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The Metabolic Drug-Drug Interaction Profile of Dabrafenib: In Vitro Investigations and Quantitative Extrapolation of the P450-Mediated DDI Risk

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ABSTRACT

Dabrafenib is a potent ATP-competitive inhibitor for the V600 mutant b-raf rapidly accelerated fibrosarcoma (b-raf) kinase currently approved in the United States for the treatment of metastatic melanoma. Studies were conducted in human liver microsomes, recombinant human cytochrome P450 (P450) enzymes, and human hepatocytes to investigate the potential of dabrafenib and its major circulating metabolites to perpetrate pharmacokinetic drug-drug interactions (DDIs) as well as have their own pharmacokinetics affected (victim) by coadministered drugs. Dabrafenib metabolism was mediated by CYP2C8 (56% to 67%) and CYP3A4 (24%); in addition, it has demonstrated inhibition of CYP2C8, 2C9, 2C19, 3A4 (atorvastatin), and (nifedipine), with calculated IC50 values of 8.2, 7.2, 22.4, 16, and 32 μM. It also demonstrated metabolism-dependent inhibition of CYP3A4 with a maximal inactivation rate constant of 0.040 minute−1 and a concentration required to achieve half-maximal inactivation for CYP3A4 of 38 μM. Hydroxy-dabrafenib inhibited CYP1A2, 2C9, and 3A4 (midazolam) with calculated IC50 values of 83, 29, and 44 μM, and carboxy-dabrafenib did not inhibit any of the P450 enzymes tested. Desmethyl-dabrafenib inhibited CYP2B6, 2C8, 2C9, 2C19, and 3A4 (midazolam, atorvastatin, and nifedipine) with calculated IC50 values of 78, 47, 6.3, 36, 17, 20, and 28 μM, respectively. At 30 μM dabrafenib showed increases in CYP2B6 and CYP3A4 mRNA expression indicative of induction. The potential clinical relevance of these findings was explored by using mechanistic static mathematical models to estimate the magnitude of change (area under the curve change) as a result of P450-mediated DDI interactions. This risk-assessment approach indicated that dabrafenib is unlikely to perpetrate any in vivo DDIs by inhibition mechanisms, but is a likely inducer of CYP3A4 and a victim of CYP3A4 and CYP2C8 inhibitors. Furthermore, inclusion of the in vitro drug interaction data for dabrafenib metabolites did not impact the overall clinical risk assessment.

Introduction

In the past 40 years, patients with advanced metastatic melanoma have had very few treatment options, with no first-line standard of care that offered a proven overall survival benefit (Eggermont et al., 2011; Heakal et al., 2011). The prognosis for these patients has been very poor, with a median survival time of 6–9 months and a 3-year survival rate of only 10% to 15% (Eggermont et al., 2011). It has been estimated that there will be approximately 76,600 new cases of melanoma and an estimated death toll of just over 9,400 in 2013 (ACS, 2013). Recently, advances have been made in the development of targeted therapies that kill melanoma cells or therapies that specifically target mutated proteins in cell signaling pathways. One pathway that has been established in mediating growth signals that drive cancer is the RAS-RAF-MEK-ERK mitogen-activated protein (MAP) kinase cascade (Roberts and Der, 2007; Heakal et al., 2011). Three genes encode the RAF-serine/threonine kinases, one of which is b-raf. b-raf is mutated in approximately 60% of melanoma patients, with 74% to 90% being a V600E point mutation that results in a constitutively active kinase that has at least 10 times higher activity compared with wild type (Maldonado et al., 2003; Flaherty and McArthur, 2010; Eggermont et al., 2011; Heakal et al., 2011; Falchuck et al., 2012). As a result of increased understanding of these pathways, several targeted therapies for b-raf have recently been approved or are currently in development (Eggermont, 2011).

Dabrafenib is a potent ATP-competitive inhibitor for the V600 mutant b-raf kinase (Hauschild et al., 2012) and is currently approved in the United States for the treatment of metastatic melanoma. During the development of dabrafenib, the earliest clinical studies were conducted in cancer patients instead of healthy volunteers. As a consequence, these

ABBREVIATIONS: b-raf, b-rapidly accelerated fibrosarcoma; CLr, intrinsic clearance; ClICYP, intrinsic clearance for recombinant P450 enzyme; DDI, drug-drug interaction; DMSO, dimethyl sulfoxide; Fa, fraction of inhibitor absorbed in blood; Fb, fraction of inhibitor absorbed into enterocyte; Fg, fraction of substrate escaping gut metabolism; fm, fraction metabolized; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HLM, human liver microsomes; HMM, hepatocyte maintenance medium; HPLC, high-performance liquid chromatography; Igut, intestinal metabolism; IndC50, mean concentration of inducer at 50% maximum induction; IndMax, maximum fold induction observed in vitro; Ka, rate of inhibitor absorption; Kdissociation constant of the inhibitor from the enzyme; Km, concentration required to achieve half-maximal inactivation; kmax, maximal inactivation rate constant; Km, concentration at half-maximal; LC/MS, liquid chromatography/mass spectrometry; LSC, liquid scintillation counting; MAP, mitogen-activated protein; MDI, metabolism-dependent inhibition; MS/MS, tandem mass spectrometry; P450, cytochrome P450; PXR, pregnane X receptor; Qg, enterocytic blood flow; Qh, liver blood flow; Rif, rifampicin; Vd, volume of distribution.
patients were likely taking comedinations for related or unrelated disease states, especially agents for the management of pain. Therefore, studies designed to assess the risk of drug interactions were conducted to investigate dabrafenib’s potential not only to cause pharmacokinetic drug interactions (perpetrator) but also have its own pharmacokinetics affected (victim) by coadministered drugs. In vitro studies, their generated results, and interpretation have become a foundation in drug development to assess potential drug-drug interactions (Venkatakrishnan et al., 2003; Bachmann and Lewis, 2005; Obach et al., 2006). The present studies have evaluated the potential of dabrafenib and its metabolites to participate in cytochrome P450 (P450)-mediated drug-drug interactions as both a victim and perpetrator. In vitro data generated from human liver microsomes, recombinant human P450 enzymes, and human hepatocytes have been used in mechanistic static mathematical models to estimate the likely magnitude of change (area under the curve [AUC] change) as a result of P450-mediated drug-drug interactions. This approach, using quantitative extrapolation, is also a key component of clinical decision-making and has recently gained support from both industry and regulatory authorities (Venkatakrishnan et al., 2003; Obach et al., 2006; Einolf, 2007; Shardlow et al., 2011; FDA, 2012). We demonstrate that the results from the in vitro studies describe the mechanisms underlying the metabolic pathway of dabrafenib, and identify the key drug-interaction risks based on static mathematical predictions. These results informed comedication inclusion criteria for early clinical studies and also influenced the nature and design of in vivo clinical drug-interaction studies.

Materials and Methods

**Radiolabeled Material and Chemicals.** [14C]-dabrafenib (GSK2118436; N-[3-5-(2-amino-4-pyrimidinyl)-2-(1,1-dimethylhydrazinyl)-1,3-thiazol-4-yl]-2-fluorophenyl)-2,6-difluorobenzensulfonamide, methanesulfonate salt), was supplied as a solid by GlaxoSmithKline Chemical Development (Stevenage, UK). The purity of the material was determined to be 99%. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, β-nicotinamide adenine dinucleotide phosphate (NADP), sulfaphenazole, furafylline, and quinidine were obtained from Sigma Chemical Company (St. Louis, MO). Azamulin and benzyltinaviranol were obtained from BD Gentest (Woburn, MA). Montelukast was obtained from Sequoia Research Products Ltd., (Oxford, UK). Scintillation cocktails, Ultima Gold, were obtained from Packard Ltd. (Meriden, CT), and IN-FLOW 2:1 was obtained from INUS Systems (Tampa, FL). Human liver microsomes (HLMs) prepared from mixed-gender pools of 15 human livers were obtained from Xenotech LLC (Lenexa, KS). Supersomes containing individually overexpressed human P450 enzymes derived from baculovirus-infected insect cells and control Supersomes lacking any native human P450 activity were obtained from BD Gentest. Supersomes expressing CYP2C8, CYP2C9, CYP2C19, and CYP1A4 coexpressed P450 reductase and cytochrome b5, whereas Supersomes expressing CYP1A2 and CYP2D6 coexpress CYP reductase only. For induction studies, human hepatocytes were obtained from CellzDirect (Pittsboro, NC), hepatocyte maintenance medium (HMM) containing 0.1 μM insulin, 0.1 μM dexamethasone, 0.5 μg/ml gentamicin, and 50 ng/ml amphotericin was obtained from Lonza (Walksville, MD), and the prototypical P450 inducers, omeprazole (CYP1A2), phenytoin (CYP2B6), and rifampicin (CYP3A4) were purchased from Sigma Aldrich. TaqMan reagents were purchased from Invitrogen (Grand Island, NY), Promega SV 96 Total RNA Isolation System was obtained from Promega (Madison, WI), and the Ribogreen assay kit was purchased from BD Gentest. All other reagents used in these investigations were reagent grade or higher and obtained from standard commercial suppliers.

**P450 Reaction Phenotyping.** The P450 enzymes involved in the metabolism of dabrafenib and its metabolites were identified by monitoring substrate-depletion kinetics using nonradiolabeled material and by metabolite formation using radiolabeled dabrafenib. The metabolic clearance (substrate depletion) of dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib (0.5 μM) was measured in incubations containing potassium phosphate buffer (50 mM, pH 7.4) with Supersomes containing 20 pmoles/ml of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4. Incubations were initiated by the addition 200 μl of cofactor and maintained at 37°C in a total incubation volume of 1.000 μl. The reactions were terminated at 0, 3, 6, 9, 12, 18, and 30 minutes by adding 100 μl of incubation mixture to 200 μl of acetonitrile with an internal standard. Control incubations were performed in the absence of oxidative cofactor in which the cofactor solution was replaced with sodium bicarbonate. Incubation contents were subsequently analyzed by liquid chromatography/mass spectrometry (LC/MS).

Metabolite formation was determined by incubating [14C]-dabrafenib (5 μM) with human liver microsomes (1.0 mg/ml protein concentration) and potassium phosphate buffer (50 mM, pH 7.4) in a total volume of 500 μl. After an equilibration period of 5 minutes at 37°C, reactions were initiated by the addition of cofactor solution (an NADPH-regenerating system containing 1.7 mg of NADP, 7.8 mg of glucose-6-phosphate, and six units of glucose-6-phosphate dehydrogenase per ml of 2% (w/v) sodium bicarbonate) and shaken in a water bath for 8 minutes. The metabolite P450 reaction phenotype was determined in incubations in the presence and absence of the P450-selective chemical inhibitors azamulin (CYP3A4, 5 μM) (Stresser et al., 2004), sulfaphenazole (CYP2C9, 10 μM) (Baldwin et al., 1995), quinidine (CYP2D6, 1 μM) (Otton et al., 1988), montelukast (CYP2C8, 1 μM) (Walsky et al., 2005), benzyltinaviranol (CYP2C19, 5 μM) (Suzuki et al., 2002), and furafylline (CYP1A2, 10 μM) (Sesardic et al., 1990). Because they are mechanism-based P450 inactivators, incubations with furafylline and azamulin were equilibrated with microsomes and buffer for 5 minutes at 37°C in a shaking water bath followed by addition of cofactor and preincubated in the presence of the NADPH-regenerating system for 10 minutes before adding [14C]-dabrafenib solution to start the reaction. To confirm the results from the use of P450-selective chemical inhibitors, metabolite formation was also monitored from incubations with human recombinant P450 enzymes (Supersomes). Each incubation sample contained 300 pmoles/ml of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 ([14C]-dabrafenib (5 μM); and an appropriate volume of potassium-phosphate buffer (50 mM, pH 7.4). Reactions were initiated in a similar manner to that described above. Control incubations were performed by withholding microsomes, supersomes, inhibitors or NADPH, respectively. All reactions described above were terminated by adding an equal volume of acetonitrile and centrifuged at 13,000 rpm. The supernatants of each sample were analyzed by radio-high-performance liquid chromatography (HPLC) and LC/MS ([Liquid Chromatography/Mass Spectrometry to the nth fragmentation].

**Radio-HPLC Metabolite Profiling and Identification.** The supernatants from [14C]-dabrafenib incubations with HLMs and recombinant enzymes were analyzed by HPLC with radiochemical detection. The HPLC system was configured with an HP-1100 binary pump from Agilent Technologies (Palo Alto, CA). Online radio detection was conducted using a β-RAM radiodetector from INUS Systems, fitted with a built-in liquid scintillant pump. Chromatography of all samples was generated on a Phenomenex Synergi Fusion RP80HA column (4 μm, 4.6 x 250 mm) and a Phenomenex Synergy Fusion RP (4.0 x 3.0 mm) guard column by injecting 100 μl of the supernatant from each incubation (Phenomenex; Torrance, CA). The mobile phase was composed of acetonitrile (solvent B) and 10 mM ammonium acetate at pH 5.5 (solvent A). The flow rate was 1.0 ml/minute at ambient temperature. A 45-minute gradient was used in the following manner: 0–35 minutes, 65%B; 35.1–40 minutes, 95%B; and 40.1–45 minutes, 15%B. All of the postcolumn eluate was directed to the β-RAM and monitored continuously with a liquid scintillation flow cell (INUS Systems). The β-RAM operated in the homogeneous liquid scintillation counting mode with the addition of 2.0 ml/minute of IN-FLOW 2:1 scintillation cocktail to the effluent. The β-RAM response was detected and recorded in counts per minute over real time.

**LC/MS Analysis for the Quantification of Dabrafenib and its Metabolites from In Vitro Samples.** Dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib were extracted by liquid-liquid extraction using ethyl acetate. Carboxy-dabrafenib was extracted by protein precipitation using 80/20 ethanol/water. All extracts were analyzed by ultra-HPLC tandem mass spectrometry (MS/MS) using a TurboIonspray interface with positive-ion multiple-reaction monitoring. Changes in peak area ratio of each analyte relative to its respective isotopically labeled internal standard ([13C8]-dabrafenib, [13C8]-hydroxy-dabrafenib, [13C8]-desmethyl-dabrafenib), and [13C8]-carboxy-dabrafenib was used to monitor depletion kinetics.
Cytochrome P450 Inhibition. The inhibition of cytochrome P450 activity was assessed using methods previously described (Shar Todd et al., 2011; Polli et al., 2013) by incubating dabrafenib or its three metabolites with human liver microsomes over a concentration range of 0.1–100 μM. Incubations were conducted in duplicate with 0.1 mg/mL of human liver microsomes in 50 mM potassium phosphate buffer (pH 7.4) at 37°C for 5 or 10 minutes with the following probe substrates at concentrations corresponding to their respective concentration at half-maximal rate (Km) values: phenacetin (CYP1A2), coumarin (CYP2A6), buspirone (CYP2B6), rosiglitazone (CYP2C8), diclofenac (CYP2C9), 5-methyltryptophan (CYP2C19), and nifedipine, atorvastatin, and midazolam (CYP3A4). Reactions were initiated using an NADPH-regenerating system containing 1.7 mg NADP, 7.8 mg glucose-6-phosphate, and six units of glucose-6-phosphate dehydrogenase per ml of 2% w/v sodium bicarbonate. P450 metabolism–dependence inhibition (MDI) was examined by preincubating dabrafenib or its metabolites with HLMs and cofactor for 20 minutes and continuing incubation with the appropriate probe substrate (described above) for 5–10 minutes. A reduction of IC50 from 1.5–2.0 fold is indicative of metabolism-dependence inhibition (Grimm et al. 2009). Incubations were stopped by the addition of an equal volume of acetonitrile and subsequently analyzed for probe substrate metabolite formation using LC/MS/MS by methods previously described (Reese et al., 2008).

Determination of P450 Inactivation Kinetic Parameters. CYP3A4 inactivation kinetic parameters were determined for dabrafenib because it demonstrated metabolism-dependence inhibition of this enzyme. Preincubations of dabrafenib with microsomes in the presence of cofactor were conducted. Residual enzyme activity was subsequently determined from secondary incubations containing fresh cofactor and midazolam (at a concentration approximately 10 times its Km). Preincubations with dabrafenib, HLMs, and cofactor were performed with a volume of 0.5 ml in a shaking water bath at 37°C. Each preincubation contained 365 μl 50 mM potassium phosphate buffer pH 7.4, 25 μl HLMs (1 mg/ml final protein concentration), and 10 μl of dabrafenib solution in methanol. After an approximately 5-minute prewarming and removal of duplicate 20 μl aliquots (0-minute control samples), reactions were initiated by the addition of 95 μl of cofactor solution to each preincubation. At 2.5, 5, 10, 15, and 20 minutes after cofactor addition, duplicate 25 μl aliquots (single aliquots at 20 minutes) were taken from each preincubation and added to a prewarmed dilution mix containing 125 μl phosphate buffer, 50 μl fresh cofactor solution, and 50 μl 125 μM midazolam (CYP3A4 probe substrate). With each transfer into the dilution mix, microsomes were diluted to a concentration of 0.1mg/ml protein, and organic solvent was maintained at <2%. The 0-minute samples removed from the preincubations before cofactor addition were also added to the dilution mix, together with an extra 5-μl cofactor. All diluted incubations proceeded for 4 minutes before termination with 250 μl acetonitrile. Incubations were analyzed for 1-hydroxymidazolam using LC/MS/MS.

Cytochrome P450 Induction. Incubations in cultured human hepatocytes with dabrafenib (0.1–100 μM) were conducted and the measured increases in mRNA levels by quantitative reverse transcriptase polymerase chain reaction were employed to assess the P450 enzyme induction potential. Cultured human hepatocytes were obtained from CellzDirect as cultured monolayers on a collagen subestratum with a Matrigel overlay. Upon arrival, shipping medium was replenished with supplemented hepatocyte maintenance medium (HMM) containing 0.1 μM insulin, 0.1 μM dexamethasone, 0.5 μg/ml gentamycin, and 50 ng/ml amphotericin from Lonza. For cryopreserved hepatocytes from a collagen substratum with a Matrigel overlay. Upon arrival, shipping medium was changed once daily for 48 hours. Total RNA was extracted from the cell homogenates by column extraction using a Promega SV96 Total RNA Isolation System. Genomic DNA was removed from the samples using DNase provided in the kit. Total RNA isolates were quantified using the RiboGreen assay kit. The specific mRNA level was quantitatively detected for the following genes: CYP1A2, 2B6, 3A4, and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Cell health of plated human hepatocytes used in the induction investigations was determined by measuring total RNA using RiboGreen. The use of total RNA levels as an indicator of cell health was validated against the use of CellTiter-Glo Luminescent Cell Viability Assay. Total RNA was determined to be a good indicator of cell health (data not shown).

Quantitative DDI Risk Assessment for Inhibition of CYPs 2C8, 2C9, and 2C19. The potential for increased exposure (AUC) of the clinical probe drug substrates, rosiglitazone (CYP2C8), warfarin (CYP2C9), or omeprazole (CYP2C19), when coadministered with dabrafenib, and considering contributing metabolites, was determined using a mechanistic static mathematical model (Rowland and Matin, 1973; Obach et al., 2006) modified to incorporate metabolite contributions (Eq. 4, Supplemental Information). For the purposes of this analysis, the mechanism of inhibition was assumed to be reversible and competitive since these enzymes were not inhibited in a metabolism-dependent manner; therefore, the measured IC50 was divided by 2 to serve as an approximate value of Ki (Cheng and Prussoff, 1973). The estimated Ki values used for dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib are described in Table 3, and all other inputs are summarized in Table 5. Carboxy-dabrafenib did not demonstrate inhibition of CYP2C8, 2C9, or 2C19 and therefore was not included in this analysis. The following parameters were used as surrogates for the concentration of inhibitor(s) available at the enzyme active site [I], and each was corrected for plasma protein binding:

1. The steady-state Cmax concentrations of dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib in humans at the intended dose of 150 mg (twice daily) as shown in Table 5 and then corrected for plasma protein binding.
2. An estimate of the hepatic portal vein (hepatic inlet) concentration for dabrafenib, including contributions from the circulating metabolites hydroxy-dabrafenib and desmethyl-dabrafenib.

a. Hepatic portal vein (hepatic inlet) concentration for the metabolites was assumed to be the metabolite steady state Cmax concentration since these are formed systemically.
3. An estimate of inhibitor concentration in the liver has been proposed as an additional surrogate for estimating drug-interaction risk (Shar Todd et al., 2011). Liver partitioning of dabrafenib-related radiocarbon from a biodistribution study conducted in rats (quantitative whole body autoradiography) indicated a liver-to-blood ratio of approximately 30:1.

a. The ratio was corrected for the blood:plasma concentration ratio (0.49) to provide a liver:plasma concentration ratio of 15:1, which was used for this analysis.
b. Because metabolite contributions to the liver:plasma ratio determined from the whole-body autoradiography study cannot be differentiated from parent, the 30:1 ratio was used and corrected for metabolites’ blood-to-plasma ratios (16.5:1 and 16.8:1 for hydroxy-dabrafenib and desmethyl-dabrafenib, respectively).

Quantitative DDI Risk Assessment for CYP3A4. The impact of dabrafenib and its metabolites on coadministered midazolam, a sensitive CYP3A substrate, was predicted using a model that considered contributions of dabrafenib and its relevant circulating metabolites and incorporated P450 inhibition, inactivation, and induction interaction mechanisms (Fahmi et al., 2008) (Eq. 6, Supplemental Information). In addition to the surrogates of dabrafenib concentrations used previously, an estimate of the enterocyte concentration for dabrafenib was used, because perturbations to CYP3A in the gut wall are an important mechanism of drug interactions (Galetin et al., 2008). All parameters are summarized in Tables 4 and 5.

Quantitative DDI Victim Risk Assessment for Dabrafenib. The potential for increased exposure (AUC) of dabrafenib when coadministered with ketoconazole, a strong CYP3A4 inhibitor, was determined using a mechanistic static mathematical model (Rowland and Matin, 1973; Obach et al., 2006) (Eq. 8, Supplemental Information). For the purposes of this analysis, the steady-state
coadministered with gemfibrozil, the steady state C max concentrations of dabrafenib and gemfibrozil 1-\(\text{O}\)-\(\beta\)-glucuronide were set to 13.47 \(\mu\)M (University of Washington drug interaction database), an estimate of the hepatic portal vein (hepatic inlet) concentration was determined using 97% protein binding (www.drugbank.ca), and an estimate of the inhibitor concentration in the liver was determined using a liver:plasma ratio of 2 (Rodgers et al., 2005a, 2005b; Rodgers and Rowland, 2006, 2007). The molecular weight (531.43 g) and rate of inhibitor absorption (Ka) (0.036 minute\(^{-1}\)) of ketoconazole were obtained from Drugbank, a knowledge base for drugs, drug actions, and drug targets (www.drugbank.ca). A dose of 400 mg ketoconazole was employed in the model, as this is a standard dose used in clinical DDI studies performed by GlaxoSmithKline. The CYP3A4 fraction metabolized (fm) for dabrafenib was determined by in vitro methodologies, and the fraction of substrate escaping gut metabolism (Fg) of dabrafenib was estimated as described in Yang et al., 2007 (Eq. 9–11, Supplemental Information). All other input parameters for the model are shown in Table 6.

While gemfibrozil has been used clinically to investigate CYP2C8-mediated DDIs, quantitative extrapolation of the potential DDI risk is complicated by both direct and mechanism-based CYP2C8 inhibition (glucuronide metabolite), as well as inhibition of drug transporters, including OATP1B1. The potential role of OATP1B1 in the disposition of dabrafenib is unknown; therefore, the risk assessment was based on CYP2C8 only, using a mathematical model previously described (Fahmi et al., 2008) (Eq. 12), (Supplemental Information). The CYP3A4 fraction metabolized (fm) for dabrafenib was determined by in vitro methodologies, and the fraction of substrate escaping gut metabolism (Fg) of dabrafenib was estimated as described in Yang et al., 2007 (Eq. 9–11, Supplemental Information).

## Results

### In Vitro Metabolism and Phenotyping Studies

The enzymes involved in the oxidative metabolism of dabrafenib and its circulating metabolites, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib were determined using recombinant P450 enzymes and pooled human liver microsomes. In experiments using recombinant P450 enzymes, the intrinsic clearance (rate of substrate depletion) was scaled using internally generated relative activity factors (Störmer et al., 2000) or intersystem extrapolation factors (Proctor et al. 2004) to place the rates within the context of anticipated human liver microsomal activity for each of the P450s. Individual scaled human

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dabrafenib % to Total Scaled</th>
<th>Desmethyl-Dabrafenib % to Total Scaled</th>
<th>Hydroxy-Dabrafenib % to Total Scaled</th>
<th>Carboxy-Dabrafenib % to Total Scaled</th>
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<tbody>
<tr>
<td>CYP1A2</td>
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<td>CYP3A4</td>
<td>23</td>
<td>69</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

In microsomes, CLint was calculated using the following formula:

\[
Cl\text{int} = \frac{rate \times ml}{min \times 0.5mg\text{protein}}
\]

In Supersomes, CLint was calculated with the formula:

\[
Cl\text{int,}rCYP = \frac{rate \times ml\text{inc}}{min \times pmol\ P450}
\]

CLint, intrinsic clearance (ml/min/mg protein).

# Table

<table>
<thead>
<tr>
<th>P450</th>
<th>%P450 Contribution Based on Metabolite Formation</th>
<th>%P450 Contribution Based on Substrate Depletion</th>
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<tr>
<td>1A2</td>
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<tr>
<td>3A4</td>
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<td>23</td>
</tr>
</tbody>
</table>

\(^{a}\)Values represent the normalized (scaled) rates of total metabolite formation (expressed as a percentage) of individual P450s with respect to the total normalized rates of metabolites from the reaction phenotyping study.

\(^{b}\)Values represent the normalized (scaled) rates of parent depletion (expressed as a percentage) of individual P450s with respect to the total normalized rates of metabolites from the intrinsic clearance study.
recombinant P450 intrinsic clearance values were added to generate a total scaled rate, and a % P450 contribution was subsequently calculated. Based on substrate depletion kinetic analysis, dabrafenib was primarily metabolized by CYP2C8 (56%) and CYP3A4 (23%), with minor contributions from CYP2C9 (10%). Hydroxy-dabrafenib was metabolized by CYP3A4 (100%), and carboxy-dabrafenib was not metabolized by any of the cytochrome P450 enzymes evaluated. Desmethyl-dabrafenib was predominantly metabolized by CYP3A4 (69%), with minor contributions from CYP2C9 (22%) and CYP2C9 (10%). (Tables 1 and 2).

Metabolite formation using [14C]-dabrafenib was determined from human recombinant P450 enzymes and pooled human liver microsomes with detection by HPLC with both radiochemical and MS/MS detection (for structural confirmation based on authentic standards). Hydroxy-dabrafenib was produced in incubations with P450s 2C8 and 3A4 (300 pmol/ml) (Fig. 2) as well as minor production observed in 2B6, 2C8, 2C9, Diclofenac, 2C19, Mephenytoin, and 2D6. Bupropion, Rosiglitazone, and Diclofenac were metabolized by CYP2C8, 2C9, and 2C19, respectively (Table 2). Therefore, mechanism-based inactivation was not observed by any of the cytochrome P450 enzymes evaluated. Desmethyl-dabrafenib nor desmethyl-dabrafenib were detected in incubations with human liver microsomes.

To summarize the results of P450 phenotyping studies, independent measurements of the depletion of dabrafenib and the formation of its metabolites produced similar outcomes that were in good agreement. Both studies confirm that dabrafenib was largely metabolized by CYP2C8 and CYP3A4, with minor contributions from CYP2C9 and 2C19 (Table 1). Hydroxy-dabrafenib, the primary oxidation product of dabrafenib, was metabolized exclusively by 3A4. Carboxy-dabrafenib, a carboxylic acid that forms via two successive oxidation steps from hydroxy-dabrafenib, was not metabolized by the P450 enzymes tested in this study. Desmethyl-dabrafenib, which appears to represent a decarboxylation of carboxy-dabrafenib, was metabolized by CYP3A4 with minor contributions from P450s 2C9 and 2C19.

In Vitro CYP Inhibition. Dabrafenib demonstrated inhibition of CYP2C8, 2C9, 2C19, 3A4 (atorvastatin), and 3A4 (nifedipine) with calculated IC50 values of 8.2, 7.2, 22.4, 16, and 32 μM, respectively (Table 3). Dabrafenib also showed metabolism-dependent inhibition of CYP3A4 (nifedipine and midazolam) with 2.1- and 2.4-fold decreases in IC50 values after a 20-minute preincubation with cofactor, respectively (Table 3). Therefore, mechanism-based inactivation was presumed and P450 inactivation kinetic parameters were subsequently determined. The maximal inactivation rate constant (k_{inact}) was 0.040 minute⁻¹ and the concentration of dabrafenib required to achieve half-maximal inactivation (K_{i}) of CYP3A4 was 38 μM (Fig. 3).

Hydroxy-dabrafenib inhibited CYP1A2, 2C9, and 3A4 (midazolam) with calculated IC50 values of >80, 29, and 44 μM, respectively, but did not inhibit 2A6, 2B6, 2C8, 2C19, 2D6, or 3A4 (atorvastatin, nifedipine) at concentrations up to 100 μM (Table 3). Carboxy-dabrafenib did not inhibit any of the P450 enzymes tested in this study. (Table 3).

TABLE 3

Inhibition of cytochrome P450 enzymes by dabrafenib and its circulating metabolites in human liver microsomes

<table>
<thead>
<tr>
<th>P450 Substrate</th>
<th>Control Pre- inc IC50 (μM)</th>
<th>NADPH Pre- inc IC50 (μM)</th>
<th>Fold Δ in IC50</th>
<th>Control Pre- inc IC50 (μM)</th>
<th>NADPH Pre- inc IC50 (μM)</th>
<th>Fold Δ in IC50</th>
<th>Control Pre- inc IC50 (μM)</th>
<th>NADPH Pre- inc IC50 (μM)</th>
<th>Fold Δ in IC50</th>
<th>Control Pre- inc IC50 (μM)</th>
<th>NADPH Pre- inc IC50 (μM)</th>
<th>Fold Δ in IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2 Phenacetin</td>
<td>&gt;80</td>
<td>&gt;60</td>
<td>1.2</td>
<td>83</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>2A6 Coumarin</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>2B6 Bupropion</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>2C8 Rosiglitazone</td>
<td>7.7</td>
<td>9.3</td>
<td>0.83</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>2C9 Diclofenac</td>
<td>7.2</td>
<td>7.2</td>
<td>1.0</td>
<td>29</td>
<td>28</td>
<td>1.0</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>2C19 Mephenytoin</td>
<td>22.4</td>
<td>24.2</td>
<td>0.93</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>2D6 Bufuralol</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.0</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>3A4 Atorvastatin</td>
<td>19</td>
<td>13</td>
<td>1.5</td>
<td>&gt;100</td>
<td>98</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>3A4 Midazolam</td>
<td>&gt;100</td>
<td>24</td>
<td>≥4.2</td>
<td>44</td>
<td>47</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>3A4 Nifedipine</td>
<td>39</td>
<td>19</td>
<td>2.1</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined; Pre-inc, preincubation.

TABLE 4

Clinical probe substrates and assumptions of f_{m} and F_{p} used in dabrafenib DDI predictions

<table>
<thead>
<tr>
<th>Clinical Probe</th>
<th>f_{m}</th>
<th>F_{p}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8 Rosiglitazone</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C9 Warfarin</td>
<td>0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C19 Omeprazole</td>
<td>0.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>CYP3A4 Midazolam</td>
<td>0.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.57&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
In Vitro CYP Induction. To investigate the effect of dabrafenib on the mRNA levels of cytochrome P450 genes (CYP1A2, 2B6, and 3A4), dabrafenib (0.1–100 μM) was incubated with human hepatocytes for 48 hours. mRNA was extracted, and specific mRNA levels were quantitatively detected for the following genes: CYP1A2, 2B6, 3A4, and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). At 30 μM, dabrafenib maximally increased CYP2B6 and CYP3A4 mRNA levels to mean ratios of treated over control of 32 and 30, corresponding to 320% and 150% increases relative to their prototypic inducers, respectively (Fig. 4). There were no observed changes in mRNA levels for CYP1A2. Loss in cell viability was observed at the highest concentration (100 μM) as measured by the reduction in total mRNA compared with controls. The possible P450 induction effects of dabrafenib metabolites have not been individually characterized to date. However, the metabolites were formed in incubations with human hepatocytes, and therefore the induction response characterized for dabrafenib may include contributions from metabolites.

### TABLE 5
Parameter inputs and assumptions used in perpetrator mechanistic static mathematical model predictions of dabrafenib DDI

<table>
<thead>
<tr>
<th>Parameter Input</th>
<th>Dabrafenib</th>
<th>Hydroxy-Dabrafenib</th>
<th>Desmethyl-Dabrafenib</th>
<th>Definition of Inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax,s (μM)</td>
<td>2.8</td>
<td>1.9</td>
<td>0.69</td>
<td>Steady-state Cmax values obtained from (Ouellet et al., 2013).</td>
</tr>
<tr>
<td>Protein Binding</td>
<td>99.7</td>
<td>96.3</td>
<td>99.9</td>
<td>Protein binding data measured separately.</td>
</tr>
<tr>
<td>Liver:Plasma Ratio</td>
<td>15:1</td>
<td>16.5:1</td>
<td>16.8:1</td>
<td>Liver:plasma ratio determined from QWBA measured separately.</td>
</tr>
<tr>
<td>Dose</td>
<td>150 mg (twice daily)</td>
<td></td>
<td></td>
<td>Obach et al., 2006.</td>
</tr>
<tr>
<td>ka</td>
<td>0.03 min⁻¹</td>
<td></td>
<td></td>
<td>U.S. Food and Drug Administration, 2012.</td>
</tr>
<tr>
<td>Qh</td>
<td>1.617 ml/min³</td>
<td></td>
<td></td>
<td>Quinney et al., 2010.</td>
</tr>
<tr>
<td>Fa</td>
<td>1.54</td>
<td>1</td>
<td></td>
<td>Yang et al., 2008.</td>
</tr>
<tr>
<td>Fg</td>
<td>0.57</td>
<td></td>
<td></td>
<td>Borges et al., 2005.</td>
</tr>
<tr>
<td>Qg</td>
<td>300 ml/min³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kdeg,μ</td>
<td>0.000413 kdeg/min³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indmax (Ψ)</td>
<td>26.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IndC50</td>
<td>1.6 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Yang et al., 2007b; Rodgers et al., 2005a, 2005b; Rodgers and Rowland, 2006, 2007; In vitro data measured separately; University of Washington Database; [www.drugbank.ca]; [RxList.com]; U.S. Food and Drug Administration, 2012; VandenBrink et al., 2011; Varma et al., 2012; Schneck et al., 2004; Ogilvie et al., 2006; Honkalammi et al., 2011; Yang et al., 2008; In Silico, Simcyp Ltd v8.20, Sheffield, UK.

### TABLE 6
Parameter inputs used in victim mechanistic static mathematical model predictions of dabrafenib DDIs

<table>
<thead>
<tr>
<th>Parameter Input</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>148 nm/s^c</td>
</tr>
<tr>
<td>Q_{abl}</td>
<td>18 l/h^a</td>
</tr>
<tr>
<td>A</td>
<td>6,600 cm^2/min^c</td>
</tr>
<tr>
<td>CYP3A4_h</td>
<td>66.2 nmoles/total gut^b</td>
</tr>
<tr>
<td>CYP3A4_g</td>
<td>142 pmole/mg protein^b</td>
</tr>
<tr>
<td>Microsomal yield</td>
<td>29.3^b</td>
</tr>
<tr>
<td>P_{eff,man}</td>
<td>0.44 e⁻³cm³/min^b</td>
</tr>
<tr>
<td>HLM C{Lin,3A4}</td>
<td>6.1 ul/min/mg protein^b</td>
</tr>
<tr>
<td>f_{m,CYP3A4}</td>
<td>0.28^c</td>
</tr>
<tr>
<td>f_{m,CYP2C8}</td>
<td>0.67^c</td>
</tr>
<tr>
<td>K_{deg,c}</td>
<td>0.000502 kdeg/min/mg^c</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.02 μM^d</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>9.3^h</td>
</tr>
<tr>
<td>Gemfibrozil-1-O-β-Glucuronide</td>
<td>10.1^i</td>
</tr>
<tr>
<td>Ki</td>
<td>0.21^i</td>
</tr>
<tr>
<td>K_{prot}</td>
<td>NA</td>
</tr>
<tr>
<td>K_{1}</td>
<td>NA</td>
</tr>
<tr>
<td>K_{2}</td>
<td>NA</td>
</tr>
<tr>
<td>f</td>
<td>0.03^i</td>
</tr>
<tr>
<td>C_{max} (μM)</td>
<td>13.17^f</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>400</td>
</tr>
<tr>
<td>Liver:plasma</td>
<td>2^b</td>
</tr>
<tr>
<td>Ka (min⁻¹)</td>
<td>0.036^f</td>
</tr>
<tr>
<td>Fa</td>
<td>0.85</td>
</tr>
<tr>
<td>Qh</td>
<td>1.617 ml/min^d</td>
</tr>
<tr>
<td>Fa</td>
<td>0.85</td>
</tr>
<tr>
<td>Fg</td>
<td>0.85</td>
</tr>
<tr>
<td>Qt</td>
<td>300 ml/min^d</td>
</tr>
</tbody>
</table>

*Yang et al., 2007b; Rodgers et al., 2005b; Rodgers and Rowland, 2006, 2007; In vitro data measured separately; University of Washington Database; [www.drugbank.ca]; [RxList.com]; U.S. Food and Drug Administration, 2012; VandenBrink et al., 2011; Varma et al., 2012; Schneck et al., 2004; Ogilvie et al., 2006; Honkalammi et al., 2011; Yang et al., 2008; In Silico, Simcyp Ltd v8.20, Sheffield, UK.
Prediction of Perpetrator DDI Risk for Dabrafenib and its Circulating Metabolites Using a Mechanistic Static Mathematical Model. The potential for increased exposure (AUC) of rosiglitazone (CYP2C8), warfarin (CYP2C9), or omeprazole (CYP2C19), if co-administered with dabrafenib, taking into account the contributions from the circulating metabolites hydroxy-dabrafenib and desmethyl-dabrafenib, was estimated using in vitro in vivo extrapolation (IVIVE) techniques. When surrogates of dabrafenib concentration and contributing metabolite concentrations were corrected for plasma protein binding, the maximum extrapolated drug interaction was a 1.0-fold change for rosiglitazone, warfarin, and omeprazole, indicative of no anticipated change of their exposure due to P450 inhibition when coadministered with dabrafenib.

Prediction of Perpetrator DDI Risk for Dabrafenib and its Circulating Metabolites Using The Net Effect Model. The CYP3A4 drug interaction profile of dabrafenib involves direct inhibition, inactivation and induction. The static mechanistic model employed for this analysis considered all of these mechanisms. The fold change in midazolam using systemic Cmax with hepatic and gut contributions was 0.63-fold. The extrapolated drug interaction with unbound hepatic inlet concentration was 0.48-fold and 0.34 with estimated free liver concentration. Following the U.S. Food and Drug Administration’s Draft Guidance for Industry, using estimated free hepatic inlet concentrations and considering the potential impact of gut wall metabolism, the fold change in dabrafenib in the presence of ketoconazole was 1.5-fold or a 50% increase in AUC. The fold change in dabrafenib using unbound hepatic inlet concentrations of gemfibrozil with contributions from its glucuronide metabolite was 1.6, or a 60% increase in AUC. Based on the different surrogates of ketoconazole or gemfibrozil tested in the static mechanistic models described, dabrafenib is a likely victim of CYP3A4 and CYP2C8 inhibition.

Prediction of Victim DDI Risk for Dabrafenib Using a Mechanistic Static Mathematical Model. The potential for increased exposure (AUC) of dabrafenib if coadministered with ketoconazole, a strong CYP3A4 inhibitor or gemfibrozil, a CYP2C8 inhibitor, was estimated using in vitro–in vivo extrapolation techniques. The fold change in dabrafenib using unbound surrogate concentrations of ketoconazole was 1.5. Following the U.S. Food and Drug Administration’s Draft Guidance for Industry, using estimated free hepatic inlet concentrations and considering the potential impact of gut wall metabolism, the fold change in dabrafenib in the presence of ketoconazole was 1.5-fold or a 50% increase in AUC. The fold change in dabrafenib using unbound hepatic inlet concentrations of gemfibrozil with contributions from its glucuronide metabolite was 1.6, or a 60% increase in AUC. Based on the different surrogates of ketoconazole or gemfibrozil tested in the static mechanistic models described, dabrafenib is a likely victim of CYP3A4 and CYP2C8 inhibition.

Discussion

In a phase I study previously conducted to assess the metabolism and excretion of [14C]dabrafenib, results showed the mean total recovery of radioactivity was 93.8%, with the majority recovered in feces (71.1% of administered dose). The mean percentage of the
The excreted dose in feces that represented oxidative metabolites was approximately 48%. Urinary excretion accounted for 22.7% of the dose, with no detection of parent drug in urine (Bershas et al., 2013). Therefore, P450-mediated oxidation likely contributes 70% to the metabolism of dabrafenib in vivo. In vitro, dabrafenib undergoes oxidative metabolism when incubated with HLMs and recombinant P450 enzymes as shown by the intrinsic clearance (Tables 1 and 2) and radiolabeled phenotyping studies (Fig. 1). Dabrafenib metabolism was primarily mediated by CYP2C8 and CYP3A4; however, the relative contributions of P450s 2C8 and 3A4 to the systemic clearance of dabrafenib have not been conclusively resolved. A determination of $K_{m}$ and $V_{max}$ for hydroxy-dabrafenib formation, which is mediated by both CYP2C8 and 3A4, has been initiated and is the subject of a further investigation of the mechanisms of dabrafenib’s oxidative metabolism (data not shown). These studies and estimates, based on the investigations described in this work, indicate that CYP2C8 contributed approximately 56% to 67%, and CYP3A4 contributed approximately 24% (Table 1). Based upon these observations it is possible that dabrafenib could be the victim of a drug-drug interaction with strong inhibitors of CYP2C8 and/or CYP3A4. Predictions using static mathematical models to assess the victim risk of dabrafenib with the probe inhibitor ketoconazole (CYP3A4) determined that the fold change in dabrafenib exposure, considering total gut concentration, was 1.5-fold. Employing the same static mathematical model to assess the victim risk in the presence of the probe inhibitor gemfibrozil (CYP2C8) is complicated by its metabolite, gemfibrozil-1-$O$-$\beta$-glucuronide, an irreversible mechanism-based inhibitor of CYP2C8 (Baer et al., 2009). As a consequence, the prediction was performed with the assumption of complete enzyme inhibition, and contributions of the inhibitory potential of the metabolite were incorporated. These predictions determined that the fold change in dabrafenib exposure using unbound hepatic inlet concentrations of inhibitor was 1.6-fold. Therefore, a clinical DDI study was recommended to further investigate the effect of the administration of 400 mg daily dosing of ketoconazole and 75 mg twice-daily dosing of dabrafenib, and a separate study arm investigated the administration of 600 mg daily of gemfibrozil and 75 mg twice-daily of dabrafenib (Suttle et al., 2014). The results of the clinical DDI study demonstrated a 71% increase in dabrafenib exposure upon repeat dosing for 22 days in the presence of ketoconazole and a 47% increase in dabrafenib exposure in the presence of gemfibrozil (Suttle et al., 2014). While the predictive performance of the mechanistic static model was in generally good agreement with the clinically observed results, it is worth noting that the static prediction underestimated the degree of interaction with ketoconazole and overestimated the degree of interaction with gemfibrozil. One possible explanation for this observation is that the in vitro measured $f_{m}$ for CYP3A4 and CYP2C8 did not translate to the in vivo $f_{m}$. Another possible explanation is that the steady-state contribution of CYP3A was higher than would be estimated from the in vitro data. Nevertheless, the model was able to identify the risk of interaction within a 2-fold range and provided the necessary information to inform the clinical development strategy. It should also be noted that under theoretical conditions in which both of these enzymes are inhibited, the increase in dabrafenib exposure would be
anticipated to be higher than when the enzymes are inhibited individually. However, based on extrapolating the in vivo fm values from the respective CYP3A4 and CYP2C8 clinical studies and assuming complete inhibition of both pathways, the anticipated AUC change would be anticipated to be less than 4-fold.

Dabrafenib demonstrated inhibition of CYP2C8, 2C9, 2C19, and 3A4 in human liver microsomes, with metabolism-dependent inhibition of CYP3A4 (Table 3). Dabrafenib was also shown to induce human CYP3A4 and CYP2B6 in hepatocytes, but no increase in CYP1A2 mRNA was observed (Fig. 4). The potential for interactions with sensitive substrates of CYP2C8, 2C9, and 2C19 was investigated by the application of a mechanistic static mathematical model for drug-drug interaction magnitude (Eq. 4, Supplemental Information), which included the contribution of relevant circulating metabolites. No increase in rosiglitazone, warfarin, or omeprazole exposure was predicted as a result of P450 inhibition by dabrafenib. However, the potential induction of the CYP2Cs has not been investigated in vitro and consequently cannot be ruled out. Therefore, a clinical DDI investigation with warfarin (CYP2C9 substrate) was recommended based on this risk assessment.

The CYP3A4 drug-interaction profile of dabrafenib is complex, involving direct inhibition, inactivation, and induction. In addition, dabrafenib has circulating metabolites that have been characterized for their effects on CYP3A4 in vitro. The net effect model employed for this analysis considered all of these effects. When considering the metabolite contributions, carboxy-dabrafenib did not inhibit CYP3A4 up to the highest concentration tested (100 μM) and a calculated IC50 could not be obtained; therefore, its contribution was not incorporated into this analysis. Independent of the surrogate concentration of dabrafenib and its metabolites used, the net effect of dabrafenib on CYP3A4 was induction. The detailed results obtained for each surrogate concentration, comparing the effects of only hepatic metabolism, was modulated versus the combined effects on both hepatic and intestinal (gut) metabolism (Table 7). All surrogates of dabrafenib concentration in this analysis have been corrected for plasma protein binding, including intestinal metabolism (Igut). However, the impact of correcting Igut for plasma protein binding versus its total concentration has been analyzed. In the case of dabrafenib, the predicted impact on midazolam AUC was maximal when both hepatic and gut effects were considered and Igut was corrected for plasma protein binding. The fold change in midazolam using systemic Cmax with hepatic and gut contributions was 0.63-fold (Table 7). The extrapolated drug interactions with unbound hepatic inlet concentration were 0.48- and 0.34-fold using estimated free liver concentration (Table 7). Following the U.S. Food and Drug Administration’s Draft Guidance for Industry (U.S. Food and Drug Administration, 2012) and taking into consideration free hepatic inlet concentrations but using total gut concentrations, the fold change in midazolam was 0.87-fold (Table 7). The impact of induction appears slightly reduced under these conditions, suggesting that maximizing theoretical gut concentration increases the impact of inhibition/inactivation of intestinal CYP3A4. The preliminary results of a clinical drug-drug interaction study of dabrafenib with midazolam were available at the time of this analysis. The clinically observed change in midazolam exposure was 0.26-fold (0.210, 0.318), corresponding to a 74% decrease (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/202806Orig1s000TOC.cfm). The most accurate prediction was made when unbound estimated liver concentrations were used, the intestinal component was considered, and Igut was corrected for plasma protein binding. Under these conditions, a 0.34-fold change was predicted. Shardlow et al. demonstrated that the most accurate predictions within their dataset were obtained using unbound liver or unbound hepatic inlet concentrations while also including the intestinal contribution (Shardlow et al., 2011). In our analysis of dabrafenib, using an estimated liver concentration corrected for protein binding as well as correcting Igut for protein binding gave the most accurate prediction of 0.34-fold change. At least one hypothesis for this observation is that current models may be overestimating the enterocyte concentration, which consequently affects the DDI impact of intestinal CYP3A4.

The available treatment options in oncology can be generally divided into two classes: chemotherapeutics and targeted therapies (Kenny et al., 2012). Targeted therapies are largely composed of kinase inhibitors, which are better tolerated with fewer side effects and usually administered for longer periods of time in comparison with chemotherapeutics (Traer and Deininger, 2010; Kenny et al., 2012). However, like their counterparts, targeted therapies have similar issues with interindividual pharmacokinetic variability and narrow therapeutic windows (Sparreboom

### TABLE 7

<table>
<thead>
<tr>
<th>Dabrafenib-HPMC Surrogate Concentration (with Metabolite Contribution)</th>
<th>Extrapolated Fold Change in AUC</th>
<th>Midazolam</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatic Component</td>
<td>Combined Gut (Igut Corrected for Plasma Protein Binding) + Hepatic Component</td>
<td>Combined Gut + Hepatic Component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound plasma Cmax</td>
<td>0.91</td>
<td>0.63</td>
<td>1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound estimated hepatic inlet concentration</td>
<td>0.69</td>
<td>0.48</td>
<td>0.87*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound estimated liver concentration</td>
<td>0.50</td>
<td>0.34</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Recommended predicted value as described in the FDA Draft Guidance for Industry (U.S. Food and Drug Administration, 2012).


Cheng Y and Prussof WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Eur J Biochem 36:254–260.


In conclusion, these studies identified the complex mechanisms of dabrafenib disposition and identified the potential risk of drug–drug interactions from both a victim and perpetrator perspective. This knowledge will enable more effective treatment and manage care of melanoma patients, who currently have very limited treatment options.

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Authorship Contributions
Participated in research design: Lawrence, Skordos.
Conducted experiments: Lawrence, Nguyen, Bowen.
Performed data analysis: Lawrence, Nguyen, Bowen, Richards-Peterson, Skordos.
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References


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Title:

The metabolic drug-drug interaction profile of dabrafenib: In vitro investigations and quantitative extrapolation of the P450-mediated DDI risk

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Drug Metabolism and Disposition
Supplemental Information

Introduction

Calculations supporting the determination of intrinsic metabolic clearance of dabrafenib, the scaling of metabolic rate data generated from recombinant CYP enzymes, and the quantitative estimation of drug interaction risk using mechanistic static mathematical models are described in this supplement.

Materials and Methods

**CALCULATIONS**

*Intrinsic clearance for phenotyping studies:* The metabolic clearance rate of dabrafenib and its circulating metabolites was determined by fitting the data to a single exponential equation as follows:

\[ y = A_0 e^{-kt} \]

where \( y \) = concentration at time \( t \), \( A_0 \) = initial concentration and \( k \) = the rate constant.

The intrinsic clearance (mL/min/mg protein) in recombinant enzymes was calculated using the following formula:

\[ \text{ClinCYP} = \frac{\text{rate}_{\text{min}} \times \frac{ml}{pmolP450}}{20 \text{ pmoles/mL}} \]

Where 20 pmoles/mL is the recombinant CYP concentration in the incubation mixture and ClinCYP is the intrinsic clearance for recombinant CYP enzyme.

*CYP Inhibition studies:* The IC_{50} values were calculated in GraFit according to one of the following equations:
\[ v = \frac{V_0}{1 + \left( \frac{[I]}{IC_{50}} \right)^s} \]

or, if a background of uninhibitable activity was observed:

\[ v = \frac{V_0}{1 + \left( \frac{[I]}{IC_{50}} \right)^s} + \text{Background} \]

Where \( V_0 \) = uninhibited control rate of substrate metabolite production, \( v \) = observed rate of substrate metabolite production, \([I]\) = inhibitor concentration, \( s \) = slope factor and Background = uninhibitable rate of substrate metabolite production.

**CYP inactivation kinetics:** For the determination of the inactivation parameters, rates of 1’-hydroxymidazolam production at each NADPH pre-incubation period and each concentration of dabrafenib or troleandomycin were expressed as a percentage of the mean uninhibited control rate.

\[ \%\text{control}_{(t)} = \frac{v_{(t)}}{v_{0(t)}} \times 100 \]

The natural logarithm of \( \%\text{control} \) was plotted against the pre-incubation time for each concentration of inhibitor and linear regression analysis of the data was performed using GraFit. The slope of the regression line gave the observed inactivation rate constant (k) at each concentration. Inactivation rate constants were then plotted against inhibitor concentrations and the kinetic constants, \( k_{\text{inact}} \) and \( K_i \), were calculated, from non-linear regression analysis using GraFit, according to the following equation:
Where \( k \) = observed inactivation rate constant (as determined above), \([I]\) = inhibitor concentration, \(k_{inact}\) = maximal rate constant of inactivation and \(K_I\) = inhibitor concentration required to achieve half-maximal rate of inactivation.

**EC50 and Emax calculations for CYP enzyme induction:** The mean mRNA level for each specific CYP was expressed as a mean ratio of treated over solvent control according to the following equation:

\[
\text{Ratio} = \left( \frac{\text{CYP mRNA copy numbers in treated cells}}{\text{CYP mRNA copy numbers in solvent control}} \right) \quad (\text{eq.} \, 2)
\]

The induction response was compared with the appropriate prototypical inducer and expressed as a percentage according to the following equation:

\[
\%\text{Max} = \left( \frac{\text{compound mean mRNA copies - solvent control mean mRNA copies}}{\text{prototypical inducer mean mRNA copies - solvent control mean mRNA copies}} \right) \times 100 \quad (\text{eq.} \, 3)
\]

**Mechanistic static model for estimation of DDI by CYP inhibition:** The potential for increased exposure (AUC) of the CYP2C probe substrates, rosiglitazone (CYP2C8), warfarin (CYP2C9) or omeprazole (CYP2C19), if co-administered with dabrafenib, taking into account any contributing metabolites, was determined using a mechanistic static mathematical model [Obach, 2006; Rowland, 1973] modified to incorporate metabolite contributions as described below [Reese, 2008; Yeung, 2011]:

\[
\frac{AUC_i}{AUC} = \frac{1}{fm + \frac{[I]}{K_I}} \quad (\text{eq.} \, 4)
\]
Where:

\( fm \) is the fractional contribution of the inhibited enzyme to the systemic clearance of the affected drug

\( I = \) the inhibitor concentration at the CYP’s active site

\( K_i = \) is the dissociation constant of the inhibitor from the enzyme.

An estimate of the hepatic portal vein (hepatic inlet) concentration for dabrafenib including contributions from the circulating metabolites hydroxy-dabrafenib and desmethyl-dabrafenib was calculated using the following equation (Shardlow, 2011):

\[
C_{max} = \frac{ka \times Fa \times Dose}{Qh} + \frac{ka \times Fa \times Dose}{Qh}
\]

(eq.5)

Where:

\( ka \) is the absorption rate constant.

\( Fa \) is the fraction absorbed and escaping metabolism in the gut wall

\( Qh \) is the hepatic blood flow

**Mechanistic static model for estimation of net DDI by CYP inhibition, inactivation and induction:** The potential change in drug interaction magnitude of dabrafenib on midazolam (CYP3A4 probe substrate) exposure was determined by employing a mechanistic static mathematical model. The model, shown below, considered contributions of dabrafenib and its metabolites, and incorporated CYP inhibition, inactivation and induction interaction mechanisms [Fahmi, 2008; Shardlow, 2011; Obach, 2007].

\[
\frac{AUC_{po}^{int}}{\text{AUC}_{po}^{int}} = \frac{CL_{in,t,h}^{int}}{CL_{in,t,h}^{int}} \times \frac{F_{g}^{int}}{F_{g}^{int}} = \left( \frac{1}{[A \times B \times C] \times f_m + (1 - f_m)} \right) \times \left( \frac{1}{[X \times Y \times Z] \times (1 - F_g) + F_g} \right)
\]

(eq.6)
\[ A = \frac{k_{\text{deg},h}}{k_{\text{deg},h} + \left[I\right]_h \times k_{\text{inact}}} \]
\[ B = 1 + \frac{d \times \psi \times \left[I\right]_h}{\left[I\right]_h + EC_{50,i}} \]
\[ C = \frac{1}{1 + \sum \frac{\left[I\right]_h}{Ki}} \]
\[ X = \frac{k_{\text{deg},g}}{k_{\text{deg},g} + \left[I\right]_g \times k_{\text{inact}}} \]
\[ Y = 1 + \frac{d \times \psi \times \left[I\right]_g}{\left[I\right]_g + EC_{50,i}} \]
\[ Z = \frac{1}{1 + \frac{\left[I\right]_g}{Ki}} \]

Where,

- \( A \) represents the time-dependent inactivation term in the hepatic intrinsic clearance of the affected drug.
- \( B \) represents the presence of induction in the hepatic intrinsic clearance of the affected drug.
- \( C \) represents the reversible inhibition term in the hepatic intrinsic clearance of the affected drug.
- \( X \) represents the time-dependent inactivation term in the intestinal intrinsic clearance of the affected drug.
- \( Y \) represents the presence of induction in the intestinal intrinsic clearance of the affected drug.
- \( Z \) represents the reversible inhibition term in the intestinal intrinsic clearance of the affected drug.
- \( fm \) is the fractional contribution of the inhibited enzyme to the systemic clearance of the affected drug.
- \( fg \) is the fractional contribution of the inhibited enzyme to the intestinal clearance of the affected drug.
- \( k_{\text{deg}} \) (\( CYP \)) is the degradation rate of the hepatic enzyme.
- \( k_{\text{deg}} \) (\( gut \)) is the degradation rate of the gut enzyme.
- \( d \) is the calibration factor for in vitro to in vivo induction scaling.
- \( \text{IndC}_{50} \) (\( EC_{50,i} \)) is the concentration of inducer at 50% maximum induction.
Ψ (E\textsubscript{max} or Ind\textsubscript{max}) is the maximum fold induction observed in vitro

I= the inhibitor concentration at the CYP’s active site

K\textsubscript{i}= is the dissociation constant of the inhibitor from the enzyme.

K\textsubscript{inact} is the maximal inactivation rate constant

K\textsubscript{I} is the concentration required to achieve half-maximal inactivation

An estimate of the enterocyte concentration for dabrafenib was determined using the equation shown below [Yang 2007b]:

\[
C_{\text{max}} + \frac{ka \ast Fa \ast Dose}{Qg}
\]

(eq.7)

Where:

ka is the absorption rate constant.

Fa is the fraction absorbed at the enterocytic site

Qg is the enterocytic blood flow

**Mechanistic static model for estimation of victim DDI by CYP3A4 inhibition:** The potential change in drug interaction magnitude of dabrafenib exposure in the presence of ketoconazole (CYP3A4 probe inhibitor) was determined by employing a mechanistic static mathematical model. The model, shown below, considered the CYP3A4 f\textsubscript{m} of dabrafenib as measured in vitro and the estimated F\textsubscript{g} of dabrafenib, calculated from in vitro permeability and intrinsic clearance determinations [Obach, 2007].

\[
\frac{AUC_i}{AUC} = \frac{1}{f_m \left(1 + \frac{[I]_{\text{in vivo}}}{K_i}\right) + (1 - f_m) x \left(1 + \frac{1 - F_g}{1 + \frac{[I]_{gut}}{K_i}}\right)}
\]

(eq.8)

Where:

f\textsubscript{m} is the fraction metabolized of dabrafenib by CYP3A4

[I] is the concentration of ketoconazole
Ki is the dissociation constant of the inhibitor from the enzyme

Fg is the fraction absorbed dose that escapes intestinal metabolism in enterocytes

The estimation of the fraction metabolized in the gut was determined using the equations shown below [Yang 2007b]:

\[
F_g = \frac{Q_{gut}}{Q_{gut} + f_{ug} \times CLu_{int,g}}
\]

(eq.9)

\[
Q_{gut} = \frac{Q_{villi} \times CL_{perm}}{Q_{villi} + CL_{perm}}
\]

(eq.10)

\[
CL_{perm} = P_{eff,man} \times A
\]

(eq.11)

Where:

Fg is the fraction absorbed dose that escapes intestinal metabolism in enterocytes

Q_{gut} is a hybrid flow term dependent upon the villous blood flow and permeability of the compound

F_{ugut} is the fraction of drug unbound in the enterocyte

CLu_{int,g} is the net intrinsic metabolic clearance in the gut based upon unbound drug concentrations

Q_{villi} is the villous blood flow

CL_{perm} is the clearance term defining permeability through the enterocyte

P_{eff,man} is the effective intestinal permeability

A is the net cylindrical small intestine surface area
Mechanistic static model for estimation of victim DDI by CYP2C8 inhibition: The potential change in drug interaction magnitude of dabrafenib exposure in the presence of gemfibrozil (CYP2C8 probe inhibitor) was determined by employing a mechanistic static mathematical model [Fahmi, 2008] which was modified to incorporate the mechanism-dependent inhibition potential of gemfibrozil-1-O–β-glucuronide metabolite. The model, shown below, considered the CYP2C8 \( f_m \) of dabrafenib as measured in vitro, the \( K_i \) of gemfibrozil and gemfibrozil glucuronide and the \( k_{inact} \), \( K_i \) of gemfibrozil glucuronide.

\[
\frac{AUC'_{po}}{AUC_{po}} = \frac{1}{k_{deg,h} + \frac{[I_{gluc}]_{h} \times k_{nact}_{gluc}}{[I_{gluc}]_{h} + K_{I_{gluc}}}} \times \frac{1}{1 + \left( \frac{[I]_{gem}}{K_{I_{gem}}} + \frac{[I]_{gluc}}{K_{I_{gluc}}} \right)} \times f_m + (1 - f_m)
\]

(eq. 12)

Where:

\( f_m \) is the fractional contribution of the inhibited enzyme to the systemic clearance of the affected drug

\( k_{deg} (CYP) \) is the degradation rate of the hepatic enzyme

\( I = \) the inhibitor concentration at the CYP’s active site

\( K_i = \) is the dissociation constant of the inhibitor from the enzyme.
$K_{\text{inact}}$ is the maximal inactivation rate constant

$K_I$ is the concentration required to achieve half-maximal inactivation

*Gem* refers to parameters associated with gemfibrozil

*Gluc* refers to parameters associated with gemfibrozil-1-O-β-glucuronide