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

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Nonstandard abbreviations: CL_i = intrinsic clearance; CliCYP = intrinsic clearance for recombinant CYP enzyme; CYP = cytochrome P450; DDI = drug drug interaction; HLM = human liver microsomes; IC_{50} = concentration required for 50% inhibition; K_i = the dissociation constant of the inhibitor from the enzyme; K_i = Concentration required to achieve half-maximal inactivation; \( k_{\text{inact}} \) = Maximal inactivation rate constant; \( K_m \) = concentration at half-maximal rate; \( V_{\text{max}} \) = maximum rate of metabolism; Ind_{max} = maximum fold induction observed in vitro; IndC50= mean concentration of inducer at 50% maximum induction; \( C_{\text{max}} \) = maximum circulating concentration in vivo; Ka=Rate of inhibitor absorption; Fa=Fraction of inhibitor absorbed in blood; Qh=Liver blood flow; Fg=Fraction of substrate escaping gut metabolism; Qg=Enterocytic blood flow; Fa=Fraction of inhibitor absorbed into enterocyte; LSC = liquid scintillation counting; MDI = metabolism-dependent inhibition; PXR = pregnane X receptor; RIF = rifampicin; Vd = volume of distribution; MAP= mitogen activated protein; HMM=hepatocyte maintenance medium; GAPDH = glyceraldehydes-3-phosphate dehydrogenase;
Dabrafenib is a potent ATP-competitive inhibitor for the V600 mutant BRAF kinase currently approved in the United States for the treatment of metastatic melanoma. Studies were conducted in HLMs, recombinant human CYP enzymes and human hepatocytes to investigate dabrafenib’s and its major circulating metabolites’ potential to perpetrate pharmacokinetic DDI’s as well as have its own pharmacokinetics affected (victim) by co-administered drugs. Dabrafenib metabolism was mediated by CYP2C8 (56-67%) and CYP3A4 (24%) and demonstrated inhibition of CYP2C8, 2C9, 2C19, 3A4 (atorvastatin) and (nifedipine), with calculated IC\textsubscript{50} values of 8.2, 7.2, 22.4, 16 and 32\textmu M. It also demonstrated MDI of CYP3A4 with a k_{\text{inact}} of 0.040 min\textsuperscript{-1} and a K\textsubscript{i} for CYP3A4 of 38\textmu M. Hydroxy-dabrafenib inhibited CYP1A2, 2C9 and 3A4 (midazolam) with calculated IC\textsubscript{50} values of 83, 29 and 44\textmu M, and carboxy-dabrafenib did not inhibit any of the CYP enzymes tested. Desmethyl-dabrafenib inhibited CYP2B6, 2C8, 2C9, 2C19 and 3A4 (midazolam, atorvastatin and nifedipine) with calculated IC\textsubscript{50} values of 78, 47, 6.3, 36, 17, 20 and 28\textmu M. At 30 \textmu M 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 (AUC change) as a result of CYP-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’s 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 [Heakal, 2011; Eggermont, 2011]. The prognosis for these patients has been very poor with a median survival time of 6-9 months and a three year survival rate of only 10-15% [Eggermont, 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 of these pathways that has been established in mediating growth signals that drive cancer is the RAS-RAF-MEK-ERK mitogen activated protein (MAP) kinase cascade [Roberts, 2007; Heakal, 2011]. Three genes encode the RAF-serine/threonine kinases, one of them being BRAF. BRAF is mutated in approximately 60% of melanoma patients, with 74-90% being a V600E point mutation which results in a constitutively active kinase that has at least ten times higher activity compared to wild type. [Falchook, 2012; Heakal, 2011; Eggermont, 2011; Flaherty, 2010; Maldonado, 2003]

As a result of increased understanding of these pathways, several targeted therapies for BRAF have recently been approved or are currently in development [Eggermont, 2011].

Dabrafenib is a potent ATP-competitive inhibitor for the V600 mutant BRAF kinase [Hauschild, 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 patients were likely taking co-medications 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 pharmacokinetics affected (victim) by co-administered drugs. In vitro studies, their generated results and interpretation have become a foundation in drug development to assess potential drug-drug interactions [Obach, 2006; Venkatakrishnan, 2003; Bachmann, 2005]. The present studies have evaluated dabrafenib and its metabolites’ potential to participate in CYP-mediated drug-drug interactions as both a victim and perpetrator. In vitro data generated from human liver microsomes, recombinant human CYP enzymes and human hepatocytes have been utilized in mechanistic static mathematical models to estimate the likely magnitude of change (AUC change) as a result of CYP-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 [FDA, 2012; Shardlow, 2011; Einolf, 2007; Obach 2006, Venkatakrishnan, 2003]. 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 co-medication 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-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl}-2,6-difluorobenzenesulfonamide, methanesulfonate salt), was supplied as a solid by GlaxoSmithKline Chemical Development. The purity of the material was determined to be 99%. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, β-nicotinamide adenine dinucleotide phosphate (NADP), sulphaphenazole, furafylline and quinidine were obtained from Sigma
Chemical Company (St. Louis, MO). Azamulin and benzyl nirvanol 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 IN/US Systems (Tampa, FL). Human liver microsomes (HLM) prepared from mixed-gender pools of 15 human livers were obtained from Xenotech LLC (Lenexa, KS). Supersomes™, containing individually over-expressed human CYP enzymes, derived from baculovirus infected insect cells, and control Supersomes™, lacking any native human CYP activity were obtained from BD Gentest (Woburn, MA). Supersomes™ expressing CYP2C8, CYP2C9, CYP2C19 and CYP3A4 co-expressed CYP reductase and cytochrome b₅, while Supersomes™ expressing CYP1A2 and CYP2D6 co-expressed 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 gentamycin, 50 ng/mL amphotericin was obtained from Lonza (Walksville, MD) and the prototypical CYP inducers, omeprazole (CYP1A2), phenytoin (CYP2B6) and rifampicin (CYP3A4) were purchased from Sigma Aldrich (St. Louis, MO). TaqMan™ reagents were purchased from Invitrogen (Grand Island, NY), Promega SV 96 Total RNA Isolation System was obtained from Promega (Madison, WI) and RiboGreen™ assay kit was purchased from BD Gentest (Woburn, MA). All other reagents used in these investigations were reagent grade or higher and obtained from standard commercial suppliers.

**CYP reaction phenotyping.** The CYP enzymes involved in the metabolism of dabrafenib and its metabolites were identified by monitoring substrate depletion kinetics using non-radiolabeled 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 1000 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 LC/MS.

Metabolite formation was determined by incubating [14C]-dabrafenib (5μM) with human liver microsomes (1.0 mg/mL protein concentration) and potassium phosphate buffer (50mM, pH 7.4) in a total volume of 500μL. Following 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 6 units of glucose-6-phosphate dehydrogenase per mL of 2% (w/v) sodium bicarbonate) and shaken in a water bath for 8min. The metabolite CYP reaction phenotype was determined in incubations in the presence and absence of the CYP-selective chemical inhibitors: azamulin (CYP3A4, 5 μM) [Stresser, 2004], sulphaphenazole (CYP2C9, 10 μM) [Baldwin, 1995], quinidine (CYP2D6, 1 μM) [Otton, 1988], montelukast (CYP2C8, 1 μM) [Walsky, 2005], benzylnirvanol (CYP2C19, 5 μM) [Suzuki, 2002], and furafylline (CYP1A2, 10 μM) [Sesardic, 1999]. Since they are mechanism-based CYP 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 co-factor and pre-incubated 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 CYP
selective chemical inhibitors, metabolite formation was also monitored from incubations with human recombinant CYP 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 as described above. Control incubations were performed in the absence of microsomes or Supersomes™, the absence of inhibitors and the absence of cofactor in which the cofactor solution was replaced with sodium bicarbonate. All reactions described above were terminated by adding an equal volume of acetonitrile and centrifuged at 13,000rpm. The supernatants of each sample were analyzed by radio-HPLC and LC/MS².

**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 and HP-1100 membrane degasser, HP-1100 autoinjector and an HP-1100 binary gradient pump from Agilent Technologies (Palo Alto, CA). On-line radiodetection was conducted using a β-RAM radiodetector from IN/US System, Inc. (Tampa, FL) fitted with a built-in liquid scintillant pump. Chromatography of all samples were generated on a Phenomenex Synergi Fusion – REP80A column (4 μm, 4.6 x 250 mm) and a Phenomenex Synergi Fusion – RP (4.0 x 3.0mM) guard column by injecting 100 μL of the supernatant from each incubation. The mobile phase was composed of acetonitrile (solvent B) and 10mM ammonium acetate at pH5.5 (solvent A). The flow rate was 1.0 mL/min at ambient temperature. A 45 min gradient was used in the following manner: 0 to 35min, 65%B; 35.1 to 40 min, 95%B; 40.1 to 45 min, 15%B. All of the post-column eluate was directed to the βRAM and monitored continuously with a liquid scintillation flow cell (IN/US Systems, Tampa, FL). The βRAM operated in the homogenous liquid scintillation counting mode with the addition of
2.0 mL/min of IN-FLOW 2:1 scintillation cocktail to the effluent. The βRAM 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 UHPLC MS/MS using a TurboIonspray™ interface with positive ion multiple reaction monitoring. Change in peak area ratio of each analyte relative to its respective isotopically labelled internal standard ([2H9] dabrafenib, [2H6 13C2] hydroxy-dabrafenib, [2H6 13C2] desmethyl- dabrafenib) and, [2H6 13C2] carboxy-dabrafenib, was utilized to monitor depletion kinetics.

**Cytochrome P450 Inhibition.** The inhibition of cytochrome P450 activity was assessed using methods previously described (Polli, 2013; Shardlow, 2011) by incubating dabrafenib or its three metabolites, with human liver microsomes over a concentration range of 0.1 to 100 μM.
Incubations were conducted in duplicate with 0.1mg/mL of human liver microsomes (HLMs) 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 K_m values: phenacetin (CYP1A2), coumarin (CYP2A6), bupropion (CYP2B6), rosiglitazone (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19) and nifedipine, atorvastatin and midazolam (CYP3A4). Reactions were initiated using an NADPH regenerating system containing 1.7mg NADP, 7.8 mg glucose-6-phosphate and 6 units of glucose-6-phosphate dehydrogenase per mL of 2% w/v sodium bicarbonate. CYP metabolism-dependent inhibition (MDI) was examined by pre-incubating dabrafenib or its metabolites with HLMs and cofactor for 20 minutes and continuing incubation with appropriate probe substrate (described above) for 5-10 minutes. A reduction of IC50
between 1.5-2.0 fold is indicative of metabolism dependent inhibition [Grimm 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, 2008].

**Determination of P450 Inactivation Kinetic Parameters.** CYP3A4 inactivation kinetic parameters were determined for dabrafenib since it demonstrated metabolism dependent inhibition of this enzyme. Pre-incubations 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 concentration approximately 10 times its km). Pre-incubations with dabrafenib, HLMs and cofactor were performed with a volume of 0.5 mL in a shaking water bath at 37°C. Each pre-incubation contained 365 μL 50 mM potassium phosphate buffer pH 7.4, 25 μL HLMs (1 mg/mL final protein concentration) and 10μL of dabrafenib solution in methanol. Following approximately 5 min pre-warming and removal of duplicate 20 μL aliquots (0 min control samples), reactions were initiated by the addition of 95 μL of cofactor solution to each pre-incubation. At 2.5, 5, 10, 15 and 20 min following cofactor addition, duplicate 25 μL aliquots (single aliquots at 20 min) were taken from each pre-incubation and added to a pre-warmed 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 min samples, removed from the pre-incubations before cofactor addition, were also added to dilution mix together with an extra 5 μL cofactor. All diluted incubations proceeded for 4 min 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 – 100μM) were conducted and the measured increases in mRNA levels by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) were employed to assess the CYP enzyme induction potential. Cultured human hepatocytes were obtained from CellzDirect™ (Pittsboro, NC) as cultured monolayers on a collagen substratum with a Matrigel overlay. Upon arrival, shipping medium was replenished with supplemented Hepatocyte Maintenance Medium (HMM) containing 0.1μM insulin, 0.1μM dexamethasone, 0.5 μg/mL gentamycin, 50 ng/mL amphotericin from Lonza (Walksville, MD). For cryopreserved hepatocytes from CellzDirect™ (Pittsboro, NC), cells were thawed and seeded according to the supplier’s protocol and cultured in an overlay configuration in HMM containing 0.25mg/mL Matrigel™. Plated hepatocytes were placed in a humidified CO₂ incubator (5% CO₂) at 37°C for 1 day prior to exposure to dabrafenib. Stock solutions of dabrafenib (0.1 – 100 μM) were prepared in dimethyl sulphoxide (DMSO) and diluted in incubation medium. Prototypical inducers of CYP1A2 (omeprazole at 50 μM), CYP2B6 (phenytoin at 50 μM) and CYP3A4 (rifampicin at 10 μM) were initially dissolved in DMSO and subsequently diluted in incubation medium with a final concentration of DMSO in all incubations of 0.1 % (v/v). The medium was removed and replaced with pre-warmed duplicate culture medium solutions containing dabrafenib (0.1-100μM), omeprazole (50 μM), phenytoin (50 μM) or rifampicin (10 μM). Hepatocytes were also exposed to solvent control in quadruplicate. All cells were then incubated with change of drug/control 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 which is 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 co-administered with dabrafenib, and considering contributing metabolites, was determined using a mechanistic static mathematical model [Obach, 2006; Rowland, 1973] 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, 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 utilized as surrogates for [I] (concentration of inhibitor(s) available at the enzyme active site), and each were 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 (BID) 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 $C_{\text{max}}$ concentrations since these are formed systemically.

3. An estimate of inhibitor concentration in the liver has recently been proposed as an additional surrogate for estimating drug interaction risk [Shardlow, 2011]. Liver partitioning of dabrafenib-related radiocarbon from a bio-distribution study conducted in rats (quantitative whole body autoradiography) indicated a liver to blood ratio of approximately 30:1.
   
   a) The ratio was corrected for blood:plasma concentration ratio (~0.49) to provide a liver:plasma concentration ratio of 15:1 which was used for this analysis.
   
   b) Since metabolite contributions to the liver:blood ratio determined from the whole body autoradioigraphy study cannot be differentiated from parent, the 30:1 ratio was utilized 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 co-administered midazolam, a sensitive CYP3A substrate, was predicted using a model which considered contributions of dabrafenib and its relevant circulating metabolites, and incorporated CYP inhibition, inactivation and induction interaction mechanisms [Fahmi, 2008;] (eq.6, Supplemental information).
In addition to the surrogates of dabrafenib concentrations utilized previously, an estimate of the enterocyte concentration for dabrafenib was used since perturbations to CYP3A in the gut wall is an important mechanism of drug interactions [Galetin, 2008]. All parameters have been summarized in Table 4 and Table 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 [Obach, 2006; Rowland, 1973] (eq. 8, Supplemental information). For the purposes of this analysis, the steady state C\text{max} concentrations of ketoconazole was set to 13.47 μM [Univ. of Washington DIDB], an estimate of the hepatic portal vein (hepatic inlet) concentration was determined using 99% protein binding [Wishart, 2008] and an estimate of the inhibitor concentration in the liver was determined using a liver:plasma ratio of 2 [Rodgers et al. 2005a, 2005b, 2006, 2007]. The molecular weight (531.43g) and ka (0.036 min\(^{-1}\)) of ketoconazole were obtained from Drugbank, a knowledge base for drugs, drug actions and drug targets [Wishart, 2008]. A dose of 400mg of ketoconazole was employed in the model as this is a standard dose used in clinical DDI studies performed by GSK. The CYP3A4 \text{fm} for dabrafenib was determined by in vitro methodologies and the Fg of dabrafenib was estimated as described in Yang, J et al. [2007b] (eq. 9-11, Supplemental information). All other input parameters for the model are shown in Table 6.

While gemfibrozil has been utilized clinically to investigate CYP2C8 mediated DDI, quantitative extrapolation of the potential DDI risk is complicated by both direct and mechanism-based CYP2C8 inhibition (glucuronide metabolite) as well as inhibitor 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, 2008] (eq. 12), (Supplemental information). To determine the potential for increased exposure (AUC) of dabrafenib when coadministered with gemfibrozil, the steady state C\textsubscript{max} concentrations of gemfibrozil and gemfibrozil 1-O–β–glucuronide was set to 102 μM [Schneck, 2004] and 56 μM [Honkalammi, 2010] respectively, an estimate of the hepatic portal vein (hepatic inlet) concentration was determined using 97% protein binding [Varma, 2012] and 88.5% [Honkalammi, 2010] respectively, and an estimate of the inhibitor concentration in the liver was determined using a liver partitioning value of 11:1 [Rodgers et al. 2005a, 2005b, 2006, 2007] which was corrected for blood:plasma concentration ratio (~0.75) [Rodgers et al. 2005a, 2005b, 2006, 2007] to provide a liver:plasma concentration ratio of 8.3:1 which was used for this analysis. The following values for molecular weight (250.34g) [Wishart, 2008] and ka (0.05 min\textsuperscript{-1}) for gemfibrozil [Varma, 2012] and a molecular weight (426.5g) [Wishart, 2008] for gemfibrozil 1-O–β–glucuronide were employed in the model. A dose of 600mg of gemfibrozil was utilized in the model as this is a standard dose used in clinical DDI studies. The CYP2C8 \textit{fm} for dabrafenib was determined by in vitro methodologies and all other parameters for the model are shown in Table 6.

RESULTS

\textit{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 CYP enzymes and pooled human liver microsomes. In experiments using recombinant CYP enzymes, the intrinsic clearance (rate of substrate depletion) was scaled using internally generated relative activity factors (RAF) [Störmer, 2000] or intersystem extrapolation factors (ISEF) [Proctor, 2004] to place the rates within the context of anticipated human liver microsomal activity for each of the
CYPs. Individual scaled human recombinant CYP intrinsic clearance values were added to generate a total scaled rate and a % CYP 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 CYP2C19 (22%) and CYP2C9 (10%). (Table 1 and 2).

Metabolite formation using [14C]-dabrafenib was determined from human recombinant CYP 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 CYPs 2C8 and 3A4 (300pmol/mL) (Fig 2) as well as minor production observed in CYP2C9 and CYP2C19 (data not shown). In addition to hydroxy-dabrafenib, carboxy-dabrafenib was also observed in incubations of recombinant human CYP3A4 where the metabolite’s structure was confirmed by LC/MS/MS and by comparison to an authentic standard. Human liver microsomal incubations only produced hydroxy-dabrafenib, and its formation was inhibited by the inclusion of the CYP selective chemical inhibitors montelukast (CYP2C8, 67% inhibition) and azamulin (CYP 3A4, 24% inhibition). Interestingly, neither carboxy-dabrafenib nor desmethyl-dabrafenib were detected in incubations with human liver microsomes.

To summarize the results of CYP 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 which forms via two successive oxidation steps from hydroxy-dabrafenib, was not metabolized by the CYP enzymes tested in this study. Desmethyl-dabrafenib which appears to represent a decarboxylation of carboxy-dabrafenib was metabolized by CYP3A4 with minor contributions from CYPs 2C9 and 2C19.

In Vitro CYP Inhibition. Dabrafenib demonstrated inhibition of CYP2C8, 2C9, 2C19, 3A4 (atorvastatin) and 3A4 (nifedipine) with calculated IC$_{50}$ values of 8.2, 7.2, 22.4, 16 and 32μM, respectively (Table 3). Dabrafenib also showed metabolism-dependent inhibition of CYP3A4 (nifedipine and midazolam) with 2.1- and ≥4.2-fold decreases in IC$_{50}$ value following a 20 minute pre-incubation with co-factor, respectively (Table 3). Therefore, mechanism-based inactivation was presumed and CYP inactivation kinetic parameters were subsequently determined. The maximal inactivation rate constant (k$_{inac}$) was 0.040 min$^{-1}$ and the concentration of dabrafenib required to achieve half-maximal inactivation (K$_{I}$) of CYP3A4 was 38μM (Figure 3).

Hydroxy-dabrafenib inhibited CYP1A2, 2C9 and 3A4 (midazolam) with calculated IC$_{50}$ values of >80, 29 and 44μM, respectively, but did not inhibit 2A6, 2B6, 2C8, 2C19, 2D6 and 3A4 (atorvastatin, nifedipine) at concentrations up to 100μM (Table 3). Carboxy-dabrafenib did not inhibit any of the CYP enzymes tested in this study. (Table 3).

Desmethyl-dabrafenib inhibited CYP2B6, 2C8, 2C9, 2C19 and 3A4 (midazolam), 3A4 (atorvastatin) and 3A4 (nifedipine) with calculated IC$_{50}$ values of >70, 47, 6.3, 36, 17, 20 and 28μM, respectively, but did not inhibit CYP1A2, 2A6 and 2D6 at concentrations up to 100μM.
Desmethyl-dabrafenib demonstrated metabolism-dependent inhibition of CYP3A4 (atorvastatin, midazolam and nifedipine) with a decrease in IC₅₀ value following a 20 minute pre-incubation with NADPH co-factor of 1.7, 2.2- and 2.3-fold, respectively. Inactivation kinetic parameters were not determined for desmethyl-dabrafenib and metabolism-dependent inhibition was not observed for any of the other CYP’s investigated (Table 3).

**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 48hrs. mRNA was extracted and specific mRNA level was 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 a mean ratio of treated over control of 32 and 30, corresponding to 320% and 150% increases relative to their prototypic inducers, respectively (Figure 4). There were no observed changes in mRNA levels for CYP1A2. Loss in cell viability was observed at the highest concentration (100μM) as measured by the reduction in total mRNA compared to control. The possible CYP induction effects of dabrafenib’s 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.

**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 CYP inhibition when co-administered 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 FDA Draft Guidance for Industry and taking into consideration free hepatic inlet concentrations but using total gut concentrations the fold change in midazolam was 0.87-fold (Table 7). All of the surrogates tested in this model indicate that dabrafenib is a likely inducer of CYP3A4 in vivo.

**Prediction of Victim DDI Risk for Dabrafenib Using a Mechanistic Static Mathematical Model.** The potential for increased exposure (AUC) of dabrafenib if co-administered with ketoconazole, a strong CYP3A4 inhibitor or gemfibrozil, a CYP2C8 inhibitor, was estimated using in vitro in vivo extrapolation (IVIVE) techniques. The fold change in dabrafenib using unbound surrogate concentrations of ketoconazole was 1.5. Following the FDA 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 upon 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 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, 2013]. Therefore CYP-mediated oxidation likely contributes >70% to the metabolism of dabrafenib in vivo. In vitro, dabrafenib undergoes oxidative metabolism when incubated with HLMs and recombinant CYP P450 enzymes as shown by the intrinsic clearance (Table 1 and 2) and radiolabeled phenotyping studies (Figure 1). Dabrafenib metabolism was primarily mediated by CYP2C8 and CYP3A4, however the relative contributions of CYPs 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–β–glucuronide, an irreversible mechanism-based inhibitor of CYP2C8 [Baer, 2009]. As a consequence, the prediction was performed in which complete enzyme inhibition was assumed 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 75mg BID of dabrafenib and a separate study arm which investigated the administration of 600mg daily of gemfibrozil and 75mg BID of dabrafenib [Suttle B, 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 B, 2014]. While the predictive performance of the mechanistic static model was in general 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 fm for CYP3A4 and CYP2C8 did not translate to the in vivo fm. 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 (Figure 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 (DDI) 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 CYP 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 which 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 IC_{50} could not be obtained, therefore, its contribution was not incorporated into this analysis. Independent of the surrogate concentration of dabrafenib and its metabolites utilized, 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 effect on both hepatic and intestinal (gut) metabolism, are shown in Table 7. All surrogates of dabrafenib concentration in this analysis have been corrected for plasma protein binding including $I_{\text{gut}}$. However the impact of correcting $I_{\text{gut}}$ 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 $I_{\text{gut}}$ 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 interaction with unbound hepatic inlet concentration was 0.48- and 0.34-fold using estimated free liver concentration (Table 7). Following the FDA Draft Guidance for Industry [FDA, 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 minimized 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) or 74% decrease [Tafinlar SBA, 2013]. The most accurate prediction was made, when unbound estimated liver concentrations were utilized, the intestinal component was considered and $I_{\text{gut}}$ 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 was obtained using unbound liver or unbound hepatic inlet concentrations while also including the intestinal contribution [Shardlow, 2011]. In our analysis of dabrafenib, using an estimated liver concentration corrected for protein binding as well as correcting $I_{\text{gut}}$ 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 different classes: chemotherapeutics and targeted therapies [Kenny, 2012]. Targeted therapies are largely comprised of kinase inhibitors which are better tolerated with fewer side effects and usually administered for longer periods of time in comparison to chemotherapeutics [Traer, 2010 and Kenny, 2012]. However, like their counterparts, targeted therapies have similar issues with inter-individual pharmacokinetic variability and narrow therapeutic windows [Sparreboom, 2009]. As a consequence, understanding the potential drug-drug interaction potential is critical in oncology patient care. We’ve described here the in vitro investigations performed to gain a better mechanistic understanding of the metabolism of dabrafenib and employ that data in the use of static mathematical modeling to predict the overall DDI risk potential in the treatment of melanoma patients. The complex drug-drug interaction profile of dabrafenib makes it an excellent candidate for the construction and deployment of a physiologically based pharmacokinetic model and this is the subject of a further investigation. This technique offers distinct advantages over the static approaches employed in this analysis. Particularly the ability to provide dynamic time-based estimates of changes to both enzyme and dabrafenib concentrations, which may improve the predictivity of clinical outcomes. However, the static models employed here are relatively simple to use and have provided an accurate assessment of the drug interaction risks based on preliminary clinical data available to date. Therefore, these tools continue to have utility during drug development.
In addition, this DDI risk analysis has provided key insight to the relevance of drug metabolites in the assessment of drug interaction risks. The recent draft FDA guidance on drug interactions indicates that in vitro studies to investigate the DDI risks must be conducted for metabolites which meet or exceed 25% of the parent drug’s systemic exposure (AUC). The metabolites discussed here represent the primary and secondary oxidation products of dabrafenib and each exceed the threshold of 25% of dabrafenib exposure. A relatively comprehensive in vitro assessment of the metabolites’ perpetrator risks were conducted and the generated data were included in the modified mathematical models to assess the combined risks associated with dabrafenib and its circulating metabolites. Interestingly, inclusion of the in vitro data for metabolites did not change the DDI risks already identified for dabrafenib when analyzed alone. This finding appears to be consistent with previous observations that the inclusion of metabolite contributions to DDI risk assessment rarely impacts the overall assessment of risk [Yeung, 2011; Yu, 2013].

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 managed care of melanoma patients who currently have very limited treatment options.
Acknowledgements

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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 and Skordos

Wrote or contributed to the writing of the manuscript: Lawrence, Nguyen, Bowen, Richards-Peterson and Skordos
References:


Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 percent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol. 22:3099-3108.


GlaxoSmithKline Study Number BRF112680. A Phase I, Open-Label, Multiple-Dose, Dose-Escalation Study to Investigate the Safety, Pharmacokinetics, and Pharmacodynamics of the BRAF Inhibitor dabrafenib in Subjects with Solid Tumors

GlaxoSmithKline Study Number BRF113468. An Open-Label Study to Examine the Effects of a High-Fat Meal and Particle Size on the Pharmacokinetics of Orally Administered GSK2118436 in Subjects with BRAF Mutation-Positive Tumor

GlaxoSmithKline Study Number BRF113771. A Three-Part, Open-Label Study to Evaluate the Effects of Repeat Dose GSK2118436 on the Single Dose Pharmacokinetics of Warfarin and the Effects of Repeat Dose Oral Ketoconazole and Oral Gemfibrozil on the Repeat Dose Pharmacokinetics of GSK2118436 in Subjects with BRAF Mutant Solid Tumors


DMD#57778


Tafinlar, Drug Approval Package
Tafinlar (dabrafenib) Capsules Company: GlaxoSmithKline, LLC. Application No.: 202806. Approval Date: 05/29/2013
http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/202806Orig1s000TOC.cfm


Figure Legends

Figure 1. Chemical Structures of Dabrafenib and its Circulating Metabolites: Hydroxy-dabrafenib, Carboxy-dabrafenib and Desmethyl-dabrafenib.

Figure 2. Radiochromatograms of Dabrafenib Incubations in Human Liver Microsomes and Supersomes™. 5.0 μM [14C]-dabrafenib was incubated in duplicate at 37°C for 8min with cofactor in 1.0 mg/mL HLMs (A), 300pmol/mL recombinant CYP2C8 (B) and CYP3A4 (C). Radiochromatograms are one representative of n=2.

Figure 3. Kinetic Constants for CYP3A4 Inactivation by Dabrafenib. DB (1.0 – 100 μM) was preincubated in HLM (1mg/mL) for 2.5, 5, 10, 15 and 20 min at 37°C. At each time point the pre-incubations were diluted into HLM (0.1mg/mL) mixtures containing a final concentration of 25 μM midazolam, DB concentrations were diluted to 0.01 – 10 μM.

Figure 4. Mean Effect of Dabrafenib Treatment on CYP3A4 mRNA Levels in Cultured Human Hepatocytes. Relative induction of CYP3A4(■) genes by dabrafenib (0.1 – 50μM) in cultured human hepatocytes relative to GAPDH control.
**Table 1. Total Percent Contribution of Individual CYP Enzymes to Dabrafenib Metabolism**

<table>
<thead>
<tr>
<th>CYP</th>
<th>% CYP Contribution(^a) based on metabolite formation</th>
<th>% CYP Contribution(^b) based on substrate depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>0</td>
<td>1.9</td>
</tr>
<tr>
<td>2C8</td>
<td>67</td>
<td>56</td>
</tr>
<tr>
<td>2C9</td>
<td>4.0</td>
<td>10</td>
</tr>
<tr>
<td>2C19</td>
<td>5.0</td>
<td>1.5</td>
</tr>
<tr>
<td>2D6</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>3A4</td>
<td>24</td>
<td>23</td>
</tr>
</tbody>
</table>

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

\(^b\) Values represent the normalized (scaled) rates of parent depletion (expressed as a percentage) of individual CYPs with respect to the total normalized rates from intrinsic clearance study.
Table 2. Relative Contribution of CYP Enzymes to Dabrafenib and its Circulating Metabolite Depletion in Supersomes<sup>TM</sup>

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Dabrafenib % to Total Scaled&lt;sup&gt;c&lt;/sup&gt; CLint of CYP450</th>
<th>Desmethyl-dabrafenib % to Total Scaled&lt;sup&gt;d&lt;/sup&gt; CLint of CYP450</th>
<th>Hydroxy-dabrafenib % to Total Scaled&lt;sup&gt;d&lt;/sup&gt; CLint of CYP450</th>
<th>Carboxy-dabrafenib % to Total Scaled&lt;sup&gt;d&lt;/sup&gt; CLint of CYP450</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>1.9%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>6.9%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>56%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>10%</td>
<td>9.6%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1.5%</td>
<td>22%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>0.4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>23%</td>
<td>69%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Clint: intrinsic clearance (mL/min/mg protein) in microsomes was calculated using the following formula:

\[ \text{Clint}_{\text{HLM}} = \frac{\text{rate}}{\text{min}} \times \frac{\text{ml}}{0.5 \text{mg protein}} \]

<sup>b</sup> Clint: intrinsic clearance (mL/min/mg protein) in supersomes was calculated using the following formula:

\[ \text{Clint}_{\text{rCYP}} = \frac{\text{rate}}{\text{min}} \times \frac{\text{ml}}{\text{pmol P450}} \]

<sup>c</sup> RAF: Relative activity factor; scaling factor used to estimate human liver microsomal Clint from recombinant CYP Clint.

<sup>d</sup> ISEF: Intersystem extrapolation factor; scaling factor used to estimate human liver microsomal Clint from recombinant CYP Clint [Proctor et al. 2004]
Table 3. Inhibition of Cytochrome P450 Enzymes by Dabrafenib and its Circulating Metabolites in Human Liver Microsomes

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

- Microsomes, buffer and compound pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.
- Microsomes, buffer and compound pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.
- Data obtained from direct inhibition assay conditions performed on a separate plate from control pre-incubation plate.
- ND = Not determined.
- Data are representative of incubations performed in duplicate.
Table 4. Clinical Probe Substrates and Assumptions of $f_m$ and $F_g$ Used in Dabrafenib DDI Predictions

<table>
<thead>
<tr>
<th>Clinical Probe</th>
<th>$f_m$</th>
<th>$F_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8</td>
<td>Rosiglitazone</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Warfarin</td>
<td>0.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Omeprazole</td>
<td>0.87&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam</td>
<td>0.89&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

$f_m$, fraction of available dose metabolized by hepatic P450; $F_g$, fraction of absorbed dose escaping gut metabolism by CYP3A4 (assumed to be 1 for all other P450’s) [Sharello, 2011]

<sup>a</sup>Baldwin, 1999; <sup>b</sup>Venkatakrishnan, 2007; <sup>c</sup>Yin, 2004; <sup>d</sup>Chen, 2006; <sup>e</sup>Obach, 2006.
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>Values</th>
<th>Definition of inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dabrafenib</td>
<td>Hydroxy-dabrafenib</td>
</tr>
<tr>
<td>Cmax&lt;sub&gt;a&lt;/sub&gt;(μM)</td>
<td>2.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Protein Binding&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.7</td>
<td>96.3</td>
</tr>
<tr>
<td>Liver:Plasma Ratio&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15:1</td>
<td>16.5:1</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>150mg (BID)</td>
<td>Rate of inhibitor absorption</td>
</tr>
<tr>
<td><strong>ka</strong></td>
<td>0.03 min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>Fraction of inhibitor absorbed in blood</td>
</tr>
<tr>
<td><strong>Fa</strong></td>
<td>1</td>
<td>Liver blood flow</td>
</tr>
<tr>
<td><strong>Qh</strong></td>
<td>1617 mL/min&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Fraction of inhibitor absorbed into the enterocyte</td>
</tr>
<tr>
<td><strong>Fa</strong></td>
<td>1</td>
<td>Enterocytic blood flow</td>
</tr>
<tr>
<td><strong>Fg</strong></td>
<td>0.57&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Degradation rate of the hepatic enzyme</td>
</tr>
<tr>
<td><strong>Qg</strong></td>
<td>300 mL/min&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Degradation rate of the intestinal enzyme</td>
</tr>
<tr>
<td><strong>K&lt;sub&gt;deg,h&lt;/sub&gt;</strong></td>
<td>0.000413 kdeg.min&lt;sup&gt;-1&lt;/sup&gt; &lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>K&lt;sub&gt;deg,g&lt;/sub&gt;</strong></td>
<td>0.000561 kdeg/min&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Indmax (Ψ)</strong></td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td><strong>IndC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>1.6 μM</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Steady state Cmax values obtained from clinical study BRF113468 [Grossman, 2012]; <sup>b</sup> Protein binding data measured separately; <sup>c</sup> Liver:plasma ratio determined from QWBA measured separately; <sup>d</sup> Obach, 2006; <sup>e</sup> FDA, 2012; <sup>f</sup> Quinney, 2010; <sup>g</sup> Yang, 2008; <sup>h</sup> Borges, 2005.
Table 6. Parameter Inputs Used in Victim Mechanistic Static Mathematical Model Predictions of Dabrafenib DDI

<table>
<thead>
<tr>
<th>Parameter Input</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>148 nm/s&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Q&lt;sub&gt;vi&lt;/sub&gt;</td>
<td>18 L/h&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>6600 cm&lt;sup&gt;2a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP3A&lt;sub&gt;4&lt;/sub&gt;</td>
<td>66.2 nmoles/total gut&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP3A&lt;sub&gt;4&lt;/sub&gt;</td>
<td>142 pmoles/mg mp&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microsomal Yield</td>
<td>29.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pa&lt;sub&gt;eff, man&lt;/sub&gt;</td>
<td>0.44 e&lt;sup&gt;-6&lt;/sup&gt; cm/s&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>HLM CL&lt;sub&gt;int&lt;/sub&gt;</td>
<td>6.1 μL/min/mg protein&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>fm&lt;sub&gt;CYP3A4&lt;/sub&gt;</td>
<td>0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>fm&lt;sub&gt;CYP2C8&lt;/sub&gt;</td>
<td>0.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>K&lt;sub&gt;deg&lt;/sub&gt;</td>
<td>0.000502 kdeg/min&lt;sup&gt;m&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ketoconazole</th>
<th>Gemfibrozil</th>
<th>Gemfibrozil-1-O-β-glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td>K&lt;sub&gt;I&lt;/sub&gt;</td>
<td>0.02 μM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>K&lt;sub&gt;act&lt;/sub&gt;</td>
<td>NA</td>
<td>NA</td>
<td>0.2&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td>K&lt;sub&gt;I&lt;/sub&gt;</td>
<td>NA</td>
<td>NA</td>
<td>10.1&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>f&lt;sub&gt;u&lt;/sub&gt;</td>
<td>0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.119&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μM)</td>
<td>13.17&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1.02&lt;sup&gt;j&lt;/sup&gt;</td>
<td>59&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>400</td>
<td>600</td>
<td>NA</td>
</tr>
<tr>
<td>Liver:plasma</td>
<td>2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>K&lt;sub&gt;a&lt;/sub&gt; (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.036&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;e&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>Fa</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qh</td>
<td>1617 mL/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fa</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fg</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qg</td>
<td>300mL/min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Yang, 2007b; Rodgers, 2005a, 2005b, 2006, 2007; c in vitro data measured separately; d University of Washington Database; e Wishart, 2007; f RxList.com; g FDA, 2012; h VandenBrink, 2011; Varma, 2012; i Schneck, 2004; j Olgowie, 2006; k Honkalampi, 2010; l Yang, 2008; m In Silico, Simcyp™ Ltd v8.20, Sheffield, UK.
Table 7. Extrapolated changes in midazolam AUC based on in vitro P450 data and surrogates of the dabrafenib-HPMC concentration with metabolite contributions at the CYP3A4 active site

| dabrafenib-HPMC Surrogate Concentration (with metabolite contribution) | Extrapolated fold Change in AUC |
|---|---|---|
| **Midazolam** | Hepatic Component | Combined Gut \((I_{\text{gut}}\), corrected for plasma protein binding) & Hepatic Component | Combined Gut & Hepatic Component |
| Unbound Plasma Cmax | 0.91 | 0.63 | 1.14 |
| Unbound Estimated Hepatic Inlet Concentration | 0.69 | 0.48 | 0.87\(^a\) |
| Unbound Estimated Liver Concentration | 0.50 | 0.34 | 0.62 |

\(a\). Recommended predicted values as described in the FDA Draft Guidance for Industry [FDA, 2012]
Figure 1.

Dabrafenib (DB)  Hydroxy-dabrafenib (HDB)

Carboxy-dabrafenib (CDB)  Desmethyl-dabrafenib (DDB)
Figure 2.

A.

B.

C.
Figure 3.

$k_{\text{inact}} = 0.040 \text{ min}^{-1}$

$K_I = 38 \mu\text{M}$
Figure 4.

CYP3A4 mRNA Level (Ratios of Treated to Control) vs [Dabrafenib] µM

Ind_{max} (ψ) = 26.9
EC_{50} = 1.6 µM
Title:

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

Authors:

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Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, King of Prussia, PA 19406

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:

\[ \text{Cl}_{i\text{CYP}} = \frac{\text{rate}}{\text{min}} \times \frac{\text{ml}}{\text{pmolP450}} \]

Where 20 pmoles/mL is the recombinant CYP concentration in the incubation mixture and \( \text{Cl}_{i\text{CYP}} \) 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 uninhibitab 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.

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

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:
(eq. 1) \[ k = \frac{k_{\text{inact}}[I]}{K_I + [I]} \]

Where \( k \) = observed inactivation rate constant (as determined above), \([I]\) = inhibitor concentration, \( k_{\text{inact}} \) = 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:

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

**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]:

\[
\frac{AUC_i}{AUC} = \frac{1}{fm + \left( 1 - fm \right)} \left( \frac{1}{1 + \sum \frac{[I]}{K_I}} \right)
\]

(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

\( Ki = \) 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):

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

(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].

\[
\frac{AUC'_{po}}{AUC_{po}} = \frac{CL_{int,h}}{CL_{int,h}} \times \frac{F_{g}'}{F_{g}} = \left( \frac{1}{[A \times B \times C] \times f_{m} + (1 - f_{m})} \right) \times \left( \frac{1}{[X \times Y \times Z] \times (1 - F_{g}) + F_{g}} \right)
\]

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

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_{max} or Ind_{max}) is the maximum fold induction observed in vitro

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

K_i= is the dissociation constant of the inhibitor from the enzyme.

K_{inact} is the maximal inactivation rate constant

K_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]:

\[
C_{max} = \frac{ka \cdot Fa \cdot Dose}{Qg}
\]

(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_m of dabrafenib as measured in vitro and the estimated F_g of dabrafenib, calculated from in vitro permeability and intrinsic clearance determinations [Obach, 2007].

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

(eq.8)

Where:

f_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]:

\[
F_g = \frac{Q_{\text{gut}}}{Q_{\text{gut}} + f_{\text{u}} g \times CL_{\text{int.g}}}
\]

(eq.9)

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

(eq.10)

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

(eq.11)

Where:

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

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

F_{\text{u}} g is the fraction of drug unbound in the enterocyte

CL_{\text{int.g}} is the net intrinsic metabolic clearance in the gut based upon unbound drug concentrations

Q_{\text{villi}} is the villous blood flow

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

P_{\text{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.

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

(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