An Innovative Approach to Characterize Clinical ADME and Pharmacokinetics of the Inhaled Drug Nemiralisib Using an Intravenous Microtracer Combined with an Inhaled Dose and an Oral Radiolabel Dose in Healthy Male Subjects [S]

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ABSTRACT

An innovative open-label, crossover clinical study was used to investigate the excretion balance, pharmacokinetics, and metabolism of nemiralisib—an inhaled phosphoinositide 3-kinase delta inhibitor being developed for respiratory diseases. Six healthy men received a single intravenous microtracer of 10 μg [^{14}C]nemiralisib with a concomitant inhaled nonradiolabeled 1000 μg dose followed by an oral 800 μg dose of [^{14}C]nemiralisib 14 days later. Complementary methods including accelerator mass spectrometry allowed characterization of a range of parameters including oral absorption (F_{abs}), proportion of nemiralisib escaping gut wall metabolism (F_g), hepatic extraction (E_h), fraction of dose absorbed from inhaled dose (F_{lung}), and renal clearance. Intravenous pharmacokinetics of nemiralisib were characterized by low blood clearance (10.0 l/h), long terminal half-life (55 hours), and high volume of distribution at steady state (728 l). Nemiralisib exhibited moderate inhaled and oral bioavailability (38% and 35%) while F_{lung} was 29%. Absorption and first-pass parameters were corrected for blood renal clearance and compared with values without correction. Any swallowed nemiralisib was relatively well absorbed (F_{abs}, 0.48) with a high fraction escaping gut wall metabolism and low extraction by the liver (F_g and E_h being 0.83 and 0.10, respectively). There were no major human plasma metabolites requiring further qualification in animal studies. Both unchanged nemiralisib and its oxidative/conjugative metabolites were secreted in bile, with nemiralisib likely subject to further metabolism through enterohepatic recirculation. Direct renal clearance and metabolism followed by renal clearance were lesser routes of elimination.

SIGNIFICANCE STATEMENT

A number of innovative features have been combined into one small clinical study enabling a comprehensive description of the human pharmacokinetics and metabolism of an inhaled molecule. Design elements included an intravenous ^{14}C tracer administration concomitant with an inhalation dose that enabled derivation of parameters such as fraction absorbed (F_{abs}), the proportion of drug escaping first-pass extraction through the gut wall and liver (F_g and F_h) and hepatic extraction (E_h). Entero-test bile sampling enabled characterization of biliary elimination pathways.

Introduction

Nemiralisib (bis(6-(1*H*-indol-4-yl)-4-(5-{[4-(propan-2-yl)piperazin-1-yl]methyl}-1,3-oxazol-2-yl)-1*H*-indazole) succinate) is a potent and selective inhaled inhibitor of phosphoinositide 3-kinase delta being developed for the treatment of chronic respiratory disorders (Down et al., 2015; Cahn et al., 2017; Begg et al., 2019). The characterization of human absorption, distribution, metabolism, and excretion (referred to as ADME) and pharmacokinetics (PK) is an important and required part of drug development. Human ADME qualifies whether the toxicology species are relevant to the safety assessment of the drug for human use and informs on the utility of specific clinical safety studies including investigations of possible drug interactions or special patient groups (e.g., hepatic or renal impaired patients). Intravenous PK is needed to characterize primary PK parameters such as bioavailability, clearance and volume of distribution, which contribute to a fundamental understanding of how the drug enters, distributes, and leaves the body. A full ADME investigation usually includes administration of a radioisotope

using the dose, route, and formulation of interest. This presents many challenges for those drugs intended to be administered by extravascular routes such as inhaled nemiralisib.

The complications associated with administering a radioisotope by inhalation are not trivial (Harrell et al., 2013). Although precedented (Affrime et al., 2000), it is challenging and often not possible to recreate the exact intended commercial form, including particle size in the intended clinical device. Other complications associated with running an inhaled human ADME study include not being able to quantify accurately the dose crucial for a full interpretation of the ADME data. For this reason, the human metabolism of nemiralisib was characterized using an intravenous microtracer administration as a surrogate for the proportion of the inhaled dose reaching the systemic circulation through the lung, while the swallowed portion was characterized through oral administration of [14C]nemiralisib (in a separate dosing period).

Traditionally, intravenous PK is defined following a two-way crossover design in which healthy subjects receive single intravenous and extravascular therapeutically relevant administrations on separate occasions. A drawback of this approach is that the therapeutic intravenous dose requires an intravenous toxicology package to support the clinical trials application. To encourage wider application of exploratory clinical studies, harmonized International Committee on Harmonization guidance was developed to allow ultra-low intravenous administration (<100 μg) to humans with a substantially reduced toxicology package (https://www.ich.org/fileadmin/PublicWebSite/ICHProducts/Guidelines/Multidisciplinary/M3R2/Step4/M3R2Guideline.pdfhttps://database.ich.org/sites/default/files/M3_R2__Guideline.pdf). Referred to as microdosing, the approach commonly involves the incorporation of a 14C-isotope into the drug so that systemic exposure can be measured by highly sensitive analytical technologies such as accelerator mass spectrometry (AMS). A further development of the microdose design is to administer the 14C-labeled microdose concomitantly with an extravascular dose in a so-called "microtracer" design, where intravenous PK can be defined at therapeutically relevant concentrations (Lappin, 2016). Herein, we develop the approach described by Ambery et al. (2018) for inhaled batefenterol, whereby an intravenous 14C-tracer dose of nemiralisib was administered concomitantly with a therapeutically relevant inhaled dose delivered using the intended commercial formulation and device.

Human biliary disposition can be investigated by noninvasive collection of human duodenal bile samples using the Entero-Test device (HDC Corp., Mountain View, CA) (Bloomer et al., 2013). Duodenal bile sampling presents many advantages over stool sampling to provide understanding of biliary secretion of parent drug and metabolites. For example, there is no need to extract drug-related material from complex fecal matter, the resultant metabolic profile is a better indicator of drug and metabolites secreted in bile and any consideration of metabolism of the drug by gut micro flora becomes unnecessary. To take full advantage of the intravenous 14C microtracer dose, the Entero-Test was deployed to fully characterize nemiralisib metabolites secreted in bile as well as provide an indication of possible enterohepatic recirculation.

It is common industry practice to estimate oral absorption (F_{abs}) from human radiolabel studies where an intravenous dose has been included and at least three different approaches can be used (Table 1). Less commonly, further derivations of these equations can be used to describe other first-pass parameters such as the proportion of nemiralisib escaping first-pass extraction through the gut wall and liver (F_g and F_h) and hepatic extraction (E_h), which, to the authors knowledge, have never been published in full within the context of a human ADME study. Herein, we review equations for the derivation of absorption and first-pass parameters and apply them to nemiralisib.

Our study includes a number of innovative approaches combined here for the first time into a single clinical study, which aimed to fully characterize and understand the human ADME and PK profile of inhaled nemiralisib.

Materials and Methods

Nemiralisib, [14C]nemiralisib, and [2H7]nemiralisib were supplied by Chemical Development, GlaxoSmithKline (GSK) R&D, Stevenage, UK. All other solvents and reagents were of analytical grade and were purchased from commercial suppliers. In all cases, nemiralisib hemisuccinate salt was administered to study participants. All dose levels herein are calculated in terms of nemiralisib free base.

Study Design

This was an open-label, single center, nonrandomized, 2-period, single-sequence crossover study in healthy men conducted at Hammersmith Medicines Research Center (London, UK) between November 2017 and December 2017 (GSK study number 206764; ClinicalTrials.gov NCT03315559). The study adhered to the Declaration of Helsinki, was approved by the London Harrow Research Ethics Committee, and each subject gave their written informed consent prior to any study procedure.

Following a screening visit, subjects underwent two treatment periods, conducted on-site, with a 14-day washout between treatments (Fig. 1). In treatment period 1, subjects inhaled 1000 μg free base of nonradiolabeled nemiralisib as a dry powder from the ELLIPTA inhaler device (GSK, Brentford, UK). Nemiralisib hemisuccinate salt was blended with lactose and magnesium stearate and packed in foil blisters containing 500 μg free base per blister, requiring two inhalation actuations to achieve the full 1000 μg dose. Within 5 minutes, each subject received 10 μg [14C]nemiralisib free base (approximately 22.2 kBq; 0.6 μCi) via intravenous microtracer infusion over 15 minutes. [14C]Nemiralisib hemisuccinate salt was dissolved in normal saline at 1 μg/ml so that 10 μg free base was administered in a volume of 10 ml. Blood, urine, and fecal samples were collected up to 168 hours (7 days) after dosing. In treatment period 2, subjects were administered 800 μg free base of [14C]nemiralisib hemisuccinate salt (1850 kBq; 50 μCi) as an oral solution in water (800 μg [14C]nemiralisib free base was dissolved in 10 ml water). Samples were collected for a minimum of 168 hours and up to 336 hours (day 15) or until the amount of radioactivity excreted by each subject was <1% per day of the administered dose on two consecutive 24-hour excreta collection intervals. Each subject was exposed to an effective radioactive dose of 0.75 mSv, well within the safety limits defined by the World Health Organization category II (0.5–5 mSv; within the dose limits for members of the public) and the International Commission on Radiologic Protection category IIa (0.1–1 mSv; minor risk) (ICRP, 1991).

Study Population

Healthy men were aged from 30 to 55 years, weighed ≥50 kg with a body mass index of 19–31 kg/m^2, and had a history of regular bowel movements. Subjects had no history of drug or alcohol abuse, no clinically relevant disease, were nonsmokers, had not taken other medications within the last 7 days (14 days if a potential enzyme inducer), and had not been exposed to significant radiation within the last 12 months. Full details of the eligibility criteria can be found on ClinicalTrials.gov; NCT03315559.

ABBREVIATIONS: ADME, absorption, distribution, metabolism and excretion; Ae, amount excreted; AMS, accelerator mass spectrometry; CL_r, renal clearance; E_h, hepatic extraction; F_{abs}, fraction absorbed following oral dose; F_g, fraction escaping gut wall metabolism; F_h, fraction escaping hepatic metabolism; F_{lung}, fraction of dose absorbed from inhaled dose; F_{oral}, oral bioavailability; LLQ, lower limit of quantification; LSC, liquid scintillation countingpg Eq/mL, picogram equivalents (radioactivity) per mL; PK, pharmacokinetics; R&D, Research & Development; $t_{1/2}$, terminal phase half-life; t_{max}, time of maximum observed concentration; UPLC, ultra-performance liquid chromatography.

TABLE 1

Equations to calculate absorption and first-pass pharmacokinetic parameters for nemiralisib

Equation 1 is a commonly used approach to estimate oral absorption (F_{abs}) whereby the % radioactive dose excreted in urine is compared with oral and intravenous administrations. This approach is not advised for molecules with low urinary excretion of total drug-related material. Equation 2 is an alternative approach published by Nomeir et al. (2009) that compares the AUC (preferably using 0_inf for both or 0_t for both if not possible) of total radioactivity (TRA) between oral and intravenous administrations. This approach is not reliant on significant recovery of the radioactive dose in urine. Equations 5–9 summarize an approach to calculating F_{abs} that also provides estimates for hepatic extraction (E_h), proportion of drug escaping first-pass extraction through the liver (F_h), and proportion of drug escaping first-pass extraction through the gut wall (F_g). To the authors' knowledge, these equations in the context of a human ADME study, although occasionally used, have never been compared and published in a peer-reviewed journal. Equations 3 and 4 calculate renal blood clearance (CL_r) from the amount of drug (unchanged nemiralisib) excreted in urine divided by the blood AUC; in this case, blood $AUC_{(0-t)}$ was used. Excretion data were used for the same sampling duration used to calculate blood AUC. In this case, oral data were used since technical limitations prevented an estimate from the intravenous dose. Equation 5 was used to calculate hepatic extraction (E_h) from intravenous total and renal blood clearance ($CL_{b\ total}$ and $CL_{b\ renal}$, respectively) and human hepatic blood flow (Q_H; assumed to be 87 l/h) (Davies and Morris, 1993). Equation 6 was used to calculate the proportion of drug escaping first-pass extraction through the liver (F_h) from E_h. Equation 7 calculates metabolite load (ML) for both oral and intravenous administrations as the proportion of AUC_{TRA} that is not associated with unchanged parent drug. Herein this was calculated for both the oral and intravenous routes. Equation 8 calculates the proportion of drug escaping first-pass extraction through the gut wall (F_g) from ML and F_h. Equation 9 calculates the fraction absorbed (F_{abs}) from F_h, oral bioavailability (F_{oral}) and F_g. All equations were applied to clinical study data outside the original study plan or post hoc.

Eq. 1	$Fabs = \%\ radioactive\ dose\ excreted\ in\ urine\ (oral)/\%\ radioactive\ dose\ excreted\ in\ urine\ (i.v.)$
Eq. 2	$F_{abs} = (AUC_{TRA}\ oral/AUC_{TRA}\ i.v.)*(Dose\ i.v./Dose\ oral)$
Eq. 3	$Ae\ urine = \%\ unchanged\ drug\ in\ urine * \%\ dose\ in\ urine * actual\ dose\ level.$
Eq. 4	$CL_r = Ae\ urine/AUC\ blood$
Eq. 5	$E_h\ (blood) = (CL_{b\ total} - CL_{b\ renal})/Q_H$
Eq. 6	$F_h = (1 - E_h\ (blood))$
Eq. 7	$ML_{route} = (AUC_{TRA\ route} - AUC_{route})/AUC_{TRA\ route}$
Eq. 8	$F_g = (1 - (ML_{oral} - ML_{i.v.}))/F_h$
Eq. 9	$F_{abs} = F_{oral}/(F_h * F_g)$

Sample Collection and Processing

Blood samples were collected up to 7 days after dosing into ethylenediaminetetraacetic acid tubes. Blood volumes were 3 ml for all pharmacokinetic sampling times with an additional 9 ml collected for metabolite sampling at specified sample times (0.5 and 4 hours after dosing for period 1 or 2, 6, and 24 hours after dosing for period 2). Plasma was harvested from blood following centrifugation. Urine and fecal samples were collected for 7 days in treatment period 1 and for between 7 and 15 days or until <1% radioactive dose was recovered in any 24-hour period in treatment period 2. Sampling of duodenal bile during treatment period 1 was conducted using the Entero-Test string device (Guiney et al., 2011), which was swallowed approximately 3.5 hours before the inhaled dose and removed about 2.5 hours after the end of the infusion (2.75 hours post infusion start). Approximately 2 hours before removal, a food cue was used to stimulate gall bladder emptying. The food cue involved providing visual images of food and asking the participants to imagine eating food, providing a sniff of orange zest and a high fat food morsel (e.g., sausage sandwich). Samples of urine, feces, and bile were stored frozen prior to shipment for analysis.

Sample Analysis

Mass Balance and Excretion. Total radioactivity excreted in urine and fecal samples was determined using AMS in samples collected during treatment period 1 (Xceleron Inc., Germantown, Maryland, USA) and by liquid scintillation counting (LSC) in samples collected during treatment period 2 (Covance Laboratories Limited, Harrogate, UK). Where levels of radioactivity were too low to be detected by LSC, samples were analyzed by AMS. No cross validation was performed between LSC and AMS because substantial literature supports the individual qualification of both techniques in isolation (Garner et al., 2000; Keck

et al., 2010). For LSC, efficiency correlation curves were routinely checked through the use of ^{14}C quenched standards. AMS instrument standards (independently internationally established by consensus) were analyzed along with process standards for the graphitization process. Urinary radioactivity was measured in triplicate weighed aliquots by LSC using a Packard TriCarb liquid scintillation counter (Canberra Packard, Pangbourne, Berkshire, UK). Feces samples were combusted using a Perkin Elmer (Pangbourne, Berkshire, UK) Sample Oxidizer. The combusted products were absorbed in Carbosorb (Perkin Elmer Life Sciences, Ltd.) and mixed with Permafluor E+ scintillation fluid (Perkin Elmer Life Sciences, Ltd.). The efficiency of oxidation was determined by combustion of quality control standards, but no corrections were made for combustion efficiency. For each individual run to be acceptable, the efficiency of oxidation measured at the beginning and end of each run was required to be 95%–105%. The lower limits of quantification (LLQ) were assigned as twice the mean background disintegrations.

For measurement of total radioactivity by AMS, urine and feces samples underwent combustion (oxidation) and graphitization (reduction) and radioactivity levels were determined using a Single Stage AMS 250KV system (NEC, Middleton, WI) (Young et al., 2008). The mean LLQ was 0.15 pg nemiralisib Eq/ml for urine measurements and 0.954 pg Eq/g in feces samples.

Pharmacokinetic Assessments. Plasma samples were analyzed for nemiralisib concentrations (Aptuit, Verona, Italy) following inhaled and oral administration of nemiralisib (periods 1 and 2) using a validated analytical method based on protein precipitation with acetonitrile containing [$^2H_7$]nemiralisib as an internal standard, followed by liquid chromatography and TurboIonSpray tandem mass spectrometric detection analysis. The LLQ was 20 pg/ml using a 50 μl aliquot of human plasma with a higher limit of quantification of 10,000 pg/ml. Typically, a 3 μl aliquot of extracted sample was injected onto a liquid chromatography system consisting of a 50 × 2.1 mm internal diameter, Agilent Zorbax SB-CN 5 μm column and a Waters Acquity UPLC chromatography system. Mobile phases of 10 mM ammonium formate and acetonitrile at a flow rate of 0.8 ml/min and a column temperature of 40°C were used to elute nemiralisib at a typical retention time of 0.9 minutes. Concentration data generated from treatment period 1 were deconvoluted to derive drug concentrations following inhalation administration by subtraction of drug concentrations determined by AMS (referred to as [^{14}C]nemiralisib concentration) from the intravenous microdose administration. Plasma total radioactivity and [^{14}C] nemiralisib were analyzed by the Department of Bioanalysis, GSK, Ware, UK. Plasma total radioactivity was assessed by direct AMS for period 1 samples (inhaled + intravenous dose), which had an LLQ of 0.629 pg Eq/ml. For period 2 plasma samples (oral dose), total radioactivity was assessed by LSC, which had an LLQ of 49.0 pg Eq/ml. Plasma [^{14}C]nemiralisib (parent drug following intravenous microdose administration) was determined using a validated analytical method based on protein precipitation, followed by ultra-high-performance
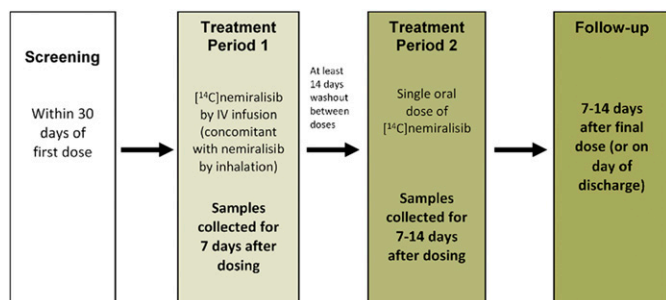

Fig. 1. Study schematic In both treatment periods, treatments were administered after overnight fasting (≥8 hours). If radioactivity was higher than 1% upon discharge, fecal samples were collected by subjects at home after day 15.

TABLE 2

Summary of PK parameters following 10 μg i.v. [^{14}C]nemiralisib +1000 μg inhaled nemiralisib and 800 μg oral [^{14}C]nemiralisib

All parameters calculated from individual data as presented in Supplemental Table 1.

PK Parameter (unit)	N	n	Route of Administration	Geometric Mean (CVb %)	95% Confidence Interval
Total radioactivity following 10 μg i.v. [^{14}C]nemiralisib + 1000 μg inhaled nemiralisib and 800 μg oral [^{14}C]nemiralisib					
C_{max} (pq/ml)	6	6	i.v.	204 (52.3)	(122, 343)
	6	6	Oral	704 (24.4)	(546, 906)
t_{max}[a] (h)	6	6	i.v.	0.233 (0.233, 0.233)	NA
	6	6	Oral	7.00 (2.00, 8.02)	NA
AUC_{0-t}[b] (h*pg Eq/ml)	6	6	i.v.	770 (17.7)	(641, 926)
	6	6	Oral	31,730 (35.7)	(22,057, 45,644)
AUC_{0-inf} (h*pg Eq/ml)	6	3	i.v.	813 (8.18)	(664, 996)
	6	1	Oral	34,749 (NE)	NE
$t_{1/2}$ (h)	6	3	i.v.	56.9 (7.70)	(47.0, 68.9)
	6	1	Oral	39.6 (NE)	NE
Ae urine[c] (total radioactivity ng Eq)	6	6	i.v.	1044 (NA)	NA
CLr (l/h) (total radioactivity)	6	6	i.v.	1.25 (16.8)	(1.05, 1.49)
Intravenous [^{14}C]nemiralisib					
Radiometric dose (μg)	6	6	i.v.	8.10 (3.2)	(7.83, 8.37)
C_{max} (pg/ml)	6	6	i.v.	206 (57.9)	(117, 362)
t_{max}[a] (h)	6	6	i.v.	0.233 (0.233, 0.233)	NA
AUC_{0-t}[d] (h*pg/ml)	6	6	i.v.	546 (45.3)	(347, 860)
AUC_{0-inf} (h*pg/ml)	6	4	i.v.	751 (48.3)	(362, 1555)
AUC_{0-t} ratio	6	6	i.v.	0.709 (29.2)	(0.525, 0.958)
AUC_{0-inf} ratio	6	2	i.v.	0.722 (50.3)	(0.010, 51.406)
$t_{1/2}$ (h)	6	4	i.v.	54.7 (36.0)	(31.4, 95.4)
CL[e] (l/h)	6	4	i.v.	10.8 (46.7)	(5.32, 21.9)
Vz (l)	6	4	i.v.	851 (14.0)	(682, 1062)
Vss (l)	6	4	i.v.	728 (11.2)	(609, 870)
Inhaled nemiralisib					
Nominal dose (μg)	6	6	Inhaled	1000 (0)	NA
C_{max} (pg/ml)	6	6	Inhaled	5259 (62.8)	(2871, 9633)
t_{max}[a] (h)	6	6	Inhaled	0.0333 (0.017, 0.050)	NA
AUC_{0-t}[f] (h*pg/ml)	6	6	Inhaled	28,426 (34.5)	(19,998, 40,406)
AUC_{0-inf} (h*pg/ml)	6	6	Inhaled	33,374 (33.2)	(23,773, 46,854)
$t_{1/2}$ (h)	6	6	Inhaled	58.4 (16.8)	(49.0, 69.7)
Oral nemiralisib					
Radiometric dose (μg)	6	6	Oral	769 (0.65)	(763, 774)
C_{max} (pg/ml)	6	6	Oral	399 (27.8)	(300, 531)
t_{max}[a] (h)	6	6	Oral	6.00 (2.00, 8.00)	NA
AUC_{0-t}[g] (h*pg/ml)	6	6	Oral	16,913 (53.8)	(9960, 28,719)
AUC_{0-inf} (h*pg/ml)	6	4	Oral	25,108 (46.3)	(12,451, 50,632)
AUC_{0-t} ratio	6	6	Oral	0.533 (24.7)	(0.413, 0.688)
AUC_{0-inf} ratio	6	1	Oral	0.377 (NE)	NE
$t_{1/2}$ (h)	6	4	Oral	50.8 (40.2)	(27.5, 94.0)
CLr (l/h) (parent nemiralisib)	6	6	Oral	0.93 (from pooled radioprofiling)	NE

N, number of subjects; *n*, number of evaluable subjects; NA, not applicable; NE, not estimable; Vz, volume of distribution during terminal phase after intravenous administration. Vss, volume of distribution at steady state

[a]t_{max} expressed as median and range.

[b]AUC_{0-t} = 168 h for intravenous and 96–168 h for oral.

[c]Ae urine, total radioactivity in urine sample (0–168 h) shown as arithmetic mean.

[d]AUC_{0-t} = 72–168 h.

[e]10.8 l/h based on plasma or (10.8 l/h/1.08 ratio) 10.0 l/h for blood, i.e., corrected for blood:plasma ratio of 1.08 (unpublished GSK data).

[f]AUC_{0-t} = 96–168 h.

[g]AUC_{0-t} = 72–168 h.

liquid chromatography + AMS. The LLQ of this method was 1.00 pg/ml using a 100 μl aliquot of plasma. Further details of the analytical methodologies are provided in the online Supplemental Methods.

Quantification and Characterization of Metabolites. Using samples taken following both dosing periods, an equal volume of plasma from each subject was pooled to produce a single representative plasma sample per time point for each dosing route. With these sample pools, a single plasma pool (representative of the area under the plasma concentration time curve from time 0 to 24 hour) was prepared for each dosing route by combining volumes in proportion to the time interval between individual samples (Hop et al., 1998). For each dosing group, a pooled urine sample was prepared for individual subjects by taking an equal percentage by weight (0.1%) from each time point cumulatively containing approximately 90% of the total radioactive drug-related material excreted by this route. These individual pools were then used to prepare a single urine pool per dose route by pooling equal volumes. Individual pooled fecal homogenate samples were prepared from both dosing periods using a similar approach. Here, individual pools were prepared by taking 1% by weight from each time-point until

the pool cumulatively contained approximately ≥90% of the excreted radioactivity for that matrix per subject. A combined fecal pool was then prepared by taking an equal weight from each individual pool. Plasma and feces samples were extracted with acetonitrile, dried using a centrifugal evaporator and reconstituted in 50/50, acetonitrile/water (v/v). Duodenal bile was extracted with acetonitrile from the Entero-Test bile strings (collected following inhaled + intravenous microtracer dosing) and the extracts were mixed with scintillation fluid prior to counting. LSC vials were then individually counted for 15 minutes duration using a Packard Tricarb low level scintillation counter. Bile string extracts from the two subjects containing the highest levels of radioactivity were pooled to determine the metabolic profile. Metabolites were identified and quantified (where possible) in plasma, urine, feces and bile string extract samples using off-line radio-UPLC with either Topcount (feces sample extracts) or AMS (plasma, urine, and bile sample extracts) as off-line radio-detector. Off-line radiodetection by Topcount was conducted by collecting fractions into 96 shallow well Luma microtiter plates (Perkin Elmer) containing solid scintillant. The liquid chromatography eluate fractions were allowed to evaporate (in air at room temperature) and the dried
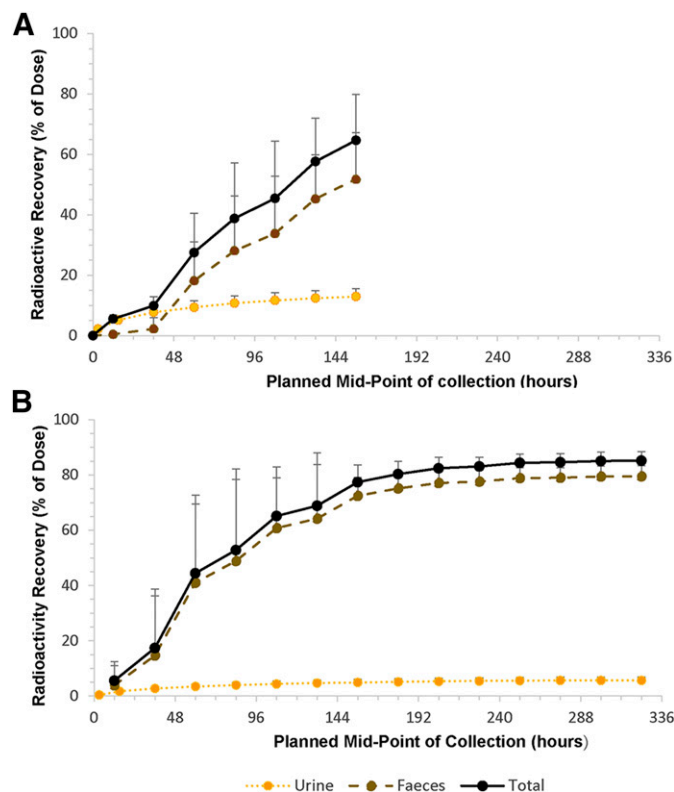
Fig. 2. Cumulative arithmetic mean (+S.D.) of radioactivity recovery over time following intravenous (A) and oral (B) administration of [^{14}C]nemiralisib.

plates were sealed prior to scintillation counting. Each well was counted using a microplate scintillation counter (Packard Topcount NXT; Perkin Elmer) with 12 independent photomultiplier tubes and no background subtraction. The scintillation data were converted into a form compatible with Laura software using its "LSC import" function to reconstruct radio-chromatograms. Off-line radio-detection by AMS was conducted based on a previously reported method used to characterize inhaled vilanterol (Harrell et al., 2013), except in the current study UPLC was deployed with a flow rate of 0.3 ml/min compared with a flow rate of 1 ml/min using conventional HPLC for vilanterol. Fractions were collected from the column eluate post sample injection using a CTC Analytics HTX Pal system. The timing for collection was pre-set depending on sample acquired. Additional details are provided in the Supplemental Methods and in published literature (Young et al., 2014). Metabolite structures were assigned by comparison of relative retention times to pre-existing data from in vitro human hepatocyte preparations (unpublished GSK data).

Safety

Safety was assessed by measurement of vital signs, 12-lead ECGs and routine laboratory tests before dosing, over the first 24 hour post-dose and prior to discharge. Adverse events were monitored throughout the study.

Pharmacokinetics and Statistical Analysis

The sample size of 6 was deemed appropriate to investigate the primary objective (Penner et al., 2009). Plasma nemiralisib (parent drug following inhaled or oral administration), [^{14}C]nemiralisib (parent drug following intravenous administration), and total radioactivity concentration-time data were analyzed by non-compartmental methods with WinNonlin Version 7.0, using the actual sampling times recorded during the study for derivation of PK parameters. Nemiralisib plasma kinetics in terms of clearance (CL), volume of distribution at steady-state, together with the bioavailability via each route (inhaled/oral) of administration were calculated.

Several parameters calculated in this publication did not form part of the original study plan and are, therefore, deemed calculated post hoc. Blood
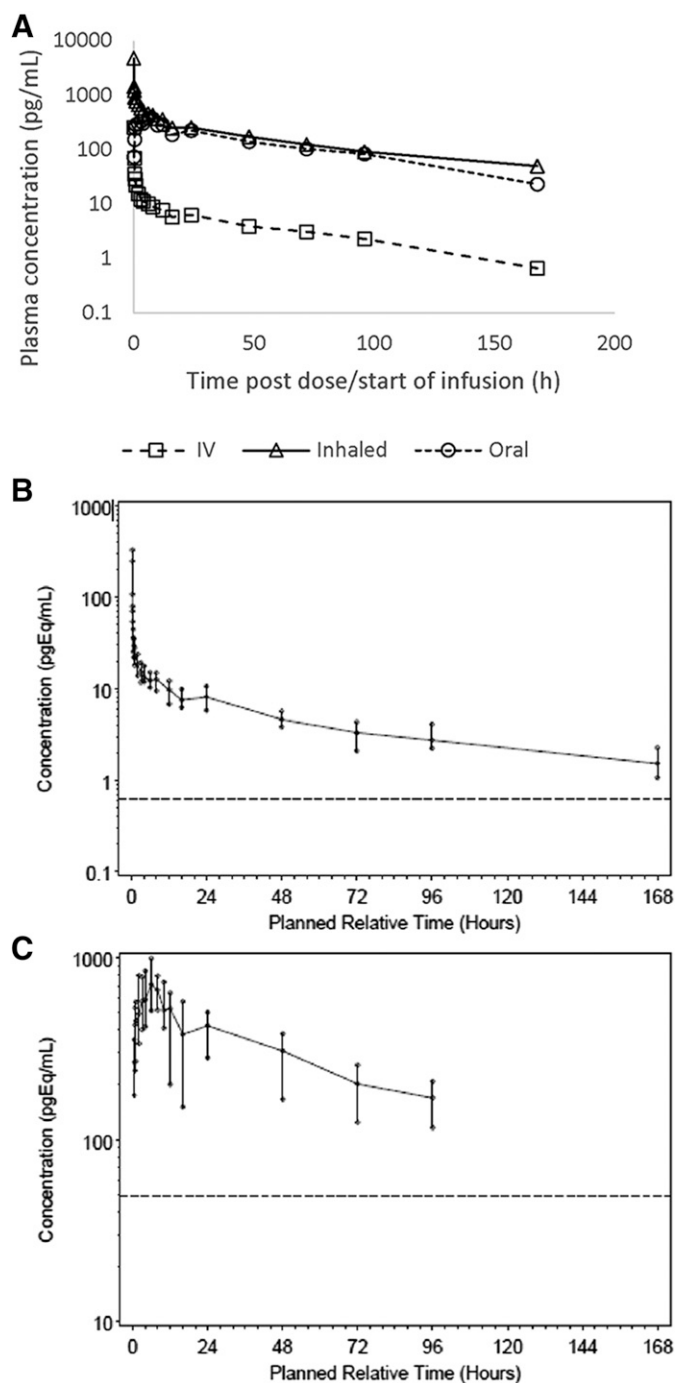

Fig. 3. Median (and range; B and C only) concentrations of (A) plasma nemiralisib following 10 μg intravenous [^{14}C]nemiralisib +1000 μg inhaled nemiralisib and 800 μg oral [^{14}C]nemiralisib; (B) total radioactivity in plasma following intravenous administration; and (C) total radioactivity in plasma following oral administration.

clearance was calculated post hoc as plasma clearance/blood:plasma ratio where blood:plasma ratio was assumed to be of 1.08 (GSK unpublished data). Percentage liver blood flow was also calculated post hoc as blood clearance/human hepatic blood flow, which was assumed to be 87 l/h (Davies and Morris, 1993). F_{lung} was calculated post hoc with reference to nemiralisib data generated in other clinical study publications. Equations used to characterize various absorption and first-pass pharmacokinetic parameters are provided in Table 1, which include those calculated post hoc. This post hoc analysis included all derivations of first-pass and absorption parameters (with the exception of ML), as

TABLE 3

Nemiralisib absolute bioavailability following inhaled delivery and oral dosing

All parameters calculated from individual data as presented in Supplemental Table 1.

Test Route of Administration	PK Parameter	n	Absolute Bioavailability (%)	
			Geometric Mean (CVb%)	95% Confidence Intervals
Inhaled	F_{0-inf}	4	38.07 (19.7)	(27.93, 51.89)
	F_{0-t}	6	42.13 (21.8)	(33.59, 52.84)
Oral	F_{0-inf}	4	35.15 (3.35)	(33.32, 37.07)
	F_{0-t}	6	32.61 (12.8)	(28.54, 37.28)

detailed in Table 4. Three different approaches were used to characterize F_{abs}. In eqs. 1 and 2, F_{abs} is calculated by comparing total drug-related material in urine (eq. 1) or plasma (eq. 2) between intravenous and oral doses. A third approach uses other first-pass parameters (eq. 9) and has been used to calculate F_{abs} with and without a consideration of renal clearance of parent nemiralisib (CL_r). CL_r was calculated using eqs. 3 and 4 from the amount of unchanged nemiralisib (amount excreted (Ae) urine – post hoc) derived from the metabolite profile collected following oral administration in preference to intravenous administration where radioactivity concentrations were substantially lower. A maximum renal clearance of total radioactivity (total drug-related material) in urine is provided for the intravenous dose in Table 2 and has not been used for any further mathematical derivations. Further equations are provided in Table 1 and the corresponding notes to characterize E_h (eq. 5), F_h (eq. 6), and F_g (eqs. 7 and 8) were all calculated post hoc. Since a value for nemiralisib renal clearance was not available, CL_r in eq. 5 (post hoc from urine metabolite profile) was initially set to zero and F_h in eq. 8 was set to 1 (using a worst-case hepatic load of 100%). E_h (eq. 5), F_h (eq. 6), and F_g (eqs. 7 and 8) were recalculated, correcting for CL_r, for comparison of these values when calculated with and without a correction for CL_r.

Results

Demographic and Baseline Characteristics. Seven men were screened, of whom six were enrolled and completed the study. Their mean (S.D.) age was 43 (10) years with a mean (S.D.) body mass index of 28 (3.8) kg/m².

Mass Balance and Excretion. The mean recovery of radioactivity over time following intravenous and oral administration of [¹⁴C] nemiralisib is presented graphically in Fig. 2. Excretion of [¹⁴C]

TABLE 5

Quantification of the major radioactive components in plasma extracts following intravenous or oral administration of [¹⁴C]nemiralisib

ID	% Sample Radioactivity (pg equivalents [¹⁴C]nemiralisib/g)	
	Plasma	
	Intravenous 0–24 h	Oral 0–24 h
P (nemiralisib)[a]	87.6 (47.3)	76.3 (511)
M1(GSK2330979) and/or M9	8.4 (4.6)	10.0 (66.7)
Total radioactive material assigned % (pg equivalents [¹⁴C]nemiralisib/g)	96.0 (51.9)	86.3 (578)
Total pg equivalents [¹⁴C]nemiralisib/g in pool	54.0	670

[a]Identified by LC-MS, all other peaks identified by comparison of relative retention times to pre-existing data from in vitro human hepatocytes.

nemiralisib-related material was slow and protracted irrespective of the route of administration. Following oral administration of [¹⁴C] nemiralisib, a total of 85% of the administered dose was recovered by 14 days after dosing with 79% being recovered in feces and 6% in the urine. Following intravenous administration of [¹⁴C]nemiralisib (in combination with an inhaled non-radiolabeled dose), 65% of the administered dose was recovered by 7 days after dosing with 52% recovered in feces (approximately 80% of the total recovered radioactivity) and 13% in the urine (approximately 20% of the total recovered radioactivity).

Pharmacokinetic Results. Following inhaled administration, maximum nemiralisib plasma concentrations (C_{max}) were attained rapidly (median t_{max}: 0.033 hours; 2 minutes) with a steep decline in concentrations immediately after dosing, followed by a slower decline and a geometric mean terminal phase half-life ($t_{1/2}$) of 58.4 hours (Fig. 3A; Table 2). The geometric mean absolute bioavailability following inhaled nemiralisib was 38% (95% CI: 28%, 52%) based on area under the plasma concentration time curve from time zero extrapolated to infinite time (Table 3). Small fluctuations (secondary peaking) in plasma concentrations were observed over the first 24 hour post dose. At the end of intravenous infusion, for both total radioactivity and nemiralisib, median t_{max} was 0.233 hour (14 minutes) with geometric mean C_{max} (204 pg Eq/ml and 206 pg/ml, respectively) being

TABLE 4

Summary of metabolic, first-pass, and absorption pharmacokinetic parameters

Parameters calculated from individual data as presented in Supplemental Table 1. Blood:plasma ratio for nemiralisib is assumed to be 1.08 (measured unpublished value). Blood:plasma ratio for nemiralisib drug-related material (radioactivity) was not measured and is assumed to be 1. CL_r was calculated from urine metabolic profile and assumed to be 0.93 l/h (Table 2).

Parameter	Treatment	Equation	N	n	Geometric Mean (CVb%)	95% Confidence Intervals
ML(i.v.)[a,b]	i.v.	eq. 7	6	6	0.252 (0.203)	(0.039, 0.465)
ML(oral)[a]	Oral	eq. 7	6	6	0.435 (33.8)	(0.308, 0.614)
First pass pharmacokinetic parameters calculated with correction for CL_r[c]						
E_h (blood)	i.v.	eq. 5	6	4	0.103 (51.2)	(0.048, 0.223)
F_h (blood)	Oral	eq. 6	6	4	0.884 (7.55)	(0.784, 0.997)
F_g (blood)	Oral	eq. 8	6	4	0.829 (27.3)	(0.541, 1.27)
F_{abs} (blood)	Oral	eq. 9	6	4	0.480 (25.1)	(0.324, 0.711)
First pass pharmacokinetic parameters calculated assuming CLr is negligible[d]						
E_h (blood)	i.v.	eq. 5	6	4	0.115 (46.7)	(0.057, 0.233)
F_h (blood)[c]	Oral	eq. 6	6	4	0.873 (7.64)	(0.774, 0.986)
F_g (blood)[e]	Oral	eq. 8	6	6	0.785 (21.3)	(0.630, 0.979)
F_{abs} (blood)	Oral	eq. 9	6	4	0.549 (23.8)	(0.378, 0.798)
Other methods to calculate F_{abs}[e]						
F_{abs}	Oral	eq. 1	Pooled Mean		0.450	—
F_{abs}	Oral	eq. 2	6	6	0.441 (25.9)	(0.337, 0.576)

N, number of subjects; n, number of evaluable subjects.

[a]When AUC_{0-inf} values were unavailable for calculating metabolite load (ML), AUC_{0-t} was used. Specifically, AUC_{0-inf} was only used for subject 5 (both routes) and subject 1 (i.v. only) – see Supplemental Table 1.

[b]ML (i.v.) is presented as an arithmetic mean, S.D., and corresponding 95% CI since geometric mean could not be calculated due to one negative value.

[c]Parameters have been corrected for renal clearance (CL_r) once parameter was available from urinary metabolism work.

[d]Parameters calculated prior to the availability of CLr. CLr is assumed "zero" for values calculated from eqs. 5, 6, and 9. Fh is assumed to be "1" for the calculation of F_{abs} using eq. 9.

[e]$F_{g\ blood}= F_{g\ plasma}$ when blood:plasma ratio of drug-related material and F_h are assumed to be 1.
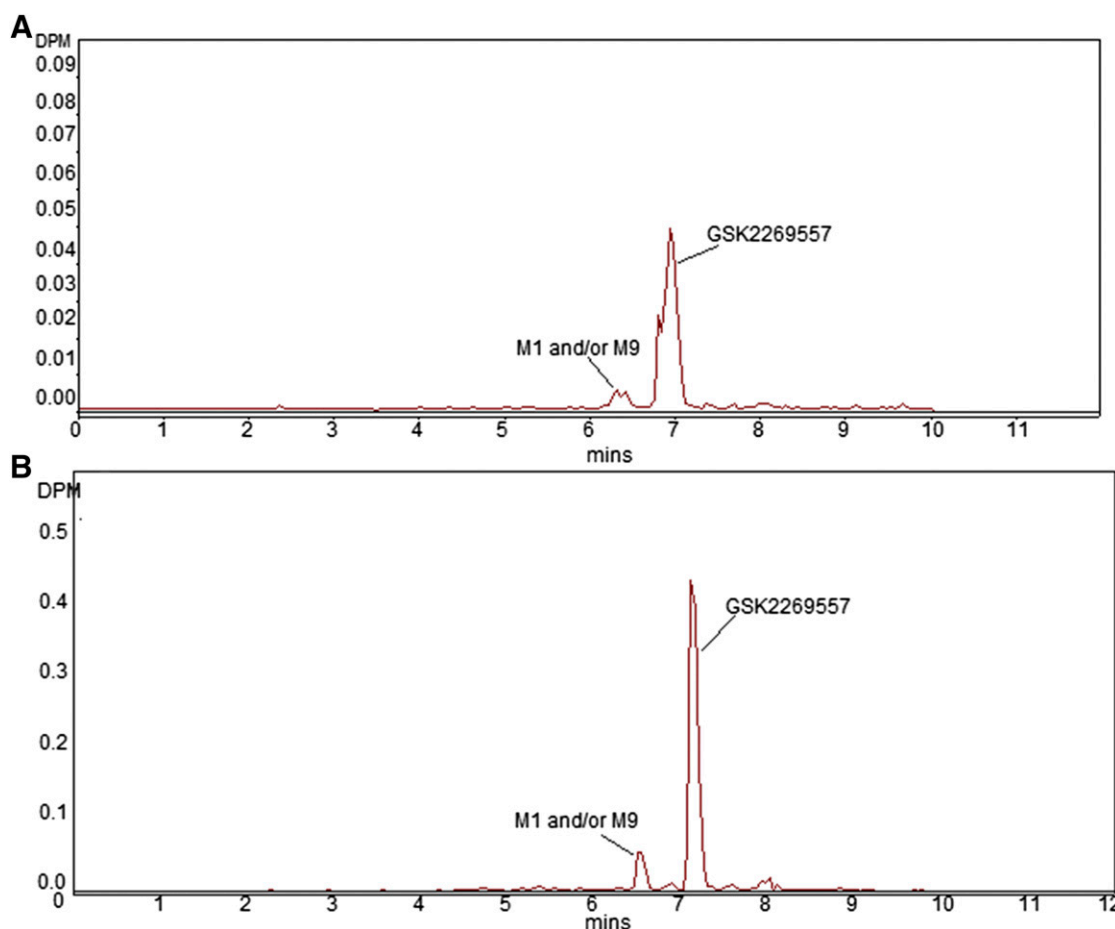
Fig. 4. Reconstructed AMS radiochromatogram of pooled human plasma extracts (0- top 24-hour pool) following intravenous (A) and oral (B) administration of [^{14}C] nemiralisib. DPM, disintegrations/min.

followed by an initial rapid then slower decline in concentrations (Fig. 3; Table 1). A small increase in nemiralisib plasma concentration (secondary peak) was observed at 24 hours. intravenous plasma nemiralisib concentrations rapidly declined from C_{max} to 32.7 pg/ml at 0.5 hour post dose with a $t_{1/2}$ of 54.7 hours. The geometric mean clearance of nemiralisib from plasma was low (10.8 l/h) and geometric mean volume of distribution at steady state was high (728 l). By correcting plasma clearance with a human blood:plasma ratio of 1.08 (unpublished GSK data), the corresponding human blood clearance is 10.0 l/h (10.8/1.08) or 11% liver blood flow compared with a human hepatic blood flow of 87 l/h (Davies and Morris, 1993).

Following oral administration, nemiralisib plasma profiles indicated a protracted absorption phase with a median time to peak levels of 6 hour post dose followed by a slow decline in drug levels. Geometric mean $t_{1/2}$ following oral dosing was 50.8 hours, within a similar range to that observed with intravenous dosing (Fig. 3; Table 1). Geometric mean absolute bioavailability of [^{14}C]nemiralisib following oral administration of an 800 μg dose (mean radiometric dose of 769 μg), was 35% (95% CI: 33%, 37%) based on area under the plasma concentration time curve from time zero extrapolated to infinite time (Table 2). Similar to the inhaled nemiralisib profile, small fluctuations in nemiralisib plasma concentrations (secondary peaking) were observed following both intravenous and oral administration. The geometric mean area under the plasma concentration time curve from time zero to last time of quantifiable concentration ratio indicated that nemiralisib accounted for 71% and 53% of total circulating radioactivity in the plasma following intravenous and oral administration, respectively.

Absorption and First-pass Pharmacokinetic Parameters. The absorption parameters of nemiralisib are described in Table 4 with geometric means being presented in the text. A value of 0.549 was originally derived for F_{abs} using eq. 9 and assuming a negligible CL_r since parent nemiralisib CL_r was not available at the time of reporting. Herein we have compared this value (0.549) to several other methods that can be used to estimate F_{abs}, including the equivalent eq. 9 but incorporating an adjustment for CL_r. Lower estimates of F_{abs} are derived when comparing urinary excretion (0.450; eq. 1) or plasma radioactivity AUC (0.441; eq. 2) between oral and intravenous administrations. When the uncorrected value (0.549) is corrected for renal blood clearance using eq. 9 the revised estimate (0.480) is closer to those following application of eqs. 1 and 2. This revised estimate (0.480) has been used for the purposes of this publication as the true estimate of F_{abs}. There were small changes to F_h, F_{oral}, and F_g depending on whether a correction for CL_r was applied or not; again, all corrections using CL_r have been applied for the purposes of this publication after the original study was reported.

Inhaled Disposition. An estimate of F_{lung} (the inhaled dose absorbed through the lung) can be calculated by integrating the data reported for this clinical study with charcoal block data from a previously published nemiralisib clinical study (Wilson et al., 2018). The fraction of drug orally absorbed following inhaled delivery represents 23% of the resulting systemic exposure (Wilson et al., 2018), which, when compared with F_{inh} (38%), means that (0.23% × 38%) 8.7% of the inhaled exposure is coming from the oral absorption making the inhaled fraction via the lung (F_{lung})
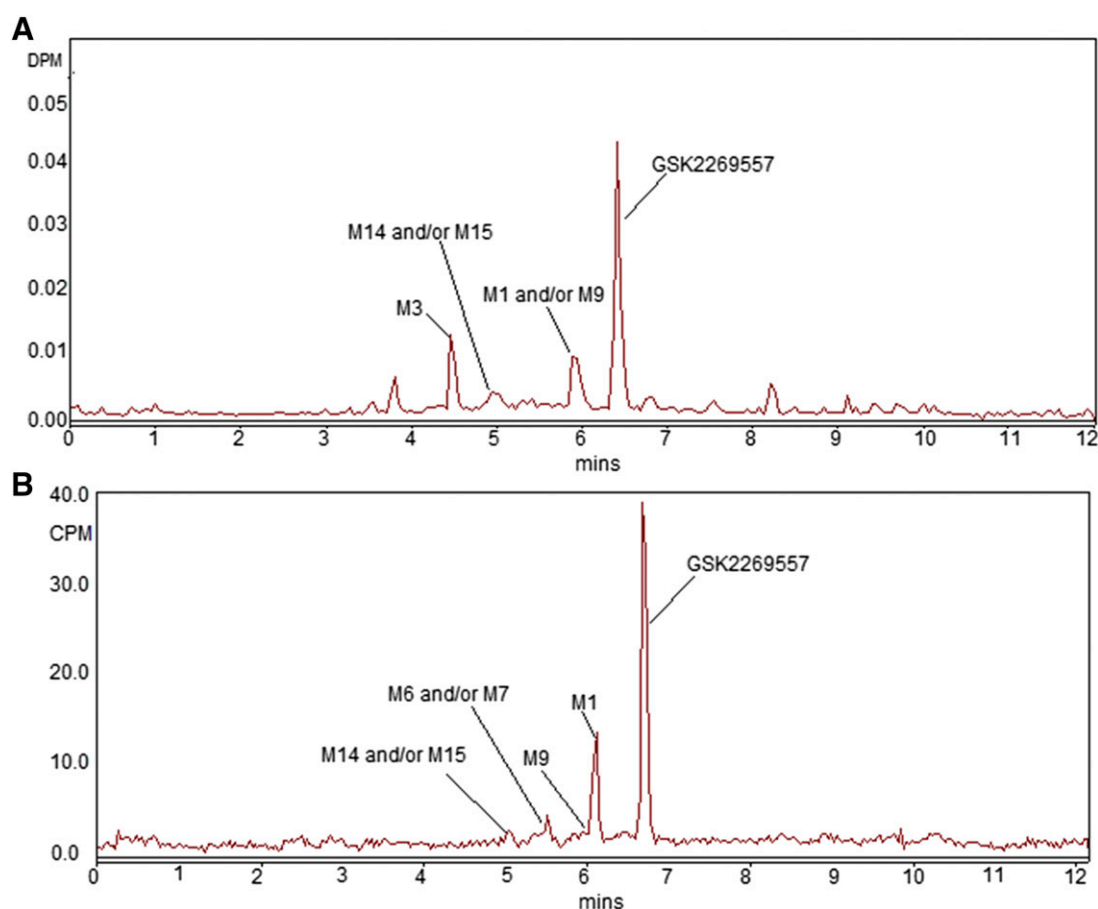
Fig. 5. (A) Reconstructed AMS radiochromatogram of pooled human urine and (B) Reconstructed TopCount radiochromatogram of pooled human feces, following oral administration of [^{14}C]nemiralisib. CPM, counts/min; DPM, disintegrations/min .

(38%–8.7%) = 29%. These calculations do not form part of the clinical study reporting but demonstrate the utility of integrating data across different studies.

Metabolism. AMS analysis of time resolved pooled plasma (0–24 hours) extracts from intravenous and oral dosed subjects showed that

nemiralisib was the major circulating component (88% and 76% of plasma radioactivity, 47.3 and 511 pgEq/ml, respectively) (Table 5). The other notable radiolabeled component (~8% and 10% of the samples radioactivity, 4.55 and 66.7 pgEq/ml, respectively) was identified as metabolite M1 (GSK2330979, formed by *N*-dealkylation) and/or M9

TABLE 6

Quantification of the major radioactive components in urine, feces and bile extracts following intravenous or oral administration of [^{14}C]nemiralisib

ID	Mean % Matrix Radioactivity (Mean % Dose)			
	Urine Oral 6-Subject pool	Feces		Bile Intravenous 6-Subject pool
		Intravenous 6-Subject pool	Oral 6-Subject pool	
P (nemiralisib)	43.3 (2.2)	13.2 (6.8)	41.0 (21.6)	52.4
M3	11.9 (0.6)	ND	ND	ND
M1(GSK2330979) and/or M9	14.7 (0.8)	8.3 (4.3)	14.7 (7.8)[a] 1.8 (1.0)[b]	12.1
M6 and/or M7	ND	8.8 (4.6)	4.4 (2.3)	6.3
M14 and/or M15	7.6 (0.4)	12.8 (6.6)	2.0 (1.1)	7.4
Total radioactive material assigned[c]	77.4 (4.0)	43.1 (22.3)	63.9 (33.7)	78.1
% Administered dose in matrix pool[d]	5.2	51.7	72.1	N/A
Total % dose excreted in matrix[e]	5.66	51.7	79.5	N/A

[a]M1 (GSK2330979).
[b]M9.
[c]Total radioactive material assigned is the sum of the % matrix radioactivity (and % dose) for all identified metabolites in the analyzed pool.
[d]% Administered dose in the matrix pool represents approximately ≥90% of the radioactivity excreted via that route and is quoted for all matrices other than bile. Bile string collection is not quantitative.
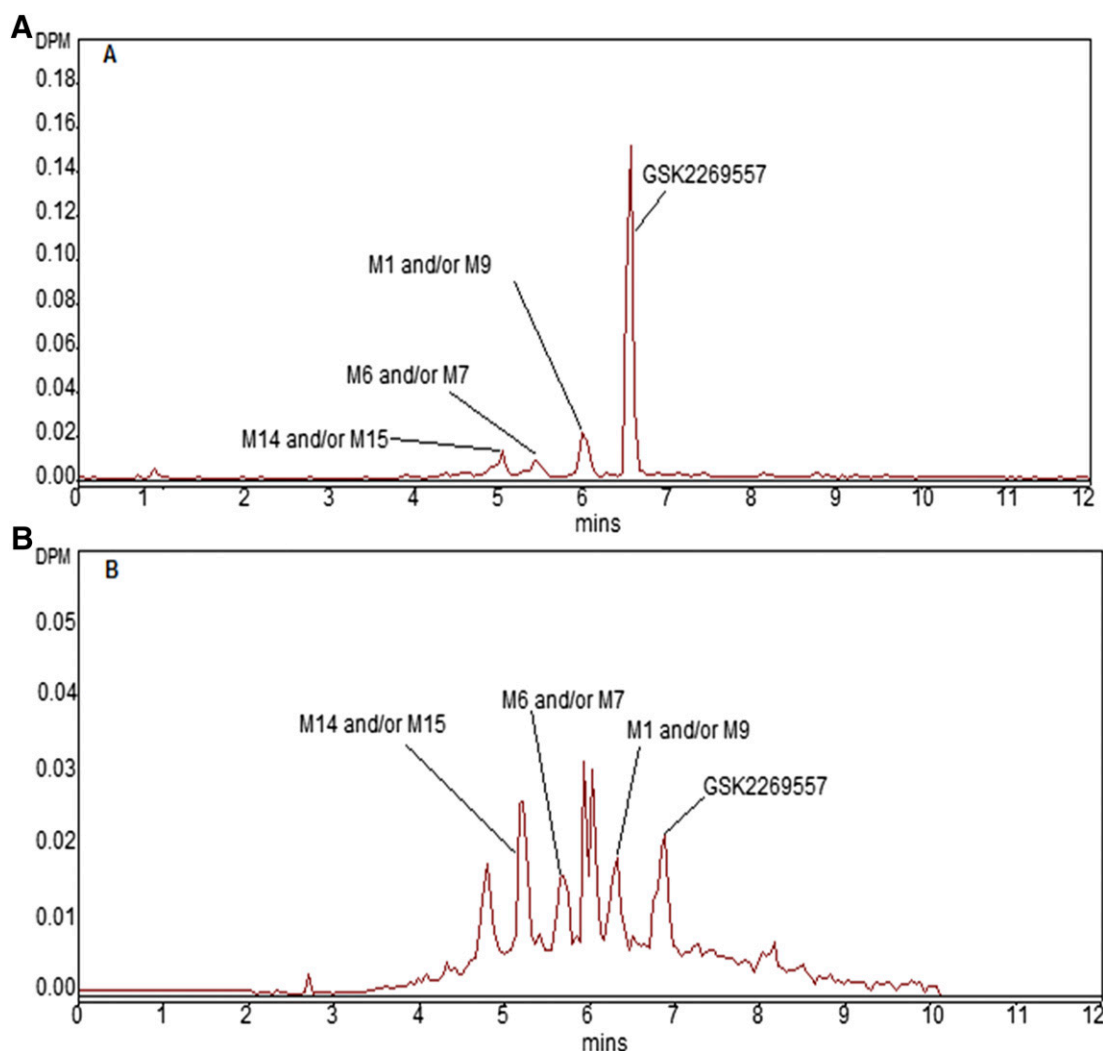[e]Total % dose excreted in matrix is the mean figure from the ADME study.

Fig. 6. Reconstructed AMS radiochromatogram of pooled human duodenal bile extract (A) and pooled human feces extract (B), following intravenous administration of [^{14}C]nemiralisib. DPM, disintegrations/min.

(formed by *N*-dealkylation and sulfate conjugation), which had both shown comparable relative retention times in human hepatocytes to that observed for the plasma component here. Representative AMS chromatograms of reconstituted pooled plasma extracts are shown in Fig. 4.

Following oral dosing, unchanged nemiralisib was the principal radioactive component in pooled urine extracts (43% of the AMS radiochromatogram, ~2% of the administered dose) and feces (41% of the radiochromatogram or ~22% of the dose) (Fig. 5; Table 6). By applying eqs. 3 and 4 (Table 1), this corresponded to a low renal blood clearance of unchanged nemiralisib of 0.93 l/h, which was in line with the overestimated renal clearance approximated from total radioactivity excreted in urine from the intravenous dose (1.25 l/h). Other notable radioactive peaks on the urine AMS radiochromatogram (~12%, 15%, and 8% of the sample radioactivity; 0.6%, 0.8%, and 0.4% of the dose, respectively) were identified as M3 (formed by oxidation and glucuronide conjugation), M1 and/or M9, and M14 (formed by direct glucuronide conjugation) and/or M15 (formed by oxidation). In feces, the *N*-dealkylated metabolite (M1, GSK2330979) was another major component (~15% of the chromatogram or 8% of the dose), while other minor metabolites represented ≤4% of the chromatogram (M9, M6, and/or M7; M14 and/or M15) (Fig. 5; Table 6).

Following intravenous dosing, unchanged nemiralisib was also the main component seen in duodenal bile extract (52% of the

chromatogram) with other minor metabolites (expressed as % of the chromatogram) identified as M1 and/or M9 (12%), M6 and/or M7 (6%), and M14 and/or M15 (7%) (Fig. 6; Table 6). Unchanged nemiralisib was a lesser component in feces extracts following intravenous administration (13% of the chromatogram, or ~7% of the administered dose) with other components (expressed as % of the chromatogram) identified as M1 and/or M9 (8%), M6 and/or M7 (9%), and M14 and/or M15 (13%) (Fig. 6; Table 6). A proposed metabolic scheme for nemiralisib is shown in Fig. 7.

Safety. No deaths, serious adverse events, or other notable adverse events were reported during the study, and no event led to withdrawal of the subject from the study or permanent discontinuation of the study treatment. Adverse events of headache (two episodes) and malaise (one episode) occurred in one of the six subjects in the study. Two subjects presented with neutrophil counts below 1.5×10^9/l not considered as adverse events or regarded as clinically significant. There were no clinically significant changes in vital signs, ECG, or urinalysis findings.

Discussion

An innovative clinical pharmacology trial design has been used to fully characterize human ADME and PK of inhaled nemiralisib in healthy men through integration of data derived from a microtracer
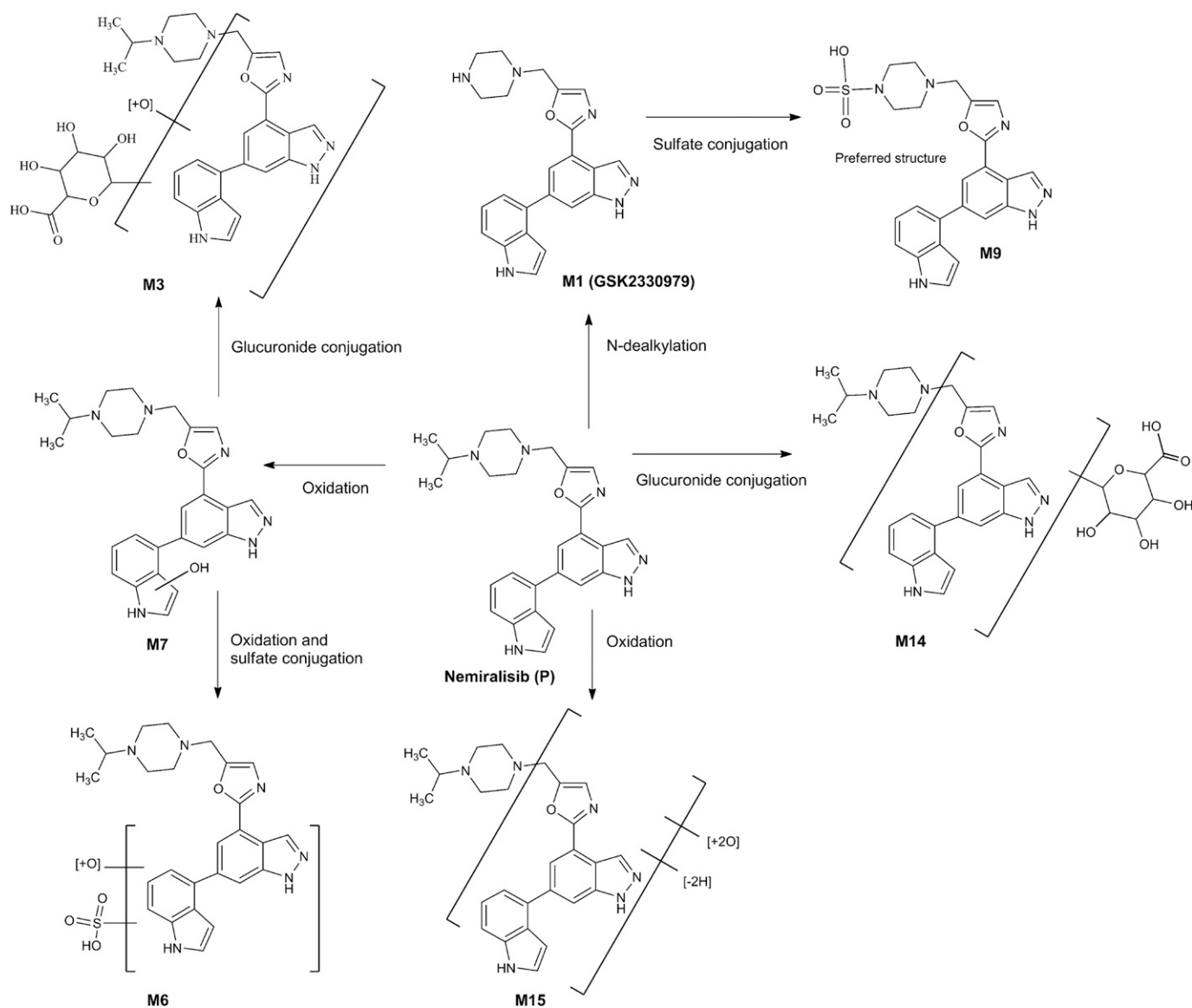
Fig. 7. Putative metabolic scheme for nemiralisib in human.

[14]C intravenous administration delivered concomitantly with an inhaled therapeutically relevant dose, followed by an oral ^{14}C administration after a washout of 14 days. The study used synergistic state-of-the-art analytical methods including AMS, which was configured to deliver highly sensitive nemiralisib specific plasma concentration levels, total drug related material concentrations in plasma, excretion balance data, and metabolite profiles in plasma, bile, and feces. The small two-part study has enabled the generation of intravenous, oral, and inhaled PK parameters including parameters such as F_{abs}, F_g, E_h, F_{lung}, and CL_r, parameters that are not easily derived using conventional clinical PK designs. The incorporation of a ^{14}C-isotope into the parent nemiralisib molecule enabled a full evaluation of nemiralisib metabolism in both plasma (to assess human metabolite exposure) and in excreta (to assess elimination pathways), with intravenous and oral administrations of [^{14}C]nemiralisib being used as surrogates for the lung-deposited and swallowed portions of an inhaled dose, respectively. Pharmacological relevance of the intravenous microtracer dose was achieved through concomitant administration with a therapeutically relevant inhalation dose. Furthermore, metabolite profiling of human duodenal bile using the Entero-Test device enabled metabolites

eliminated through biliary elimination to be characterized, giving a more thorough understanding of elimination pathways, including enterohepatic recirculation. The microtracer study design published for inhaled batefenterol (Ambery et al., 2018) has been developed further to include the calculation of additional parameters and a more comprehensive metabolism assessment and is, to our knowledge, the first of its kind.

Consistent with its long terminal PK half-life, nemiralisib demonstrated a slow and protracted elimination of radioactivity. A retrospective analysis of total recoveries published for mass balance radioactive clinical studies concludes that acceptable target recoveries should be >80% of the administered radioactive dose (Roffey et al., 2007). The total recovery of radioactivity achieved following oral administration of 1850 kBq radioactivity in this study was 85%, which is, therefore, acceptable. Although all subjects met the predetermined stopping criteria by day 15 (<1% dose recovered in a 24-hour collection interval), the slow protracted elimination meant that further sample collections may well have improved this total recovery further. The lower radioactive dose (22.2 kBq) delivered by intravenous administration, for logistical reasons
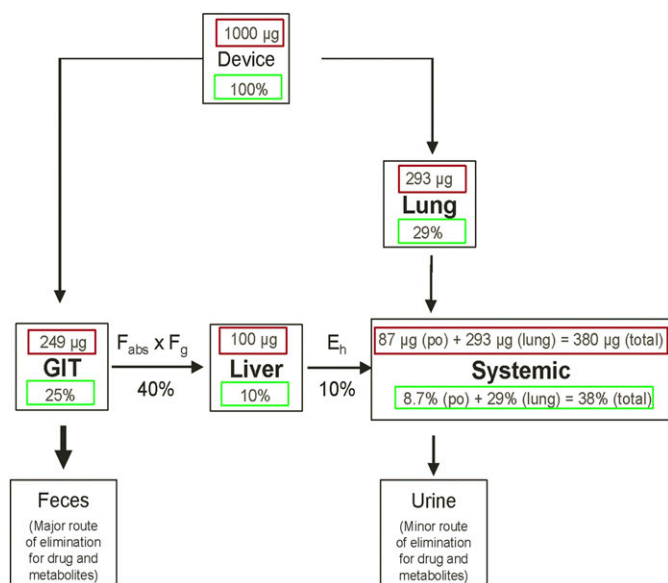
Fig. 8. Disposition scheme for inhaled nemiralisib based on the pharmacokinetic parameters derived following intravenous, oral (po), and inhalation dosing of nemiralisib to healthy men. The disposition scheme was constructed by first assuming that if $F_{inh} = 0.38$, then 38% (380 μg) reaches the systemic compartment from an inhaled dose of 1000 μg. If 23% of the systemic exposure is due to a swallowed component ($F_{swallowed}$; Wilson et al., 2018) then 9% (380 μg * 23% = 87 μg) of systemic drug is derived from that swallowed component. The difference then by subtraction is 29% (380–87 μg = 293 μg), which must reach the systemic compartment through the lung (F_{lung}). If F_{oral} is 0.35, then 25% (87 μg/0.35 = 249 μg) must each reach the gastrointestinal tract (GIT) as swallowed drug. The systemic exposure derived from the oral component can also be estimated using absorption and first-pass parameters. By multiplying F_{abs} * F_g; then 40% of swallowed nemiralisib escapes the GIT. Assuming 90% escapes first-pass clearance $(1 - E_h)$, then 8.9% (89 μg) reaches the systemic compartment from the swallowed dose, which compares well with 8.7% (87 μg) derived using F_{inh} and $F_{swallowed}$. With this approach, a total of 46% dose or 458 μg (1000 μg − 293 μg − 249 μg) is unaccounted for, presumed remaining in device or exhaled.

precluded rapid data turnaround to allow recovery to be used as a release criterion, so a fixed duration collection of 7 days post dose was protocolled. Using AMS as a highly sensitive method to determine radioactive recovery, the total recovery from the intravenous administration was 65%, which was approximately in line with the recovery over a similar period following oral administration.

Three of the approaches used to calculate F_{abs} for nemiralisib in Table 4 were in close agreement (0.450, 0.441, and 0.480 using eqs. 1, 2, and 9 correcting for CL_r). These three approaches provided a better estimate of F_{abs} than the uncorrected F_{abs} value of 0.549, which was overestimated because it did not consider CL_r. This difference highlights the importance of correcting for CL_r when using absorption and first-pass equations and is particularly important for molecules with higher CL_r. Nemiralisib was determined to be moderately well absorbed, regardless of the approach used to calculate F_{abs}.

Intravenous administration of [^{14}C]nemiralisib resulted in plasma concentration/time profiles of unchanged nemiralisib characterized by multiexponential distribution and elimination. Nemiralisib was rapidly distributed followed by slower distribution profiles with volume of distribution at steady state (728 l) exceeding total body water (42 l; Davies and Morris, 1993), suggestive of extensive distribution of the drug beyond the plasma compartment. The drug exhibited relatively low blood clearance corresponding to 11% human liver blood flow. Oral administration resulted in a protracted absorption phase followed by

a biexponential decline in drug concentrations. Any swallowed nemiralisib, including that from an inhaled dose, is likely to be relatively well absorbed across the gastrointestinal (GI) tract ($F_{abs} = 0.44$–0.48, Table 4; excluding values where CLr is assumed negligible) and then subject to poor first-pass extraction by the gut wall ($F_g = 0.83$) and liver ($E_h = 0.10$; $F_h = 0.88$). Breaking down the oral absorption into specific components (i.e., absorption, gut, and hepatic extraction) allows a better understanding of the most influential factors driving oral bioavailability (F_{oral}). In addition, it gives an understanding of the first-pass drug burden to the GI tract and liver, which helps to develop improved physiologically based PK models to assist modeling of drug-drug interactions, for example. The low clearance and moderate oral bioavailability of nemiralisib (35%) is regarded as suboptimal for inhaled drugs, which exert pharmacological action through first-pass topical delivery. Negligible oral bioavailability is generally preferred to minimize systemic toxicities from the swallowed portion of the dose (Cooper et al., 2012).

Following inhaled administration of nemiralisib, the time course of plasma concentrations of parent drug showed distinctly different profiles compared with oral dosing. The inhaled PK profiles showed rapid absorption, initial rapid distribution, and biexponential decline with marginally longer terminal phase elimination half-life ($t_{1/2}$ 58.4 hours) than obtained following oral and intravenous administration (50.8 and 54.7 hours, respectively). The inhaled absolute bioavailability of nemiralisib of 38% is likely to constitute drug derived via both an inhaled and swallowed component. Indeed, a previous study evaluating the absorption of nemiralisib through the lung by blocking absorption via the gut using charcoal indicated that 23% of the total systemic exposure after inhalation from the ELLIPTA device was attributable to orally absorbed drug (Wilson et al., 2018).

Parameters measured in this study can be pieced together into an understanding of the disposition of nemiralisib following inhalation administration. Such a scheme is presented in Fig. 8, which was constructed initially assuming inhaled and oral bioavailability of 38% and 35%, respectively, with 23% of systemic exposure being derived from the swallowed component (Wilson et al., 2018). With this, the fraction of nemiralisib bioavailable via the lung ($F_{lung} = 29\%$) and the swallowed proportion (25%) of the total device dose can be estimated. Using this approach, there is a proportion of drug (approximately 46%) that is unaccounted for. It is unclear why the calculation methods deployed in this work have resulted in such a low total accountability, although they compare well with values reported by Newman and Busse (2002),who quote dose emissions from the Rotahaler (GSK, Brentford, UK) Diskhaler (GSK, Brentford, UK), and Turbohaler (AstraZeneca, Cambridge, UK) dry powder inhalers to be in the range of 52%–63% of label claim and an F_{lung} range of 6.2%–40.5% across a range of different devices. An alternative approach to forming such a scheme, without reliance on in vivo charcoal block data, would be to predict the proportion of dose swallowed and deposited in the lung using in vitro aerodynamic particle size distribution data (Wilson et al., 2019).

Following intravenous and oral dosing, the major circulating drug-related component in plasma was unchanged nemiralisib. A single radioactive peak representing approximately 8%–10% of sample radioactivity was composed of up to two metabolites resulting from N-dealkylation and N-dealkylation with sulfate conjugation. The proportion of individual metabolite, therefore, did not exceed the 10% threshold outlined by International Committee on Harmonization M3(R2) harmonized guidance (2009), above which additional qualification work in animals might be warranted. The chemical structures of the metabolites identified resulted from common

oxidative and conjugative routes and are regarded as innocuous and unlikely to be associated with reactivity. M1 was approximately 16-fold less potent against pi3Kd compared with nemiralisib (IC50 of 3.2 nM compared with 0.2 nM – GSK unpublished data) and unlikely, therefore, to result in systemic toxicities at low inhalation dose levels (<1 mg). The study has, therefore, not revealed any human nemiralisib metabolites needing further qualification in either in-vitro or further animal studies.

Unchanged nemiralisib was a major component in duodenal bile following intravenous administration but was a lesser component in feces extracts (Fig. 6), suggesting that enterohepatic recirculation of nemiralisib is a likely explanation for the secondary peaks observed in the PK plasma profiles and a contributing factor to the long terminal half-life and protracted elimination of the drug. Following oral administration, 24% of the administered dose was associated with unchanged parent in urine and feces (Table 6). Routes of metabolism included various oxidations including N-dealkylation and oxidation of the indole ring, sulfate conjugation, glucuronide conjugation, and combinations thereof. The renal blood clearance of nemiralisib was low (0.93 l/h), calculated using eq. 4 (Table 1). Overall, these data indicate that nemiralisib and oxidative/conjugative metabolites are secreted into the bile to be excreted in feces. A high proportion of metabolic elimination is likely to result from enterohepatic recirculation of unchanged nemiralisib. Direct renal clearance and metabolism followed by renal clearance are lesser routes of elimination.

In conclusion, an innovative small two-part clinical pharmacology study employing concomitant dose routes, state-of-the-art technology and comprehensive estimations of ADME-PK parameters has enabled the inhaled disposition of nemiralisib to be rigorously characterized.

Authorship Contributions

Participated in research design: Harrell, Wilson, Man, Riddell, Jarvis, Young, Crossman, Kenworthy, Marotti, Wilkes, Hessel, Fahy.

Conducted experiments: Chambers, Crossman, Pereira, Wilkes.

Performed data analysis: Wilson, Man, Jarvis, Chambers, Crossman, Kenworthy, Beaumont, Marotti, Fahy.

Wrote or contributed to the writing of the manuscript: All authors.

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