatic clearance did not differ greatly (i.e., CV less than 50%) between healthy volunteer subjects in separate studies. This supported assuming hepatic clearance and Fh from a separate study (Fh=0.87; where FH = 1-EH; EH = CLs/QH,blood; QH = 20.7 mL/min/kg (87 L/h) for a 70 kg person (Davis and Morris, 1993); because the blood to plasma ratio of cobimetinib was 0.98, hepatic extraction was directly determined from total systemic plasma CL) for subjects in the current study. Taking the fraction of the oral dose absorbed (Fa) to be 0.88 ± 0.02 (mean ± SD) (described above), Fg was estimated to be 0.37 ± 0.14.

While the PBPK model was successful in describing the PK profile of cobimetinib after IV administration in the absolute bioavailability study described by Musib et al., 2013 (Figure 8A), simulation of the oral PK profile with the same parameters (predicted Fa=1.0) overestimated the observed Cmax and AUC by 6-fold (59.2 vs. 10.0 ng/ml) and 3-fold (1450 vs. 495 µg.h/ml), respectively (Figure 8B). Previously-described sensitivity analysis (testing ~10-fold lower and higher than the observed/experimental value) indicated that permeability (0.1 to 10 cm/s ×10^4), particle size (2.5 to 250 µM) and solubility (0.079 to 7.9 mg/mL) had little impact on the PK profile of cobimetinib (Musib et al., 2013). We did note that the observed mean AUC in the current study was ~1.5-fold lower than in the absolute bioavailability study, which was likely due to inter-subject variability; however, the base PBPK model overestimated exposures by 2- to 3-fold in both cases. To better describe the PK data (to within 1.5 fold of observed AUC), an addition of intestinal CL (to best fit) was necessary (Figure 8C). Using the refined model, the simulations described the elimination half-life (61 h, predicted vs. 75.5 h, observed) and AUC_{0-τ} (420 ng.h/mL, predicted vs. 495 ng.h/mL, observed) with good success.
DMD # 66282

Discussion

The objective of the current study was to characterize the metabolism and excretion of cobimetinib in humans following a single 20 mg oral dose. The radioactive dose was fully recovered with the majority being eliminated in feces (76.5% of the dose) and lesser amounts eliminated in urine (17.8% of the dose). Most of the radioactivity (>80% of the dose) was recovered in the first 7 d following dosing. Cobimetinib was well-absorbed with the extent of absorption estimated to be 88%. This was consistent with findings from nonclinical radiolabeled studies where 70-80% of the administered radioactivity was recovered in urine and bile from bile-duct cannulated rats and dogs (Choo et al., 2012). In the preclinical species, metabolites were predominantly excreted in bile (76.5% of the administered dose in rats and 60.3% of the administered dose in dogs), which was consistent with, in humans, the majority of radioactivity being recovered as metabolites in feces. Metabolite profiling of human samples indicated unchanged cobimetinib was the most abundant drug-derived species in plasma. Prior to elimination, cobimetinib was extensively metabolized and unchanged drug in urine and feces accounted for 1.6% and 6.6% of the dose, respectively.

Given the low intersubject variability in total radioactivity PK profiles (CV for AUC and C_{max} were 24 and 28%, respectively), plasma were pooled across subjects at 1, 2, 4, 6, 24, and 48 h post-dose to characterize the circulating radioactivity and estimate metabolite exposures. Total radioactivity in plasma declined more slowly than unchanged drug, which indicated that one or more components of the circulating radioactivity were not unchanged drug. Unchanged cobimetinib and M16 were the major circulating components up to 48 h post-dose (AUC_{0-48 h}), accounting for 20.5 and 18.3% of the circulating drug-related material, respectively. No other metabolite in plasma approached 10% of total drug-related material. After 48 h post-dose, cobimetinib and M16 declined in parallel indicating that M16 clearance was formation rate-limited. The total radioactivity in plasma declined to 22% of C_{max} at 48 h (61.7 ng eq/ml at 2 h and 13.6 ng eq/ml at 48 h) but the unextractable radioactivity showed little decline after 2 h and was approximately the same concentration at 48 h (7.33 ng eq/ml at 2 h and 7.96 ng eq/ml at 48 h).
Thus, the unextractable portion was an increasing percentage of the plasma radioactivity at later time points. These observations were consistent with radioactivity becoming associated with plasma protein(s) and once it was associated, its elimination was determined by the catabolism cycle for the protein, which was long. This became the predominant determinant for the observed long elimination phase for total radioactivity. Over the first 48 h, approximately 31.5% of the radioactivity in plasma was unextractable. Converting this to an amount, based on a total plasma volume of 3000 ml for humans (Davies and Morris, 1993), less than 100 µg or less than 0.1% of the administered dose was in plasma and unextracted. This was consistent with fully recovering the administered radioactive dose in urine and feces over the 17-day study period. To test the hypothesis that the long lived radioactivity was associated with plasma proteins, we employed targeted MS analyses to identify cobimetinib-related adducts of proteins or amino acid residues, but were unsuccessful, presumably due to the low percentage of modified protein and the limitations of detection of the analytical methods. Similar observations of incomplete recoveries of radioactivity from plasma have been reported for lapatinib in humans, though in that case, only unchanged drug was a detectable radiopeak for plasma samples (Castellino et al., 2012).

The metabolic profiles for human samples indicated extensive metabolism of cobimetinib and there were only minor quantitative differences observed for the six subjects. There were a few metabolites that were identified for the first time in this study, but they were found at trace levels (≤2% of the administered dose) except for M56 (tri-oxidation), which accounted for 5% of the dose and was in feces. The proposed structures for these metabolites also suggested they formed from primary metabolites that were common between humans and the preclinical species. Overall, the metabolism in humans was described by predominantly oxidative biotransformations that occurred in successive steps. Oxidation reactions modified the aromatic portion, including deiodination reactions, and at the aliphatic portion, which lead to piperidine ring-opening and further modification. Conjugative reactions, mostly glucuronidation, were also evident for cobimetinib and its oxidative metabolites. In mass balance studies in rats and dogs, cobimetinib was well-absorbed following oral administration and extensively
metabolized, and the metabolic pathways were largely overlapping with humans (manuscript in preparation). Further, the abundant circulating metabolites in humans were observed in one or more tested preclinical species, which validated the choice of these nonclinical species for toxicological studies of cobimetinib. In vitro CYP reaction phenotyping described CYP3A4 as the predominant enzyme for cobimetinib metabolism. For the direct glucuronidation of cobimetinib (to form M15), UGT2B7 catalyzed the conjugation reaction. With HLM, glucuronidation was a minor contribution to the complete metabolism. This was consistent with excreta and plasma profiles, which showed at least 63.5% of the dose was transformed by oxidative pathways. M15 was a minor analyte in excreta (2.1% of the dose in urine) and plasma (7.4% of drug-related material). Thus, it appeared that the predominant pathway for cobimetinib in vivo clearance was oxidative metabolism that was catalyzed by CYP3A4.

In the current study, the absolute bioavailability was determined to be 28%. Since drug absorption did not seem to be a barrier (F_a=0.88 from this study), the leading hypothesis was that extraction of the cobimetinib by the liver and particularly by the gut had limited oral exposures. This hypothesis was consistent with the extensive metabolism observed and the enzymology data that indicated the major metabolic pathways for cobimetinib were CYP3A4-mediated oxidation and, to a lesser extent, UGT2B7-mediated glucuronidation. These enzymes are expressed in the intestine and liver, and have been implicated as major barriers to oral bioavailability by extracting drug by first-pass metabolism (Cubitt et al., 2009; Gertz et al., 2010). Given that the metabolites generated by the intestine could not be distinguished from those generated by hepatic metabolism, we examined the current data set to estimate the extent of gut metabolism that attenuated the systemic oral exposures. First, taking F and F_a from the current study and applying the hepatic clearance determined from the IV dosing study (F_h=0.87) (Musib et al., 2013), F_g was calculated to be 0.37, which was indicative that intestinal metabolism contributed significantly to the oral bioavailability of cobimetinib. Consistent with this premise, there was good correlation between F vs F_g (r^2=0.996), whereas a correlation would not be expected for a compound for which F is only limited by hepatic extraction/CL_systemic/total (Supplementary Figure S2). The inter-
individual and study-to-study variability in mean exposure observed for the current study and the absolute bioavailability study may be a consequence of the variability in the intestinal gut expression of CYP3A4 in individuals. A 6-fold range in exposures is in keeping with observations for other drugs that are CYP3A4 substrates and that are metabolized by the gut (Masica et al., 2004).

Given the added knowledge of factors that impact cobimetinib disposition, we have tested the reliability of a transgenic mouse model with differential expression of CYP3A4 in the gut and/or liver for predicting the relative contribution of hepatic and intestinal metabolism (Choo et al., 2015). The results showed good concurrence with the inference from the human radiolabeled study that cobimetinib was extensively extracted by metabolism at the gut compared to at the liver. Across structurally diverse and structurally highly related species, the relationship between extraction due to metabolism at the gut and liver has not been clear. With benzodiazepines, $F_g$ estimates for alprazolam, triazolam, and midazolam are 0.05, 0.6, and 0.5, respectively, despite only a single atom difference for triazolam and alprazolam (Masica et al., 2004). The challenges in predicting intestinal metabolism are further exemplified by several drugs that are predominantly cleared by CYP3A4 (e.g., simvastatin, buspirone, lovastatin, and tacrolimus), which have low-to-moderate hepatic clearance (as determined with liver microsomes), but are extracted by the gut very extensively (Yang et al., 2007). Clearly, developing reliable methods for predicting the contribution of intestinal extraction to determining bioavailability is an area requiring further study.

With increased understanding of the ADME properties of cobimetinib from the $^{14}$C study – specifically, the estimate for $F_a$ and key insight/confirmation on the involvement of intestinal metabolism – the PBPK model for cobimetinib was refined and utilized to describe the observed PK. With the hepatic clearance, the IV profile of cobimetinib was well described (Figure 8A). However, this base (IV) model did not adequately describe the oral concentration profile of cobimetinib (~3-fold discrepancy in AUC between observed vs. simulated) (Figure 8B). Therefore, parameters that influenced the oral disposition of cobimetinib were evaluated. While various scenarios were simulated (see Supplementary Table S1), the
addition of gut metabolism (to best fit; entered in the model as $K_m$ and $V_{max}$) best described the oral profile of cobimetinib (Figure 8C), which nicely corroborated its relatively large (~40%) role in the oral disposition of cobimetinib. Furthermore, in the clinical interaction study of cobimetinib with itraconazole, 6.7- and 3.2-fold increases in cobimetinib AUC and $C_{max}$, respectively, were observed. To describe the itraconazole DDI, $f_m$ CYP3A of 0.78 and $F_g$ of 0.45 were estimated. Subsequently, PBPK simulations were used to describe the magnitude of interactions from moderate and weak CYP3A4 inhibitors and inducers (American Society of Clinical Pharmacology and Therapeutics Annual Conference, New Orleans, 2015).

In summary, data from the mass balance study indicated that the oral dose of cobimetinib was well-absorbed and extensively metabolized in humans. Unchanged cobimetinib was the main circulating species and there were no human unique metabolites observed in circulation. The majority of the drug-related radioactivity was excreted as metabolites in the feces with minor renal elimination. Cobimetinib metabolism was mediated primarily by CYP3A4, which catalyzed multiple oxidative pathways with extensive sequential metabolism. The oxidative modifications occurred most extensively at the piperidine ring, which resulted in several ring-opened metabolites, and to a lesser extent at the aromatic core of cobimetinib. In addition to characterizing the metabolic fate for cobimetinib, this study allowed the estimation of $F_a$ and, in turn, supported that intestinal metabolism contributes to limiting oral bioavailability. These findings increased our understanding of the disposition and total metabolism of cobimetinib and had implications for interpreting and predicting DDI.
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**Author contributions:**

Participated in research design: Takahashi, Choo, Ma, Wong, Halladay, Rooney, Gates, Dresser, Musib

Conducted experiments: Takahashi, Choo, Wong

Contributed new reagents or analytic tools: Takahashi, Deng

Performed data interpretation: Takahashi, Choo, Ma, Wong, Halladay, Musib

Contributed to the writing of the manuscript: Takahashi, Choo, Ma, Rooney, Gates, Hop, Khojasteh, Musib
References


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Current affiliation for M.J.D.: Denali Therapeutics Inc., South San Francisco, CA

Legends for Figures

Figure 1. Chemical structure of [14C]cobimetinib. The asterisk denotes the location of the 14C radiolabel, which was uniformly distributed throughout the fluoro-iodoaniline ring.

Figure 2. Concentration-time profiles of total radioactivity (solid squares) and cobimetinib (open diamonds) in plasma following a single oral dose of [14C]cobimetinib (20 mg, 200 µCi) given to healthy male subjects. Cobimetinib concentrations were determined by LC-MS/MS analysis. Data points are mean values and error bars are SD for n=6 subjects.

Figure 3. Cumulative excretion of total radioactivity following a single oral dose of [14C]cobimetinib (20 mg, 200 µCi) given to healthy male subjects. Open diamonds are radioactivity in urine, open squares are radioactivity in feces, and solid squares are the sum of urine and feces. Data points are mean values and error bars are SD for n=6 subjects.

Figure 4. Representative metabolite radiochromatograms for plasma pooled from six subjects following a single oral dose of [14C]cobimetinib at 1 h (top), 4 h (middle), and 24 h (bottom) post-dose. Signals that were assigned to metabolite structures have been labeled. The signal at ~100 min was judged to be an artifact because it was also observed in the radioprofile for predose plasma.

Figure 5. Representative metabolite radiochromatograms for urine (upper) and feces (lower) from an individual subject following a single oral dose of [14C]cobimetinib.
Figure 6. Proposed metabolic pathways of cobimetinib in healthy male subjects following a single 20 mg oral dose. Excreted metabolites that accounted for greater than 5% of the dose and all circulating metabolites are described. M17, M32, and the piperidine monooxidation metabolites were not observed in the current study, but are included to describe the likely sequential metabolism of cobimetinib. Samples where a metabolite was observed are indicated in brackets with P for plasma, U for urine, and F for feces.

Figure 7. Reaction phenotyping for cobimetinib. (A) Effect of selective P450 chemical inhibitors on cobimetinib metabolism in pooled HLM. (B) Cobimetinib metabolism by expressed recombinant P450 Supersomes. (C) Formation of M15 by alamethicin-activated HLM and expressed recombinant UGT (solid bars) and effect of fluconazole (UGT2B7 inhibitor, light bars). Data bars are mean values and error bars are SD from triplicate incubations.

Figure 8. The observed PK profile of cobimetinib (symbols, mean±SD) and PBPK fit (line). (A) 2 mg IV fit, (B) single 20 mg oral dose; using parameters from IV fit, (C) single 20 mg oral dose with addition of intestinal CL.
Tables

Table 1. Pharmacokinetic parameters for cobimetinib and total drug-related radioactivity following a single oral dose of [14C]cobimetinib (20 mg, 200 µCi) given to healthy male subjects. Data for all parameters are mean (SD) except $T_{\text{max}}$, which is median (min, max); n=6.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cobimetinib*</th>
<th>Total Plasma Radioactivity</th>
<th>Total Blood Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng-eq/ml)</td>
<td>10.0 (2.70)</td>
<td>68.2 (9.67)</td>
<td>54.9 (5.20)</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.02 (1.00, 8.00)</td>
<td>2.00 (2.00, 2.05)</td>
<td>2.00 (2.00, 2.05)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-t}$ (ng-eq h/ml)</td>
<td>455 (179)</td>
<td>3533 (812)</td>
<td>2925 (497)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (ng-eq h/ml)</td>
<td>495 (183)</td>
<td>4621 (1256)</td>
<td>4012 (759)**</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>75.5 (21.9)</td>
<td>141 (56.9)</td>
<td>97.9 (32.1)**</td>
</tr>
</tbody>
</table>

* units of ng/mL for $C_{\text{max}}$ or ng.h/ml for AUC, ** n=4
Table 2. Plasma concentrations and percentage of total radioactivity AUC\(_{0-48\,\text{h}}\) for cobimetinib and its metabolites following a single oral dose of [\(^{14}\text{C}\)]cobimetinib (20 mg, 200 µCi) given to healthy male subjects. Plasma were pooled across subjects (n=6) at individual time points for metabolite profiling.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Plasma Concentration (ng-eq/ml)</th>
<th>Percentage of Total Radioactivity AUC(_{0-48,\text{h}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Cobimetinib</td>
<td>7.92</td>
<td>10.51</td>
</tr>
<tr>
<td>M6</td>
<td>3.76</td>
<td>4.65</td>
</tr>
<tr>
<td>M12</td>
<td>4.80</td>
<td>5.96</td>
</tr>
<tr>
<td>M15</td>
<td>5.32</td>
<td>7.46</td>
</tr>
<tr>
<td>M16</td>
<td>3.63</td>
<td>6.60</td>
</tr>
<tr>
<td>M18</td>
<td>2.34</td>
<td>3.15</td>
</tr>
<tr>
<td>M19</td>
<td>1.43</td>
<td>2.12</td>
</tr>
<tr>
<td>M20</td>
<td>0.39</td>
<td>1.23</td>
</tr>
<tr>
<td>M21</td>
<td>1.43</td>
<td>1.20</td>
</tr>
<tr>
<td>M40</td>
<td>2.34</td>
<td>1.16</td>
</tr>
<tr>
<td>M44</td>
<td>1.82</td>
<td>2.22</td>
</tr>
<tr>
<td>M45</td>
<td>1.82</td>
<td>2.29</td>
</tr>
<tr>
<td>M57</td>
<td>1.04</td>
<td>1.37</td>
</tr>
<tr>
<td>M59</td>
<td>2.86</td>
<td>1.74</td>
</tr>
<tr>
<td>M60</td>
<td>1.56</td>
<td>0.92</td>
</tr>
<tr>
<td>Not extracted</td>
<td>3.48</td>
<td>7.33</td>
</tr>
<tr>
<td>Total Radioactivity</td>
<td>48.1</td>
<td>61.7</td>
</tr>
</tbody>
</table>

ND, not detected
Table 3. Percentages of administered radioactive dose for cobimetinib and its metabolites in urine and feces following a single oral dose of [14C]cobimetinib (20 mg, 200 µCi) given to healthy male subjects. Metabolites that accounted for less than 2% of the administered dose are not individually listed. Values are mean (SD) for 6 subjects.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Percentage Excreted</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>% of Dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobimetinib</td>
<td>1.6 (0.4)</td>
<td>6.6 (1.7)</td>
<td>8.2 (1.9)</td>
</tr>
<tr>
<td>M5</td>
<td>D</td>
<td>5.2 (0.6)</td>
<td>5.2 (0.6)</td>
</tr>
<tr>
<td>M10</td>
<td>0.3 (0.1)</td>
<td>10.3 (2.1)</td>
<td>10.7 (2.1)</td>
</tr>
<tr>
<td>M15</td>
<td>2.1 (0.9)</td>
<td>ND</td>
<td>2.1 (0.9)</td>
</tr>
<tr>
<td>M21</td>
<td>0.9 (0.1)</td>
<td>2.7 (0.6)</td>
<td>3.6 (0.6)</td>
</tr>
<tr>
<td>M28</td>
<td>ND</td>
<td>2.7 (0.7)</td>
<td>2.7 (0.7)</td>
</tr>
<tr>
<td>M29</td>
<td>0.3 (0.1)</td>
<td>6.9 (2.4)*</td>
<td>7.9 (2.4)</td>
</tr>
<tr>
<td>M62</td>
<td>0.7 (0.1)</td>
<td>6.9 (2.4)*</td>
<td>7.9 (2.4)</td>
</tr>
<tr>
<td>M37</td>
<td>0.3 (0.1)</td>
<td>1.9 (0.6)</td>
<td>2.1 (0.6)</td>
</tr>
<tr>
<td>M40</td>
<td>0.2 (0.1)</td>
<td>3.3 (0.6)</td>
<td>3.5 (0.5)</td>
</tr>
<tr>
<td>M49</td>
<td>0.7 (0.1)</td>
<td>1.8 (0.3)</td>
<td>2.5 (0.3)</td>
</tr>
<tr>
<td>M52</td>
<td>D</td>
<td>2.4 (0.6)</td>
<td>2.4 (0.6)</td>
</tr>
<tr>
<td>M53</td>
<td>D</td>
<td>3.1 (0.4)</td>
<td>3.1 (0.4)</td>
</tr>
<tr>
<td>M55</td>
<td>D</td>
<td>2.1 (0.6)</td>
<td>2.1 (0.6)</td>
</tr>
<tr>
<td>M56</td>
<td>ND</td>
<td>5.3 (2.6)</td>
<td>5.3 (2.6)</td>
</tr>
<tr>
<td>Minor metabolites with oxidative deiodination</td>
<td>2.5 (0.5)</td>
<td>4.9 (0.7)</td>
<td>7.3 (1.1)</td>
</tr>
<tr>
<td>Minor metabolites with oxidation at aromatic</td>
<td>1.3 (0.2)</td>
<td>4.0 (0.9)</td>
<td>5.3 (0.7)</td>
</tr>
<tr>
<td>Other minor metabolites</td>
<td>3.2 (0.7)</td>
<td>7.1 (0.9)</td>
<td>10.3 (1.1)</td>
</tr>
<tr>
<td>Total cobimetinib and identified metabolites</td>
<td>14.0 (2.0)</td>
<td>70.2 (2.2)</td>
<td>84.1 (2.6)</td>
</tr>
<tr>
<td>Total radioactivity</td>
<td>17.8 (2.5)</td>
<td>76.5 (2.3)</td>
<td>94.3 (1.6)</td>
</tr>
</tbody>
</table>

D, detected only by mass spectrometry; ND, not detectable.

* M29 and M62 co-eluted and the sum of the two metabolites is presented.
Figure 2

The graph shows the plasma concentration (ng (Eq)/ml) over time (h) for different conditions. The y-axis represents the plasma concentration ranging from 0.01 to 100.00, while the x-axis represents time in hours from 0 to 264. Two distinct lines with error bars indicate the variability in the data points over time.
Figure 7

A

% Remaining

+NADPH -NADPH +ABT (P450) +tirafusivine (1A2) +ticlopidine (2A6) +sulaphenazole (2C9) +quercetin (2C9) +quinidine (2D6) +ketoconazole (3A4/5) +roleandomycin (3A4/5)

B

% Remaining

rCYP Enzyme

1A2 2A6 2B6 2C8 2C9 2C19 206 2E1 3A4 3A5

C

M15 formed

HLM 1A1 1A3 1A4 1A6 1A7 1A9 1A10 2B4 2B7 2B15 2B17

UGT Enzyme