Metabolism, Excretion, and Pharmacokinetics of Selumetinib, an MEK1/2 inhibitor, in Healthy Adult Male Subjects

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ABSTRACT

Purpose: Selumetinib (AZD6244, ARRY-142886), an oral mitogen activated kinase 1/2 inhibitor, is in clinical development for the treatment of a variety of different tumor types. Herein, we report a study that determined the distribution, metabolism, and excretion of selumetinib in healthy male volunteers.

Methods: In this open-label, single-center, Phase I clinical trial, 6 subjects received a single 75-mg dose of [14C]-selumetinib. Blood and excreta samples were collected for pharmacokinetic and radiometric analyses. Tolerability monitoring was performed throughout the study.

Findings: The Cmax of plasma selumetinib was 1520 ng/mL at 1 hour postdose and declined with a t1/2 of 13.7 hours. Over a 216-hour postdose collection period, total dose recovery was 93% of the radioactive dose, with 59% recovered from feces and 33% from urine. Circulating drug-related material was primarily associated with plasma, with minimal distribution into red blood cells. Selumetinib was the major circulating drug-related component and accounted for 40% of the plasma radioactivity (mean of AUC0–72h pool). The major circulating metabolite (M2; accounting for 22% of the plasma radioactivity) resulted from multiple biotransformation pathways, including loss of the ethanediol moiety in combination with glucuronidation. A further 6 circulating metabolites were identified, each accounting for between 2% and 7% of plasma radioactivity. Selumetinib was a minor component in urine, accounting for ≤1% of the dose. M2 was the most abundant metabolite in urine, accounting for 10% of the dose, and there were 5 other metabolites accounting for between 1% and 10% of the dose. In feces, selumetinib accounted for a mean of 19% of the dose. Also present were 7 metabolites accounting for between 1% and 9% of the dose. The majority of the dose was recovered as metabolites, indicating that the liver is the major route of drug elimination. There were no tolerability concerns.

Implications: The findings from this study will inform the label and will contribute to the understanding of the clinical pharmacology of selumetinib. ClinicalTrials.gov identifier: NCT01931761. (Clin Ther. 2016;38:2447–2458) © 2016 The Authors. Published by Elsevier HS Journals, Inc.

Key words: excretion, metabolism, pharmacokinetics, selumetinib.

INTRODUCTION

Selumetinib (AZD6244, ARRY-142886; Figure 1) is a marker extraction kernel 1/2 inhibitor with a short t1/2. It has shown activity in a variety of different tumor types and is currently being investigated in a Phase III trial for differentiated thyroid cancer and in a Phase II registration trial for neurofibromatosis type 1 (ClinicalTrials.gov identifiers: NCT01843062, NCT01492918).

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and NCT01362803, respectively). It is a regulatory expectation and important to characterize the absorption, distribution, metabolism, and excretion of investigational drugs. Such information will inform both the risks of giving the drug to renally and hepatically impaired patients and the risk for drug–drug interactions. An excretion balance study in humans receiving radiolabeled drug is the usual way that this is performed. Based on in vitro data, selumetinib is believed to be mainly metabolized by cytochrome P450 (CYP) enzymes (ie, CYP3A4 and -2C19) and by the uridine diphosphate glucuronosyltransferase (UGT) 1A1 (report no. [be000021-21], data on file, [AstraZeneca, Cheshire, UK]); consequently, metabolism is expected to play a key role in the clearance of selumetinib.

In pivotal studies, selumetinib is administered twice daily at 75 mg under fasted conditions. Absorption is relatively rapid, with the T_max typically being 1.5 hours and t₁/₂ being ~13 hours. An N-desmethyl metabolite (Figure 1) that, is ~3- to 5-fold more potent than selumetinib based on in vitro cellular ERK1/2 phosphorylation inhibition assays, has been characterized in previous studies (study NCT02093728 and NCT02046850, data on file, [AstraZeneca, Cheshire, UK]). This metabolite has been shown to be produced in vitro by a number of enzymes, including CYP2C19, -2C9, -2C8, and -1A2, and typically circulates at concentrations of ~7% relative to selumetinib in patients.

We report here the results from a Phase I study that evaluated the routes and rates of excretion of [¹⁴C]-radiolabeled selumetinib and N-desmethyl selumetinib in plasma and the percentages of the radioactive dose recovered from urine and feces in healthy male subjects. The pharmacokinetic (PK) properties of selumetinib and N-desmethyl selumetinib in plasma were determined, and the metabolite profiles and distribution, elimination, excretion in plasma and excreta samples were characterized. In addition, the tolerability of a single dose of [¹⁴C]-selumetinib was evaluated.

SUBJECTS AND METHODS
This Phase I, open-label, single-center, single-dose study was designed to examine the PK properties, distribution, metabolism, excretion, and tolerability of selumetinib in healthy male subjects (ClinicalTrials.gov identifier: NCT01931761). The study was performed in accordance with the standards of the Declaration of Helsinki and the International Conference on Harmonisation/Good Clinical Practice, and was approved by an institutional review board. Written informed consent was obtained from all subjects prior to study entry.

Participants
The key inclusion criteria were as follows: male subjects aged 50 to 65 years (inclusive), with regular bowel movements and identified as healthy based on their medical history and physical examination, laboratory parameters, ECG, echocardiography, and eye examination (all performed prior to dosing); a body mass index of 18 to 32 kg/m² (inclusive); a body weight of 50 to 100 kg (inclusive); and a creatinine clearance rate >50 mL/min, as calculated using the Cockcroft-Gault formula.

Subjects were ineligible for inclusion based on the following criteria: history or presence of central serous retinopathy or retinal vein thrombosis; intraocular pressure of >21 mm Hg and/or uncontrolled glaucoma; radiation exposure from clinical studies of >5 mSv in the preceding 12 months or of >10 mSv in the preceding 5 years; left ventricular ejection fraction of <55%; history of any clinically important disease or
disorder; history or presence of gastrointestinal, hepatic, and/or renal disease and/or any other condition known to interfere with the absorption, distribution, metabolism, or excretion of drugs; current smokers or those who have smoked or used nicotine products within the previous 3 months; presence or history of alcohol abuse; excessive intake of caffeine; use of any prescribed or nonprescribed medication, particularly one with enzyme-inducing properties, in the 4 weeks prior to study drug administration; and/or any intake of grapefruit, grapefruit juice, Seville oranges, or related products within 7 days prior to study admission.

**Study Design**

Six healthy male subjects received 3 × 25 mg (free base equivalents; capsule formulation used in ongoing Phase III studies) of [14C]-selumetinib Hyd-Sulfate oral capsules, each containing 5.69 MBq, for a total radioactivity of 17.07 MBq (target amount per capsule, 7.52 MBq) as a single dose on day 1. Subjects were fasted from 10 hours prior to the administration of selumetinib and remained fasted for 4 hours postdose. No fluids were allowed from 1 hour prior to selumetinib administration until 1 hour postdose, except the water needed (240 mL) to consume selumetinib; thereafter, subjects were allowed free access to water. Subjects were to remain at the study center for up to 7 days following receipt of selumetinib, for the collection of blood, urine, and fecal samples and for medical monitoring. The length of the residential period could be reduced or extended if deemed appropriate based on emerging data.

**Sample Collection**

Blood, plasma, urine, and fecal samples were collected to determine drug concentrations and total radioactivity. Samples were initially planned to be collected up to 168 hours; however, as excretion was protracted in 1 subject, the collection of excreta was extended to at least 216 hours for all subjects. Plasma and fecal samples were stored at −20°C, and blood and urine samples were stored at 4°C until analysis.

Blood samples for analysis of plasma selumetinib and N-desmethyl selumetinib, plasma radioactivity, and blood radioactivity were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 hours. Due to the extended residence time, additional samples (168, 192, 216, and 240 hours) were taken to monitor the concentration of compound-related material while the subjects remained at the clinic. Blood samples for metabolite profiling and identification were taken at 0, 2, 4, 12, 24, 48, and 72 hours. Urine samples were taken at 0–6, 6–12, and 12–24 hours and then daily until a minimum of 216 hours postdose. Fecal samples were taken at 0–12 and 12–24 hours and then daily until a minimum of 216 hours postdose. Urine and fecal samples beyond 216 hours postdose were taken to achieve higher recovery of the dose if appropriate.

**Bioanalysis**

Plasma samples (K2EDTA anticoagulant) were kept frozen at −20°C or colder. Samples from the study were analyzed for selumetinib and N-desmethyl selumetinib concentrations using an LC-MS/MS method, with concentration ranges of 2 to 2000 ng/mL for selumetinib and 2 to 500 ng/mL for N-desmethyl selumetinib. Samples were spiked with 13C6 stable label internal standards, and extracted by protein precipitation using 4 volumes of methanol:acetoni-trile. After centrifugation, the supernatants were reduced to dryness under nitrogen at 50°C, and reconstituted in methanol:water before analysis. The extract was subjected to HPLC analysis using a Luna C18 50 × 2 mm column (Phenomenex, Torrance, California); the 5-µm particle size analytical column was maintained at 30°C. A gradient mobile phase system was used with acetonitrile with a flow rate of 0.500 mL/min and the gradient running from 25% to 65% over 2 minutes, then increasing to 95% over 0.1 minute and held for 0.5 minutes before re-equilibration.

Quantification was accomplished using either an API 5000 or 5500 triple quadruple mass spectrometer (AB Sciex, Foster City, California). Mass spectrometry was run in positive electrospray, multiple-reaction monitoring, with an ion spray voltage of 5500 V and TurbolonSpray temperature of 600°C (AB Sciex). The following transitions were monitored for the analytes: selumetinib, 459.0/397.2; N-desmethyl selumetinib, 445.3/383.1. The accuracy and precision of the analytical method were previously described.

For selumetinib, the within-batch mean accuracy ranged from 94.0% to 104.0%, and the within-batch precision (%CV) ranged from 3.3% to 6.7%. The between-batch mean accuracy ranged from 92.7% to 100.0%, and the between-batch precision ranged from 3.3% to 7.0%.
For N-desmethyl selumetinib, the within-batch mean accuracy ranged from 93.1% to 112%, and the within-batch precision (%CV) ranged from 2.2% to 5.0%. The between-batch mean accuracy ranged from 98.3% to 103.5%, and the between-batch precision ranged from 3.2% to 10.1%.

Radiometric Analysis
Duplicate samples of urine (~1 g) and plasma (0.1–0.5 mL) were taken for direct determination of radioactivity. Fecal samples were weighed and homogenized with a volume of water sufficient to produce a paste, using a commercial blender. Radioactivity levels in fecal homogenate and whole blood were determined after combustion in oxygen using an automatic sample oxidizer (model 307; PerkinElmer, Waltham, Massachusetts). The combustion products were absorbed into CarboSorb (PerkinElmer) and mixed with the scintillator cocktail PermaFluor E⁺ (PerkinElmer) for measurement of radioactivity. The efficiency of the oxidizer was checked using carbon-14 standards (Spec-Check; PerkinElmer) and was > 94%. Each batch of sample results was corrected for the oxidizer efficiency.

Radioactivity levels in urine, plasma, and dose-bottle rinses were quantified directly using liquid scintillation counting with a liquid scintillation counter with automatic external standard quench correction. Urine and dose-bottle rinse samples were mixed with Ultima Gold XR scintillant and counted on a 2300TR Tri-Carb Scintillation Counter (PerkinElmer). Plasma samples were mixed with Ultima Gold scintillant and counted on a 1414 Guardian Scintillation Counter (PerkinElmer). Detected counts per minute were converted to disintegrations per minute using quench correction.

Metabolite Profiling and Identification
Sample Preparation
Time-normalized plasma samples (0–72 hours) were prepared for each subject using the approach described by Hamilton et al.¹¹ to produce samples representative of the AUC. Each pool was treated with acetonitrile (1:2 vol/vol) and centrifuged (2000g for 10 minutes at 4°C) and the supernatant removed. To recover additional drug-related material, the plasma pellets were further washed using mixtures (~5 mL) of either acetonitrile/water (9:1 vol/vol or 4:1 vol/vol) or methanol/water (4:1 vol/vol). The combined supernatants were reduced to dryness under a stream of nitrogen (35°C) and reconstituted in 10% acetonitrile:90% water (vol/vol) prior to analysis by HPLC with MS detection and quantification via fraction collection and off-line 96-well microplate scintillation analysis (HPLC-RAD-MS²).

Urine was pooled for each subject by combining 5% by weight of each time point sample. Samples from individual time points were included if they contained ≥1% of the dosed radioactivity. The urine pools were centrifuged (2000g for 10 minutes at 4°C) prior to analysis by HPLC-RAD-MS².

Fecal homogenates were pooled for each subject by combining 5% by weight of each time point sample. Samples from an individual time point were included if they contained ≥1% of the dosed radioactivity. Aliquots of the pooled fecal samples were treated with acetonitrile (3:5 wt/vol), mixed, sonicated, and centrifuged (2000g for 10 minutes at 4°C) and the supernatant removed. Additional extractions were performed using the same approach but with mixtures (~5 mL) of acetonitrile and water (4:1 vol/vol and 1:1 vol/vol). The combined supernatants were reduced to dryness under a stream of nitrogen (35°C) and reconstituted in 10% acetonitrile:90% water prior to analysis by HPLC-RAD-MS².

Chromatography and Mass Spectrometry
Plasma and fecal extracts and centrifuged urine samples were injected onto a variable-gradient reverse-phase HPLC system, which utilized a CSH C₁₈ column (2.5 μm, 150 x 4.6 mm; Waters, Milford, Massachusetts), with 2 mobile phases consisting of 5% acetonitrile:95% water (vol/vol) plus 0.1% formic acid (vol/vol) and acetonitrile plus 0.1% formic acid (vol/vol), at a total flow rate of 1 mL/min. The postcolumn eluent was split (4:1), with the majority of the flow directed to a liquid handler for fractionation into 96-well ScintiPlates (PerkinElmer); the remainder of the flow was directed to an LTQ Orbitrap XL linear ion-trap mass spectrometer (Thermo Fisher Scientific, Kalamazoo, Michigan), which was equipped with an electrospray interface operating in positive ion mode. Full scan data were acquired over the mass range 100 to 1000 amu. MS2 and MS3 data for unchanged drug and metabolites were acquired with normalized collision energies of 35 and 40 eV, respectively. Reconstructed radiochromatograms were produced following import of 96-well...
ScintiPlate count data into Laura software version 4.1.7.70 (LabLogic Systems, Brandon, Florida). The count data were collected using a Microbeta scintillation counter (PerkinElmer) following removal of solvent from the plates. Data from the LTQ Orbitrap XL mass spectrometer were acquired and processed using Xcalibur software version 2.0.7 (Thermo Fisher Scientific).

Pharmacokinetic Parameters
PK parameters were determined by noncompartmental analysis using Phoenix WinNonlin version 6.3 (Certara, Princeton, New Jersey). PK parameters were derived by the following methods: $C_{\text{max}}$ and $T_{\text{max}}$ were determined by inspection of the concentration–time profiles; $\lambda_z$ was calculated by log-linear regression of the terminal portion of the plasma concentration–time profiles; terminal half-life ($t_{1/2}\lambda_z$) was calculated as $\ln(2)/\lambda_z$; apparent clearance was determined from the ratio of dose/AUC. Apparent volume of distribution was determined from the mean residence time/C2 apparent clearance.

Tolerability Assessments
Each subject underwent screening in the 28 days prior to dosing to confirm eligibility. Medical evaluations were performed on day −1 (baseline), on the day of dosing (predose), and at follow-up/discharge (on day 7 or on the last day of urine/fecal collection, whichever was later). Assessments included recording of concurrent medications, a complete physical examination, 12-lead ECG, echocardiography, clinical chemistry, hematology, urinalysis, supine blood pressure and pulse rate, body weight, and recording of any adverse events (AEs) and serious AEs. Vomit within 6 hours of dosing was analyzed for radioactivity.

Statistical Analysis
Given the exploratory nature of the study, no formal statistical hypothesis testing was performed. The statistical analysis was descriptive and consisted of subject listings, graphs, and summary statistics comprising geometric means, %CVs, arithmetic means, SDs, medians, and minimal and maximal values as appropriate.

RESULTS
Subjects’ Demography and Baseline Characteristics
Six white men were recruited and received study treatment. Mean baseline demographic characteristics were as follows: age, 56 years (range, 52–61 years); weight, 83.1 kg (range, 68.6–91.6 kg); and body mass index, 27.2 kg/m² (range, 23.7–29.6 kg/m²).

Plasma Pharmacokinetics
Plasma concentration–time plots of selumetinib and N-desmethyl selumetinib are presented in Figure 2, and the key PK parameters are presented in Table I. The geometric mean $C_{\text{max}}$ of selumetinib was 1520 ng/mL, and AUC, 4510 ng h/mL. Selumetinib was rapidly absorbed, with a median $T_{\text{max}}$ of 1 hour (range, 1–1.5 hours). Concentrations of selumetinib declined over time, with a mean terminal $t_{1/2}$ of 13.7 hours. Concentrations of the active metabolite N-desmethyl selumetinib were ~7% relative to selumetinib.

Renal clearance was not formally calculated, but the mean estimate of renal clearance (fraction of dose excreted <1% × apparent clearance 15.7 L/h) was <0.157 L/h, which is in agreement with findings from a renal impairment clinical study (study no. [NCT02063204], data on file, Dymond et al, [AstraZeneca], [Cheshire, UK], [state]).

![Figure 2. Profile of plasma and blood radioactivity and selumetinib and N-desmethyl selumetinib in plasma over time (semilogarithmic scale).](image-url)
Plasma and Blood Radioactivity

A full profile of plasma samples was collected for radiometric analysis; however, due to bioanalytical challenges, the plasma PK radioactivity parameters derived from the full profile of plasma samples were considered to be unreliable, and therefore a reduced number of plasma samples collected for metabolite profiling was used for radiometric analysis instead. Using this reduced plasma sample set, a comparison of the radioactivity concentration–time plot with the plasma selumetinib concentration–time plot demonstrated that total plasma radioactivity levels were consistently higher and declined more slowly (Figure 2). This finding is consistent with the presence of circulating metabolites.

Whole-blood radioactivity concentrations were lower than was plasma radioactivity at equivalent times, ranging from 60% to 70%, and did not appear to change with time (Table II). It was estimated that the association of the radioactivity with the red blood cell component of blood was ~6%. There was no indication of a trend in the change in the distribution with time.

Excretion Balance

Excretion of the dose in urine and feces over time is presented in Figure 3. One subject was excluded from the summaries of fecal and total excretion because he did not produce fecal samples in the first week after dosing. The vast majority of the total radioactive dose was recovered in urine by 96 hours and in feces by 144 hours, with near-complete recovery of the dose (93%) within a 9-day collection period. Fecal excretion (mean, 59%) was the predominant elimination

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma Selumetinib</th>
<th>Plasma N-desmethyl Selumetinib</th>
<th>Whole Blood Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC, GM (%CV), ng h/mL</td>
<td>4510 (19.2)</td>
<td>351 (23.1)</td>
<td>8200 (26.8)</td>
</tr>
<tr>
<td>C_max, GM (%CV), ng/mL</td>
<td>1520 (28.5)</td>
<td>109 (23.9)</td>
<td>1390 (17.6)</td>
</tr>
<tr>
<td>T_max, median (range), h</td>
<td>1.00 (1.00-1.50)</td>
<td>1.00 (1.00-1.50)</td>
<td>1.00 (1.00-1.50)</td>
</tr>
<tr>
<td>t_1/2, mean (SD), h</td>
<td>13.7 (5.04)</td>
<td>9.40 (1.01)</td>
<td>56.8 (48.0)</td>
</tr>
<tr>
<td>CL/F, mean (SD), L/h</td>
<td>15.7 (2.87)</td>
<td>NR</td>
<td>8.78 (2.07)</td>
</tr>
<tr>
<td>V/F, mean (SD), L</td>
<td>146 (48.5)</td>
<td>NR</td>
<td>359 (232)</td>
</tr>
</tbody>
</table>

CL/F = apparent clearance; GM = geometric mean; NR = not reported; V/F = apparent volume of distribution.

Table II. Concentration ratios (%) to total plasma radioactivity (N = 6). Data are given as geometric mean (%CV).

<table>
<thead>
<tr>
<th>Ratio</th>
<th>2 h</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma selumetinib</td>
<td>54.2 (6.0)</td>
<td>50.0 (7.1)</td>
<td>37.7 (14.9)</td>
<td>24.1 (5.6)</td>
<td>15.1 (19.6)</td>
<td>ND (ND)</td>
</tr>
<tr>
<td>Plasma N-desmethyl selumetinib</td>
<td>3.98 (14.3)</td>
<td>3.72 (11.7)</td>
<td>3.54 (9.9)</td>
<td>3.11 (20.4)</td>
<td>ND (ND)</td>
<td>ND (ND)</td>
</tr>
<tr>
<td>Whole blood</td>
<td>60.3 (6.6)</td>
<td>59.7 (6.6)</td>
<td>69.7 (7.5)</td>
<td>61.0 (3.9)</td>
<td>61.5 (4.5)</td>
<td>60.4 (4.1)</td>
</tr>
<tr>
<td>Distribution into RBCs</td>
<td>5.94 (75.7)</td>
<td>3.60 (109.5)</td>
<td>17.9 (25.0)</td>
<td>5.59 (87.0)</td>
<td>6.18 (77.3)</td>
<td>6.74 (45.7)</td>
</tr>
</tbody>
</table>

ND = not determined; RBCs = red blood cells.
*The distribution into RBCs was determined by [(Cwb – (1 – Ht)*Cp)/Cwb]*100, where Cwb is concentration in whole blood, Cp is concentration in plasma, and Ht is hematocrit (Ht was the mean value of the laboratory Ht data obtained from Day -1 and follow-up).
route of drug-related material, with moderate elimination in urine (mean, 33%).

Metabolite Profiling and Identification

Examples of the representative pooled metabolite profiles are presented in plasma, urine, and feces in Figure 4. The proposed metabolic pathways of selumetinib are shown in Figure 5.

Metabolites in Plasma

Selumetinib was the major circulating drug-related component, accounting for 40% of the plasma radioactivity (mean of data from all subjects, AUCO–72h pool). The major circulating metabolite, an amide glucuronide, accounted for 22% of the plasma radioactivity (mean of data from all subjects) and was postulated to result from loss of the ethanediol moiety in combination with glucuronidation and an additional loss of 2 mass units, possibly due to further oxidation of the N-methylbenzimidazole moiety or intramolecular cyclization (M2; m/z 571). A further 6 metabolites were identified, each accounting for between 2% and 7% of the circulating drug-related material (mean of data from all subjects), including metabolites resulting from N-demethylation in combination with the loss of the ethanediol moiety, glucuronidation, and further oxidation of the benzimidazole moiety or intramolecular cyclization (M1; m/z 557); N-demethylation in combination with gluconidation (M3; m/z 619); direct glucuronidation of selumetinib (M4; m/z 633); N-demethylation (M8; m/z 433); N-demethylation in combination with oxidation of the primary alcohol group to a carboxylic acid (M11; m/z 457); and a carboxylic acid resulting from hydrolysis of the amide group (M15; m/z 398). A number of trace drug-related components (<2% of the circulating radioactivity) were identified, including the primary amide resulting from loss of the ethanediol moiety (M14; m/z 397) and components involving N-demethylation, glucuronidation, loss of the ethanediol moiety, mono-oxidation, and a loss of 2 mass units, possibly due to further oxidation of the N-methylbenzimidazole moiety or intramolecular cyclization.

Metabolites in Urine

Selumetinib was a minor drug-related component in urine, accounting for ≤1% of the dose in all subjects. The most abundant metabolite identified in urine was M2 (m/z 571), which accounted for 10% of the dose (mean of data from all subjects). Metabolites accounting for between 1% and 10% of the dose in the urine (mean of data from all subjects) included M1 (m/z 557), M3 (m/z 619), M4 and another direct glucuronide conjugate of selumetinib, M7 (both, m/z 633), and M8 (m/z 433). Trace drug-related components included the primary amide (M14; m/z 397) and carboxylic acid (M15; m/z 398) metabolites.

Metabolites in Feces

Selumetinib accounted for between 6% and 34% of the dose in the feces of individual subjects, with the exception of the subject who provided limited fecal samples up to 168 hours postdose, in whom unchanged selumetinib accounted for <1% of the dose. Mean percentages of selumetinib dose recovered in feces were 23% and 19% when this subject’s data were excluded and included, respectively. Seven metabolites accounted for between 1% and 9% of the dose in feces (mean of data from all subjects): the primary amide metabolite (M14; m/z 397), the N-desmethyl metabolite (M8; m/z 433), the carboxylic acid metabolite (M15; m/z 398), and product resulting from loss of the ethanediol moiety in combination with N-demethylation (M12; m/z 383), loss of the ethanediol moiety in combination with N-demethylation and mono-oxidation (M13; m/z 399), loss of the ethanediol moiety in combination with
Figure 4. Reconstructed radiochromatography of AUC in pooled human plasma (A), urine (B), and feces (C) after a single 75-mg oral dose of [14C]-selumetinib in a representative subject.
N-demethylation and ribose conjugation (M9; m/z 515), and loss of the ethanediol moiety in combination with a loss of 2 mass units, possibly due to further oxidation of the N-methylbenzimidazole moiety or intramolecular cyclization (M10; m/z 395).

**Tolerability**

Two of the six subjects in the study reported ≥ 1 AE: 1 subject reported lymph node pain and vomiting (at 168–192 hours after dosing, and therefore was considered unlikely to contain significant amounts of radioactivity), while the other subject reported upper abdominal pain and spurious diarrhea. All of the AEs reported in the study were assessed by the Investigator as being mild in intensity and not related to selumetinib. All AEs were resolved by the end of the study.

**DISCUSSION**

This Phase I study examined the absorption, distribution, and metabolism of selumetinib by investigation of the routes and rates of excretion of [14C]-radio-labeled selumetinib and its metabolite, N-desmethyl selumetinib in healthy male volunteers. Almost complete recovery of the total radioactive dose (93%) was from urine and feces within a 9-day collection period.
Moderate elimination in urine (33%) as metabolites of selumetinib was detected (the most abundant metabolite, at 10% of the dose, was the amide glucuronide [M2]), with <1% as unchanged selumetinib. The fraction of dose recovered in feces was 59%, with selumetinib accounting for a mean of 19% of the dose and 7 metabolites each accounting for between 1% and 9% of the dose. Hence, the majority of the radioactive dose was recovered as metabolites of selumetinib, indicating that the liver plays a major role in the elimination of selumetinib and its metabolites.

In this study, of the 19% of dose recovered as unchanged selumetinib in feces, it was not possible to differentiate the portion that was extracted from the systemic circulation from the amount of unabsorbed dose or that which had resulted from gut microbial degradation of metabolites back to the parent drug. In a separate study, the absolute bioavailability of a single oral dose of selumetinib 75 mg in healthy subjects was 62% (90% CI, 60.1–64.1), as determined by coadministration of an intravenous 80-μg microradiolabeled dose of [14C]-selumetinib (study no. 2014-002993-35, data on file, EudraCT, London, UK). The fraction absorbed was estimated to be 71%, based on the intravenous clearance and assuming normal hepatic blood flow in a 70-kg human to be 87 L/h based on the approach of Gabrielsson and Weiner. Based on these data, it is estimated that ~10% of a 75-mg oral dose of selumetinib was removed by first-pass effects, which suggests that this mechanism and the amount of unabsorbed drug contribute equally to the amount of unchanged selumetinib recovered in feces.

The plasma PK of selumetinib and N-desmethyl selumetinib in this study were similar to those reported in previous studies of single-dose selumetinib Hyd-Sulfate 75-mg capsules PO in healthy subjects (study nos. NCT02093728 and NCT02046850, data on file, [AstraZeneca, Cheshire, UK]). Based on time-matched concentration ratios, selumetinib accounted for 54.2% of total plasma radioactivity at 2 hours, reducing to 15.1% at 48 hours, while N-desmethyl selumetinib accounted for 3.98% at 2 hours, reducing to 3.11% at 24 hours; hence, total plasma radioactivity concentrations appeared to show a slower decline than plasma selumetinib and N-desmethyl selumetinib concentrations, indicating the presence of other circulating metabolites with greater systemic retention than selumetinib. The concentration ratios of unchanged and N-desmethyl metabolites accounted for 51% and 9% of the dose, respectively, and 25% of the dose was accounted for as other circulating metabolites, which are a result of metabolism of the ethanediol moiety in combination with glucuronidation. A further 6 circulating metabolites were identified, each accounting for between 2% and 7% of plasma radioactivity.

In vitro studies indicated that the phase I metabolism of selumetinib was predominantly via CYP3A4, with some contribution from CYP2C19 and -1A2. Investigations into the relative contribution of CYP and UGT enzymes to the overall clearance of selumetinib indicated that ~56% of the observed in vitro intrinsic clearance of selumetinib could be attributed to CYP metabolism, and ~29%, attributed to direct glucuronidation by UGT enzymes (ie, excluding pathways in which phase I oxidation occurs before glucuronidation). The UGT isoforms involved in turnover of selumetinib were found to be UGT1A1 and -1A3. Based on findings from in vitro experiments, the active N-desmethyl metabolite was metabolized by conjugation (glucuronidation) and CYPs.

Due to the lower systemic exposure of the N-desmethyl metabolite in relation to selumetinib, plasma concentrations were measurable only to 24 hours, whereas detectable concentrations of selumetinib were achieved to 48 hours. The apparent difference in the derived elimination half-life between the parent and metabolite was likely due to the different amounts of data used to define the elimination phase, and it is possible that the elimination half-life of the metabolite could have been underestimated. The shapes of the curves of selumetinib and N-desmethyl metabolite are similar.
oxidation or/and glucuronidation and so these findings are in agreement with the key metabolic routes identified in the in vitro experiments.

The results from this study are based on data from healthy subjects with normal renal and hepatic function. The metabolism, excretion, and PK findings reported here may differ in patients in whom disease and any associated difference in hepatic or renal function could alter the disposition of the drug (study nos. [NCT02063204 and NCT02063230], data on file, Dymond et al, [AstraZeneca], [Cheshire, UK]). Also, this was a single-dose study, whereas long-term dosing is expected in clinical practice. Accepting the limitations of the study, we believe the study provides good insight into the disposition of the drug in patients in clinical practice.

Issues were encountered in some of the analyses of radioactivity in plasma and whole blood. These issues manifested as high variability in replicates and unusual kinetic trends. Further analysis was performed on specific whole-blood and plasma samples (40% and 27%, respectively). It was observed that, in some instances, the variance between the first 3 aliquots was >15%. It was also observed that there was an increase in concentration in whole blood that was not reflected in the plasma. An additional 2 aliquots of whole blood and plasma were analyzed and counted on the liquid scintillation counter in a 10-minute protocol. A further aliquot of whole blood was analyzed in order to confirm the additional results. The whole-blood results generated from aliquots in 2 specific liquid scintillation counting batches have been excluded, and a mean of all other data has been calculated and reported. High variability between aliquot results subsequently reduced from 40% to 14%, with all of the remaining high-variability results being near the lower limit of quantification. The results of the plasma analysis and further analysis were considered unreliable, and therefore a lower number of plasma samples, initially intended for metabolite profiling, were analyzed to derive quantitative radioactivity data. Variability and kinetic trends in this analysis appeared reliable. This observation suggests that there were no issues with the sample-collection procedures but that a technical problem was associated with the original analysis.

For metabolite profiling, time-normalized plasma samples (0–72 hours) from each subject were prepared using the approach described by Hamilton et al to produce a composite of aliquots from samples collected at the various time points; this composite was representative of the AUC in that period. This is an accepted method for providing a representative relative abundance of the metabolites formed in a set time period. However, the pooled-sample technique will not allow for the characterization of any change in the metabolite profile with time.

The current study was limited in that it was performed in 6 subjects; this small sample size was chosen to restrict the number of healthy subjects exposed to a radioactive dose. This sample size is typically used in human mass balance studies and is accepted by regulatory authorities (www.ema.europa.eu/docs/en_GB/document...guideline/2012/07/WC500129606.pdf EMA guidance: Guideline on the investigation of drug interactions (CPMP/EWP/560/95/Rev. 1 Corr. 2**). June 2012).

CONCLUSIONS

In this PK study of selumetinib in healthy male volunteers, ~93% of the dose was recovered within 9 days, with 59% in feces and 33% in urine. Urinary elimination of unchanged selumetinib was minimal (<1%). There were circulating metabolites of selumetinib and minimal distribution of drug-related material in red blood cells. Metabolism of selumetinib included N-demethylation, direct glucuronidation of selumetinib, and conjugation of phase I metabolites. Profiling of the metabolites circulating in plasma and excreted in urine and feces indicated that oxidative and glucuronidation mechanisms are both important metabolic routes. [14C]-selumetinib Hyd-Sulfate appeared to have been well tolerated. The findings from this study will inform the label and will contribute to the understanding of the clinical pharmacology of selumetinib.

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All of the authors had full access to the data, contributed to the writing of the manuscript, and approved the final manuscript for submission. Additionally, the authors were involved as follows: A.W. Dymond, C. Pattison, and P. Martin: study conception and design, data analysis and interpretation; C. Howes, K. So, and G. Mariani: data analysis and interpretation; G. Ford: data acquisition;
M. Savage: data acquisition, analysis and interpretation; and S. Mair (principal investigator): study conception and design; data acquisition, analysis, and interpretation.

CONFLICTS OF INTEREST STATEMENT
This study and medical editing were funded by AstraZeneca (Macclesfield, UK). The sponsor contributed to the study design; collection, analysis, and interpretation of the data; writing of the manuscript; and the decision to submit the manuscript for publication. C. Howes, K. So, and G. Mariani are employees of, and may hold stock options in, AstraZeneca. A.W. Dymond, C. Pattison and P. Martin were employees of AstraZeneca at the time of study conduct and may hold stock options in AstraZeneca. M. Savage is employed by York Bioanalytical Solutions, contracted by AstraZeneca to provide metabolite characterization for this study; S. Mair is employed by Quotient Clinical, contracted by AstraZeneca to conduct the study; and G. Ford is employed by Quotient Bioresearch Ltd, contracted by AstraZeneca to provide quantitative radiochemical analysis. The authors have indicated that they have no other conflicts of interest with regard to the content of this article.

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