Metabolism and Disposition of Oral Dabrafenib in Cancer Patients: Proposed Participation of Aryl Nitrogen in Carbon-Carbon Bond Cleavage via Decarboxylation following Enzymatic Oxidation

David A. Bershas, Daniele Ouellet, Donna B. Mamaril-Fishman, Noelia Nebot, Stanley W. Carson, Samuel C. Blackman, Royce A. Morrison, Jerry L. Adams, Kristen E. Jurusik, Dana M. Knecht, Peter D. Gorycki, and Lauren E. Richards-Peterson


Primary laboratory of origin: Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline.
Running Title

Dabrafenib Disposition in Cancer Patients

Corresponding Author:

Lauren E. Richards-Peterson, Ph.D.

Department of Drug Metabolism and Pharmacokinetics

GlaxoSmithKline UW2730

709 Swedeland Road

P.O. Box 1539

King of Prussia, Pennsylvania, 19406

Tel: 610-270-4548

Fax: 610-270-5041

Email: Lauren.E.Richards-Peterson@gsk.com

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Non-standard Abbreviations:

ADME absorption, distribution, metabolism and excretion
AUC area under the curve
<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>DPM</td>
<td>disintegrations per minute</td>
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<tr>
<td>DRM</td>
<td>drug-related material</td>
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<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>LLQ</td>
<td>lower limit of quantitation</td>
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<tr>
<td>LSC</td>
<td>liquid scintillation counting</td>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>MS/MS</td>
<td>tandem mass spectrometry</td>
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<td>UHPLC</td>
<td>ultra-high-performance liquid chromatography</td>
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Abstract

A Phase I study was conducted to assess the metabolism and excretion of [14C]dabrafenib (GSK2118436), a BRAF inhibitor, in four patients with BRAF V600 mutation positive tumors after a single oral dose of 95 mg (80 µCi). Assessments included the following: 1) plasma concentrations of dabrafenib and metabolites using validated UHPLC-MS/MS methods, 2) plasma and blood radioactivity, 3) urinary and fecal radioactivity, and 4) metabolite profiling. Results showed the mean total recovery of radioactivity was 93.8%, with the majority recovered in feces (71.1% of administered dose). Urinary excretion accounted for 22.7% of dose, with no detection of parent drug in urine. Dabrafenib is metabolized primarily via oxidation of the t-butyl group to form hydroxy-dabrafenib. Hydroxy-dabrafenib undergoes further oxidation to carboxy-dabrafenib, which subsequently converts to desmethyl-dabrafenib via a pH-dependent decarboxylation. The half-lives for carboxy- and desmethyl-dabrafenib were longer than for parent and hydroxy-dabrafenib (18-20h vs. 5-6h). Based on plasma AUC, dabrafenib, hydroxy-, carboxy- and desmethyl-dabrafenib, accounted for 11%, 8%, 54% and 3% of the plasma radioactivity, respectively. These results demonstrate that the major route of elimination of dabrafenib is via oxidative metabolism (48% of the dose) and biliary excretion. Based on our understanding of the decarboxylation of carboxy-dabrafenib, a low pH-driven, nonenzymatic mechanism involving participation of the aryl nitrogen is proposed to allow prediction of metabolic oxidation and decarboxylation of drugs containing an aryl nitrogen positioned alpha to an alkyl (ethyl or t-butyl) side chain.
Introduction

Dabrafenib (TAFINLAR™, GSK2118436; N-{3-[5-(2-Amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-y1]-2-fluorophenyl}-2,6-difluorobenzene sulfonamide, methane sulfonate salt) is an orally bioavailable, potent and selective RAF kinase inhibitor. The RAS/RAF/MEK/ERK pathway is a critical proliferation pathway in many human cancers and BRAF mutations have been identified in approximately 40 to 60% of melanoma (Davies et al., 2002; Rubinstein et al., 2010). Dabrafenib has been approved for the treatment of BRAF V600E mutation-positive tumors as monotherapy and is currently under development for use in combination therapy with trametinib, an allosteric non-competitive inhibitor of MEK1/MEK2 activation and kinase activity (Flaherty et al., 2012).

The pharmacokinetics (PK) of dabrafenib after single and repeat dosing have been investigated as part of a first-time-in-human (FTIH) study, where it has been shown that after single dose administration, dabrafenib concentrations peaked about 2 hours post-dose and declined thereafter, with a terminal half-life of approximately 5 hours (Falchook et al., 2012). Most of the human dabrafenib studies conducted to date have included an assessment of parent drug concentration, along with the concentration of the three main circulating metabolites, hydroxy-, carboxy- and desmethyl-dabrafenib, in order to better define the clinical pharmacokinetics of the drug. These three metabolites were identified as being of potential clinical importance based on preclinical pharmacology as they were shown to be active in preclinical models, albeit with different potency, and metabolic data suggested that they represented a major pathway of elimination. Preliminary human metabolite information obtained using plasma samples from the FTIH study confirmed the importance in terms of exposure of these 3 metabolites.
The metabolite profile and routes of excretion following oral administration have yet to be fully elucidated in humans. Here we report our findings from a study of the metabolism and excretion of [14C]dabrafenib in subjects with BRAF V600 mutation-positive tumors after a single radiolabeled oral dose of 95 mg. In addition to metabolite profiling and determination of total radioactivity concentrations in plasma, assessment of the concentrations of dabrafenib and its three main metabolites using validated UHPLC-MS/MS methods allowed for characterization of the full PK profiles, calculation of the PK parameters, and determination of the fraction of the area under the curve (AUC) accounted for by dabrafenib combined with these three metabolites, relative to the total concentration of radioactivity in plasma. Results of in vitro experiments to understand the pH-dependency of the decarboxylation of carboxy-dabrafenib to desmethyl-dabrafenib are presented, and a mechanism is proposed for metabolic oxidation and decarboxylation of drugs containing an aryl nitrogen alpha to an ethyl or t-butyl side chain. Taken together, these results provide a full understanding of dabrafenib disposition in humans.
Materials and Methods

Chemicals and Reagents. $[^14C]$Dabrafenib (N-[3-5-(2-Amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl]-2,6-difluorobenzene sulfonamide) methane sulfonate salt (non-micronized), radiochemical purity of 96.9% and specific activity of 111.2 $\mu$Ci/mg) was synthesized by Isotope Chemistry, GlaxoSmithKline, Stevenage, UK. A single $^{14}$C label was positioned in the thiazole moiety. Unlabeled dabrafenib and metabolite standards, hydroxy-dabrafenib [M7], carboxy-dabrafenib [M4] and desmethyl-dabrafenib [M8], were prepared by Product Development, GlaxoSmithKline, King of Prussia, PA and by Pharmaron, Beijing, China (Rheault et al., 2013; Adams et al., 2009). Chemicals and solvents of reagent or HPLC grade were purchased from commercial sources.

Subjects and Study Design: The study (ClinicalTrials.gov registration identifier NCT01262963) was a Phase I, single-center, open-label study in subjects with BRAF V600 mutation-positive tumors. Male or female subjects were eligible to be enrolled in the study if they were at least 18 years of age and had a BRAF V600-positive tumor as determined via relevant genetic testing. Additional relevant inclusion criteria included having an Eastern Cooperative Oncology Group (ECOG) performance status $\leq 1$ at screening, body weight $\geq 45$ kg, a body mass index $\geq 19$ kg/m$^2$ and $\leq 35$ kg/m$^2$ and adequate organ function. Medications and food or drinks known to be inhibitors or inducers of CYP2C8 or CYP3A4 were prohibited during the study. All subjects (three male and one female) received an oral radiolabeled dose as a suspension on Day 1. Blood samples were obtained for PK, total radioactivity and metabolite radioprofiling analysis (details below). Safety and tolerability assessments (including adverse events, vital signs and electrocardiograms) were also collected throughout the study.
The study protocol was approved by the Alpha Independent Review Board (San Clemente, CA), and the study was conducted at Comprehensive Clinical Development NW, Inc. (formerly Charles River Clinical Services, Tacoma, WA). This study was conducted in accordance with good clinical practice (GCP) and all applicable regulatory requirements, and the guiding principles of the Declaration of Helsinki. All subjects provided written informed consent before study entry. Following completion of the study, eligible subjects had the option to enter an open-label rollover study of dabrafenib to receive continued treatment (ClinicalTrials.gov registration identifier NCT01231594).

**Radiation Safety of Subjects.** The expected radiation exposure of a subject receiving an 80 μCi oral dose of \([^{14}C]\)dabrafenib was estimated prognostically according to regulatory guidelines (Code of Federal Regulations (Revised as of April 1, 2013)). Data from male and female rat whole body autoradiography, and excretion studies following oral administration of \([^{14}C]\)dabrafenib were subject to relative organ mass scaling and physiological time scaling and fit using non-compartmental analysis. The human absorbed dose estimates were calculated following the medical internal radiation dose (MIRD) schema using the MIRDOSE3.1 software package (Oak Ridge Associated Universities, 1994). Oral administration of 80 μCi of \([^{14}C]\)dabrafenib to adult male and female subjects was expected to result in an effective dose to the whole body of less than 100 mRem (1 mSv), within Risk Category IIa in accordance with the International Commission on Radiological Protection. Hence, the radiation risks in the current study were estimated to be low.

**Dosing and Sample Collection.** Subjects received the equivalent of 95 mg of dabrafenib free base, which was administered as the methanesulfonate salt in an oral suspension of 0.5%
hydroxypropyl methylcellulose and 0.1% polysorbate-80 solution. Each dose contained approximately 80 μCi of radiolabeled carbon. The radiopurity of the dose ranged from 98.9 to 99.5%. The single oral dose was administered in the morning of Day 1.

Blood samples were collected on Day -1, Day 1, pre-dose (for determination of background radioactivity) and then at the following times post-dose: 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours, for determination of blood radioactivity, plasma radioactivity, and plasma concentrations of dabrafenib and its three main metabolites (hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib), with additional plasma obtained at select time points (2, 6, 10 and 24 hours post-dose) for radioprofiling. Urine (0–6-hour, 6–12-hour and 12–24-hour intervals followed by 24-hour intervals) and feces (24-hour intervals) were collected pre-dose and for a minimum of 96 hours and a maximum of 240 hours after dosing. Collection was stopped after 96 hours if > 90% of the administered dose was recovered and total radioactivity in feces and urine was ≤ 1% of the administered dose in two consecutive collections.

**Sample Analysis for Radioactivity.** Analysis of total radioactivity in blood and plasma at all time points was conducted internally at GlaxoSmithKline (King of Prussia, PA). Triplicate aliquots of blood (0.2–0.4 g) were combusted in a Model 307A Oxidizer (PerkinElmer Inc, Waltham, MA). The resulting \(^{14}\text{CO}_2\) was trapped and scintillation cocktail (Perkin Elmer Carbosorb E/Permafluor E+) added. Triplicate aliquots of plasma (0.1–0.3 g) were transferred to scintillation vials and 10 mL of scintillation cocktail (Perkin Elmer Ultima Gold) added. Samples were assayed for radioactivity by liquid scintillation counting (LSC) using a PerkinElmer Tri-
Carb 3170 TR low-level liquid scintillation counter. The total blood and plasma radioactivity concentrations were calculated by converting the LSC data (disintegrations per minute, [DPM]) to concentrations (ng dabrafenib equivalent/g or nM) using the specific activity (0.84 μCi/mg or 0.44 μCi/μmol, respectively) of [14C]dabrafenib. The assumption was made that 1 g of plasma is equivalent to 1 mL of plasma, based on historic experimental precedent.

Analyses of total radioactivity content in collected excreta samples were conducted at Covance Laboratories Inc. (Madison, WI). Triplicate aliquots of fecal homogenate sample (0.3 g per aliquot) were combusted in a Model 307 Sample Oxidizer (Packard Instrument Co., Downers Grove, IL). The resulting 14CO2 was trapped, scintillation cocktail added, and the radioactivity content was quantified by LSC. Triplicate aliquots of urine sample (1.0 g per aliquot) were transferred to scintillation vials, and scintillation cocktail was added. The vials were then counted in a scintillation counter for 5 minutes or 100,000 counts.

Quantification of Dabrafenib, Hydroxy-dabrafenib, Desmethyl-dabrafenib and Carboxy-dabrafenib in Plasma. Two separate validated assays were used to measure dabrafenib and its three metabolites. Concentrations of dabrafenib, hydroxy-dabrafenib and desmethyl-dabrafenib in plasma samples at all time points were determined with a validated analytical UHPLC-MS/MS method, over the range of 1 to 1000 ng/mL. Dabrafenib, hydroxy-dabrafenib and desmethyl-dabrafenib were extracted from 50 μL of human plasma by liquid-liquid extraction with 1 mL ethyl acetate after the addition of isotopically labeled internal standards ([2H9]dabrafenib, [2H6 13C2]hydroxy-dabrafenib and [2H6 13C2]desmethyl-dabrafenib). Extracts (4 μL) were injected onto a Waters Acquity BEH C18 column (1.7 μm, 50 x 2.1 mm; Waters,
Milford, MA) maintained at 55 °C. The mobile phase consisted of water-formic acid (0.1%, v/v) (solvent A) and acetonitrile (solvent B). A flow rate of 0.8 mL/min was maintained for the entire run. The following 1.4-min linear gradient was used: start at 30% B, increase to 50% B over 0.5 min, maintain 50% B for 0.3 min, increase to 80% B over 0.2 min and maintain at 80% B for 0.3 min. The column was re-equilibrated after each injection. Detection was performed by positive ion MS/MS using a TurboIon-Spray interface on an API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Canada) with multiple reaction monitoring (m/z 520-m/z 277 for dabrafenib, m/z 529-m/z 280 for [2H6]-dabrafenib, m/z 536-m/z 323 for hydroxy-dabrafenib, m/z 544-m/z 331 for [2H6 13C2]-hydroxy-dabrafenib, m/z 506-m/z 277 for desmethyl-dabrafenib and m/z 514-m/z 280 for [2H6 13C2]-desmethyl-dabrafenib). Concentrations of carboxy-dabrafenib in plasma samples were determined with a separate validated analytical UHPLC-MS/MS method, over the range of 5 to 5000 ng/mL. Carboxy-dabrafenib was extracted from 25 μL of human plasma by protein precipitation using 80/20 ethyl alcohol/Milli-Q water containing an isotopically labeled internal standard ([2H6 13C2]-carboxy-dabrafenib). Extracts (4 μL) were injected onto a Waters Acquity BEH Phenyl column (1.7 μm, 50 x 2.1 mm) maintained at 55 °C. The mobile phase consisted of water-formic acid (0.1%, v/v) (solvent A) and acetonitrile (solvent B). A flow rate of 1 mL/min was maintained for the entire run. The following 1.4-min linear gradient was used: start at 30% B, increase to 50% B over 0.5 min, maintain 50% B for 0.3 min, increase to 80% B over 0.2 min and maintain at 80% B for 0.3 min. The column was re-equilibrated after each injection. Detection was performed by positive ion MS/MS using a TurboIon-Spray interface on an API 4000 mass spectrometer with multiple reaction monitoring (m/z 550-m/z 506 for carboxy-dabrafenib and m/z 558-m/z 514 for [2H6 13C2]-carboxy-dabrafenib).
**Pharmacokinetic Analysis.** PK parameters were determined for blood and plasma total radioactivity and plasma dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib concentrations. PK parameters were calculated by standard non-compartmental analysis with WinNonlin Pro 5.2 (Pharsight, Mountain View, CA) by ICON Development Solutions (Marlow, UK). All calculations of non-compartmental parameters were based on actual sampling times. The PK endpoints of interest were area under the plasma-concentration time curve (AUC$_{0-t}$ and AUC$_{0-\infty}$), maximum plasma concentration (C$_{max}$), time to C$_{max}$ ($t_{max}$) and half-life ($t_{1/2}$). Metabolite-to-parent ratios were calculated after correcting for differences in molecular weight.

The ratio of blood-to-plasma total radioactivity concentrations at each PK sampling time point was calculated to assess partitioning of dabrafenib-related material into red blood cells. Plasma ratio of AUC$_{0-\infty}$ of dabrafenib and metabolites (corrected for differences in molecular weight) to total radiocarbon were determined.

**Sample Preparation for Metabolite Profiling.**

*Plasma.* The plasma samples collected for metabolite profiling at selected time points (2, 6, 10, and 24 hours) were analyzed individually. Each sample (2 mL) was first extracted with 0.5 volumes of methanol, vortex mixed, and sonicated in a Covaris sonicator (E210 series, Covaris Inc., Woburn, MA). Then, 0.5 volumes of acetonitrile was added to each sample which was then vortex mixed, sonicated, and centrifuged at 3500 g$_{av}$ at room temperature for 10 minutes. The supernatants were removed and combined. The pellet was then extracted once more with 0.5 volumes of methanol and acetonitrile in an identical procedure with the resulting supernatants.
combined with the first extracts. Duplicate aliquots were removed from the combined extract for LSC to determine its radioactivity content and extraction recoveries. The combined extract was then concentrated to approximately 3 mL using a vacuum centrifuge. The samples were laterally mixed for 30 minutes at 750 rpm and at 10°C, sonicated using the Covaris, and centrifuged at 3500 g<br>av at room temperature for 10 minutes. Duplicate aliquots were removed from the concentrated supernatants for LSC to determine concentration efficiency prior to analysis by quantitative radio-HPLC (radio-HPLC, described below).

**Urine.** Urine samples from collections representing ≥ 2 % of the administered dose were pooled for each patient based on the total sample weight collected from each interval to produce a single representative sample for the patient. Aliquots of the pooled samples were centrifuged at 3500 g<br>av at room temperature for 5 minutes. The supernatants were then analyzed by radio-HPLC.

**Feces.** Fecal homogenate samples from collections representing ≥ 2 % of the administered dose were pooled for each patient based on the total sample weight collected from each interval to produce a single pooled representative sample for the patient. The pooled fecal homogenate samples were extracted with 3 volumes of 3:1 (v/v) methanol:water, sonicated at room temperature for 15 minutes, vortex mixed at room temperature for 20 minutes, and centrifuged at 3000 g<br>av at room temperature for 5 minutes. The supernatants were removed and combined. The residual pellets were extracted twice more with the same solvent in an identical procedure with the resulting supernatants combined with the first extracts. The combined extract was then evaporated to dryness under a stream of nitrogen gas and reconstituted in 1:1 (v/v) methanol:water. The reconstituted sample was sonicated for 15 minutes, vortex mixed for 20
minutes, and centrifuged at 3000 gav at room temperature for 5 minutes prior to analysis by radio-HPLC.

**Quantitative Radio-HPLC Analysis.** Radio-HPLC analysis was conducted on selected urine, fecal and plasma samples on Agilent 1100 and 1200 systems (Agilent Technologies, Palo Alto, CA) consisting of a pump (G1312A binary), an autosampler (G1329A) and a Phenomenex Synergi Polar RP column (4.6 x 250 mm, 4μ). Aliquots (≤ 900 μL) of samples were injected and eluted at 1 mL/min with 10 mM ammonium acetate, pH 5.5 (Solvent A) and acetonitrile (Solvent B). The following gradient was used: 0–5 min, isocratic at 5% B in A; 5–12 min, 5 to 20% B in A; 12–40 min, 20 to 35% B in A; 40–60 min, 35 to 95% B in A; 60–64 min, isocratic at 95% B in A; 64–65 min, 95 to 5% B in A. Column eluate for the fecal samples was collected into an online radiodetector, Radiomatic Flow Scintillation Analyzer 625TR (PerkinElmer Inc., Shelton, CT). The column eluate for the urine and plasma samples was collected into 96-deep well scintillator-coated microtiter plates using Agilent 1100 and 1200 fraction collectors. The plates were dried using a vacuum centrifuge and sealed using Top-Seal A (PerkinElmer). The sealed plates were analyzed in a TriLux 1450 Microbeta scintillation counter (PerkinElmer). Each well was counted for 15 minutes with no background subtraction. The resulting data was used to reconstruct radio-HPLC chromatograms. Each radioactive peak was calculated as the percentage of the total counts detected and expressed as nanogram equivalents of dabrafenib per gram of plasma or the percentage of the dose recovered in urine and feces (all corrected for the overall recovery of extraction and reconstitution). The lower limit of quantification (LLQ) was defined as one times the background area integrated in each chromatogram.
Mass Spectroscopic Analysis LC/MS\textsuperscript{n} was used to analyze representative samples of plasma extracts, urine and fecal homogenate extracts according to the conditions described above. During the LC separation, a post-column split was used to direct approximately 15\% of the sample to an LTQ-Orbitrap XL (ThermoFisher, San Jose, CA) mass spectrometer equipped with an electrospray ionization (ESI) source, or an LTQ XL (ThermoFisher) mass spectrometer equipped with an ESI source, implementing data dependent scanning by using a parent mass list. The parent mass list consisted of masses of all known and probable metabolites. A full scan mass spectrum (at resolution 30,000 for the Orbitrap) was collected and the data interrogated in real time to identify mass peaks corresponding to masses in the parent mass list. If present, the parent mass peaks were selected as target peaks for subsequent MS\textsuperscript{n} scans. The remaining LC eluate from the post-column split was directed into a Gilson 215 liquid handler with a collection time of approximately 11 seconds per well. All instruments utilized a LEAP autosampler for sample introduction. Data were acquired and processed using Xcalibur software (version 2.1).

Effect of pH on the Conversion of Carboxy-Dabrafenib to Desmethyl-Dabrafenib

Carboxy-dabrafenib (20 \(\mu\)g/mL) was incubated at 37\(^\circ\)C in buffers of varying pH (7, 5, 4 and 3) for up to 1h. Each buffer (4.9 mL) was added to separate 15 mL tubes and pre-heated in a ThermoFisher reciprocal shaking bath for 15 minutes at 37\(^\circ\)C. An aliquot (0.1 mL) of a 1 mg/mL carboxy-dabrafenib solution was added to each tube. Aliquots of the incubates were removed immediately and after approximately 1h and were then analyzed by LC/UV/MS. Peak areas from the UV signals corresponding to the carboxy- and desmethyl-dabrafenib retention times and MS signals versus authentic standards were determined and are presented as a percentage of the total amount of carboxy-dabrafenib in the pH 7 incubate aliquot at t=0 or t=1h.
Results

Subject Demographics and Safety. Four subjects were enrolled and completed the study. Two subjects remained on study for 11 days after dosing while the other two subjects were discharged on Day 9 and Day 10 after > 90% of the total dose was recovered, although one subject did not meet the early stopping criteria of two consecutive 24-hour excreta collections containing \( \leq 1\% \) of the dose (the final two 24-hour fecal collections for this subject contained 1.62 and 0.23\%). All subjects were Caucasian, three male and one female, with ages ranging from 23 to 57 years and body weights ranging from 57.0 to 94.9 kg. All subjects had stage IV, metastatic BRAF V600 mutation-positive melanoma with an ECOG performance status of 0 (n=1) or 1 (n=3) at screening.

All adverse events were Grade 1 or Grade 2 except for one adverse event of dehydration, which was Grade 3 and not considered related to study drug. The most common adverse event was diarrhea, which was reported in two out of four subjects. There were no serious adverse events, no withdrawals due to adverse events, and no adverse events related to changes in clinical laboratory parameters, electrocardiograms or vital signs.

Pharmacokinetics. The PK parameters for dabrafenib and its metabolites, and for total radioactivity (blood and plasma) are summarized in Table 1; the PK profiles are shown in Figure 1. The median time to peak dabrafenib concentration was 1.0 hour after administration of the oral suspension. The median t\(_{\text{max}}\) for radioactivity in blood and plasma was delayed relative to
dabrafenib in plasma, consistent with the formation and circulation of metabolites. The maximum plasma concentrations of metabolites hydroxy-, carboxy- and desmethyl-dabrafenib were achieved at 3.0, 10 and 30 hours post-dose, respectively. The geometric mean plasma half-life of dabrafenib and its primary metabolite hydroxy-dabrafenib were similar (5.3 hours and 5.7 hours, respectively). Metabolites carboxy-dabrafenib and desmethyl-dabrafenib had longer half-lives (17.5 hours and 20.4 hours, respectively) relative to parent (Figure 1, upper panel). The metabolite to parent AUC$_{0-\infty}$ ratios for hydroxy-, carboxy- and desmethyl-dabrafenib were 0.75, 4.9 and 0.26, respectively, with carboxy-dabrafenib being the most abundant metabolite in circulation.

The contribution of parent drug and its metabolites to total plasma radioactivity is illustrated in Figure 1 (lower panel), which shows the plasma concentration-time profiles of dabrafenib, the total concentration of dabrafenib plus its three main metabolites with metabolite concentrations adjusted for differences in molecular weights, and total radioactivity. As shown, dabrafenib and its three metabolites account for the majority of the plasma radioactivity profile. Using the geometric mean plasma AUC$_{0-\infty}$ ratios of dabrafenib to total plasma radioactivity, the percentage of total radioactivity in the form of parent compound was approximately 11%. The metabolites hydroxy-, carboxy- and desmethyl-dabrafenib accounted for approximately 8%, 54% and 3% of plasma radioactivity, respectively.

The mean blood-to-plasma ratio of total radioactivity concentration ranged from 0.58 to 0.70 across the four subjects, suggesting minimal association of dabrafenib drug-related material (DRM) with red blood cells.
Metabolite Profiles in Plasma. The relative amounts of dabrafenib and quantifiable metabolites in plasma after oral administration of [14C]dabrafenib are shown in Table 2. Recovery of radioactivity from the plasma following extraction with organic solvent was high (range of 88% to 99%). Consistent with the concentration data determined using the validated UHPLC-MS/MS assay, dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib were notable radiolabeled components in plasma extracts from all four subjects. Dabrafenib was the predominant circulating component at 2 hours post-dose, with carboxy-dabrafenib becoming the predominant component by 10 hours post-dose. At 24 hours post-dose, concentrations of dabrafenib represented less than 1.4% of circulating DRM, with carboxy-dabrafenib as the predominant component in all subjects (44.8%). The remaining radioactivity not accounted for by dabrafenib and its metabolite can be accounted for by circulating downstream metabolites of desmethyl-dabrafenib (M26, M28, M29, M30 and M31), observed mostly at the 24-hour timepoint, plus any trace metabolites not detected by HPLC radioprofiling.

There were no significant qualitative or quantitative differences between the radioprofiles of plasma samples from Subjects 401, 403 and 404, with representative profiles depicted in Figure 2. Subject 406 had a different radioprofile, notable for additional oxidative metabolites and higher levels of the downstream metabolites of desmethyl-dabrafenib at the later plasma time points. Moreover, at 24 hours post-dose, carboxy-dabrafenib, desmethyl-dabrafenib, along with two additional metabolites (M26 and M31), were all predominant circulating species (10–29% DRM) in this subject. Several minor metabolites (below LLQ), derived from the further oxidation of desmethyl-dabrafenib, were also detected (M28, M29 and M30). The sequential
metabolic transformation in Subject 406 appeared to be more efficient with more rapid appearance of carboxy- and desmethyl-dabrafenib in this subject relative to other subjects. Carboxy- and desmethyl-dabrafenib had shorter $T_{\text{max}}$ (6 vs. 10–12 hours and 12 vs. 24–34 hours, respectively) and half-lives (11 vs. 16–24 hours and 16 vs. 20–26 hours) in Subject 406 compared to the other subjects, but the metabolite-to-parent AUC ratios were similar across all four subjects.

Mass Balance. The mean cumulative percentage radioactive dose recovered in urine and feces is illustrated in Figure 3. [14C]Dabrafenib-related material was predominantly excreted in feces with a mean ± SD recovery of 71.1 ± 8.1% of the administered dose. Urinary excretion accounted for 22.7 ± 4.7% of the dose. Total recovery of radioactivity in all excreta varied from 88.4% to 100% (mean recovery of 93.8%), with the majority recovered within the first 4 days (85.6%).

Metabolite Profiles in Urine. Representative radio-HPLC chromatograms from human urine are shown in Supplementary Figure 1. Individual and mean quantification data for the major metabolites, expressed as a percentage of the administered dose, are given in Supplementary Table 1. Unchanged dabrafenib was not detected in urine, while M3 (a glucuronide of hydroxy-dabrafenib) was detected only in urine. In Subjects 401, 403 and 404, carboxy-dabrafenib was the predominant radiocomponent in the pooled urine samples (6–12% of the dose), with the other urinary radiocomponents each accounting for less than 3.5% of the dose in these 3 subjects. There were minimal differences, qualitatively and quantitatively, in the radioprofiles between Subjects 401, 403 and 404. In Subject 406, carboxy-dabrafenib, M28/M29 and M26 were the
predominant urinary radiocomponents (3.7–5.9% of the dose), with the other radiocomponents each accounting for < 2% of the dose.

**Metabolite Profiles in Feces.** Representative radio-HPLC chromatograms obtained from analysis of human fecal extracts are shown in Supplementary Figure 2. Individual and mean quantification data for the major metabolites are provided in Supplementary Table 1. Similar to the data obtained from analysis of urine, the radioprofiles of fecal extracts from Subjects 401, 403 and 404 were qualitatively and quantitatively similar, while the profile for Subject 406 had a smaller presence of parent compound and greater amounts of downstream oxidative metabolites of desmethyl-dabrafenib. The percentage of the administered dose excreted as oxidative metabolites was calculated to be 48% of the dose by summing the mean percent dose of all metabolites in urine and feces derived from oxidative pathways (i.e., all metabolites).

Dabrafenib was the predominant radiocomponent in feces from Subjects 401, 403 and 404, accounting for 19 to 35% of the dose, while desmethyl-dabrafenib was the predominant component (15.7% of dose) in feces from Subject 406. The percentage dose of desmethyl-dabrafenib excreted from Subjects 401, 403 and 404 was, on average, similar to that from Subject 406 (13.9%), while fecal excretion of dabrafenib from Subject 406 accounted for only 1.8% of the dose. The metabolites carboxy-dabrafenib and hydroxy-dabrafenib were detected in fecal samples from all four subjects. Four minor metabolites, products of further oxidation of desmethyl-dabrafenib (i.e. M26, M28, M29 and M30), were only detected in the feces of Subject 406, with each accounting for <5% of the dose. As the synthetic standard of carboxy-dabrafenib has been shown to undergo decarboxylation at low pH or in the presence of aprotic solvents, it is
possible that \textit{ex vivo} decarboxylation of carboxy-dabrafenib to form desmethyl-dabrafenib may have contributed to the levels of desmethyl-dabrafenib detected in feces.

**Structural Characterization of Metabolites.** Metabolite structures were characterized by LC/MS\textsuperscript{n} analysis from plasma, urine and fecal homogenate extracts (a summary of metabolite structures and MS\textsuperscript{n} fragmentation data is available in Supplementary Table 2). Similarities in HPLC retention times and mass spectrometric fragmentation patterns with those obtained from the available standards facilitated the identification of carboxy-dabrafenib, hydroxy-dabrafenib and desmethyl-dabrafenib. Structures of other metabolites were proposed based on their mass spectrometric fragmentation patterns relative to dabrafenib or these known metabolites.

M30 was characterized as an oxidation product of desmethyl-dabrafenib. MS/MS generated a fragment ion 44 atomic mass units (amu) less than the protonated molecular ion, indicating a loss of carbon dioxide (carboxy-dabrafenib exhibited a similar fragmentation). Further fragmentation generated an ion with an additional loss of 220 amu. This corresponds to cleavage of the isopropyl and the difluorophenyl sulphone moieties; accurate mass analysis of this ion does not indicate presence of any oxygen molecules. Therefore, M30 results from oxidation of both isopropyl terminal methyl groups, one to the alcohol, and the other to the acid.

M31 can be assigned as the unsaturated (propenyl) derivative, since the isopropyl moiety is the only position that would support the loss of two hydrogen atoms. There was insufficient spectroscopic data for definitive structural assignments of the other downstream oxidative metabolites of desmethyl-dabrafenib.
Effect of pH on Carboxy-Dabrafenib to Desmethyl-Dabrafenib Conversion

While carboxy-dabrafenib was stable at pH 7, this metabolite decarboxylated to generate desmethyl-dabrafenib at pH 4 and pH 3, with some conversion noted at pH 5 (Figure 4). Conversion of carboxy-dabrafenib to desmethyl-dabrafenib was greater following incubation for 1h at 37°C.
Discussion

Disposition of Dabrafenib in Humans

The human biotransformation of dabrafenib is illustrated in Figure 5. Following oral absorption, dabrafenib is primarily transformed via oxidation of the t-butyl group to form hydroxy-dabrafenib. Hydroxy-dabrafenib is further oxidized to the carboxylic acid derivative, carboxy-dabrafenib and, based on urinary data, can also undergo Phase II conjugation to generate hydroxy-dabrafenib O-glucuronide (M3). Carboxy-dabrafenib circulates until it undergoes decarboxylation to form desmethyl-dabrafenib via a non-enzymatic process or is excreted in urine or bile. Following biliary secretion, it is possible that carboxy-dabrafenib may also undergo decarboxylation in the gut to form desmethyl-dabrafenib, which is either excreted in feces or potentially absorbed back into the bloodstream. Circulating desmethyl-dabrafenib is further metabolized to downstream oxidative metabolites.

The main circulating drug-related components were dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib and desmethyl-dabrafenib, which together account for 76% of circulating radioactivity in humans. As demonstrated in the PK profiles, dabrafenib was the predominant circulating component at earlier times post-dose, with carboxy-dabrafenib becoming the predominant component at later time points. The terminal phase of hydroxy-dabrafenib parallels that of parent, suggesting that it is a formation rate-limited metabolite. In contrast, for carboxy-dabrafenib, its concentrations peaked later, it exhibited a longer half-life, and it is considered to be elimination rate-limited. The desmethyl-dabrafenib terminal phase parallels that of carboxy-dabrafenib and is thought to be formation rate-limited relative to carboxy-dabrafenib. Both carboxy- and desmethyl-dabrafenib are predicted to accumulate with repeat dosing.
The pharmacology of dabrafenib metabolites was evaluated using preclinical assays in BRAF V600 mutant enzymes or cell lines (Adams et al., 2009). Relative potency generally ranked in the following order: dabrafenib > hydroxy-dabrafenib ~ desmethyl-dabrafenib >> carboxy-dabrafenib. Based on our current understanding, the relative contribution of metabolites to clinical activity depends on the steady-state exposure of metabolites and parent. Dabrafenib, hydroxy-dabrafenib and desmethyl-dabrafenib may contribute to clinical activity while contribution of carboxy-dabrafenib is assumed to be minimal despite high plasma exposure at steady-state.

These metabolites were identified early in the program in preclinical studies and have been measured in patients starting with the first-time-in-human study (Study BRF112680) to better understand their disposition and contribution to the clinical efficacy and safety profile of dabrafenib. The biotransformation of dabrafenib was qualitatively similar between human and the non-clinical toxicology species (i.e., rat and dog), however, to establish coverage for the main circulating metabolites, additional studies were conducted in the mouse (Richards-Peterson et al., 2013).

**Excretion of Dabrafenib in Humans and Clinical Implications**

Following a single oral administration of [14C]dabrafenib, fecal excretion is the major route of elimination accounting for 71.1% of radioactive dose while urinary excretion accounted for 22.7% of administered radioactivity. The predominant radiolabelled component in urine was carboxy-dabrafenib accounting for 7% of the dose, while M28 and M29 were predominant in 1 subject (6% of the dose, together). In addition, hydroxy-dabrafenib, desmethyl-dabrafenib, M3,
M26 and M30 were detected in urine samples (<2.1% for each) while unchanged dabrafenib was not observed in urine.

In feces, dabrafenib was predominant, accounting for 21.8% of the radiolabelled suspension dose, while desmethyl-dabrafenib, carboxy-dabrafenib, and hydroxy-dabrafenib accounted for 14.4%, 9.5%, and 4.5% of the dose, respectively. Other metabolites including M26, M28/M29 and M30, each representing 2.1%-3.1% of the administered dose, were detected in feces in only 1 subject. Of the total amount of unchanged parent excreted in the feces, it is unclear what proportion was due to unabsorbed drug versus biliary elimination of dabrafenib. The elimination of dabrafenib was predominantly by oxidative metabolism (48% of the dose), based on the total recovery of oxidative metabolites in urine and feces. Thus, renal impairment is unlikely to significantly impact exposure to parent dabrafenib, while hepatic impairment may alter its exposure.

Investigation of the decarboxylation of carboxy-dabrafenib to desmethyl-dabrafenib

The first two steps of the metabolism of dabrafenib are oxidative (i.e., hydroxy-, then carboxy-dabrafenib formation) and mediated by CYP enzymes (GlaxoSmithKline, 2013), while the final step (decarboxylation) does not require oxidative enzymes. The decarboxylation of carboxy-dabrafenib to generate desmethyl-dabrafenib, was investigated in vitro by incubation of the synthetic carboxy-dabrafenib standard in buffers of pH 7, 5, 4, and 3. While some conversion was seen immediately at pH 3 and 4, extensive conversion was detected following a 1h incubation, as well as some conversion at pH 5 (Figure 4). Decarboxylation in the range of pH 3-4 corresponds to the pKa of the carboxylic acid of carboxy-dabrafenib, calculated to be 4.24
Therefore, decarboxylation of carboxy-dabrafenib is catalyzed by acidic, but not neutral, aqueous conditions. Based on these data, we have proposed an acid-catalyzed mechanism of carboxy-dabrafenib decarboxylation to generate desmethyl-dabrafenib (Figure 6), involving participation of the aryl nitrogen of the thiazole ring as a Lewis base for the carboxylic acid proton (available only near or below its pKa). A 6-membered cyclic transition state would drive the decarboxylation of the protonated carboxylic acid. This proposed mechanism is a novel variant of the accepted one for decarboxylation of 1,3-dicarboxylic acids or beta-keto acids.

Carboxy-dabrafenib was stable in cofactor fortified human liver microsomes, yet was converted to desmethyl-dabrafenib in hepatocytes from human and nonclinical species (data not shown). Thus, the decarboxylation likely occurs non-enzymatically in an acidic hepatocellular compartment (e.g., lysosome)(Chan et al., 2006), however, contribution from cytosolic enzymes cannot be ruled out.

Another example of non-enzymatic decarboxylation of a carboxylic acid metabolite was described for LC15-0133, a t-butyl oxadiazole-containing compound previously in development for treatment of diabetes, using in vitro liver metabolism systems (Yoo et al., 2008). When incubated with D₂O, the uptake of deuterium occurred on the resulting isopropyl methine carbon in rat liver microsomes. Also, the isolated carboxylic acid metabolite of LC15-0133 decarboxylated non-enzymatically in solution at high pH or under heated conditions, although we believe that the proposed base-catalyzed mechanism is unlikely to occur in a biological system. Rather, a possible mechanism of LC15-0133 metabolite decarboxylation would involve protonation of the carboxylic acid and participation of the oxadiazole nitrogen as a Lewis base, analogous to what we have proposed for carboxy-dabrafenib. Indeed, the microsomal incubation
was quenched with 1% acetic acid, potentially catalyzing some decarboxylation post-incubation. Further evidence of non-enzymatic, acid-catalyzed decarboxylation (at room temperature) has been described in the chemistry literature for benzo-thiazol-2-yl glyoxylic acid, and the proposed mechanism involves participation of the adjacent thiazole nitrogen (Baudet and Otten, 1970).

Carbon-carbon bond cleavage of an aryl-alkyl side chain was also described for torcetrapib in preclinical species, in which an aryl-ethyl to aryl-methyl conversion occurred from an intermediate metabolite containing a quinoline ring (Dalvie et al., 2008). The authors proposed a mechanism of oxidation of the terminal carbon of the ethyl group to a carboxylic acid intermediate with subsequent decarboxylation. We suggest that this intermediate is well-positioned for a 6-membered cyclic decarboxylation transition state with the quinoline nitrogen as a Lewis base, analogous to the transition state proposed for carboxy-dabrafenib decarboxylation.

In the absence of an aryl nitrogen, C-demethylation of an alkyl side chain following enzymatic oxidation still may occur, as demonstrated for a prostaglandin antagonist, CP-533,536, but would proceed via an alternative mechanism not involving decarboxylation (Prakash et al., 2008). An example of aryl t-butyl carboxylic acid formation without subsequent C-demethylation is illustrated by terfenadine (Seldane), for which a t-butyl moiety is metabolically oxidized to a stable carboxylic acid, fexofenadine (Allegra), in the absence of a nitrogen in the adjacent aryl ring (Garteiz et al., 1982).
These examples illustrate that a drug structure containing an aryl nitrogen moiety with an alkyl (ethyl or t-butyl) side chain in the alpha position can undergo carbon-carbon bond cleavage of the alkyl terminal methyl. This occurs by enzymatic oxidation with subsequent pH-dependent decarboxylation via a 6-membered cyclic transition state involving the aryl nitrogen.

**Oxidative metabolism of desmethyl-dabrafenib**

Downstream oxidative metabolites of desmethyl-dabrafenib were detected at low concentrations in human plasma, urine and/or feces. The formation of M31, the propenyl-substituted thiazole, is likely derived from oxidation of the isopropyl methine carbon in a manner analogous to that described for ezlopitant (Obach, 2001), while M30 results from oxidation of the terminal isopropyl methyls to an alcohol and a carboxylic acid. The remaining oxidative metabolites (M26, M28, and M29) could not be definitively identified with the available data.

**Conclusions**

In conclusion, the metabolic pathway and excretion of dabrafenib, including characterization of its major circulating metabolites, are well described based on results from this study. Dabrafenib is sequentially oxidized to hydroxy- and carboxy-dabrafenib which then undergoes decarboxylation to form desmethyl-dabrafenib. The proposed pH-dependent decarboxylation mechanism following metabolic oxidation of the t-butyl methyl of dabrafenib allows for the prediction of alkyl carbon-carbon bond cleavage (C-demethylation) for drugs containing an aryl nitrogen positioned alpha to an ethyl or t-butyl side chain. Furthermore, the thorough understanding of human biotransformation and disposition this study provided was crucial in guiding the nonclinical and clinical development of dabrafenib, especially in establishing the
relative pharmacokinetic exposures of the metabolites in relevant nonclinical toxicology species. This understanding allowed for more complete management of the clinical usage of this agent for disease treatment, robust modeling for the prediction of DDIs with concomitant medications and informed recommendations for hepatic or renal impairment patients.
Acknowledgments

The authors would like to acknowledge Chet Bowen and Martin Rogers for technical contributions, Stephen Castellino and Ernest Schubert for helpful discussions, Tara Rheault for the initial synthesis of dabrafenib and desmethyl-dabrafenib standards, Arun Kaura for the initial synthesis of the hydroxy- and carboxy-dabrafenib standards, Ken Lawrie, Geoff Badman and Calvin Manning for preparation of the [14C]dabrafenib as well as stable-labeled standards for the UHPLC-MS/MS assay, Catrin Dalton and Christopher Jefferds (Icon Clinical Research) for clinical study operations, and Kenneth Martin (Comprehensive Clinical Development NW) for preparation of the [14C]dabrafenib oral dosing suspension. Editorial support in the form of collating comments for the authors and graphic services was provided by Clinical Thinking and was funded by GSK.
Authorship Contribution

Participated in research design: Ouellet, Carson, Blackman, Morrison, Gorycki, Richards-Peterson

Conducted experiments: Bershas, Mamaril-Fishman, Morrison, Jurusik, Knecht

Performed data analysis: Bershas, Ouellet, Mamaril-Fishman, Nebot, Adams, Jurusik, Gorycki, Richards-Peterson

Wrote or contributed to the writing of the manuscript: Bershas, Ouellet, Mamaril-Fishman, Nebot, Carson, Blackman, Morrison, Adams, Jurusik, Knecht, Gorycki, Richards-Peterson
References


Code of Federal Regulations (Revised as of April 1, 2013). Radioactive drugs for certain research uses. 21CFR361.1.


metabolites by high-resolution liquid chromatography-tandem mass spectrometry and liquid chromatography/mass spectrometry-nuclear magnetic resonance. *Drug Metab Dispos* **36:**2093-2103.


Footnotes

This work was supported by GlaxoSmithKline.

Address correspondence to: Dr. Lauren Richards-Peterson, Drug Metabolism and Pharmacokinetics, GlaxoSmithKline UW2730, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939. E-mail: Lauren.E.Richards-Peterson@gsk.com
**Figure 1** Pharmacokinetic concentration-time profiles. Top: Mean plasma concentration-time profile of dabrafenib and each metabolite after a single oral administration of 95 mg [14C]dabrafenib Bottom: Mean plasma concentration-time profile of dabrafenib alone, dabrafenib and its three main circulating metabolites, and total radioactivity;

**Figure 2** Representative HPLC radiochromatograms of plasma extracts after a single oral administration of [14C]dabrafenib (95 mg)

**Figure 3** Mean cumulative percent radioactive dose recovered in urine and feces at specified intervals after a single 95 mg (80 μCi) oral dose of [14C]dabrafenib (n=4)

**Figure 4** Carboxy-dabrafenib conversion to desmethyl-dabrafenib

**Figure 5** Proposed metabolic pathways of [14C]dabrafenib in cancer patients

**Figure 6** Proposed mechanism of carbon-carbon bond cleavage of dabrafenib via pH dependent decarboxylation following metabolic oxidation
### Table 1

**Summary of pharmacokinetic parameters**<sup>a</sup> (n=4)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;, hr</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;, ng/mL or ng/g</th>
<th>AUC(0-∞), ng<em>hr/mL or ng</em>hr/g</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt;, hr</th>
<th>Ratio M:P</th>
<th>Ratio C:TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Radioactivity</td>
<td>5.0 (2.0–8.0)</td>
<td>1616 (27)</td>
<td>37028 (53)</td>
<td>20.8 (56)</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Plasma Radioactivity</td>
<td>3.0 (2.0–8.0)</td>
<td>2364 (18)</td>
<td>59447 (48)</td>
<td>26.4 (44)</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Plasma Dabrafenib</td>
<td>1.0 (0.6–2.0)</td>
<td>1662 (31)</td>
<td>6535 (28)</td>
<td>5.3 (28)</td>
<td>N.A.</td>
<td>0.11</td>
</tr>
<tr>
<td>Plasma Hydroxy-Dabrafenib</td>
<td>3.0 (2.0–4.0)</td>
<td>666 (29)</td>
<td>5022 (31)</td>
<td>5.7 (34)</td>
<td>0.75</td>
<td>0.08</td>
</tr>
<tr>
<td>Plasma Carboxy-Dabrafenib</td>
<td>10.0 (6.1–12.0)</td>
<td>1283 (23)</td>
<td>33948 (57)</td>
<td>17.5 (36)</td>
<td>4.91</td>
<td>0.54</td>
</tr>
<tr>
<td>Plasma Desmethyl-Dabrafenib</td>
<td>30.0 (12.0–36.0)</td>
<td>36 (87)</td>
<td>1674 (59)</td>
<td>20.4 (21)</td>
<td>0.26</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> PK parameters are presented as geometric mean (CV%). T<sub>max</sub> is reported as median (range). Radioactivity concentrations are expressed as ng equivalents dabrafenib/g. M, metabolite AUC<sub>(0-∞)</sub>; P, Parent (dabrafenib) AUC<sub>(0-∞)</sub>; C, compound (Parent or Metabolite) AUC<sub>(0-∞)</sub>; TR, total radioactivity in plasma AUC<sub>(0-∞)</sub>. 
## Table 2

Average levels of circulating dabrafenib and metabolites in humans after a single oral administration of 95 mg of [14C]dabrafenib

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% Plasma Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2h</td>
</tr>
<tr>
<td>Dabrafenib&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.6</td>
</tr>
<tr>
<td>Hydroxy-dabrafenib&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.4</td>
</tr>
<tr>
<td>Carboxy-dabrafenib&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.1</td>
</tr>
<tr>
<td>Desmethyl-dabrafenib</td>
<td>N.D.</td>
</tr>
<tr>
<td>M26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>N.D.</td>
</tr>
<tr>
<td>M28/M29</td>
<td>N.D.</td>
</tr>
<tr>
<td>M30</td>
<td>N.D.</td>
</tr>
<tr>
<td>M31&lt;sup&gt;e&lt;/sup&gt;</td>
<td>N.D.</td>
</tr>
<tr>
<td>Total quantified</td>
<td>88.1</td>
</tr>
</tbody>
</table>

Data are mean (n=4)

N.D., not detected; N.Q., below level of quantitation by radioprofiling.

<sup>a</sup> Dabrafenib was quantifiable by radioprofiling only in Subjects 401 (3.4%) and 404 (2.2%) at 24 h and was not quantifiable by radioprofiling in other subjects.
Hydroxy-dabrafenib was quantifiable by radioprofiling only Subjects 401 (3.6%) and 404 (2.6%) at 24 h and was not quantifiable by radioprofiling in other subjects.

Carboxy-dabrafenib was quantifiable by radioprofiling only in Subjects 403 (5.3%) and 406 (11.2%) at 2 h and was not quantifiable by radioprofiling in other subjects.

M26 was quantifiable by radioprofiling only in Subject 406 at 10 h (1.9%) and at 24 h (10.1%) and was not quantifiable by radioprofiling in other subjects.

M31 was quantifiable by radioprofiling only in Subject 406 at 10 h (3.4%) and was not quantifiable by radioprofiling in other subjects.
Figure 1

- Dabrafenib
- Desmethyl-dabrafenib
- Hydroxy-dabrafenib
- Carboxy-dabrafenib

Mean concentration (ng/mL) vs Time post-dose (hours)

Mean concentration (nM) vs Time post-dose (hours)
DAB = dabrafenib; CDAB = Carboxy-dabrafenib; DDAB = Desmethyl-dabrafenib; HDAB = Hydroxy-dabrafenib; M26, M28, M29, M30 and M31 = circulating downstream metabolites of desmethyl-dabrafenib

Subjects 401, 403 and 404 are qualitatively and quantitatively similar. A representative radioprofile from each timepoint is included here.

Subject 406 plasma at 24 hours post-dose

Subject 406 24-hour plasma is included to show the qualitative and quantitative differences from Subjects 401, 403 and 404.
Figure 3

Percent of dose administered

Time post-dose (hours)

Urine
Feces
Total

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Figure 4
Conversion of carboxy-dabrafenib to desmethyl-dabrafenib, t=0

Conversion of carboxy-dabrafenib to desmethyl-dabrafenib, t=1h
These pathways were proposed based on the metabolites characterized in plasma, urine and feces. Metabolites were characterized spectroscopically by LC-MS^n only. The identities of hydroxy-, carboxy-, and desmethyl-dabrafenib were further confirmed by HPLC retention time comparison to authentic standards. Structures in brackets are putative.

P = plasma  F = feces  U = urine

* - Denotes $^{14}$C position
Figure 6

- Butyl $\rightarrow$ i-Propyl

Dabrafenib $\rightarrow$ desmethyl-dabrafenib $\rightarrow$ hydroxy-dabrafenib $\rightarrow$ carboxy-dabrafenib

CYP: Cytochrome P450 enzyme system
Supplementary Materials

Metabolism and Disposition of Oral Dabrafenib in Cancer Patients: Proposed Participation of Aryl Nitrogen in Carbon-Carbon Bond Cleavage via Decarboxylation following Enzymatic Oxidation

David A. Bershas, Daniele Ouellet, Donna B. Mamaril-Fishman, Noelia Nebot, Stanley W. Carson, Samuel C. Blackman, Royce A. Morrison, Jerry L. Adams, Kristen E. Jurusik, Dana M. Knecht, Peter D. Gorycki, and Lauren E. Richards-Peterson
Supplementary Figure 1 HPLC radiochromatograms of pooled urine samples after a single oral administration of [\(^{14}\)C]dabrafenib (95 mg)
Subjects 401, 403 and 404 are qualitatively and quantitatively similar.

Subject 406 is included to show the qualitative and quantitative differences from Subjects 401, 403 and 404.

DAB = dabrafenib; CDAB = Carboxy-dabrafenib; DDAB = Desmethyl-dabrafenib; HDAB = Hydroxy-dabrafenib; M26, M28, M29 and M30 = circulating downstream metabolites of desmethyl-dabrafenib. M3 = a glucuronide of hydroxy-dabrafenib
**Supplementary Figure 2** HPLC radiochromatograms of pooled feces extract samples after a single oral administration of $[^{14}\text{C}]$dabrafenib (95 mg)
Subjects 401, 403 and 404 are qualitatively and quantitatively similar.

Subject 406 is included to show the qualitative and quantitative differences from Subjects 401, 403 and 404.

DAB = dabrafenib; CDAB = Carboxy-dabrafenib; DDAB = Desmethyl-dabrafenib; HDAB = Hydroxy-dabrafenib; M26, M28, M29 and M30 = circulating downstream metabolites of desmethyl-dabrafenib
Supplementary Table 1

Distribution of dabrafenib and metabolites in urine and feces from patients following a single oral administration of 95 mg of [14C]dabrafenib

<table>
<thead>
<tr>
<th>Matrix/Component</th>
<th>% Administered Dose in Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>401</td>
</tr>
<tr>
<td>Urine&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>0.0</td>
</tr>
<tr>
<td>M3</td>
<td>0.88</td>
</tr>
<tr>
<td>Hydroxy-dabrafenib</td>
<td>1.19</td>
</tr>
<tr>
<td>Carboxy-dabrafenib</td>
<td>6.27</td>
</tr>
<tr>
<td>Desmethyl-dabrafenib</td>
<td>2.57</td>
</tr>
<tr>
<td>M26</td>
<td>0.94</td>
</tr>
<tr>
<td>M28, M29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61</td>
</tr>
<tr>
<td>M30</td>
<td>0.32</td>
</tr>
<tr>
<td>Total quantified</td>
<td>13.8</td>
</tr>
<tr>
<td>Total dose</td>
<td>21.3</td>
</tr>
</tbody>
</table>
Feces

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>401 (6–72)</th>
<th>403 (0–48)</th>
<th>404 (6–96)</th>
<th>406 (6–48)</th>
<th>408 (0–48)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabrafenib</td>
<td>31.3</td>
<td>35.4</td>
<td>18.9</td>
<td>1.76</td>
<td>21.8</td>
<td>15.1</td>
</tr>
<tr>
<td>Hydroxy-dabrafenib</td>
<td>2.97</td>
<td>4.24</td>
<td>6.06</td>
<td>4.80</td>
<td>4.52</td>
<td>1.28</td>
</tr>
<tr>
<td>Carboxy-dabrafenib</td>
<td>3.98</td>
<td>15.2</td>
<td>10.0</td>
<td>8.70</td>
<td>9.48</td>
<td>4.61</td>
</tr>
<tr>
<td>Desmethyl-dabrafenib</td>
<td>12.7</td>
<td>13.0</td>
<td>16.1</td>
<td>15.7</td>
<td>14.4</td>
<td>1.77</td>
</tr>
<tr>
<td>M26</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.49</td>
<td>0.62</td>
<td>1.25</td>
</tr>
<tr>
<td>M28, M29</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>3.06</td>
<td>0.77</td>
<td>1.53</td>
</tr>
<tr>
<td>M30</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.11</td>
<td>0.53</td>
<td>1.06</td>
</tr>
<tr>
<td>Total quantified</td>
<td>50.9</td>
<td>67.8</td>
<td>51.1</td>
<td>38.6</td>
<td>52.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Total dose</td>
<td>69.7</td>
<td>82.9</td>
<td>66.9</td>
<td>65.0</td>
<td>71.1</td>
<td>8.08</td>
</tr>
</tbody>
</table>

N.D., Not detected, considered zero for calculation of mean and SD.

a Pooled collection period for radioprofiling (hours): 401 (6–72), 403 (0–48), 404 (6–96), 406 (6–48).

b Metabolites M28 and M29 coeluted.

c Pooled collection period for radio profiling (hours): 401 (24–144), 403 (0–96), 404 (0–120), 406 (0–72).
**Supplementary Table 2**

MS/MS data for dabrafenib and its metabolites

<table>
<thead>
<tr>
<th>Proposed Structure</th>
<th>([\text{M+H}]^+) ((m/z))</th>
<th>Characteristic Fragment Ions ((m/z))</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Dabrafenib Structure" /></td>
<td>520</td>
<td>343, 323, 307</td>
</tr>
<tr>
<td><img src="image" alt="M3 Structure" /></td>
<td>712</td>
<td>536, 506, 359, 329, 323, 293</td>
</tr>
<tr>
<td>Proposed Structure</td>
<td>[M+H]$^+$ (m/z)</td>
<td>Characteristic Fragment Ions (m/z)</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td><strong>Hydroxy-dabrafenib (M7)</strong></td>
<td><img src="image" alt="Structure" /></td>
<td>536 506, 359, 329, 323, 293</td>
</tr>
<tr>
<td><strong>Carboxy-dabrafenib (M4)</strong></td>
<td><img src="image" alt="Structure" /></td>
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</tr>
<tr>
<td><strong>Desmethyl-dabrafenib (M8)</strong></td>
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</tr>
<tr>
<td>Proposed Structure</td>
<td>[M+H]$^+ (m/z)$</td>
<td>Characteristic Fragment Ions (m/z)</td>
</tr>
<tr>
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<td>-----------------</td>
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<tr>
<td><img src="image" alt="M26 Structure" /></td>
<td>522</td>
<td>504, 464, 327, 309, 291</td>
</tr>
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<td><img src="image" alt="M28 Structure" /></td>
<td>538</td>
<td>520, 508, 492, 464, 361, 330, 325</td>
</tr>
<tr>
<td>Proposed Structure</td>
<td>[M+H]$^+$ (m/z)</td>
<td>Characteristic Fragment Ions (m/z)</td>
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<td><img src="image" alt="M29 Diagram" /></td>
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<td>508, 488, 331, 330, 311, 310, 295, 288, 268</td>
</tr>
<tr>
<td><img src="image" alt="M31 Diagram" /></td>
<td>504</td>
<td>390, 327, 307, 291</td>
</tr>
</tbody>
</table>