Absorption, Distribution, Metabolism and Excretion of [14C]-Sotorasib in Rats and Dogs: Interspecies Differences in Absorption, Protein Conjugation and Metabolism

Upendra P. Dahal, Brooke M. Rock, John Rodgers, Xiaomeng Shen, Zhe Wang, Jan L. Wahlstrom

Pharmacokinetics and Drug Metabolism, Amgen, Inc., South San Francisco, CA
Running title: ADME of sotorasib in rats and dogs

Correspondence and reprint requests to:
Jan L. Wahlstrom, Amgen, Inc, One Amgen Center Drive, Thousand Oaks, CA 91362
Phone: (805)447-6155
E-mail: janw@amgen.com

Number of text pages: 37
Number of tables: 5
Number of figures: 7
Number of references: 37
Number of words in the abstract: 186
Number of words in the introduction: 341
Number of words in the discussion: 1474

Keywords: sotorasib, mass balance, biotransformation, absorption, distribution, metabolism, excretion, protein adducts
**Abbreviations**

AUC: Area under the plasma concentration versus time curve

BDC: Bile duct cannulated

BDI: Bile duct intact

CDNB: 1-chloro-2,4-dinitrobenzene

C\textsubscript{max}: Maximum plasma concentration

DPBS: Dulbecco's phosphate buffered saline

GGT: gamma-glutamyl-transferase

KRAS: Kirsten rat sarcoma viral oncogene homolog

LSC: Liquid scintillation counting

MPPO: Methylpiperazinylpropanone

PAR: Peak area ratio

PBMCs: Peripheral blood mononuclear cells

PK: Pharmacokinetics

RBCs: Red blood cells
Abstract:

Sotorasib is a first-in-class, targeted covalent inhibitor of KRAS$^{G12C}$ approved by the FDA to treat patients with locally advanced or metastatic non-small cell lung cancer with the KRAS$^{G12C}$ mutation. The mass balance, excretion and metabolism of $[^{14}C]$-sotorasib was characterized in rats and dogs after a single dose of 60 or 500 mg/kg, respectively. Mean recovery was >90% for both species. Excretion of unchanged sotorasib was a minor pathway in rat, accounting for <4% of administered dose in urine and <7% of administered dose in feces. Approximately 66% of administered dose was recovered in the bile from bile duct cannulated rats as metabolites. Excretion of unchanged sotorasib was the major excretion pathway in dog, likely caused by solubility-limited absorption. Major pathways of sotorasib biotransformation included glutathione conjugation and oxidative metabolism. In vitro experiments demonstrated that non-enzymatic conjugation (Michael addition) was the primary mechanism of the reaction with glutathione. Extended radioactivity profiles in blood and plasma were observed in rat, but not dog, after dosing with $[^{14}C]$-sotorasib. In vitro experiments demonstrated that sotorasib-protein adducts were observed with both rat hemoglobin and serum albumin, explaining the extended radioactivity profile.
Significance statement:

This study characterized the mass balance, excretion and metabolism of [14C]-sotorasib, a covalent KRAS<sup>G12C</sup> inhibitor, in rats and dogs. Rapid absorption and extensive metabolism of sotorasib was observed in rat, while sotorasib was primarily excreted unchanged in dog feces, likely due to solubility-limited absorption. Protein adducts with both rat hemoglobin and serum albumin were characterized, explaining observed extended blood and plasma radioactivity profiles. The primary biotransformation pathway, glutathione conjugation, was demonstrated to be mediated through non-enzymatic conjugation in vitro.
Introduction

KRAS (Kirsten rat sarcoma viral oncogene homolog) encodes a guanosine 5'-triphosphate hydrolase (GTPase) protein also called KRAS (Chang et al., 1982). KRAS is a mediator of intracellular signaling integral to cell growth, proliferation and survival (Simanshu et al., 2017). KRAS cycles between an active GTP-bound and an inactive GDP-bound state (Milburn et al., 1990). Interconversion of the bound and unbound states loads GTP to activate KRAS and GTPase-activating proteins, catalyzing hydrolysis to inactivate KRAS (Pai et al., 1989). GTP binding to KRAS promotes effector binding which in turn triggers signal transduction pathways. When a protein in the transduction pathway is mutated, it can stay in an active or inactive state, which is required for the development of many cancers (Jancik et al., 2010). KRAS mutations are associated with drug resistance and poor outcomes in patients. Until recently, no selective KRAS inhibitor had been approved despite decades of scientific research (Hong et al., 2020).

KRAS\textsuperscript{G12C} is found in 13% of lung adenocarcinoma and 2% of other solid tumors (Consortium, 2017). The \textit{KRAS p.G12C} mutation has only been reported to exist in tumors, not normal tissue (Roman et al., 2018). Sotorasib (formerly AMG 510) is a first-in-class targeted covalent KRAS\textsuperscript{G12C} inhibitor, which binds with KRAS\textsuperscript{G12C} protein covalently and locks the protein in its inactive form to block oncogenic signaling (Canon et al., 2019; Lanman et al., 2020). Sotorasib has demonstrated positive results in clinical trials with durable anticancer activity in patients with KRAS\textsuperscript{G12C} mutant cancers (Skoulidis et al., 2021). Recently, the U.S. Food and Drug Administration approved sotorasib for treatment of patients with locally advanced or metastatic non-small cell lung cancer with the KRAS\textsuperscript{G12C} mutation (Blair, 2021).

The primary aim of these studies was to characterize the mass balance, routes of excretion, and metabolic pathways of \textsuperscript{14}C-sotorasib when administered orally to rats or dogs, the species used for the nonclinical toxicology studies. Mechanistic in vitro experiments to
characterize reactivity of the acrylamide warhead and the potential to form protein adducts were also carried out, because sotorasib is a targeted covalent inhibitor of KRAS<sup>G12C</sup>. 
Materials and Methods

Materials

Radioactive $^{14}$C-sotorasib was synthesized by Almac (Craigavon, UK). The radiochemical purity and the chemical purity (by UV detection) were 99.7 and 99.2%, respectively. The specific activity of $^{14}$C-sotorasib was 79.1 µCi/mg. Sotorasib and metabolites M10 (cysteine conjugation), M12 (glutathione conjugation), M18 (hydroxylation) and M24 (diketone formation) were synthesized at Amgen Inc (Thousand Oaks, CA); metabolite characterization in the Supplemental Materials. Pooled mixed gender plasma and blood of rats was purchased from Bioreclamation (Westbury, NY). Pooled rat and dog liver and kidney S9 was purchased from Xenotech (Lenexa, KS). Acivicin, ethacrynic acid, Ficoll-Paque and Ficoll-Paque Plus media were purchased from Sigma-Aldrich (St Louis, MO).

In vivo mass balance studies design and sample collection

Nonclinical in vivo studies were conducted at Covance Laboratories (now Labcorp Drug Discovery, Madison, WI) in compliance with all Covance SOPs and approval by its Institutional Animal Care and Use Committee.

Test Article and Dose Formulation Analysis

The HPLC analyses performed showed the radiopurity of the blended $^{14}$C-sotorasib to be 99.9% prior to dose preparation. The mean radiopurity values from HPLC analysis of predose and postdose aliquots were ≥ 99.6%. The liquid scintillation counting (LSC) results indicate the dose was homogeneous during the dosing periods.

Rats

Male or female Sprague-Dawley (SD) rats, bile duct intact (BDI) and bile duct cannulated (BDC), were received from Envigo RMS, Inc. At dosing, animals were 8 to 14 weeks of age.
Animals were individually housed in Nalgene cages designed to enable the collection of urine and feces. Rats were assigned to three groups (Group 1: 16 males and 16 females, Group 2: 3 males and 3 females, and group 3: 3 BDC males). A single dose of 60 mg/kg (203 µCi/kg) \[^{14}\text{C}]\)-sotorasib was dosed orally as a suspension using 10 mL/kg dose volume to the rats. At designated times following dosing, blood (Group 1), urine (Group 2 and 3), feces (Group 2 and 3), and bile (Group 3) for radioanalysis were collected. The vehicle for the oral dose was 20% Captisol, pH 2.2, in reverse osmosis water. All animals were fasted overnight. The dose was administered by oral gavage.

**Dogs**

Male or female purebred beagle dogs were purchased from Covance Research Products (Cumberland, VA). Animals were individually housed in metabolism cages to enable collection of urine and feces. Dogs (three males and three females) were assigned to one group for this study. The dogs were dosed 500 mg/kg \[^{14}\text{C}]\)-sotorasib (38.4 µCi/kg) orally as a suspension using a 5 mL/kg dose volume. At designated times following dosing, blood, urine, and feces were collected. The vehicle for the oral dose was 2% (w/v) HPMC, 1% (v/v) Tween-80 in reverse osmosis water. The volume of radiolabeled dose formulation to be administered to each animal was calculated based on body weights recorded on the day of dose administration. All animals were fasted overnight. The dose was administered by oral gavage.

**Analysis of radioactivity**

Samples were analyzed for radioactivity using either a Perkin Elmer Model 2900TR or a 2910TR liquid scintillation counter (Waltham, MA) for at least 5 minutes or 100,000 counts. Each sample was homogenized before radioanalysis. Samples were analyzed in duplicate. Scintillation counting data (cpm) were automatically corrected for counting efficiency.
Radioactivity recoveries, metabolite profiling and metabolite identification in rat and dog samples

Plasma

Plasma samples obtained from male and female rats in Group 1 at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 (females only) hours postdose were pooled by sex and time point, including equal volumes (0.1 to 1 mL equivalent volume per time point) of each sample. Plasma samples obtained from male and female dogs in Group 1 at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 hours postdose were pooled by sex and time point, including equal weights of each sample within a pool. Samples were further pooled by sex to generate two 0.25- to 8-hour AUC-representative pooled samples using time-weighted pooling (Hop et al., 1998). Liquid scintillation counting (LSC) was used to determine radioactivity in the samples. The pooled plasma sample was combined with three to four times the volume of 0.1% (v:v) formic acid in acetonitrile. The mixtures were sonicated, vortex mixed, and centrifuged, and the supernatants were removed. The extraction was repeated, and the respective supernatants were combined. Due to low recoveries in rat plasma, the extraction process was repeated with 0.1% (v/v) formic acid in methanol. Duplicate subsamples were analyzed by LSC to determine extraction recoveries. The plasma pellets were solubilized with 1N sodium hydroxide at 40°C. Duplicate subsamples were analyzed by LSC to determine plasma pellet recoveries. Combined supernatants were evaporated using nitrogen at ambient temperature; residues were reconstituted in 300 µL of reverse osmosis water:acetonitrile (2:1, v:v). Samples were sonicated, vortex mixed, and centrifuged.

Urine

Urine samples collected from male and female rats in Group 2 and male rats in Group 3 at 0 to 8, 8 to 24, 24 to 48, 48 to 72, and 72 to 96 (Group 2 only) hours postdose were pooled by
collection interval, sex, and group to generate 0- to 96-hour (Group 2) and 0- to 72-hour (Group 3) pooled samples, including 7% to 12% (equivalent percent by interval) of each sample by weight. Urine samples collected in Group 1 were pooled by weight. LSC was used to determine radioactivity in each sample. Aliquots of each pooled urine sample were centrifuged, and duplicate subsamples of the centrifuged urine pool were analyzed by LSC to determine the recoveries of radioactivity.

**Bile**

Bile samples collected from male rats in Group 3 at 0 to 2, 2 to 4, 4 to 8, and 8 to 24 hours post dosing were pooled by collection interval to create a 0- to 24-hour pooled sample of all Group 3 animals. LSC was used to determine radioactivity in each sample. A 1-mL subsample of each pooled bile sample was centrifuged, and duplicate subsamples of the centrifuged bile pool were analyzed by LSC to determine the recoveries of radioactivity.

**Feces**

Feces samples collected from rats in Group 2 and Group 3 at 0 to 24, 24 to 48, 48 to 72, and 72 to 120 (Group 3 only) hours postdose were pooled by collection interval, sex, and group to generate 0- to 120-hour (Group 3) and 0- to 72-hour (Group 2) pooled samples. Feces samples were collected from dogs in Group 1 at 0 to 24, 24 to 48, 48 to 72 (females only), and 72 to 96 (females only) hours postdose. The feces samples were pooled by sex to create a 0- to 48-hour (males) and a 0- to 96-hour (females) set of pooled samples. Subsamples of each pool were solubilized by the addition of sodium hydroxide at approximately 40°C. The solubilized samples were analyzed by LSC.

Approximately 1.0 g of each pooled feces sample was combined with three to four times the volume of 0.1% (v:v) formic acid in acetonitrile. The mixtures were sonicated, vortex mixed, and centrifuged; after repeated extraction, representative supernatants were combined.
Duplicate subsamples were analyzed by LSC to determine extraction recoveries. Due to low recoveries, the extraction process was repeated as previously described with 0.1% (v:v) formic acid in methanol. Duplicate subsamples were analyzed by LSC to determine extraction recoveries. The feces pellets were further solubilized at 40°C by the addition of sodium hydroxide. Duplicate subsamples were analyzed by LSC to determine solubilization recoveries.

The combined supernatants were evaporated to dryness under nitrogen at ambient temperature and reconstituted in 300 µL of methanol. Samples were sonicated, vortex mixed, and centrifuged; LSC was used to determine radioactivity recovery. The reconstituted plasma samples, pooled urine samples, pooled bile samples and reconstituted feces samples were analyzed by liquid chromatography mass spectrometry (LC-MS). Fractions were collected every 10 seconds using 96-well plates, where each well was filled with solid scintillant. Radioactivity was quantitated using a MicroBeta2 (PerkinElmer, Waltham, MA) and the counts were used to generate radiochemical profiles.

**Radioactivity extraction recoveries**

The rat plasma extract radioactivity recovery was up to 78% and the remaining radioactivity was recovered from the plasma pellet. The radioactivity in dog plasma extract was above 95% and plasma pellet had minimal radioactivity (~5%). The radioactivity recoveries in urine of rat and dog was above 98 and 88%, respectively. Feces extract recovery in rat was up to 77%, and the remaining radioactivity was recovered from the feces pellet. The feces extract radioactivity recovery in dogs was more than 95% and recovery in feces pellet was minimal (<5%). Rat bile radioactivity recovery was approximately 100%.

**Pharmacokinetics analysis**

Noncompartmental analysis (Gabrielsson and Weiner, 2012) was applied to the composite blood and plasma radioactivity concentration data for male and female rats.
Pharmacokinetics (PK) parameters were estimated whenever possible. Nominal doses and sampling times were used. Concentration values below the lower limit of quantitation (< 51.8 and 50.4 ng equivalents/g for rat blood and plasma, < 2120 and 2140 ng equivalents/g for dog blood and plasma, respectively) were treated as zero for descriptive statistics and pharmacokinetic analysis.

**Metabolite Profiling and Identification**

Radioactivity profiles were used to quantitate radiolabeled constituents in plasma, urine, bile and feces. The limit of quantitation for radioactivity in plasma, urine, bile and feces was set at 1% of each chromatographic run and 10 cpm peak height; radioactive levels that were less than these thresholds were considered as not detected (ND). For a given component and matrix, if at least one sample had a reportable value above the limit of quantitation, then other samples would report a value below the limit of quantitation. The cut-off for identification of metabolites was 1% of the sample radioactivity for plasma, urine, bile, and feces.

The LC system consisted of a Shimadzu CBM-20A controller, LC-30AD pumps, SIL-30ACMP autoinjector, CTO-20ACMP column oven, and DGU-20A5R degasser (Columbia, MD) in tandem with a Thermo Fisher Q-Exactive (Waltham, MA). Separation was achieved using a Waters Atlantis T3 column, 4.6 x 250 mm, 5µm particle size (Milford, MA) maintained at 50°C. Mobile phase A consisted of 0.1% (v/v) formic acid in water, while mobile phase B consisted of 0.1% (v/v) formic acid in acetonitrile. Gradient elution was used at a flow rate of 1 mL/min, with 100% mobile phase A to 5 minutes, 80% mobile phase A for 5 minutes, 60% mobile phase A for 40 min, and 5% mobile phase A for 5 minutes. Positive ion detection mode was employed. The ESI source parameters were: spray voltage 3.5 kV, capillary temperature 320°C, S-Lens RF level 51.00 and a normalized collision energy 40. Full scan FTMS data was collected at 70,000 resolution.
Formation of M10 and M12

Formation of M12

Conversion of sotorasib to M12 (glutathione conjugate) was monitored over a 10-minute incubation in the presence or absence of 50 µM ethacrynic acid. Here, 50 µM sotorasib was combined with glutathione (5mM) and either 0.1 mg/mL pooled rat or dog S9 liver fraction or vehicle (67 mM potassium phosphate buffer, pH 7.4) in a 37°C shaking incubator. At the end of the reaction, 50 µL of incubate was diluted into 1 equivalent of quenching solution containing 0.1% formic acid in acetonitrile along with 0.5 µg/mL tolbutamide to be used as internal standard. Quenched samples were centrifuged at 3220 x g for 15 minutes before LC-MS/MS analysis. Glutathione-S-transferase (GST) activity was verified in both rat and dog liver S9 fractions using a 1-chloro-2,4-dinitrobenzene (CDNB) control included in an assay kit purchased from Cayman Chemical (Ann Arbor, MI).

Formation of M10

Conversion of the glutathione-conjugated metabolite M12 to the cysteinylated metabolite M10 was studied using the S9 fractions of dog or rat kidney. Kidney S9 (0.1 mg/mL) was prewarmed in the presence or absence of the gamma-glutamyl-transferase (GGT) inhibitor acivicin (1 mM) and potassium phosphate buffer (67 mM, pH 7.4) at 37°C in a shaking incubator for 15 minutes. The reaction was initiated with an addition of M12 (50 µM) and proceeded for 60 minutes before 100 µL of reaction solution was quenched with 1.25 equivalents of quenching solution. Samples were centrifuged at 3220 x g for 15 minutes before LC-MS/MS analysis. Acivicin inhibition and GGT activity was confirmed using a colorimetric GGT Activity Assay Kit purchased from BioVision (Milpitas, CA).

Proteomic analysis of rat plasma and blood

Blood in-vitro incubation and generation of plasma and red blood cells
Whole blood samples were incubated in 37°C water bath for 3 hours with sotorasib at 20 µg/mL (~36 µM). The whole blood samples were diluted with Dulbecco’s phosphate buffered saline (DPBS) to the final volume of 7 mL and were layered over 7mL of Ficoll-Paque (1.084g/mL) and 7mL of Ficoll-Paque PLUS (1.077g/ml), respectively. The tubes were then centrifuged at 400g for 40 min at room temperature. After centrifugation, plasma was carefully collected, and peripheral blood mononuclear cells (PBMCs) were removed. Before collecting the red blood cells (RBCs), the top layer of RBCs was removed to get rid of neutrophils. The collected RBCs were washed in DPBS once and then were lysed in RIPA buffer with proteinase inhibitor. All the plasma and RBC lysis samples were immediately frozen at -80°C until further use.

Digestion of plasma and RBC proteome

Proteins were extracted from samples by acetone precipitation. Specifically, about 200 µg of proteins was diluted with 6x volume of ice-cold acetone and was vortexed for 15 minutes at 1400 rpm. The samples were then centrifuged at 4000 rpm for 15 minutes. The supernatant was discarded, and the remaining pellets were washed twice with 6x volume of ice cold methanol. The pellets were then dissolved in 100 µL of 8M urea with sonication in a 37°C water bath for 10 minutes. The samples were reduced by incubating with 2 µL of 500 mM TCEP at 37°C for 30 minutes. Subsequently, 8 µL of 250 mM iodoacetamide was added to the samples and the samples were incubated at RT for 45 minutes in dark. The samples were diluted with 500 µL of 50 mM ammonium bicarbonate and digested by adding 30 µg of trypsin at 37°C overnight. The plasma and RBC lysate digests were acidified to the final concentration of trifluoroacetic acid as 0.1% for downstream analysis.

High-pH reversed-phase peptide fractionation and untargeted bottom-up proteomics
The samples were equally aliquoted into two tubes. One aliquot of each sample was cleaned up using solid phase extraction for desalting and analyzed using LC-MS/MS for the bottom-up proteomics analysis of unfractionated samples. The other aliquot of the samples was fractionated into 8 fractions using a high-pH RP peptide fractionation kit according to the manufacturer instructions (Pierce (Thermo Fisher), Waltham, MA). The fractionated samples were also analyzed using LC-MS/MS using the exact same method for the unfractionated samples. Untargeted bottom-up proteomics analysis was done using a Waters M-class nanoAcquity nano UPLC (Milford, MA) coupled to an Orbitrap Lumos mass spectrometer (Thermo Fisher, Waltham, MA). An EASY-spray C18 column (500 mm x 75 μm, 2 μm) was used at a flow rate of 350 nL/min. The mobile phase A was 0.1% formic acid in water, and the mobile phase B was 0.1% formic acid in acetonitrile. The samples were loaded onto a C18 trapping column (20 mm x 180 μm, 5 μm) at a flow rate of 8 μL/min with 2% mobile phase B for 4 minutes. A linear gradient was used for the separation with the increase of mobile phase B from 2% to 36% over 140 minutes. The gradient was then ramped to 90% of mobile phase B in 5 minutes and was kept at 90% for 10 minutes. The mobile phase B was then increased back to 2% in 2 minutes and was kept at 2% for 20 minutes for column re-equilibration. An EASY-spray nanoESI interface was used, and +1850V was applied to trig the ESI. The ion transfer tube temperature was set to 275 °C. LC-MS/MS data were collected in positive ion mode with data dependent acquisition. The full mass scan was collected using orbitrap with the mass resolution of 120,000 at m/z 200. The m/z scan range was set to 375-1500. The RF Lens level was 30%. The AGC target was set to 4e5 with the maximum injection time set to 50 ms. The ions with 2 to 6 charges were isolated with an isolation window of 1.6 m/z for MS/MS analysis. The MS/MS was performed using HCD with the collision energy of 30%. The data were collected using orbitrap with the mass resolution of 15,000 at m/z 200. The AGC target was set to 5e4 with the maximum injection time of 40 ms. The cycle time between each full mass master scan was set to 3 seconds.
Data analysis

Data analysis was performed using Proteome Discoverer 2.3 (Thermo Fisher). For rat proteomics analysis, the UniProt up000002494 of Rattus norvegicus database (29934 entries) was used for database search. To reduce database search complexity and focus on most abundant sotorasib adducted peptides, a survey database search was performed using SEQUEST HT without considering the sotorasib adduct. The most abundant 50 proteins were used to make a “reduced database”. All the raw files were searched against the reduced database using the Sequest HT processing workflow with the settings as follows: 1) precursor ion tolerance: 10 ppm; production ion tolerance: 0.02 Da, and 2) variable modification: oxidation on methionine, sotorasib modification on cysteine and lysine, carbamidomethyl on cysteine, and acetylation on N-terminus. The identification confidence was set to 5% false discovery rate. A Consensus workflow was used to process all the raw files collected for the same sample (i.e. rat RBC or plasma) with all three biological replicates using a consensus workflow CWF_Comprehensive_Enhanced_Annotation. All the MS² spectra with a positive hit as potential sotorasib adducts were manually checked to ensure 100% confidence.
Results

Pharmacokinetic parameters in rats and dogs

The pharmacokinetic (PK) parameters for radioactivity in rat blood and plasma are presented in Table 1. The mean blood and plasma concentration versus time profiles for rat are presented graphically in Figure 1. [14C]-sotorasib-related radioactivity was rapidly absorbed with mean $C_{\text{max}}$ values observed at 0.5 hours postdose in males and females. After reaching $C_{\text{max}}$, blood and plasma concentrations of radioactivity declined, with blood and plasma concentrations still at measurable levels through the last sampling time of 144 hours postdose for both sexes. Plasma $t_{1/2}$ of total radioactivity was 43.9 hours for males and 46.7 hours for females. Blood $t_{1/2}$ and $\text{AUC}_{0-\infty}$ values for males and females were not determined because a definitive elimination phase was not apparent. Based on pharmacokinetic data, a sex-dependent difference was not apparent.

The pharmacokinetic parameters for radioactivity in dog blood and plasma are also presented in Table 1. The mean blood and plasma concentration versus time profiles male and female dogs are presented graphically in Figure 1. Following an oral dose of [14C]-sotorasib to dogs, radioactivity was absorbed with mean $C_{\text{max}}$ blood and plasma concentration values occurring from 2.3 to 4.0 hours postdose, respectively. Female dogs had approximately 3-fold higher mean plasma exposures (based on $\text{AUC}_{0-t}$) to total radioactivity than males while mean blood exposure differences were only 1.6-fold. $C_{\text{max}}$ levels in blood and plasma between sexes were within 2-fold.

In vivo blood to plasma partitioning in rats and dogs

In rats, mean blood to plasma concentration ratios for radioactivity were similar between sexes and ranged from 0.696 to 32.7 in males and from 0.677 to 21.4 in females through 144 hours postdose (Supplemental Table 1). At the earlier sampling times, blood to plasma...
ratios suggested low distribution of radioactivity into the cellular component of blood for both sexes of rats (through approximately 2 hours postdose). After 2 hours postdose, blood to plasma ratios increased through 144 hours postdose, which suggested selective retention of radioactivity in the cellular fraction of blood compared with plasma over time. In dogs, mean blood to plasma radioactivity concentration ratios ranged from 0.642 to 0.850, suggesting no preferential association with blood cells (Supplemental Table 1).

**Excretion and mass balance [\(^{14}\text{C}\)-sotorasib in rats and dogs.**

Cumulative percent of radioactivity excreted in urine, feces and bile (BDC rat only) after a dosing [\(^{14}\text{C}\)-sotorasib to rats and dogs are depicted in Figure 2.

**SD Male and Female Intact Rats (Group 2).**

The mean overall recoveries of radioactive dose in intact male and female SD rats were 89.6 to 91.6%, respectively, through 168 hours postdose after a 60-mg/kg oral dose of [\(^{14}\text{C}\)-sotorasib. Radioactivity was excreted fairly rapidly from intact rats after an oral dose, with approximately 80% of the administered radioactivity recovered by 48 hours postdose. Fecal excretion of radioactivity was the major route of elimination in rats, which accounted for approximately 79% of dose by 168 hours postdose. Urinary excretion of radioactivity was a minor elimination pathway and accounted for approximately 3% of the radioactive dose through 168 hours postdose. Carcasses accounted for approximately 2% of the radioactive dose in rats. Based on the observed excretion profiles between males and females, a sex-dependent difference was not apparent in rats for the elimination of [\(^{14}\text{C}\)-sotorasib-related radioactivity.

**SD Male BDC Rats (Group 3)**

The mean overall recovery of radioactive dose in BDC male SD rats was 95.2% through 120 hours after orally dosing [\(^{14}\text{C}\)-sotorasib (60 mg/kg). Radioactivity was excreted fairly rapidly from BDC rats after an oral dose, with 84.8% of the administered radioactivity recovered
by 48 hours postdose. Biliary excretion of the radioactivity was the major route of elimination which accounted for a mean of 66.3% of dose through 120 hours postdose. Fecal excretion of radioactivity accounted approximately 14.6% of dose. Urinary excretion of radioactivity was the minor elimination pathway and accounted for a mean of 6.2% of the radioactive dose. Carcasses accounted for approximately 2.0% of the radioactive dose.

**Beagle dogs**

Following a dosing of [\(^{14}\)C]-sotorasib to dogs, excretion of [\(^{14}\)C]-sotorasib-related radioactivity after an oral dose was predominately by the fecal route, accounting for a mean of approximately 89.6 and 92.7% (male and female) of the administered dose. Urinary excretion was minor and accounted for a mean of 3.1 and 2.2% (male and female) of the administered radioactive dose. A majority of the radioactivity was recovered in the first 48 hours (approximately 92.7 and 83.4% in male and female dogs, respectively). The overall mean recovery of radioactivity was 95.1 and 96.1% of the radioactivity administered dose, in males and females, respectively.

**Metabolite Profiling**

The eluents for representative plasma, urine, bile, and feces samples were collected and analyzed by LSC to determine the column recoveries, which was above 90% in all the samples analyzed. Analysis of rat plasma, urine, bile and feces samples quantified and identified sotorasib and 30 metabolites. Analysis of dog plasma, urine, and feces samples quantified and identified sotorasib and 11 metabolites. Four metabolites [sotorasib-cysteine conjugate (M10), sotorasib-glutathione conjugate (M12), hydroxy-sotorasib (M18) and des(MPPO)-sotorasib dione (M24)] were synthesized chemically based on in vitro metabolite profile study (structural characterization data are presented in supplemental materials) and were used as authentic standards in these studies. A summary of [\(^{14}\)C]-sotorasib and its excreted metabolites are
presented in Table 2. A summary of [\textsuperscript{14}C]-sotorasib and its metabolites circulating in rat or dog plasma are presented in Table 3. The major metabolic pathways of sotorasib in rat and dog are depicted in Figure 3. Parent masses and characteristic product ions of sotorasib and its metabolites are presented in Table 4. Although extensive metabolism was observed in rats, many metabolites were detected only in trace amount (<2% of dose in excreta and <2% of radioactivity in plasma, hence only prominent metabolites (>2% of radioactivity in plasma or >2% of dose in excreta) in rat or dog samples are discussed here.

**Rat Plasma**

High performance liquid chromatography (HPLC) with radiochemical and high-resolution mass spectrometry (HRMS) analyses of plasma after a 60-mg/kg oral dose of [\textsuperscript{14}C]-sotorasib quantified sotorasib and its metabolites are shown Figure 4A. Sotorasib glutathione conjugate M12, sotorasib cysteine conjugate M10, and des(MPO)-sotorasib dione metabolite M24 were the most abundant plasma metabolites in both sexes. Sotorasib accounted for approximately 17 to 21% of the total plasma radioactivity.

**Rat Excreta**

HPLC with radiochemical and HRMS analyses of urine, feces and bile from male and female bile duct-intact (BDI) and male bile duct-cannulated (BDC) rats quantified and identified sotorasib and multiple trace (<2% of dose) metabolites. Representative urine, feces and bile metabolite profiles (radiochromatograms) are presented in Figure 4B, 4C, and 4D. No substantive differences in metabolite profiles were evident across sexes and dose groups.

**Proposed Biotransformation Pathways in Rats**

Sotorasib underwent extensive metabolism in rats after a 60-mg/kg oral dose of [\textsuperscript{14}C]-sotorasib to 30 identified metabolites. Primary metabolism was through glutathione conjugation
and oxidation. Secondary metabolism included cleavage of cysteine conjugates and glucuronidation. The glutathione conjugation pathway accounted for 21% to 33% of dose in bile duct intact (BDI) rats and 41% of dose in BDC rats. Oxidative metabolites accounted for 10 and 21% of dose for BDC and BDI rats, respectively. Acrolein reduction represented 10% of dose in BDI rats. Dealkylation adjacent to piperazine moiety ranged from 6 to 13% of dose. Biliary excretion was the primary route of elimination for BDC rats (56% of dose). Feces and urine were minor pathways for excretion at 4 and 2% of dose, respectively.

Sotorasib in excreta accounted for 6.3% and 12.2% of dose in intact male and female rats, respectively, with urinary and fecal excretion of sotorasib accounting for 0.57% and 5.7% of dose, respectively, in intact male rats and for 1.1% and 11.1% of dose, respectively, in intact female rats. Similar to intact male rats, excretion of sotorasib in BDC male rats was minor and accounted for 3.4% of dose in urine and for 6.7% of dose in feces. Sotorasib was not quantifiable in bile, indicating no biliary clearance of sotorasib. The primary biotransformation pathways of [14C]-sotorasib in rat are presented in Figure 3.

**Dog plasma**

High performance liquid chromatography (HPLC) with radiochemical and high-resolution mass spectrometry (HRMS) analyses of plasma after a 500 mg/kg oral dose of [14C]-sotorasib quantified sotorasib and its metabolites as shown Figure 5A. Metabolite profiles of sotorasib-derived metabolites were generally similar across sexes. Sotorasib cysteine conjugate M10 and des(MPPO)-sotorasib dione M24 were the most abundant radiolabeled components in plasma for both sexes; sotorasib glutathione conjugate M12 and sotorasib cysteinylglycine conjugates and M30 were minor components. Sotorasib accounted for approximately 7% to 9% of the total plasma radioactivity AUC.

**Dog Excreta**
HPLC with radiochemical and HRMS analyses of urine and feces from male and female dogs quantified and identified sotorasib and multiple trace (<2% of dose) metabolites. Representative urine and feces metabolite profile (radiochromatograms) are presented in Figure 5B and 5C.

**Biotransformation Pathways in Dogs**

Sotorasib underwent metabolism in dogs after a 500 mg/kg oral dose of $^{14}$C-sotorasib to 11 identified metabolites. Biotransformation was primarily through glutathione conjugation. Secondary metabolism proceeded through oxidation, glucuronidation, and cleavage of the cysteine conjugate. In excreta, biotransformation through the glutathione conjugation pathway accounted for approximately 2.5% and 7.2% of dose from male and female dogs, respectively. Unchanged sotorasib in excreta accounted for a majority of dose in male and female dogs, accounting for approximately 100% of the radioactive dose. Sotorasib accounted for 0.06% of the radioactive dose recovered in urine in dogs.

**Mechanism for formation of glutathione conjugate M12 and cysteine conjugate M10**

Formation of glutathione metabolite M12 was characterized to understand the potential role of glutathione-S-transferases (GSTs) in sotorasib biotransformation. The production of M12 was studied in vitro using rat and dog hepatic S9 fractions that were fortified with up to 5 mM glutathione and compared with buffer fortified with the same concentration of glutathione. Formation of M12 in the glutathione-buffer mixture produced a measurable amount of M12, indicating that non-enzymatic conjugation of glutathione to sotorasib via Michael addition is one of the M12 formation mechanisms. Incubations including hepatic S9 fractions produced a greater amount of M12 than glutathione-buffer alone. Addition of ethacrynic acid to the hepatic S9 incubations reduced the amount of M12 to a comparable amount seen in buffer (Figure 6A), indicating that the metabolic activity of GSTs represent an additional M12 formation mechanism.
Downstream metabolism involving the production of the cysteinylated metabolite M10 from M12 was interrogated in the context of gamma-glutamyl-transferase (GGT) activity. Formation of M10 from M12 in the presence or absence of the GGT-selective chemical inhibitor acivicin was studied in both rat and dog kidney S9 fractions. Even though GGT activity in each S9 matrix was confirmed by an orthogonal control assay, there was a marked difference in M10 formation between dog and rat matrices. GGT in rat kidney S9 was concluded to play a key role in M10 production, while dog kidney S9 produced M10 from M12 to a limited extent (Figure 6B). Incubation with acivicin reduced M10 production in rat kidney S9 to that of the uninhibited levels observed in dog, supporting the role of GGT as the metabolic enzyme responsible for conversion of the glutathione conjugate to the cysteine conjugated metabolite.

**Proteomic analysis of rat blood and plasma**

The most abundant adducted proteins (via peptides) observed primarily in rat blood and plasma were identified using proteomics experiments from in vitro experiments incubating either rat blood or plasma with sotorasib. The peptide/protein database was built based on a survey database search without considering the sotorasib adduct; the top 50 most abundant proteins were selected based on the number of peptide spectral matches. In addition to the automatic peptide filtering by scoring provided by the Proteome Discoverer, all the positive hits were manually checked. The true positive hit met three criteria: 1) unmodified production ion could be located in the MS² spectra of unmodified counterpart; 2) retention time shift was observed in the modified peptide vs. the unmodified counterpart; 3) the intensity ranking of shared product ions of modified and unmodified peptides are similar. Rat serum albumin and rat hemoglobin were identified as the primary sotorasib adducts in plasma and blood, respectively (Table 5). A representative extracted ion chromatogram for a sotorasib-modified, rat hemoglobin adduct peptide (EFTPC*[125]AQAAFQK) identified in rat RBC lysis is shown in Figure 7A. The MS/MS spectra of unmodified, alkylated, and sotorasib-modified peptides are shown in Figure 7B.
positive identification of the peptide and modifications was supported by the informative y fragment ions and the correct mass shift of +560 Da, which accounted for the adduction of one sotorasib molecule. The sequence alignment of rat, dog and human hemoglobin subunit β-2 was performed and demonstrated that C125 was unique to rat hemoglobin subunit β-2 (Supplemental Figure 1). A structural representation of the sotorasib-modified rat hemoglobin-C125 adduct is shown in Figure 7C.

Discussion

In vivo mass balance studies were conducted by dosing [14C]-sotorasib in rats (60 mg/kg) and dogs (500 mg/kg) to characterize the metabolism and disposition of sotorasib. Doses were selected to match those used in the 28-day rat and dog toxicology studies. In rats, sotorasib-related radioactivity was absorbed extensively and rapidly, achieving $C_{\text{max}}$ within 0.5 hours after dosing. After reaching $C_{\text{max}}$, blood and plasma concentration of sotorasib declined quickly. However, quantifiable levels of radioactivity were observed in both blood and plasma for 144 hours postdose (Figure 1A and 1B). In contrast, absorption of sotorasib-related radioactivity in dogs was slower, achieving $C_{\text{max}}$ at 3-4 hours after dosing. After reaching $C_{\text{max}}$, blood and plasma concentration in dogs decreased quickly and radioactivity was not quantifiable after 8 hours (Figure 1C and 1D).

In vitro, sotorasib demonstrated concentration-independent plasma protein binding and blood to plasma ratios in rats and dogs at the concentrations evaluated (Supplemental Table 2). In vitro assays indicated sotorasib preferentially distributed in the plasma compartment with blood to plasma partition ratios of approximately 0.6 and 0.8 in rats and dogs, respectively. This result is consistent with the dog mass balance study where blood to plasma ratio ranged from 0.6 to 0.9 (Supplemental Table 1). The rat blood to plasma ratio was less than one for up to 1-hour postdose in the rat mass balance study, but increased gradually, reaching 32 at 144 hours postdose. The increase in blood to plasma ratio over time as well as the extended retention of
radioactivity in rat blood and plasma (Figure 1A and 1B) may be indicative of covalent binding with proteins. Covalent inhibitors, such as acalbrutinib, afatinib, ibrutinib, neratinib and osimertinib, are known to react with cellular proteins (Chandrasekaran et al., 2010; Stopfer et al., 2012; Scheers et al., 2015; Dickinson et al., 2016; Podoll et al., 2019).

Proteomic analysis was performed in rat blood and plasma samples after incubating with sotorasib to identify potential protein-adducts. A primary sotorasib protein adduct, EFTPC*AQAADFQK, was identified in rat RBC lysis, corresponding to adduction with a cysteine residue in the β-2 subunit of rat hemoglobin (Table 5). This cysteine (C125) has previously been identified to exhibit low pKa (~6.9) and high accessibility, which enables reactivity with electrophiles (Rossi et al., 1998; Rossi et al., 2001). A sequence alignment of rat, dog and human hemoglobin subunit β-2 was performed, and this cysteine was found to be unique to rat (Supplemental Figure 1). The most accessible cysteine in dog and human hemoglobin (C93) has a higher pKa (~8.5 to 9.0), leading to reduced reactivity with electrophiles; this may explain why a sotorasib-hemoglobin adduct was not observed in dog and is not expected in human (Okonjo and Aboluwoye, 1992; Okonjo and Adejoro, 1993). Low level adducts of sotorasib to other rat hemoglobin residues (K82 and C93) were also observed (Supplemental Figures 2 and 3). While a minimal to moderate decrease in RBC mass (hemoglobin and hematocrit) was observed in the 28-day and 3-month rat toxicology studies, no clinical observations were associated with this finding (Ishida et al., 2021).

Proteomic analysis of plasma samples also indicated formation of sotorasib protein adducts to rat serum albumin (Table 5). Cysteine-34 has been identified as a residue of reactivity for serum albumin due to low pKa (~6.5) and solvent accessibility (Sabbioni and Turesky, 2017). However, other nucleophilic residues such as the ε-amino group of lysines may interact with electrophiles as well (Baillie, 2020). Unexpectantly, the rat serum albumin adduct was observed at lysine residue 549, not C34 (Supplemental Figure 4). The K549 residue has been implicated as a primary site of glycation (non-enzymatic glycosylation) in patients with type
2 diabetes mellitus and carbamylation (non-enzymatic reaction with urea) in patients with kidney failure (Frolov and Hoffmann, 2010; Berg et al., 2013). The reasons for the preference in reactivity at the K549 site are unclear, although they may involve a unique combination of reactivity and accessibility.

The relative abundance of circulating metabolites was similar between rat and dog, with cysteine metabolite M10 as the primary circulating metabolite, followed by oxidative metabolite M24, glutathione metabolite M12 and cysteinylglycine metabolite M30. Sotorasib was the main circulating constituent in rat plasma and was a minor circulating constituent of dog plasma. However, marked differences between rats and dogs were observed in drug disposition. Sotorasib was quickly absorbed after oral dosing to rats, underwent extensive metabolism, and was eliminated primarily by metabolic clearance. In BDC rats, $^{[14]C}$-sotorasib-related radioactivity was excreted primarily via biliary excretion (66%) with lesser contribution from urinary (~6%) and fecal (~15%) pathways. Unchanged sotorasib accounted for <4% of the administered dose in urine and less than 12% of the administered dose in feces for BDC rats; unchanged sotorasib was not detected in bile. Metabolism was mediated primarily by glutathione conjugation and downstream pathways, as well as oxidation.

In contrast, $^{[14]C}$-sotorasib-related radioactivity was minimally absorbed by dogs. The fraction of sotorasib that was absorbed underwent extensive biotransformation, primarily through glutathione conjugation and subsequent downstream pathways, as well as oxidative metabolism. In dogs, $^{[14]C}$-sotorasib-related radioactivity was excreted primarily via fecal elimination (~92%) with lesser contribution from urinary excretion (~3%). Unchanged sotorasib accounted for <0.1% of the administered dose in urine and approximately 100% of the administered dose in feces. The low absorption of sotorasib in dogs may be explained by solubility-limited absorption. Sotorasib has pKa values of 8.06 and 4.56. The solubility of sotorasib in aqueous solution decreases over the pH range of 1.2 to 6.8 from 1.3 mg/mL to 0.03
mg/mL (https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/214665s000lbl.pdf).

Solubility-limited absorption of sotorasib may be occurring in dog due to markedly higher physiological pH along the GI tract when compared to rat (Lui et al., 1986; McConnell et al., 2008). While it is possible that biliary excretion of unchanged sotorasib is contributing to the high fecal recovery, limited increases in sotorasib exposure were observed with increasing dose in the 28-day oral toxicology studies in dog, consistent with solubility-limited absorption (Ishida et al., 2021).

In vitro experiments were conducted to characterize formation of glutathione adduct M12 and further downstream metabolites. Incubations fortified with GSH (5 mM) were carried out using rat and dog liver S9 fractions and compared to buffer controls. Marked M12 formation was observed in the buffer control containing only GSH (Figure 6A), indicating non-enzymatic conjugation (Michael addition) as a primary mechanism for glutathione conjugation. Addition of ethacrynic acid, a selective GST inhibitor, reduced formation of M12 to buffer control levels using the liver S9 fractions, confirming some contribution of GSTs to M12 formation, particularly for dog (Ahokas et al., 1985). Lack of primary GST involvement in the biotransformation of sotorasib eliminates potential concerns observed with the development of some targeted covalent inhibitors, including null GST phenotypes that may lead to marked human pharmacokinetic variability and the possible need to overcome GST activity as a resistance mechanism in tumors (Ginsberg et al., 2009; Pljesa-Ercegovac et al., 2018).

Additional in vitro experiments were carried out to determine whether enzymes were involved in the biotransformation of GSH adduct M12 to cysteine adduct M10. Biotransformation from glutathione conjugates to cysteinylglycine conjugates is typically catalyzed by gamma-glutamyltransferase (GGT) followed by further transformation by aminotransferases (Cooper and Hanigan, 2010). As the highest levels of GGT expression are observed with the renal proximal tubule surface, rat or dog kidney S9 fractions were used as the
matrices to examine the conversion of M12 to M10. Substantial amounts of M10 were formed from M12 using rat kidney S9 fractions; addition of acivicin, a known irreversible inhibitor of GGT (Gardell and Tate, 1980), markedly reduced the biotransformation of M12 to M10 in this system (Figure 6B). Limited formation of M10 from M12 was observed using dog kidney S9 fractions.

Formation of glutathione adducts or the haptenization of endogenous proteins as a hazard signal for idiosyncratic toxicity or drug-induced liver injury has been a theoretical concern for targeted covalent inhibitors. Targeted covalent inhibitors of tyrosine kinases with acrylamide warheads, such acalbrutinib, afatinib, ibrutinib, neratinib and osimertinib, have demonstrated reasonable safety profiles in the clinical setting and marked idiosyncratic toxicity has not been observed (Baillie, 2020). However, recent reports have hypothesized a role for GSH adducts in rat-specific renal toxicity observed with sotorasib and ibrutinib (Rood et al., 2021; Werner et al., 2021). Necrosis was observed only on the proximal tubular epithelium outer stripe of the outer medulla, suggesting a potential role for metabolism. Cysteine metabolites in the renal tubular epithelial cells could be further metabolized by renal β-lyase, where the resulting sulfur-containing metabolites may cause tubular epithelial cell degeneration (Werner et al., 2021). The major circulating metabolites of sotorasib (cysteine adduct M10 and oxidative dione metabolite M24) are observed in rat, dog and human (Ishida et al., 2021). The markedly higher capability of rat kidney S9 fractions to convert M12 to M10 when compared to dog kidney S9 may help to explain the species selectivity of the kidney toxicity observation.
Acknowledgements

The authors thank Eric Ballard and Dean Hickman for their assistance in designing and coordinating the rat and dog mass balance studies, John Roberts and Xuhai Be for performing the in vitro plasma protein binding and red blood cell partitioning experiments, Hao Wu for performing the whole blood incubation assay and preparing RBC lysate for proteomics and Nan Bai for generating the sotorasib-rat hemoglobin figure.
Authors’ Contributions

Participated in research design: UD, BR, JR, XS, JW, ZW

Conducted experiments: JR, XS, ZW

Performed data analysis: UD, BR, JR, XS, JW, ZW

Wrote or contributed to the writing of the manuscript: UD, BR, JR, XS, JW, ZW
References


Okonjo KO and Aboluwoye CO (1992) Ionizable groups linked to the reaction of 2,2'-dithiobispyridine with hemoglobin. *Biochim Biophys Acta* **1159**:303-310.


*Cell* **170**:17-33.


Footnotes

- These studies were funded by Amgen, Inc
- UD, BR, JR, XS, JW, and ZW are employees and shareholders of Amgen, Inc.
Figure Legends

**Figure 1.** Concentrations of radioactivity in blood and plasma at specified times after a single oral administration of $^{14}$C-AMG510 to Sprague Dawley rats and beagle dog: (A) male rat, (B) female rat, (C) male dog, and (D) female dog. Solid circles represent radioactivity in blood and solid squares represent radioactivity in plasma.

**Figure 2.** Mean cumulative percent of radioactive dose in urine, feces, and bile (BDC rat only) at specified intervals after a single oral administration of $^{14}$C-sotorasib to rats and dogs: (A) intact male SD rats, (B) intact female SD rats, (C) BDC male SD rats, (D) male beagle dogs, and (E) female beagle dogs. Closed circles represent urine samples, open circles represent fecal samples, open triangles represent bile samples and closed triangles represent combined total radioactivity in all the excreta.

**Figure 3.** Proposed metabolic pathways of sotorasib in nonclinical species.

**Figure 4.** Radiochromatograms from the rat ADME study of sotorasib: (A) representative 0.5 h pooled plasma sample from intact male rats, (B) 0 to 96-hour pooled urine sample from intact male rats, (C) 0 to 72-hour pooled feces sample from intact male rats, and (D) 0 to 24-hour pooled bile sample from BDC rats.

**Figure 5.** Radiochromatograms from the dog ADME study of sotorasib: (A) representative AUC-pooled plasma sample from female dogs, (B) 0 to 8-hour pooled urine sample from male dogs, and (C) 0- to 96-hour pooled feces sample from female dogs.

**Figure 6.** In vitro characterization of the glutathione pathway for sotorasib: (A) formation of M12 using rat and dog hepatic S9 fractions in comparison to buffer using mass spectrometry peak area ratio (PAR) and (B) formation of M10 from M12 in rat and dog kidney S9 fractions using mass spectrometry peak area ratio (PAR).

**Figure 7.** Characterization of the sotorasib-rat hemoglobin adduct: (A) extracted ion chromatograms, (B) representative HCD MS/MS spectra of the peptide, EFTPCQAQAFQK, with and without modifications, (C) proposed structural representation of the sotorasib-rat hemoglobin adduct.
### Tables

**Table 1. Pharmacokinetic parameters for radioactivity in blood and plasma collected from male and female Sprague Dawley rats after a single oral administration of $[^{14}C]$-AMG510 (60 mg/kg) or male and female beagle dogs after a single oral administration of $[^{14}C]$-AMG 510 (500 mg/kg)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Matrix</th>
<th>Sex</th>
<th>$T_{\text{max}}$ (hours)</th>
<th>$C_{\text{max}}$ (ng eq/g)</th>
<th>$t_{\frac{1}{2}}$ (hours)</th>
<th>$\text{AUC}_{0-t}$ (ng eq·hours/g)</th>
<th>$\text{AUC}_{0-\infty}$ (ng eq·hours/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Blood</td>
<td>M</td>
<td>0.5</td>
<td>19200</td>
<td>NR</td>
<td>551000</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.5</td>
<td>27200</td>
<td>NR</td>
<td>861000</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>M</td>
<td>0.5</td>
<td>27700</td>
<td>43.9</td>
<td>110000</td>
<td>117000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.5</td>
<td>37200</td>
<td>46.7</td>
<td>177000</td>
<td>192000</td>
</tr>
<tr>
<td>Dog</td>
<td>Blood</td>
<td>M</td>
<td>2.3</td>
<td>11700</td>
<td>1.71</td>
<td>54600</td>
<td>105000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>4.0</td>
<td>21700</td>
<td>NR</td>
<td>86200</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>M</td>
<td>2.7</td>
<td>20100</td>
<td>2.11</td>
<td>61000</td>
<td>163000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>4.0</td>
<td>33000</td>
<td>NR</td>
<td>186000</td>
<td>NR</td>
</tr>
</tbody>
</table>

$\text{AUC}_{0-\infty}$ obtained by extrapolation was >20%,  eq = Equivalents $^{14}C$-AMG510, F = Female, M = Male, NR = Not reportable.
Table 2. Mean percentage of Sotorasib and its Metabolites Recovered in Excreta after a single PO dose of $[^{14}\text{C}]$-Sotorasib

<table>
<thead>
<tr>
<th>Designation</th>
<th>Percent of administered $[^{14}\text{C}]$-sotorasib dose</th>
<th>Rat</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine (M)</td>
<td>Bile (M)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M (BDC)</td>
<td>M (BDC)</td>
</tr>
<tr>
<td>Sotorasib</td>
<td>0.6 1.1 3.4</td>
<td>ND</td>
<td>5.7 11.1 6.7</td>
</tr>
<tr>
<td>M3 (+oxy +gluc)</td>
<td>ND ND ND</td>
<td>2.7 ND ND ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M6 (dione + oxy)</td>
<td>ND ND ND</td>
<td>ND 4.2 2.9 ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M9 (+oxy)</td>
<td>0.03 ND 0.1</td>
<td>ND 8.0 4.3 ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M10 (cysteine adduct)</td>
<td>0.3 0.4 0.4</td>
<td>7.8 4.1 3.4 0.2</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M12 (glutathione adduct)</td>
<td>ND ND ND</td>
<td>10.3 ND ND ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M18 (+oxy)</td>
<td>0.1 0.2 0.1</td>
<td>ND 10 7.4 0.6</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M20 (mercapturate adduct)</td>
<td>0.3 0.9 0.3</td>
<td>12.8 5.7 6.3 ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M21 (alkene reduction)</td>
<td>ND ND ND</td>
<td>ND 1.0 1.8 2.1</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M22 (dione + oxy)</td>
<td>ND ND ND</td>
<td>ND 8.4 6.8 ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M30 (cysteinylglycine adduct)</td>
<td>0.2 0.2 0.2</td>
<td>3.9 ND ND ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M37 (dione + oxy + gluc)</td>
<td>ND ND ND</td>
<td>3.1 ND ND ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M52 (alkene reduction + oxy)</td>
<td>ND ND ND</td>
<td>ND 3.2 1.8 ND</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M56 (methylthio adduct)</td>
<td>ND 0.04 ND</td>
<td>ND 2.4 6.0 0.3</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M60 (thiosotorasib dimer)</td>
<td>ND ND ND</td>
<td>ND 4.8 13 0.3</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>M61 (hydroxycysteinylglycine adduct)</td>
<td>0.3 0.1 0.5</td>
<td>2.3 ND ND ND</td>
<td>ND ND ND</td>
</tr>
</tbody>
</table>

BDC = bile duct cannulated, F = female, gluc = glucuronidation, M = male, ND = not detected, oxy = monohydroxylation
Table 3. Summary of Sotorasib and Metabolite Components in Rat and Dog Plasma

<table>
<thead>
<tr>
<th>Designation</th>
<th>Male rat</th>
<th>Female rat</th>
<th>Male dog</th>
<th>Female dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sotorasib</td>
<td>20.7</td>
<td>16.7</td>
<td>6.7</td>
<td>9.2</td>
</tr>
<tr>
<td>M10 (cysteine adduct)</td>
<td>12.3</td>
<td>18.4</td>
<td>64.4</td>
<td>60.2</td>
</tr>
<tr>
<td>M12 (glutathione adduct)</td>
<td>3.8</td>
<td>8.6</td>
<td>3.5</td>
<td>6.2</td>
</tr>
<tr>
<td>M13 (+oxy + gluc)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.7</td>
</tr>
<tr>
<td>M20 (mercapturate adduct)</td>
<td>0.6</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M22 (dione + oxy)</td>
<td>1.3</td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
</tr>
<tr>
<td>M24 (dione)</td>
<td>9.7</td>
<td>8.7</td>
<td>21.3</td>
<td>20.2</td>
</tr>
<tr>
<td>M30 (cysteinylglycine adduct)</td>
<td>0.6</td>
<td>0.9</td>
<td>2.8</td>
<td>7.5</td>
</tr>
<tr>
<td>M37 (dione + oxy + gluc)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.4</td>
</tr>
<tr>
<td>M56 (methylthio adduct)</td>
<td>ND</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

AUC₀⁻ᵗ = area under the concentration-time curve, gluc = glucuronidation, ND = peak not detected, oxy = monohydroxylation
Table 4. Summary of High-Resolution Mass Spectrometry Data for Sotorasib and its Metabolites

<table>
<thead>
<tr>
<th>Designation</th>
<th>Measured Mass</th>
<th>Theoretical Mass</th>
<th>Δ ppm</th>
<th>Characteristic Product Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sotorasib</td>
<td>561.2411</td>
<td>561.242</td>
<td>-1.6</td>
<td>507, 450, 428, 409, 374, 317, 276, 138, 134, 84</td>
</tr>
<tr>
<td>M3</td>
<td>753.2685</td>
<td>753.269</td>
<td>-0.7</td>
<td>577, 466, 444, 425, 390, 333, 292, 138, 134, 84</td>
</tr>
<tr>
<td>M6</td>
<td>441.1363</td>
<td>441.1369</td>
<td>-1.4</td>
<td>308, 134</td>
</tr>
<tr>
<td>M9</td>
<td>577.2367</td>
<td>577.2369</td>
<td>-0.3</td>
<td>425, 390, 333, 292, 138, 134, 84</td>
</tr>
<tr>
<td>M10</td>
<td>682.2609</td>
<td>682.2618</td>
<td>-1.3</td>
<td>595, 593, 561, 507, 490, 450, 374, 317, 172, 138, 134, 84</td>
</tr>
<tr>
<td>M12</td>
<td>868.3251</td>
<td>868.3258</td>
<td>-0.8</td>
<td>595, 593, 561, 507, 490, 450, 374, 317, 172, 138, 134, 84</td>
</tr>
<tr>
<td>M13</td>
<td>737.2732</td>
<td>737.2741</td>
<td>-1.2</td>
<td>561, 507, 450, 428, 409, 374, 317, 276, 138, 134, 84</td>
</tr>
<tr>
<td>M18</td>
<td>577.2366</td>
<td>577.2369</td>
<td>-0.5</td>
<td>523, 466, 425, 390, 333, 292, 138, 134, 84</td>
</tr>
<tr>
<td>M20</td>
<td>724.2721</td>
<td>724.2723</td>
<td>-0.3</td>
<td>636, 595, 593, 561, 507, 450, 407, 374, 317, 172, 138, 134, 84</td>
</tr>
<tr>
<td>M21</td>
<td>563.2583</td>
<td>563.2577</td>
<td>1.1</td>
<td>507, 450, 430, 409, 374, 317, 276, 140, 134, 84</td>
</tr>
<tr>
<td>M22</td>
<td>441.1363</td>
<td>441.1369</td>
<td>-1.4</td>
<td>308, 134</td>
</tr>
<tr>
<td>M24</td>
<td>425.1407</td>
<td>425.142</td>
<td>-3.1</td>
<td>292, 275, 249, 134</td>
</tr>
<tr>
<td>M30</td>
<td>739.2821</td>
<td>739.2832</td>
<td>-1.5</td>
<td>593, 561, 507, 450, 407, 374, 317, 170, 138, 134, 84</td>
</tr>
<tr>
<td>M37</td>
<td>617.1681</td>
<td>617.169</td>
<td>-1.5</td>
<td>441, 308, 134</td>
</tr>
<tr>
<td>M52</td>
<td>579.2526</td>
<td>579.2526</td>
<td>0</td>
<td>446, 425, 390, 333, 292, 140, 134, 84</td>
</tr>
<tr>
<td>M56</td>
<td>609.245</td>
<td>609.2454</td>
<td>-0.7</td>
<td>409, 374, 317, 291, 186, 138, 134, 84</td>
</tr>
<tr>
<td>M60</td>
<td>594.221</td>
<td>594.2219</td>
<td>-1.5</td>
<td>561, 462, 450, 448, 436, 317, 303, 138, 134</td>
</tr>
<tr>
<td>M61</td>
<td>740.2667</td>
<td>740.2673</td>
<td>-0.8</td>
<td>561, 507, 450, 409, 374, 317, 170, 138, 134, 84</td>
</tr>
</tbody>
</table>
Table 5. Identified peptides with sotorasib adduct in rat blood or plasma

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td><strong>Peptide</strong></td>
<td><strong>Protein</strong></td>
</tr>
<tr>
<td>Albumin</td>
<td>K[549]QTALAEVK</td>
<td>Hemoglobin subunit β-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2

A

% of Radioactive Dose

B

% of Radioactive Dose

C

% of Radioactive Dose

D

% of Radioactive Dose

E

% of Radioactive Dose

- Urine
- Feces
- Bile
- Total
Figure 4

A

B

C

D

M1, M10, M12, M18, M20, M22, M30, M61, AMG-510

M18, M19, M20, M22, M30, M31, M32, M40, M41, M61, AMG-510

M6, M10, M11, M12, M19, M20, M22, M24, AMG-510, M57, M59

M13, M15, M17, M18, M20, M22, M30, M32, M34, M40, M42, M55, M57, M59, M61
Figure 6

(A) M12 Formation (Amount based on mass spectrometry PAR)

(B) M10 Formation (Amount based on mass spectrometry PAR)