Metabolic Disposition of Osimertinib in Rat, Dog, and Man: insights into a drug designed to bind covalently to a cysteine residue of EGFR

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Abbreviations: ADME absorption, distribution, metabolism, and excretion; AUC area under the concentration-time curve; CLint intrinsic clearance; EGFR epidermal growth factor receptor; FDA food and drug administration; FMO flavin-containing monooxygenase; HPLC high-performance liquid chromatography; LC liquid chromatography; LSC liquid scintillation counting; MS mass spectrometry; AMS accelerator mass spectrometry; MS/MS tandem mass spectrometry; nmol/eq nmolar equivalent; NSCLC non-small cell lung cancer; QWBA quantitative whole-body autoradiography; TKI tyrosine kinase inhibitor; T\textsubscript{max} time to maximum plasma concentration; UV ultraviolet
Abstract

Preclinical and clinical studies were conducted to determine the metabolism and/or pharmacokinetics of osimertinib and key metabolites, AZ5104 and AZ7550. Osimertinib was designed to covalently bind to EGFR allowing it to achieve nanomolar cellular potency (Finlay et al., 2014). Covalent binding was observed in incubations of radiolabeled-osimertinib with human and rat hepatocytes, human and rat plasma, and human serum albumin. Osimertinib, AZ5104, and AZ7550 were predominantly metabolized by CYP3A. Seven metabolites were detected in human hepatocytes; also observed in rat or dog hepatocytes at similar/higher levels. Following oral administration of radiolabeled-osimertinib to rats, drug-related material was widely distributed, with the highest radioactivity concentrations measured at 6 hours postdose in most tissues; radioactivity was detectable in 42% of tissues 60 days postdose. Concentrations of [14C]-radioactivity in blood were lower than in most tissues. Following administration of a single oral dose of radiolabeled-osimertinib 20 mg to healthy male volunteers, ~19% of the dose was recovered by 3 days postdose. At 84 days postdose, mean total radioactivity recovery was 14.2 and 67.8% of the dose in urine and feces. The most abundant metabolite identified in feces was AZ5104 (~6% of dose). Osimertinib accounted for ~1% of total radioactivity in the plasma of non-small cell lung cancer patients following 22 days of osimertinib 80 mg once daily treatment; the most abundant circulatory metabolites were AZ7550 and AZ5104 (<10% of total osimertinib-related material). Osimertinib is extensively distributed and metabolized in humans, and is primarily eliminated via the fecal route.
Introduction

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) (i.e., gefitinib, erlotinib, and afatinib) are recommended as first-line therapy for patients with advanced non-small cell lung cancer (NSCLC) that harbors an EGFR sensitizing mutation (Keedy et al., 2011; Reck et al., 2014). However, tumors of most patients treated first-line with a currently approved EGFR-TKI develop resistance, leading to disease progression (Kobayashi et al., 2005; Pao et al., 2005; Sequist et al., 2011). The EGFR T790M mutation is found in approximately 60% of cases that have developed resistance to EGFR-TKIs (Yu et al., 2013; Oxnard et al., 2011; Arcila et al., 2011; Kuiper et al., 2014; Li et al., 2014).

Osimertinib (AZD9291) is a novel oral, potent, irreversible EGFR-TKI that is structurally and pharmacologically distinct from all other licensed EGFR-TKIs (AstraZeneca Pharmaceuticals LP). Osimertinib is selective for EGFR-TKI sensitizing mutations and the T790M resistance mutation, with lower activity against wild-type EGFR compared with currently approved EGFR-TKIs (Cross et al., 2014). Osimertinib was recently approved by the US FDA for the treatment of patients with metastatic EGFR T790M mutation-positive NSCLC who have progressed on or after EGFR-TKI therapy. Osimertinib clinical activity has been demonstrated in the treatment of patients with EGFR mutant NSCLC following progression on a previous EGFR-TKI due to the EGFR T790M mutation in the ongoing AURA (NCT01802632) and AURA2 (NCT02094261) studies (Jänne et al., 2015a; Jänne et al., 2015b; Yang et al., 2015; Mitsudomi et al., 2015). Preclinical data suggest that osimertinib is principally metabolized by cytochrome P450 (CYP3A4) and produces at least two circulating active metabolites: AZ5104 and AZ7550 (Cross et al., 2014; Planchard et al., 2014).

We conducted a series of studies in laboratory animals and clinical studies in healthy volunteers and patients with NSCLC, in order to characterize the absorption, distribution, metabolism, and excretion characteristics of osimertinib. The results of these studies are reported herein.
Materials and Methods

Study conduct

The clinical studies described here were conducted in accordance with the International Conference on Harmonisation–Good Clinical Practice, the ethical principles that have their origin in the Declaration of Helsinki, applicable regulatory requirements, and the AstraZeneca policy on Bioethics and Human Biological Samples.

Where applicable, the preclinical studies described here were performed in accordance with the Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice.

Radiolabeled osimertinib and reference compounds

Radiolabeled osimertinib [(N-2-[2-(dimethylamino)ethyl-methyl-amino]-4-methoxy-5-[4-(1-methylindol-3-yl)pyrimidin-2-yl]amino)phenyl]prop-2-enamide] was synthesized by the Isotope Chemistry Section of AstraZeneca R&D (Macclesfield, UK). The structures of [14C]- and [3H]-osimertinib are illustrated in Supplementary Figure 1. [3H]-osimertinib was used to determine the covalent binding of osimertinib and/or its metabolites to human hepatic proteins (specific activity 1802 MBq/mg; purity >98%) and rat hepatic proteins (specific activity 1817 MBq/mg; purity >98%). [14C]-osimertinib was used to determine the covalent binding of osimertinib and/or its metabolites to human and rat plasma and human serum albumin (specific activity 4.44 MBq/mg; purity >98%). [14C]-osimertinib was also used to determine tissue distribution of osimertinib in the rat (specific activity 4.44 MBq/mg; purity >98%) and to determine the rates and routes of elimination of osimertinib in healthy male volunteers (specific activity 0.0303 MBq/mg; purity 99%).

Non-radiolabeled osimertinib and reference compounds (AZ5104, AZ7550, and M1) were synthesized by AstraZeneca R&D (Macclesfield, UK). The structure and purity of these compounds were assessed by high-performance liquid chromatography (HPLC) with
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ultraviolet (UV) light spectroscopy detection, mass spectrometry (MS), and nuclear magnetic resonance spectroscopy. Synthetic routes for these compounds have been previously described (Finlay et al., 2014).

**Plasma stability**

Frozen plasma and protein solutions from the following species were used for stability assessment: mouse (CD-1, male, n>3), rat (Han Wistar, male, n>3), dog (Beagle, male, n>3), human (Asian, mixed sex, n>20), human serum albumin (45 mg/mL) human α1-acid glycoprotein (0.70 mg/mL). Stock solutions of osimertinib were dissolved in DMSO then diluted into plasma or protein solutions to achieve concentrations from 0.1 to 100 µM containing 1% DMSO. Samples were vortexed and incubated at 37°C for up to 6 hours. Aliquots (50 µL) were mixed with water (50 µL) and the reaction was terminated by addition of acetonitrile (400 µL), vortexed, centrifuged and the supernatant was analyzed by HPLC-MS/MS.

**Covalent binding in human and rat hepatocytes**

[^3H]-osimertinib was incubated in triplicate at a nominal concentration of 10 µM with cryopreserved rat and human hepatocytes (0.9 million cells/mL) at 37°C for 4 hours under a 5% CO₂ atmosphere. Positive control incubations with[^3H]-labeled zomepirac were conducted in parallel in both species. Rat and human hepatocytes (combined male and female donors, n =10) were obtained from Celsis IVT (USA).

For covalent binding assessment, aliquots were removed at the beginning (0 minutes) and the end (4 hours) of the incubation, quenched with acetone and vortex mixed. Samples were kept at 8°C for 1 hour prior to protein harvesting onto filter paper using a Brandel Cell Harvester and washed with methanol. Immobilized protein was solubilized in 5% sodium dodecyl sulfate at 55°C for 20 hours. Protein content was determined using the Thermo...
Scientific™ Pierce BCA Protein Assay Kit and the concentration of radioactivity was determined by liquid scintillation counting (LSC).

For estimation of the turnover of parent osimertinib, separate aliquots were removed at 0 and 4 hours, quenched with acetonitrile containing 0.8% formic acid and kept at –20°C for more than 20 minutes before centrifugation at 4000 rpm at 4°C. The supernatants were diluted 1:1 with ultra pure water and analyzed by liquid chromatography (LC) with tandem mass spectrometry (MS/MS).

**Covalent binding in rat and human plasma and human serum albumin**

[^14C]-osimertinib was incubated in singlicate at nominal concentrations of 10 μM in rat (male, Harlan, UK) and human (males, n = 3, Medicines Evaluation Unit, Manchester, UK) plasma, and in human serum albumin (40 mg/mL) in phosphate buffered saline at 37°C. Phosphate buffered saline was used as a negative control.

For covalent binding assessment, aliquots were removed at various time points up to 6 hours. Protein was precipitated with acetone and washed extensively with methanol until the radioactivity present in the methanol supernatant was less than approximately three times the background radioactivity (as assessed by LSC). Protein was solubilized in 5% sodium dodecyl sulfate followed by incubation overnight at 55°C. Protein content was determined using the Thermo Scientific™ Pierce BCA Protein Assay Kit and the concentration of radioactivity was determined by LSC.

**Rat quantitative whole-body autoradiography**

The tissue distribution of radioactivity in male partially pigmented (Lister-Hooded) and male and female albino (Wistar Han) rats was determined following a single oral dose (4 mg/kg [8 μmol/kg]) of[^14C]-osimertinib (1.85 MBq/mg) using quantitative whole-body autoradiography (QWBA). Following osimertinib dosing, animals were euthanized from 0.5 hours postdose and at intervals up to 60 days postdose (Table 1). Sagittal sections (nominal
thickness 30 μM) were obtained through the carcass to include the following tissues: exorbital lachrymal gland (males) or ovaries (females), intra-orbital lachrymal gland, Harderian gland, adrenal gland, thyroid, brain, and spinal cord. Sections were freeze-dried and placed in contact with imaging plates (Raytek Scientific Ltd., Sheffield, UK). [14C]-Blood standards of appropriate activity (also sectioned at a nominal thickness of 30 μm) were placed in contact with all imaging plates. The sampling time after administration of [14C]-osimertinib was 0.5, 1, 6, and 24 hours, and 2, 7, 21, and 60 days in partially pigmented male rats and 1, 6, and 24 hours in albino male and female rats. Concentrations of radioactivity in tissues were quantified from whole-body autoradiograms by Covance Laboratories Ltd. (Harrogate, UK) using a validated image analysis system. The lower limits of quantification were 1186.4 dpm/g (0.023 nmol/g) in the male partially pigmented animals, 860.9 dpm/g (0.017 nmol/g) in male albino rats, and 670.8 dpm/g (0.013 nmol/g) in female albino rats. The upper limits of quantification were 7,437,550 dpm/g (145 nmol/g) in all three groups.

**Metabolism in recombinant expressed CYP isozymes**

The metabolic turnover of osimertinib was assessed by incubation of osimertinib or positive control substrates with microsomes prepared from insect cell lines that heterologously expressed the individual recombinant human CYP isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 (BD Gentest™, USA). Metabolic stability incubations were performed at 37°C. The incubations contained 0.1 M phosphate buffer (pH 7.4), microsomes (0.5 mg/mL), and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH, 5 mM final concentration), and were started by the addition of osimertinib or positive control substrates (final incubation concentration 1 μM) after a 10-minute pre-incubation period. Aliquots were removed into acetonitrile (containing internal standard) at various time points up to 30 minutes, diluted with water and centrifuged. The concentration in the supernatant was determined by HPLC-MS/MS.
The metabolism of AZ5104 and AZ7550 was assessed by incubation of AZ5104/AZ7550 or positive control substrates with bactosomes prepared from *Escherichia coli* bacteria. Heterologously expressed individual CYP isoforms (100 pmol/mL final concentration) were pre-incubated in the presence of AZ5104/AZ7550 (2 µM) or positive control substrates for 15 minutes at 37°C. Incubations were conducted in 0.1 M potassium phosphate buffer (pH 7.4) with a final solvent concentration <1%. Incubations were initiated by addition of NADPH (1 mM final concentration) and termination was by removal of samples into acetonitrile (containing internal standard). Samples were taken at up to 30 minutes and were analyzed by ultra-performance liquid chromatography with MS detection.

A substrate depletion approach was used to determine the intrinsic clearance (CLint) for each isoform. The contribution of each isoform (relative to the ten isoforms described) was calculated from the mean CLint using inter-system extrapolation factors and relative abundance factors. Additionally, in a separate experiment the extent of formation of AZ5104 and AZ7550 by each CYP isoform (relative to the formation by CYP3A4) was assessed following incubation of osimertinib (1 µM) in recombinant expressed CYP isozymes.

**Metabolism and metabolite formation in mouse, rat, dog, and human hepatocytes**

An *in vitro* study to investigate the metabolism of osimertinib following incubation with mouse (CD1), rat (Han Wistar, male), dog (Beagle, male), and human hepatocytes was undertaken. Mouse hepatocytes were obtained in-house at AstraZeneca, with rat, dog and human hepatocytes from Celsis IVT (USA). Osimertinib was incubated (5 µM) with mouse, rat, dog, and human hepatocytes (1 million cells/mL) at 37°C for 1 hour. Incubations were stopped using two volumes of acetonitrile. Samples were spun at 4000 g for 15 minutes and the supernatant was stored at −20°C until required for analysis. Semi-quantitative assessment of osimertinib metabolite profiles were made by analysis of UV traces at 320–330 nm to determine the proportion of the total area that was represented by each component. The limit of quantification for metabolites by LC-UV was 1% relative to osimertinib.
An *in vitro* study was undertaken to investigate which human CYP isoforms form the metabolites AZ5104 and AZ7550 in human hepatocytes; the study also investigated whether flavin-containing monooxygenases (FMOs) produce the metabolite M1. Metabolic stability incubations at 37°C included human hepatocytes at 1 million cells/mL and a selective inhibitor (or dimethyl sulfoxide for control incubations) for a 10-minute pre-incubation period. Incubations were started by the addition of osimertinib or a control substrate (1 µM final concentration) for 120 minutes, with sampling at suitable time points. Samples were diluted with water, centrifuged at 3000 rpm for 15 minutes, and the concentration in the supernatant was determined by HPLC-MS/MS. The mean rate of formation (pmol/min/million cells) for each metabolite was calculated. Additionally, the substrate depletion method was used to determine CLint.

**Absorption, distribution, metabolism, and excretion of osimertinib in healthy volunteers**

A phase I, open-label, single-center pharmacokinetic and mass balance study (ClinicalTrials.gov identifier NCT02096679) was undertaken in healthy male volunteers. The objective was to determine the rates and routes of excretion of [14C]-osimertinib by assessment of concentrations of total [14C] radioactivity and of osimertinib and its metabolites in whole blood, plasma, and urine as well as percentage recovery of the radioactive dose in urine and feces.

Healthy male volunteers aged 30–65 years were recruited at a single site (Quotient Clinical Ltd., Nottingham, UK). Inclusion criteria included regular bowel movements (i.e., average production of at least one stool per day). The use of prescribed or non-prescribed concomitant medications was not permitted in the 4 weeks (or longer depending on the medication’s half-life) prior to the first administration of osimertinib (except for paracetamol and non-steroidal decongestants at the discretion of the investigator). In addition, the use of medications with CYP450 enzyme-inducing properties was not permitted in the 4 weeks.
prior to the first administration of osimertinib. Subjects were also excluded if they had any intake of any product containing grapefruit or Seville oranges within 7 days of the first administration of osimertinib.

Eligible subjects were admitted to the study center on day –1. Subjects fasted overnight for 10 hours before administration of the investigational product on day 1. Each subject received a single 20 mg dose of [\(^{14}\)C]-osimertinib as an oral solution (free base equivalent) containing a nominal dose of 0.037 MBq (1 \(\mu\)Ci) activity. Volunteers were resident at the study center for 21 days following administration of [\(^{14}\)C]-osimertinib. During residency, samples of blood, urine, and feces were collected and safety assessments were undertaken. Volunteers returned for further 24-hour residency periods to allow additional safety assessments and collection of blood, urine, and feces samples (days 28–29, days 35–36, days 42–43, and days 84–85). Prior to dosing subjects, dosimetry calculation were performed by Public Health England (Didcot, UK) using the rat mass balance and QWBA study results as data inputs. This dosimetry assessment indicated that the committed effective dose was 1.0 \(\times\) 10\(^{-9}\) Sv/Bq and a radioactive dose of up to 5 MBq (132 \(\mu\)Ci) would have been the maximum for WHO Category II (World Health Organization, 1977) in males. However, this was estimated to be an insufficient radioactive dose to determine metabolites present in plasma at 10% of parent by conventional radio-detection techniques. Consequently, accelerator mass spectrometry (AMS) detection was chosen as the quantification tool. As this technique is much more sensitive, a much lower radioactive dose was feasible. This further reduced the risk to subjects and also the need to excessively dilute samples.

**Pharmacokinetic analysis**

Appropriate pharmacokinetic parameters for plasma osimertinib, AZ7550, and AZ5104 and whole blood and plasma [\(^{14}\)C] radioactivity were assessed.

The pharmacokinetic analyses were performed at Quintiles (Overland Park, KS, USA). Pharmacokinetic parameters were derived using standard non-compartmental methods with
Phoenix® WinNonLin® Professional Version 6.3 (Pharsight Corporation, Mountain View, CA, USA). Actual sampling times were used in the parameter calculations. Area under the concentration-time curve (AUC) was calculated using the linear up/log down method. Where appropriate, the AUC from time zero to the time of the last quantifiable concentration after single dosing was extrapolated to infinity using $\lambda z$ to obtain AUC.

**Mass balance**

Radioactive [$^{14}$C] recovery in feces was calculated conventionally by the summation of the amount excreted in each collection interval during the residential period (up to 504 hours postdose). Recovery of fecal radioactivity during the outpatient portion of the study (504–2016 hours postdose) was calculated from the amount recovered in the four scheduled 24-hour collection intervals using the area under the excretion rate–time curve method. Two subjects had their urine mixed during the 24 to 48 hours postdose collection period and for a further two subjects their urine was mixed during the 480 to 504 hours postdose collection period; thus radioactivity recovery in urine for the entire duration of the study was calculated using the area under the excretion rate–time curve method excluding the urine samples that had been mixed.

**Metabolite identification**

Following analysis of plasma, fecal, and urine samples by AMS for total radioactive content, appropriate samples were pooled across volunteers and time points to give one plasma, one urine, and one fecal sample for metabolite profile analyses at Xceleron Inc. (Germantown, MD, USA) and one each for metabolite identification at AstraZeneca (Macclesfield, UK). Based on the levels of radioactivity observed, 24–168 hour pooled samples were created, which balanced characterizing as much of the AUC as possible with retaining reasonable sensitivity. Osimertinib and its metabolites were extracted from plasma using acetonitrile and from fecal homogenate using acetonitrile followed by acetonitrile water and then water, while urine was analyzed without extraction. The metabolite profiles in pooled urine and feces
were determined using HPLC-UV combined with offline radioactivity monitoring by AMS for detection/quantification, MS for structural elucidation, and UV for retention time matching between HPLC-UV fractionation for AMS and HPLC-UV-MS systems.

**Sample extraction**

The pooled plasma sample was extracted by adding acetonitrile (750 µL) to plasma (300 µL), the mixture was vortexed (1 minute), centrifuged (3000 g, 5 minutes at room temperature) and the supernatant reduced to 50 µL under a stream of nitrogen. The extract was then reconstituted in acetonitrile/water (25/75 v/v, 300 µL) and the radioactivity measured by AMS and compared against an unextracted sample.

The cross-subject and time-pooled fecal homogenate sample (300 mg) was mixed with acetonitrile (750 µL), vortexed (1 minute), centrifuged (3000 g, 5 minutes, room temperature) and supernatant transferred to a clean vial. The remaining pellet was then mixed with acetonitrile/water (50/50 v/v, 750 µL), vortexed, and centrifuged as previously with the supernatant combined with the acetonitrile fraction. This combined fraction was diluted with water to achieve a final composition of 20/80 v/v acetonitrile/water, vortexed and analyzed by HPLC-AMS and HPLC-MS/MS. The column recovery from this fraction was 93%. The remaining pellet was also extracted with water (750 µL) where this fraction was only used to assess the amount of radioactivity recovered from the sample.

The pooled urine sample was centrifuged (1924 g, 10 minutes, 4°C) prior to direct HPLC-AMS and HPLC-MS/MS analysis and LSC for column recovery (100% recovered).

**Circulating metabolites in patients at steady state**

The objectives of this study were to detect, characterize, and semi-quantify metabolites of non-radiolabeled osimertinib in human plasma.
Steady-state (day 22) plasma samples were obtained following daily oral dosing of osimertinib at 80 mg to fasted patients with NSCLC in the AURA phase I study (Planchard et al., 2014). Plasma samples were selected and pooled across six individual subjects using the method of Hamilton and colleagues (Hamilton et al., 1981) to create a sample representative of the exposure to osimertinib within the pooling window. Osimertinib and related metabolites were detected, semi-quantified (as a percentage of total detected drug-related material circulating in plasma), and the structure elucidated by HPLC-UV-MS.

**Bioanalysis**

The analysis of radioactivity was performed by Xceleron Inc. (Germantown, MD, USA). Urine and freeze-dried feces samples were analyzed for carbon content using a Perkin Elmer 24000 Series 2 C, H, N analyzer. The carbon content of each sample was calculated using urea calibration standards. The \([^{14}\text{C}]:[^{12}\text{C}]\) ratio was measured in urine and freeze-dried feces by AMS and the concentration of radioactivity in each sample was calculated.

Plasma samples were analyzed by Covance Laboratories Ltd. (Harrogate, North Yorkshire, UK) for osimertinib, AZ5104, and AZ7550, simultaneously after protein precipitation using acetonitrile. The lower limit of quantification was 0.05, 0.0515, and 0.0515 nM for osimertinib, AZ5104, and AZ7550, respectively. Accuracy for osimertinib ranged from 101 to 103.3% and precision (relative standard deviation) ranged from 3.4 to 7.2. Similar method performance was observed for the two metabolites.

**Results**

**Protein binding experiments**

The stability of osimertinib was assessed from 0.1 to 100 µM in plasma incubations of up to 6 hours at 37°C to determine whether equilibrium dialysis was a suitable technique. However, the extraction efficiency of osimertinib was particularly low in human plasma and
human serum albumin (<1% and <15% respectively, Table 2) compared with dog and α1-acid glycoprotein (both >80%) or in mouse and rat (15 to 43%). As a consequence of this low extraction efficiency in human plasma, it was considered that equilibrium dialysis was not a suitable technique and consequently plasma protein binding was not determined in any species.

Ultrafiltration was considered as an alternative technique requiring a shorter incubation. However, experiments (data not shown) indicated that the non-specific binding to the ultrafiltration collection cups was too high to use this technique.

As a consequence, the plasma protein binding of osimertinib has not been determined. In the absence of a measured protein binding for AZD9291 and AZ7550, a computational approach was used to estimate binding values in human plasma. An AstraZeneca proprietary machine learning algorithm (Wood et al., 2011) for predicting the extent of protein binding in human plasma estimates the log K_{B/F} (Human) of osimertinib to be 1.95 or 99% bound. Similarly, for AZ5104 and AZ7550, estimates of human plasma protein binding were 98% for both. While this methodology is un-validated, the learning algorithm has been trained on a large and diverse set of in-house and commercial compounds (currently n >50,000, and covers a wide dynamic LogD range of -1.5 to 4.5).

**Covalent binding studies**

After incubation of [³H]-osimertinib with human hepatocytes, the amount of material bound to hepatic proteins at 0 and 4 hours was 16.7 and 693 pmol eq/mg protein, respectively. After incubation of [³H]-osimertinib with rat hepatocytes, the amount of material bound to hepatic proteins at 0 and 4 hours was 10.9 and 228 pmol eq/mg protein, respectively. The turnover of [³H]-osimertinib during human and rat hepatocyte incubations was estimated to be 15 and 71%, respectively, resulting in a fractional covalent binding of 0.29 and 0.02, respectively.
Similar levels of covalent binding were measured in rat and human plasma and in human serum albumin. For all samples, the covalent binding measured at the initial time point was around 1 pmol eq/mg protein or less. The extent of covalent binding increased linearly over time in all matrices to 159, 196, and 158 pmol eq/mg protein in rat plasma, human plasma, and human serum albumin, respectively, after a 6-hour incubation (after accounting for binding at the initial time point). There was no evidence that osimertinib covalent binding was reversible.

Rat QWBA

Following oral administration of [14C]-osimertinib to male partially pigmented animals, drug-related material was rapidly absorbed and widely distributed at the early sampling time points, with the highest concentrations of radioactivity measured at 6 hours postdose in most tissues. Radioactivity was detectable in 42% of tissues 60 days after dosing with the terminal half-life of radioactivity greater than 100 hours in most tissues [data not shown]. The pattern of distribution in male albino animals was similar to that observed in male partially pigmented rats at comparable time points, with the exception of melanin-containing tissues. Specifically, levels of radioactivity in the uvea plus retinal pigment epithelium of albino animals were many-fold lower than those in partially pigmented animals.

Peak radioactivity concentrations in both male and female albino rats occurred primarily at 6 hours postdose. The distribution in female albino rats was very similar to that in male albino rats, although the concentration of radioactivity in the circulating blood was generally higher in female than in male rats at corresponding time points. Concentrations of radioactivity in the blood of animals from each dose group were lower (at early time points) than in most tissues; this was indicated by a substantial number of tissue:blood concentration ratios that were above unity (Table 1). At later time points, the tissue:blood ratio was below one, which may reflect differential irreversible binding preferential in blood/plasma compared to tissues. The radioactive half-life in rat blood was approximately 200 hours, which is consistent with
the half-life of erythrocytes. The brain contained a tissue:blood radioactivity ratio greater than one up to six hours postdose, resulting in a brain to blood AUC ratio (0–504 hours) of approximately 0.3 after this single oral dose. Radioactivity was observed in the bile ducts up to and including 7 days after dose administration, and was also observed in the renal pyramid and urine. There was evidence of a possible minor sex-related difference in the distribution of osimertinib, with tissues of female animals generally containing slightly higher levels than tissues in male animals.

**Metabolism in recombinant expressed CYP isozymes**

Osimertinib, AZ5104, and AZ7550 were predominantly metabolized by CYP3A4/5 (54.0, 95.1, and 99.9%, respectively, Table 3). The extent of formation of AZ5104 and AZ7550 following incubation of osimertinib in recombinant expressed CYP isozymes indicated that both were primarily formed by CYP3A4 and CYP3A5 (Supplemental Table 1). The metabolic products from these incubations have not been determined.

**Metabolism and metabolite formation in mouse, rat, dog, and human hepatocytes**

Across all species, 16 different metabolites were identified. Seven metabolites were detected in human hepatocytes; these were also detected in either rat or dog hepatocytes at similar or higher levels. The metabolic profile in mouse was similar to that in rat. Two metabolites were detected above the limit of quantification by UV in human hepatocytes (Table 4). A metabolic scheme summarizing the metabolites observed is shown in Figure 1.

The rate of turnover of osimertinib in human hepatocytes was insufficient (<2 µL/min/M cells) for accurate assessment of the impact of CYP or FMO inhibition on osimertinib turnover in vitro, although it was possible to assess the impact of inhibitors on the formation of metabolites AZ5104 and AZ7550. After incubation of osimertinib with human hepatocytes, the mean rate of formation of AZ5104 was 0.044 pmol/min/million cells. The mean rate of formation did not decrease when inhibitors for CYP2C9, CYP2C19, CYP2C8, and FMO were
included in the incubation. Inclusion of the CYP3A4/5 inhibitor ketoconazole decreased the mean rate of formation of AZ5104 to 0.028 pmol/min/million cells and inclusion of the pan P450 inhibitor 1-aminobenzotriazole decreased the mean rate of formation to 0.013 pmol/min/million cells. Similar results were observed for the formation of AZ7550 (Supplemental Table 2).

Absorption, distribution, metabolism, and excretion of osimertinib in healthy volunteers

Eight healthy male subjects with a mean age of 42 years and a mean body mass index of 26.9 kg/m² were included in the study. Overall, five of the volunteers were White and three were Black or African American.

Pharmacokinetics

Pharmacokinetic parameters for plasma osimertinib, AZ5104, AZ7550, and plasma and whole blood radioactivity are summarized in Table 5. Following a single oral dose of 20 mg [14C]-osimertinib, the median time to maximum plasma concentration (tmax) for osimertinib and AZ5104 was 6.00 hours, while tmax for AZ7550 was 36 hours. In contrast, the median tmax for radioactivity in plasma was 144 hours and in blood was 132 hours. Plasma osimertinib, AZ5104, and AZ7550 accounted for only 0.8, 0.08, and 0.07%, respectively, of total plasma radioactivity based on geometric mean AUC ratios. The geometric mean AUC ratio for whole blood total radioactivity to plasma total radioactivity was near unity (0.917). The mean plasma terminal half-life for osimertinib was 61.2 hours, compared with a mean plasma terminal half-life of 474 hours in plasma and 562 hours in blood. Mean apparent total clearance and apparent volume of distribution for osimertinib was 26.7 L/h and 2260 L, respectively, compared with 0.213 L/h and 146 L, respectively, for total plasma radioactivity.
Mass balance

The mean cumulative total amount of radioactivity recovered in urine, feces, and both combined in nmolar equivalents as well as the percentage of total radioactive dose administered are summarized in Supplemental Table 3. In addition, the arithmetic mean cumulative dose recovered over time in urine, feces, and in total is shown in Figure 2. By the end of the study (84 days postdose), arithmetic mean total cumulative recovery (urine and feces combined) as a percentage of dose was 81.9% (range 77.2–89.8%). On average, approximately 47% of the dose was recovered by 7 days postdose with 68.9% recovered by the end of the residential period (21 days). Approximately 12% of the dose was recovered in feces by 3 days. Mean recovery of total radioactivity was 14.2% of the dose (range 10.5–17.1%) in urine and 67.8% of the dose (range 62.9–74.4%) in feces.

Geometric mean plasma osimertinib and total plasma radioactivity concentration–time profiles are displayed graphically in Figure 3. Total radioactivity concentrations in plasma and whole blood were similar throughout the time course; the ratio of whole blood to plasma total radioactivity ranged from 0.720 to 1.30 over 83 days.

Based on the molar equivalent, approximately 1.7% of the dose was excreted in urine as osimertinib, AZ5104, and AZ7550 (Supplemental Table 4). Consequently, renal clearance of osimertinib was low with mean (± standard deviation) renal clearance of 0.235 ± 0.116 L/h (Supplemental Table 4).

Metabolites

The extraction procedure from feces was optimized for both the solvent extraction and reconstitution solvents to ensure the maximum recovery of radioactivity and minimization of peak broadening on the HPLC column. The use of sequential acetonitrile, acetonitrile/water and water washes ensured that greater than 98% of [14C]-osimertinib and metabolites AZ5104 and AZ7550 spiked into control fecal homogenate could be recovered using the
extraction procedure. Indeed for these components around 90% was extracted in the acetonitrile fraction. The optimum reconstitution solvent was found to be acetonitrile/water (25/75 v/v) where 98% of radioactivity from the extracts was recovered and peak shape remained sharp. With a reconstitution solvent containing only 5% acetonitrile, recoveries were typically around 50%. In addition, the column recovery for radioactivity from dosed sample extracts was 100%. Consequently, the optimized extraction procedure was able to quantitatively recover osimertinib and the two metabolite standards (representative of the multiple metabolites identified from in vitro metabolism studies) and therefore was considered suitable for the extraction of fecal samples from osimertinib dosed human volunteers.

Metabolite identification investigations concentrated on samples pooled for the first seven days, where approximately 47% of the dose was excreted (40% in feces and 7% in urine). Only a further approximately 22% was excreted in samples from Day 7 to 21, the end of the residential period. Consequently, the fecal samples from 0-24 h (<1% excreted) and 7 to 21 days were not included in the pool as these would have significantly diluted the detectable material and compromised the ability to determine metabolic transformations. In addition, the extraction efficiency for samples from different time pools (Days 1-7, 7-14 and 14-21) was determined to be similar at around 65% in the HPLC-AMS fraction, with only an additional 3% extracted in the water fraction; this was not included in the HPLC-AMS fraction as it would have reduced sensitivity for metabolite identification. Consequently, the extraction efficiency was lower from dosed volunteer samples (~70%) than from fecal homogenates spiked with osimertinib and metabolites (~100%) which, considering the irreversible binding nature of the molecules, strongly suggests that in dosed samples around 30% of the radioactivity is unextracted due to being covalently bound to proteinaceous material which is a clearance mechanism in its own right. As the extraction efficiency was similar for each of the time window samples, this suggests that there was no significant difference in the extent of irreversible binding of fecal material between different time-pooled fecal samples and that
the metabolic profile may be similar across these time periods. Consequently further work
concentrated on analyzing the 24–168 hour fecal sample where 40% of the dose was
excreted as this maximized the metabolite concentrations and minimized dilution effects of
including later time points samples.

In this 24–168 hour pooled and concentrated fecal sample, less than 70% of the radioactivity
was extracted (representing around only 100 dpm of radioactivity) with the remainder
associated with the fecal pellet, most likely covalently bound to proteinaceous material.
Therefore, as 40% of the radioactive dose was in this sample and around 35% unextractable
due to covalent binding, this left 26% (40 x 65%) of the dosed radioactivity in the extract and
available for metabolite profiling and identification studies. In this 24-168 h fecal extract,
eight osimertinib related products were identified (Table 6, Figure 4, Figure 5), with AZ5104
being most abundant (5.6% of dose). In total, 14% of the administered radioactive dose
could be accounted for as specific metabolites in this pooled fecal sample extract containing
26% of the extractable dosed radioactivity. Therefore, we have been able to identify 55%
(14/26%) of the extractable metabolites in this sample with the remaining extracted
radioactivity associated with minor components each <1% of the total dose in the AMS
fractions (Figure 5). In the 0–168 hour urine sample, five osimertinib-related products were
identified (Table 6, Figure 4). The major product was M25 (1.9% of dose, structure
unknown), the remaining products each accounted for <1% of the total administered dose.
The total metabolites identified in the pooled urine sample (4.4%) represent approximately
63% of the radioactivity in the sample, with the remaining minor components unidentified in
the radiochromatogram.

Therefore, in both fecal and urinary extracts from samples taken up to 7 days post-dose,
where approximately 47% of the dose was excreted, metabolites could be identified to
account for a total of 18% of the radioactive dose administered, which represents 38% of the
total radioactivity and 55% of the extractable radioactivity in these samples. The remaining
radioactivity was associated with either minor identified metabolites each representing <1% of dosed material (~30%) or covalently bound to proteinaceous material (~30%).

The plasma metabolite profile from the human ADME study was not investigated as the extraction efficiency in the Day 1 to 7 pooled sample was too low (8%) to adequately determine metabolites at a concentration of 10% of osimertinib. In total, this plasma pool contained only 9.5 dpm/g, with 0.65 dpm/g extracted. Instead, a plasma metabolic profile was determined and metabolites identified from a pooled patient steady-state sample where metabolite concentrations are higher because accumulation has occurred (Planchard et al., 2016). However, the low plasma extraction efficiency from in vivo and in vitro samples, coupled with the demonstrable covalent binding to plasma proteins suggest that the majority of radioactivity in plasma from humans dosed with osimertinib is, indeed, irreversibly bound to plasma proteins, most likely serum albumin.

Circulating metabolites in patients at steady state

Seven circulatory metabolites were characterized from patient plasma following 22 days of continuous dosing with osimertinib 80 mg, where the two most abundant metabolites were AZ5104 and AZ7550, observed at 8 and 6% of total (Table 6; Figures 6 and 7). No metabolite was determined to be >10% of the total circulating osimertinib-related material. Proposed excretory and circulatory metabolite schemes are displayed in Figures 4 and 6, respectively.
Discussion

This manuscript describes preclinical and clinical studies undertaken to determine the metabolism and pharmacokinetics of osimertinib and its metabolites. These results help us understand the absorption, distribution, metabolism, and excretion (ADME) for osimertinib for the treatment of patients with advanced EGFR-mutant NSCLC who have progressed following prior therapy with an EGFR-TKI.

The covalent binding of osimertinib to EGFR via the cysteine residue 797 was a deliberate compound design goal (Finlay et al., 2014). Osimertinib reactivity against the target protein was optimized versus less selective binding to cysteine residues on other proteins whilst still maintaining nanomolar cellular potency against the target protein (Finlay et al., 2014). Studies were undertaken to determine the covalent binding of osimertinib and/or its metabolites to various biological matrices. Covalent binding was observed in incubations containing [³H]-osimertinib and cryopreserved human and rat hepatocytes and in incubations containing [¹⁴C]-osimertinib and rat and human plasma, and human serum albumin (with similar levels of covalent binding measured in human plasma, rat plasma, and human serum albumin). In addition, the radioactive extraction efficiency was much lower from human plasma (8%) than from feces (~70%), which suggests that covalent binding of osimertinib-related material to plasma proteins is far more extensive than to excreted proteins.

A clinical ADME study has also been reported for afatinib (Stopfer et al., 2012) another covalent binding EGFR-TKI. Similar to the data reported here, [¹⁴C]-afatinib was excreted predominately in feces (85.4% of dose) with urinary elimination a minor route. In contrast to osimertinib and unusually for a TKI, afatinib was excreted predominantly as the intact parent molecule. The minor metabolism of afatinib observed in excreta was mainly through conjugation of the Michael acceptor to give glutathione, cysteine-glycine or cysteine adducts in addition to N-oxidation of the dimethyl amino functionality. In plasma, afatinib accounted for the majority of the circulating drug related material (72.9% over 24 hours) although this
proportion decreased with time as the half-life of plasma radioactivity (118 hours) was substantially longer than that of afatinib (33.9 hours) due to afatinib components covalently binding to albumin. Unlike osimertinib, afatinib covalent binding to human serum albumin has been reported to be reversible (CHMP, 2013).

Shibata and Chiba have investigated the correlation between clearance predicted from hepatocyte incubations and total body clearance for three covalent binding TKIs (afatinib, ibrutinib, and neratinib). The authors noted a disconnect between in vivo and in vitro clearance attributed to extra-hepatic (covalent) conjugation to glutathione not captured in the hepatocyte system (Shibata and Chiba, 2015). For osimertinib, the in vitro studies indicated that direct conjugation with glutathione would be a minor metabolic pathway in humans. However, it is clear from the human ADME study that a high proportion of [14C]-osimertinib related material is excreted bound to proteinaceous material contributing a substantial fraction of overall clearance. As such, this suggests that preclinical hepatocyte and recombinant CYP studies may overestimate the contribution of cytochrome P450 metabolism to the clearance of osimertinib in the clinic. Hence, osimertinib, and other irreversible TKIs eliminated through non-specific covalent binding, may be less susceptible to cytochrome P450 mediated drug–drug interactions than predicted from hepatocyte experiments.

The metabolism of osimertinib in mouse, rat, dog, and human hepatocytes was primarily to oxidative and dealkylated products with direct conjugation to a range of glutathione (GSH), cysteine-glycine, glucuronide, and sulfate conjugates. All metabolites that formed in human hepatocytes were also seen in incubations with rat or dog hepatocytes. Direct conjugation with GSH and cysteine-glycine in human hepatocyte incubations indicates potential elimination pathways other than CYP. Experiments on osimertinib analogues suggest that GSH conjugates are formed chemically and not catalyzed by glutathione-S-transferase (AZ data on file). GSH conjugates represented a small fraction of the identified metabolites in the
human ADME study, which may reflect that osimertinib has higher affinity for protein nucleophiles than GSH.

Important information on the metabolic pathways and drug-metabolizing CYP enzymes involved in the metabolic clearance of osimertinib was gathered in a series of in vitro reaction phenotyping studies. While covalent binding may contribute to the in vitro turnover, the reduction in formation rate of AZ5104 and AZ7550 in hepatocyte incubations with a CYP3A inhibitor and the formation of AZ5104 and AZ7550 by CYP3A isoforms suggests that the turnover observed in the recombinant CYP enzyme experiments is, at least in part, due to oxidative metabolism. From these studies, and considering the major metabolites in circulation and excreted, it was concluded that CYP3A4/5 were the principal CYP enzymes responsible for metabolism of osimertinib, AZ5104, and AZ7550. While other CYPs may contribute to the metabolism of osimertinib in terms of potential drug–drug interactions, co-administration of osimertinib with a potent inhibitor or inducer of CYP3A4/5 may affect the exposure of osimertinib, AZ5104, and AZ7550. However, this would be moderated by the existence of alternative metabolic and elimination routes, including covalent binding to proteins. From the human ADME study, it would appear that around 30% of the dose is excreted in feces bound to proteinaceous material, which is considered a major eliminatory pathway.

Data from the rat QWBA study provided useful information regarding the tissue distribution of osimertinib and its metabolites, and informed the micro-radiolabeled dose and sample collection design of the ADME study undertaken in healthy volunteers. Penetration of osimertinib drug-related radioactivity across the blood–brain barrier occurred, and may provide therapeutic advantage in clinical indications where brain metastases are present (Ahn et al., 2015). The potential of osimertinib in the treatment of leptomeningeal disease is being investigated in a phase I study in patients with leptomeningeal disease (BLOOM, NCT02228369) (Lee et al., 2015). Radioactivity was observed in the bile ducts up to and including 7 days after dose administration, suggesting that drug-related material was
secreted in the bile and probably contributed to the overall fecal elimination. Additionally, radioactivity in the renal pyramid and urine suggests that renal elimination was a probable route of excretion. This was confirmed in the ADME study where the mean total osimertinib radioactivity in healthy volunteers was 14.2% of the dose in urine and 67.8% of the dose in feces at 84 days postdose. Of the excreted radioactivity, osimertinib constituted approximately 1.2% in feces and 0.71% in urine (in samples up to 7 days) indicating that the majority of the elimination was via metabolism (either enzymatic or covalent binding to proteins).

Data from the ADME study in healthy volunteers provided additional information about the distribution and excretion of osimertinib and its metabolites. On average, only approximately 12% of the dose was recovered in feces by -3 days postdose; this is much longer than gastrointestinal transit, suggesting that osimertinib was well absorbed (at least 88%) and the radioactivity subsequently found in feces was not a consequence of poor drug absorption, but was related to excretion following drug absorption. The geometric mean AUC ratio for whole blood to plasma total radioactivity ratio was near unity, suggesting that osimertinib and its metabolites were distributed in whole blood and plasma equally. The mean terminal half-life for osimertinib, AZ5104, and AZ7550 was 61.2, 55.2, and 82.0 hours, respectively; this was compared with 474 hours for total plasma radioactivity. The long radioactive half-life is not unexpected based on the findings of the rat QWBA study, which also showed prolonged retention of osimertinib-related material. The prolonged elimination of total plasma radioactivity was likely because osimertinib binds covalently to human plasma components, as demonstrated in the studies investigating covalent binding of osimertinib and/or its metabolites to human hepatic proteins, human plasma, and human serum albumin. Indeed, covalently bound radioactivity was likely a major component of plasma, as demonstrated by osimertinib, AZ5104, and AZ7550 accounting for 0.8, 0.08, and 0.07% of total plasma radioactivity, respectively, based on geometric mean AUC ratios. This would explain the low percentage of radioactivity that could be extracted from plasma samples (8%) in contrast to
feces (~70%). Taken together, these findings strongly suggest that the difference in AUC between radioactivity and osimertinib, AZ5104, and AZ7550 is due to osimertinib-related material bound to plasma proteins especially albumin. There are no findings in the clinical or preclinical studies to date to indicate that covalent binding of osimertinib and/or its metabolites to proteins is associated with any toxicological sequelae, such as idiosyncratic liver or immune-mediated toxicity (Zhou et al., 2005; Park et al., 2005). In humans, the most abundant circulating metabolites observed in steady-state plasma extracts from patients dosed with non-radiolabeled osimertinib at 80 mg were AZ5104 (N-demethylation on the indole) and AZ7550 (N-demethylation on the dimethyl amine), representing 8 and 6% of total drug-related material, respectively. No metabolite was more than 10% of total circulating osimertinib-related material. This is consistent with the most abundant metabolites identified in human excreta samples and mouse, rat, dog and human hepatocyte preparations. The series of studies described here provide important information on the metabolism and pharmacokinetics of osimertinib and its metabolites, AZ5104 and AZ7550, in laboratory animals, healthy volunteers, and in patients with NSCLC. Further clinical development of osimertinib is ongoing.
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Contributed new reagents or analytical tools: S. Martin
Performed data analysis: P. Ballard, M. Cantarini, P. Dickinson, P. Frewer, S. Martin, K. Pickup
Wrote or contributed to writing of the manuscript: P. Ballard, M. Cantarini, P. Dickinson, J. Collier, K. Pickup
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AstraZeneca Pharmaceuticals LP TAGRISSO™ (osimertinib) tablet: highlights of prescribing information. Available at [http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208065s000lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208065s000lbl.pdf), accessed on 11 December 2015.


Footnotes

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Conflicts of Interest

P. Dickinson: Shares in AstraZeneca, Director of Seda Pharma Development Services with a contract to provide services to AstraZeneca, ex-employee of AstraZeneca.

Figure legends

Figure 1. Metabolic scheme for osimertinib incubated in mouse (M), rat (R), dog (D), and human (H) hepatocytes

Figure 2. Arithmetic mean cumulative total radioactivity recoveries in urine, feces, and both combined in healthy male volunteers after a single oral dose of [\(^{14}\)C]-osimertinib 20 mg

Figure 3. Arithmetic mean concentration–time profiles of osimertinib and radioactivity in plasma in healthy male volunteers after a single oral dose of [\(^{14}\)C]-osimertinib 20 mg (semi-logarithmic scale)

Figure 4. Proposed metabolic scheme for osimertinib excreted in feces and urine up to 7 days (168 hours) in healthy male volunteers after a single oral dose of [\(^{14}\)C]-osimertinib 20 mg

Figure 5. Reconstructed AMS chromatogram of faeces extract following a single oral administration of [\(^{14}\)C]-osimertinib to healthy male volunteers (20 mg, 36.1KBq) indicating metabolites identified.

Figure 6. Proposed metabolic scheme for osimertinib in plasma in patients with non-small cell lung cancer following oral administration of multiple doses of osimertinib (80 mg/day, day 22)

Figure 7. HPLC-UV (310-350 nm) chromatogram of the AUC\(_{(0-24h)}\) human plasma pooled extract following oral dosing of osimertinib (80 mg multiple dose, Day 22, patients with advanced non-small cell lung cancer) indicating metabolites identified
Table 1. Tissue:blood ratios of radioactivity in selected tissues following oral administration of [14C]-osimertinib (4 mg/kg) to male partially pigmented rats and male and female albino rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Male partially pigmented rats</th>
<th>Male albino rats</th>
<th>Female albino rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5  1  6  24  2  7  21  60  1  6  24</td>
<td>1  6  24</td>
<td>1  6  24</td>
</tr>
<tr>
<td></td>
<td>hou hou hou day day da ho hou hou hou hou</td>
<td>rs ur rs rs ss ss ss ss</td>
<td>rs ur rs ur rs rs</td>
</tr>
<tr>
<td>Bile ducts</td>
<td>63.4 28.7 2.63</td>
<td>0.3 NC NC</td>
<td>66. 27.4 2.99 0.3</td>
</tr>
<tr>
<td>Blood</td>
<td>1.00 1.00 NC</td>
<td>1.0 NC NC</td>
<td>1.00 1.00 NC</td>
</tr>
<tr>
<td>Brain</td>
<td>1.06 1.85 0.37</td>
<td>0.1 0.2 0.2</td>
<td>1.4 NC NC</td>
</tr>
<tr>
<td>Liver</td>
<td>32.6 13.3 3.23</td>
<td>2.1 1.0 0.4</td>
<td>29. 17.3 4.47 0.3</td>
</tr>
<tr>
<td>Lung</td>
<td>14.0 20.4 3.11</td>
<td>1.2 0.8 0.8</td>
<td>25. 33.9 3.21 0.2</td>
</tr>
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<td>Muscle</td>
<td>0.90 2.01 0.46</td>
<td>0.3 0.4 0.2</td>
<td>1.9 NC NC</td>
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<tr>
<td>Ovary</td>
<td>ND ND ND ND ND ND ND ND ND ND ND ND ND</td>
<td>ND ND ND ND ND ND ND ND ND ND ND ND ND</td>
<td>ND ND ND ND ND ND ND ND ND ND ND ND ND</td>
</tr>
<tr>
<td>Pituitary</td>
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<td>2.3 1.2 1.0</td>
<td>14. 32.9 14.3 0.3</td>
</tr>
<tr>
<td></td>
<td>Male partially pigmented rats</td>
<td>Male albino rats</td>
<td>Female albino rats</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------</td>
<td>-----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>hou</td>
<td>ho</td>
<td>hou</td>
</tr>
<tr>
<td></td>
<td>rs</td>
<td>ur</td>
<td>rs</td>
</tr>
<tr>
<td>Renal cortex</td>
<td>11.7</td>
<td>15.</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
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<td>Renal medulla</td>
<td>3.82</td>
<td>9.3</td>
<td>11.5</td>
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<td>0</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Skin (non-pigmented)</td>
<td>0.64</td>
<td>1.4</td>
<td>0.57</td>
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<tr>
<td>Skin (pigmented)</td>
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<td>2.87</td>
</tr>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Spleen</td>
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<td>4</td>
<td>22.7</td>
</tr>
<tr>
<td>Testis</td>
<td>0.44</td>
<td>0.7</td>
<td>2.47</td>
</tr>
<tr>
<td>Thyroid</td>
<td>13.9</td>
<td>12.</td>
<td>10.8</td>
</tr>
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<table>
<thead>
<tr>
<th>Male partially pigmented rats</th>
<th>Male albino rats</th>
<th>Female albino rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 hou rs 1 ur 6 rs 24 rs 24</td>
<td>1 hou 7 hou 6 day 21 day 60 da 1 hou 6 hou 24 da 24</td>
<td>1 hou 6 hou 24 da 1 hou 6 hou 24 da</td>
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<td>Uvea and RPE 7.62 0 15. 97.2 173 151 180 NC 3.3 4 3.72 1.06 4 1.39 0.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NC, not calculable; ND, not done; RPE, retinal pigment epithelium*
Table 2. Mean (n=3) osimertinib remaining after incubation at 37°C for 6h in mouse, rat, dog and human plasma, human serum albumin (HSA) solution and human α1-acid glycoprotein solution (AGP)

<table>
<thead>
<tr>
<th>Osimertinib concentration (μM)</th>
<th>Osimertinib remaining after 6h (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse</td>
<td>Rat</td>
<td>Dog</td>
<td>Human</td>
<td>HSA</td>
<td>AGP</td>
</tr>
<tr>
<td>0.1</td>
<td>15</td>
<td>36</td>
<td>90</td>
<td>&lt;1</td>
<td>11</td>
<td>101</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>43</td>
<td>88</td>
<td>&lt;0.1</td>
<td>11</td>
<td>108</td>
</tr>
<tr>
<td>10</td>
<td>43</td>
<td>34</td>
<td>83</td>
<td>&lt;0.1</td>
<td>9.7</td>
<td>105</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>32</td>
<td>98</td>
<td>0.09</td>
<td>14</td>
<td>104</td>
</tr>
</tbody>
</table>
Table 3. Percentage of metabolism\(^a\) of osimertinib, AZ5104 and AZ7750 through recombinant expressed CYP isoforms

<table>
<thead>
<tr>
<th>CYP isoform</th>
<th>Osimertinib</th>
<th>AZ5104</th>
<th>AZ7750</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>12.0</td>
<td>0.373</td>
<td>ND</td>
</tr>
<tr>
<td>2A6</td>
<td>15.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2B6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2C8</td>
<td>ND</td>
<td>3.19</td>
<td>0.129</td>
</tr>
<tr>
<td>2C9</td>
<td>15.5</td>
<td>0.160</td>
<td>ND</td>
</tr>
<tr>
<td>2C19</td>
<td>ND</td>
<td>0.958</td>
<td>ND</td>
</tr>
<tr>
<td>2D6</td>
<td>ND</td>
<td>0.285</td>
<td>ND</td>
</tr>
<tr>
<td>2E1</td>
<td>3.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3A4</td>
<td>44.4</td>
<td>67.0</td>
<td>99.9</td>
</tr>
<tr>
<td>3A5</td>
<td>9.6</td>
<td>28.1</td>
<td>ND</td>
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</table>

\(^a\)relative contribution for the ten displayed CYP isoforms only

ND, not detected
Table 4. Semi-quantification by ultraviolet (UV) spectroscopy of osimertinib metabolites in mouse, rat, dog, and human hepatocytes

<table>
<thead>
<tr>
<th>Peak ID</th>
<th>Proposed structure</th>
<th>Proportion (%) of UV chromatogram (320–330 nm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
</tr>
<tr>
<td>M1</td>
<td>Oxidation (+O)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M2</td>
<td>Dealkylation (-C₄H₉N)</td>
<td>ND</td>
</tr>
<tr>
<td>AZ7550</td>
<td>Demethylation (-CH₂)</td>
<td>1–10</td>
</tr>
<tr>
<td>M4</td>
<td>Oxidation (+O)</td>
<td>1–10</td>
</tr>
<tr>
<td>M5</td>
<td>Oxidation (+O₂)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>AZ5104</td>
<td>Demethylation (-CH₃)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M7</td>
<td>Oxidation (+O)</td>
<td>ND</td>
</tr>
<tr>
<td>M8</td>
<td>Cysteine-glycine adduct</td>
<td>ND</td>
</tr>
<tr>
<td>M9</td>
<td>Demethylation (-CH₂) + oxidation (+O)</td>
<td>1–10</td>
</tr>
<tr>
<td>M10</td>
<td>Glutathione adduct</td>
<td>1–10</td>
</tr>
<tr>
<td>M11</td>
<td>Acetylation or deamination + glutathione</td>
<td>1–10</td>
</tr>
<tr>
<td>M12</td>
<td>Dealkylation (-C₄H₉N) + glutathione</td>
<td>1–10</td>
</tr>
<tr>
<td>M13</td>
<td>Oxidation (+O) + glutathione</td>
<td>1–10</td>
</tr>
<tr>
<td>M14</td>
<td>Oxidation (+O) + sulfation</td>
<td>ND</td>
</tr>
<tr>
<td>M15</td>
<td>Glutathione adduct</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M16</td>
<td>Oxidation (+O) + glucuronidation</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

% represents percentage of parent (osimertinib) UV response
ND, not detectable by mass spectrometry
Table 5. Summary of osimertinib, AZ5104, AZ7550, and plasma and whole blood radioactivity pharmacokinetic parameters in healthy male volunteers after a single oral dose of [14C]-osimertinib 20 mg

<table>
<thead>
<tr>
<th></th>
<th>Osimertinib (N=8)</th>
<th>AZ5104 (N=8)</th>
<th>AZ7550 (N=8)</th>
<th>[14C] Plasma (N=8)</th>
<th>[14C] Blood (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC, (^a) \text{nM} \cdot \text{h}</td>
<td>Geometric mean  1590</td>
<td>143</td>
<td>132</td>
<td>190,000</td>
<td>174,000</td>
</tr>
<tr>
<td></td>
<td>(geometric CV, %) (range) (N=7)</td>
<td>(29.6)</td>
<td>(92.5–170)</td>
<td>(153,000–143,000)</td>
<td>(242,000–219,000)</td>
</tr>
<tr>
<td>AUC(0–t), (^a) \text{nM} \cdot \text{h}</td>
<td>Geometric mean  1010</td>
<td>72.1</td>
<td>50.9</td>
<td>9670</td>
<td>10,700</td>
</tr>
<tr>
<td></td>
<td>(range) (N=8)</td>
<td>(632–1490)</td>
<td>(53.8–108)</td>
<td>(8060–8240)</td>
<td>(12,200–13,200)</td>
</tr>
<tr>
<td>C_{\text{max}}, \text{nM}</td>
<td>Geometric mean  29.9</td>
<td>1.41</td>
<td>0.957</td>
<td>217</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>(geometric CV, %) (range) (N=8)</td>
<td>(21.5)</td>
<td>(0.918–2.09)</td>
<td>(189–280)</td>
<td>(187–330)</td>
</tr>
<tr>
<td>T_{\text{max}}, h</td>
<td>Median (range)  6.00</td>
<td>6.00</td>
<td>36.00</td>
<td>144.00</td>
<td>132.02</td>
</tr>
<tr>
<td></td>
<td>(range) (N=8)</td>
<td>(6.00–8.12)</td>
<td>(6.00–36.00)</td>
<td>(96.00–168.00)</td>
<td>(96.00–168.07)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Median</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>(N=7)</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>-------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>$T_{lag}$, h</td>
<td>0.00 (0.00–0.00)</td>
<td>0.51 (0.50–1.00)</td>
<td>0.76 (0.00–1.50)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.25 (0.00–0.50)</td>
</tr>
<tr>
<td>$t_{1/2}$, h</td>
<td>61.2 (48.2–77.5)</td>
<td>55.2 (41.3–77.0)</td>
<td>82.0 (60.2–100)</td>
<td>474 (415–537)</td>
<td>562 (477–685)</td>
</tr>
<tr>
<td>CL/F, L/h</td>
<td>26.7 (16.1–49.9)</td>
<td>N/A</td>
<td>N/A</td>
<td>0.213 (0.165–0.262)</td>
<td>0.231 (0.183–0.280)</td>
</tr>
<tr>
<td>Vz/F, L</td>
<td>2260 (1630–3600)</td>
<td>N/A</td>
<td>N/A</td>
<td>146 (117–168)</td>
<td>191 (128–230)</td>
</tr>
<tr>
<td>MR$_{AUC}^b$</td>
<td>Geometric</td>
<td>N/A</td>
<td>0.0909 (0.0566–0.180)</td>
<td>0.0848 (0.0635–0.162)</td>
<td>N/A</td>
</tr>
<tr>
<td>MR$<em>{C</em>{max}}^b$</td>
<td>Geometric</td>
<td>N/A</td>
<td>0.0471 (0.0346–0.0937)</td>
<td>0.0319 (0.0232–0.05502)</td>
<td>N/A</td>
</tr>
<tr>
<td>R$_{AUC}^c$</td>
<td>Geometric</td>
<td>0.00826 (0.00429–0.0163)</td>
<td>0.000751 (0.000508–0.00158)</td>
<td>0.000698 (0.000566–0.00103)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*For radioactivity, AUC units are nmol/eq•h and $C_{max}$ units are nmol/eq.
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b Metabolite (AZ5104 or AZ7550) to parent (osimertinib) ratio of AUC or C\text{max}

c Ratio of AUC plasma osimertinib, AZ5104, AZ7550, or whole blood radioactivity to plasma total

AUC, area under the concentration–time curve; CL/F, apparent total clearance; C\text{max}, maximum drug concentration; CV, coefficient of variation; N/A, not applicable; t\text{\lambda z}, terminal half-life; t\text{lag}, lag time; t\text{max}, time to maximum concentration; Vz/F, apparent volume of distribution during terminal phase.
Table 6. Quantitative estimate of radioactivity of metabolites in excreta in healthy male volunteers after a single oral dose of $^{[14C]}$-osimertinib 20 mg and semi-quantification by ultraviolet (UV) light spectroscopy of circulating metabolites in AUC$_{(0-24h)}$ human plasma pool extract in patients with NSCLC following oral administration of multiple doses of osimertinib (80 mg/day, day 22).

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Modification</th>
<th>Percentage of administered dose in healthy volunteers</th>
<th>Percentage relative to total drug-related UV response in patients(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feces (pooled 24–168 hours)</td>
<td>Urine (pooled 0–168 hours)</td>
</tr>
<tr>
<td>Parent</td>
<td>Osimertinib</td>
<td>NA</td>
<td>1.2</td>
<td>0.71</td>
</tr>
<tr>
<td>M1</td>
<td>Oxidation</td>
<td>$+O$</td>
<td>ND</td>
<td>0.13</td>
</tr>
<tr>
<td>M2</td>
<td>Dealkylation</td>
<td>$-\text{C}_2\text{H}_9\text{N}$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AZ7550</td>
<td>Demethylation</td>
<td>$-\text{CH}_2$</td>
<td>2.3</td>
<td>0.44</td>
</tr>
<tr>
<td>AZ5104</td>
<td>Demethylation</td>
<td>$-\text{CH}_2$</td>
<td>5.6</td>
<td>0.96</td>
</tr>
<tr>
<td>M8</td>
<td>Cysteine-glycine adduct</td>
<td>$+\text{C}<em>6\text{H}</em>{10}\text{O}_3\text{N}_2\text{S}$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M17</td>
<td>Oxidation</td>
<td>$+O$</td>
<td>0.33</td>
<td>ND</td>
</tr>
<tr>
<td>M18</td>
<td>Oxidation and glucuronidation</td>
<td>$+\text{C}_6\text{H}_4\text{O}_7$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M19</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0.80</td>
<td>ND</td>
</tr>
<tr>
<td>Component</td>
<td>Description</td>
<td>Modification</td>
<td>Percentage of administered dose in healthy volunteers</td>
<td>Percentage relative to total drug-related UV response in patients&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>--------------</td>
<td>-----------------------------------------------------</td>
<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td>M20</td>
<td>Oxidation</td>
<td>+O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M21</td>
<td>Cysteinyll adduct</td>
<td>+C&lt;sub&gt;3&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;NS</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>M22</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M23</td>
<td>Dealkylation + oxidation</td>
<td>-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;N, +O</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>M24</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M25</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA, not applicable, ND, not detected

<sup>a</sup> Peak in accelerator mass spectrometry (AMS) profile had similar retention to M20 in feces AMS profile but no mass ion to confirm identity

<sup>b</sup> Lower limit of quantitation = 2% of osimertinib UV (310–350 nm) response

<sup>c</sup> Metabolites confirmed with authentic standard
Figure 1

M = Mouse; R = Rat; H = Human; D = Dog
Figure 2

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Figure 3

- • Osimertinib (n=8)
- ○ Total plasma radioactivity (n=8)
Figure 5

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Figure 7

UV response (μAU)

Time (min)

M18
M17
M8
AZ5104
AZ7550
M2
osimertinib
bilirubin

-860000
-862000
-864000
-866000
-868000
-870000
-872000
-874000
-876000
-878000
-880000
-882000
-884000
-886000
-888000
-890000

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