Mass Balance and Absorption, Distribution, Metabolism, and Excretion Properties of Balcinrenone following Oral Administration in Combination with Intravenous Microtracer in Healthy Subjects

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ABSTRACT

An absorption, distribution, metabolism, and excretion study was performed to determine the basic pharmacokinetic parameters, mass balance, and metabolite profiles of balcinrenone, a mineralocorticoid receptor modulator, in humans. This open-label, single-center, nonrandomized study had a two-period design. In period 1, eight healthy male subjects were dosed with a microtracer intravenous infusion of [14C]balcinrenone shortly after receiving an oral dose of unlabeled balcinrenone in a capsule. Following a 7-day washout, the same group of subjects subsequently received an oral dose of [14C]balcinrenone as a suspension in period 2. Clearance and absolute bioavailability of balcinrenone were determined to be 14.2 l/h and 52%, respectively. Renal clearance was determined to be 5.4 l/h (fu • glomerular filtration rate), indicating elimination via active tubular secretion, which was potentially mediated by P-glycoprotein 1 and/or organic anion transporter 3, according to in vitro transporter data. In total, 94.1% of the oral dose was recovered: 45.2% in the urine and 48.9% in the feces. Balcinrenone was primarily metabolized via oxidation, and in vitro data suggest that cytochrome P450 3A4 was the main enzyme responsible. Intact [14C]balcinrenone accounted for 55% of drug-related material in the plasma; four metabolites were identified, each representing <6% of the total plasma radioactivity. In conclusion, this two-period study has determined the basic pharmacokinetic parameters of balcinrenone in humans, including absolute bioavailability and disposition. No metabolites warranted further evaluation on account of their low representation, and any contribution to the pharmacodynamic response or potential drug-drug interactions was deemed negligible.

SIGNIFICANCE STATEMENT

This study provides a detailed understanding of the pharmacokinetics, disposition, and metabolism of balcinrenone following oral and microtracer intravenous administration in humans. In vitro phenotyping and transporter data granted mechanistic insights into the absorption, distribution, metabolism, and excretion properties of balcinrenone. This knowledge will guide future nonclinical and clinical studies evaluating drug-drug interactions, organ dysfunction, and safety of metabolites.

Introduction

Balcinrenone is a nonsteroidal, selective mineralocorticoid receptor (MR) modulator under development in combination with the sodium-glucose cotransporter-2 inhibitor dapagliflozin for the treatment of heart failure and comorbid chronic kidney disease. Dapagliflozin is a marketed product that has demonstrated renal and/or cardiovascular benefits in patients with type 2 diabetes mellitus (Neuen et al., 2019; Wiviott et al., 2019), heart failure (Kosiborod et al., 2017; McMurray et al., 2019), and chronic kidney disease (Heerspink et al., 2020), whereas balcinrenone is a new chemical entity. The marketed MR antagonists eplerenone and spironolactone have been shown to reduce mortality and hospitalization in patients with congestive heart failure (Pitt et al., 1999; Pitt et al., 2003; Zannad et al., 2011); however, their use is limited by the compound class-inherent risk of hyperkalemia in patients with declining kidney function (Juurlink et al., 2004; Lazich and Bakris, 2014).

Unlike other MR antagonists, balcinrenone is only a partial antagonist of MR due to its unique interaction with the receptor (Bamberg et al., 2018; Granberg et al., 2019). In preclinical studies, it has been
demonstrated that the differentiated mode of action of balcinrenone delivers organ-protective effects that can be separated from acute effects on urinary electrolyte levels (Bamberg et al., 2018). This suggests that balcinrenone has the potential to mitigate the risk of hyperkalemia in patients who are at high risk, such as those receiving treatment of heart failure and comorbid chronic kidney disease.

In phase I studies in healthy male subjects, single doses up to 1200 mg and multiple ascending doses up to 300 mg twice daily of balcinrenone in suspension have been shown to be well tolerated, with no safety concerns (Erlandsson et al., 2018; Whittaker et al., 2020). In the multiple ascending doses study, balcinrenone was rapidly absorbed, with C<sub>max</sub> reached in a median time (T<sub>max</sub>) of <1 hour and a terminal half-life of 4–10 hours; exposure [area under the curve (AUC) and C<sub>max</sub>] increased in a dose-proportional manner up to doses of 200 mg, and target engagement was confirmed by a robust dose-dependent rise in mean serum aldosterone levels while serum electrolyte levels remained stable (Whittaker et al., 2020). These observations in humans are therefore consistent with data from preclinical studies and highlight the therapeutic potential of MR blockade with balcinrenone, coupled with a low risk of hyperkalemia.

Human absorption, distribution, metabolism, and excretion (ADME) studies are central among the clinical pharmacology studies performed during drug development for a new chemical entity. They provide quantitative information about absorption, routes and rates of elimination, and circulating as well as excreted metabolites. Results from human ADME studies can provide valuable insights and help to guide further studies if these are warranted, such as studies in organ-impaired subjects, drug-drug interaction studies, and preclinical safety studies (Coppola et al., 2019). Even though balcinrenone is not being developed as a monotherapy, a separate human ADME study is still required according to the European Medicines Agency’s “guideline on clinical development of fixed combination medicinal products” (www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-development-fixed-combination-medicinal-products-revision-2_en.pdf).

In this study, the mass balance, pharmacokinetic (PK) parameters, and metabolic fate of balcinrenone were evaluated in healthy male subjects. Basic PK parameters, including absolute bioavailability, were evaluated following an intravenous microtracer of [14C]<sub>C</sub>balcinrenone administered shortly after an oral dose of unlabeled balcinrenone. In vitro phenotyping data were generated to quantify the routes of balcinrenone elimination mediated by individual cytochrome P450 (P450) enzymes, and in vivo transporter data were generated to help obtain a mechanistic understanding of the renal elimination process.

### Materials and Methods

[14C]<sub>C</sub>Balcinrenone was manufactured by Eurofins Selcia (Essen, UK) on behalf of AstraZeneca. Capsules containing pellets of balcinrenone were manufactured by AstraZeneca (Gothenburg, Sweden). Quotient Sciences (Nottingham, UK) formulated the [14C]<sub>C</sub>balcinrenone microtracer intravenous solution and the oral suspension of [14C]<sub>C</sub>balcinrenone. Synthetic standards of six balcinrenone metabolites (M2 and M5–9) were supplied by AstraZeneca (Gothenburg, Sweden).

Ultima Gold LSC-cocktail was obtained from PerkinElmer (Waltham, MA). Specific chemicals used for analysis by accelerator mass spectrometry (AMS), conducted by TNO (Metabolic Health Research, Leiden, Netherlands), included ANU sucrose-8542 with a certified 14C/12C ratio purchased from the National Institute of Standards and Technology (Gaithersburg, MD), and acetanilide and paracetamol purchased from Sigma-Aldrich (Saint Louis, MO). All other solvents and reagents were of analytical or higher grade and acquired from commercial suppliers.

### Clinical Study Design

This was an open-label, single-center, 2-period, nonrandomized study of healthy subjects conducted at Quotient Sciences between December 21, 2020, and February 4, 2021 (NCT04686591) (Fig. 1). The study adhered to the Declaration of Helsinki and was approved by the London-Surrey Borders Research Ethics Committee. Each subject provided written informed consent prior to commencing any of the study procedures.

In period 1, the absolute bioavailability of a single oral dose of balcinrenone and the PK parameters of [14C]<sub>C</sub>balcinrenone after intravenous infusion were assessed. Following an overnight fast (minimum 10 hours), subjects received a single oral dose of 100 mg balcinrenone in a capsule; 2 hours and 15 minutes later, subjects then received 100 μg [14C]<sub>C</sub>balcinrenone, containing 30.7 kBq (0.8 μCi) [14C]<sub>C</sub>balcinrenone as a continuous 15-minute intravenous infusion. The end of the infusion coincided with the expected oral T<sub>max</sub> of balcinrenone. Subjects remained resident in the clinical unit until up to 72 hours after the oral dose (up to day 4). In period 2, the ADME properties of oral [14C]<sub>C</sub>balcinrenone were assessed. Following an overnight fast (minimum 10 hours), subjects received a single oral dose of 100 mg [14C]<sub>C</sub>balcinrenone, containing 8 MBq [14C]<sub>C</sub> (216 μCi) as an oral suspension. Subjects remained resident in the clinical unit up to 168 hours postdose (up to day 8) and were discharged as a group after meeting all discharge criteria. The same subjects took part in both study periods. There was a minimum washout period of 7 days between dosing in period 1 and period 2.

### Rationale for Dose

A single oral dose of 100 mg was evaluated in the current study, which is within the dose range evaluated in early clinical development and ongoing studies (Erlandsson et al., 2018; Whittaker et al., 2020). Dose levels up to 1200 mg were evaluated during the early clinical studies of balcinrenone, with no tolerability or safety concerns noted and dose-linear pharmacokinetics observed up to 200 mg (Erlandsson et al., 2018; Whittaker et al., 2020). To achieve the primary objectives of the study, the oral radioactive dose of [14C]<sub>C</sub>balcinrenone was...
proposed to be as low as possible but still sufficient enough to allow an adequate assessment of its metabolite profile by radioactivity detection (RAD). The intravenous dose of 100 μg [14C]balcinrenone is in line with the International Council for Harmonization (ICH) M3 definition of a microdose (a dose that is ≤1/100th of the pharmacologically active dose, up to a maximum dose of 100 μg) (www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation_en.pdf). Estimates of the expected total radiation exposure following intravenous and oral doses of [14C]balcinrenone resulted in a committed effective dose equivalent of 1.55 mSv, which fell within International Commission on Radiologic Protection risk category Ib (1–10 mSv) for a radioactive dose (ICRP, 1991).

Study Population
Healthy men aged 30–60 years who were nonsmokers and had a body mass index of 18–30 kg/m² (body weight, ≥50 kg) were included. Only male subjects were eligible to participate in this study as full reproduction toxicology data in female animals were not available.

Collection of Blood Samples and Excreta
Blood and urine samples (periods 1 and 2) and fecal samples (period 2 only) were collected at regular intervals for PK, safety, and mass balance analysis from day 1 to the follow-up visit. In period 1, blood samples were collected pre-oral dose and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, 24, 30, 36, 48, 60, and 72 hours post-oral dose for analysis of balcinrenone concentrations in plasma. Blood samples were also collected at 2 (predose for intravenous infusion), 2.25, 2.5, 2.75, 2.5, 2.67, 2.83, 3, 3.5, 4, 5, 6, 8, 12, 16, 24, 30, 36, 48, 60, and 72 hours post-oral dose for the analysis of [14C]balcinrenone and total radioactivity in plasma. Urine samples were obtained pre-oral dose and 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours postdose for analyses of balcinrenone in plasma, total radioactivity in plasma and whole blood, and metabolite profiling and characterization in plasma. Urine samples were collected at admission, predose, and 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours postdose for analyses of balcinrenone, total radioactivity, and for metabolite profiling and characterization. Fecal samples were collected predose (between 24 hours prior to dosing and predose) and 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours postdose. Fecal homogenates were analyzed for total radioactivity and metabolite profiling and characterization. Samples for safety assessment were collected predose and 24 and 168 hours postdose.

Bioanalysis of Unlabeled Balcinrenone, [14C]Balcinrenone, and Total Radioactivity
Analysis of Unlabeled Balcinrenone by High-Performance Liquid Chromatography with Tandem Mass Spectrometry. Concentrations of unlabeled balcinrenone in plasma and urine were determined by Covance Laboratories Limited (Harrogate, UK) using high-performance liquid chromatography with tandem mass spectrometry as previously described (Whittaker et al., 2020).

Analysis of [14C]balcinrenone and total 14C by AMS. Following microtracer intravenous administration of [14C]balcinrenone, total 14C radioactivity in plasma was analyzed by TNO (Leiden, Netherlands) using AMS. The concentrations of [14C]balcinrenone in plasma and urine were also determined by AMS after sample fractionation using high-performance liquid chromatography. In brief, plasma samples were added into tin foil cups, dried, and combusted using an elemental analyzer (vario MICRO; Elementar, Langenselbold, Germany), and total 14C was analyzed using 1 MV multielement AMS (model 4110 Bo, High Voltage Engineering, Amersfoort, Netherlands). For the determination of [14C]balcinrenone, plasma samples were extracted using protein precipitation and the supernatant separated and concentrated under a gentle nitrogen stream. Plasma extracts and urine samples were then injected into an ultraperformance liquid chromatography (UPLC) column, and the liquid chromatography (LC) eluate of [14C]balcinrenone was fractionally collected in foil cups for AMS analysis. The LC separation of [14C]balcinrenone from the matrices was achieved using an Acquity UPLC HSS C18 1.8 μm, 2.1 mm × 150 mm Waters Corp., Milford, Massachusetts) at 45°C. Mobile phases A and B were 0.1% formic acid in water and acetonitrile, respectively. The flow rate was 0.4 ml/min, with a linear gradient from 15% B to 40% B in 17.5 minutes, followed by a ramp to 90% B in 1.4 minutes and hold-up for 4 minutes before decreasing back to 15% B at 23 minutes. A photodiode-array detector was used to monitor the balcinrenone peak. The fraction of [14C]balcinrenone eluate was collected at the retention time between 10.1 and 10.5 minutes. AMS-specific instrument setup, calibration, and data processing for 14C determination were performed as previously described (Bauman et al., 2022) and were applied to the quantification of total 14C in plasma and [14C]balcinrenone fraction in plasma and urine.

Analysis of Total Radioactivity by Liquid Scintillation Counting. Following oral administration of [14C]balcinrenone in Period 2, total 14C radioactivity in plasma, whole blood, urine, and feces was analyzed by Pharmaron (Northamptonshire, UK) using liquid scintillation counting (LSC). Scintillation cocktail was added directly to plasma and urine samples, whereas samples of whole blood were solubilized and then decolored before adding scintillation fluid. Fecal samples were homogenized, and subsamples of each homogenate were dried and combusted before the addition of scintillation cocktail and analysis by LSC. A detailed summary of the procedure used for detection of radioactivity is included in Supplemental Methods. Samples were analyzed in duplicate on a PerkinElmer Tri-Carb 3100 scintillation counter (PerkinElmer Inc., Waltham, MA), with automatic external standard quench correction to determine total 14C radioactivity. The limit of quantification using LSC was taken as twice the background dpm value for samples of the same type.

Metabolite Profiling in Plasma, Urine, and Feces
Metabolite identification and profiling (period 2 only) were conducted at AstraZeneca Gothenburg, Sweden, using LC combined with RAD for quantification and high-resolution mass spectrometry (HRMS) for structural elucidation.

Sample Preparation. Pooled plasma, urine, and fecal homogenates across seven subjects were prepared for metabolite profiling and characterization. Plasma samples were first pooled across subjects at each timepoint between 0 and 24 hours by equivolumetric mixing. Subsequently, one single pool was prepared with the volumes proportional to the sampling timespan to give one AU14C24h pool as previously described (Hamilton et al., 1981). For urine and feces, individual samples were pooled across subjects and collection intervals in proportion to the total volume or weight of the excreta collected. The obtained pools of urine120h and feces120h were profiled, which accounted for 95% and 96% of the total excreted radioactivity in urine and feces, respectively, over the entire collection period (0–168 hours).

Plasma. To 1 ml pooled human plasma, 3 ml methanol/acetonitrile (1:1, v/v) was added. The sample was vortex mixed for 2 minutes and centrifuged at 10,000g, 4°C, for 10 minutes. The supernatant was transferred to a new tube and concentrated to approximately 100 μl under a gentle nitrogen flow at room temperature. An aliquot of 100 μl of 40% acetonitrile aqueous solution was added to the residue, vortex mixed for 1 minute, and then centrifuged at 10,000g, 4°C, for 10 minutes. The supernatants were analyzed by LSC to determine the extraction efficiency. An aliquot sample extract was injected onto the LC column for LC-HRMS analysis and off-line radioactivity measurements.

Urine. To 200 μl pooled human urine, 50 μl methanol/acetonitrile (1:1, v/v) was added. The sample was vortex mixed and centrifuged at 10,000g, 4°C, for 10 minutes. The supernatant was analyzed by LSC to determine the extraction recovery, and LC-HRMS and off-line radioactivity detection for metabolite identification and profiling.

Feces. Approximately 0.3 g pooled fecal homogenates were weighed and transferred into Precellys 2-ml reinforced tubes (Bertin Corp., MD) precooled with six 3-mm diameter ceramic balls in each tube. An extraction mixture of methanol/acetonitrile (1:1, v/v) was added to sample tubes at a ratio of 1:4 of fecal homogenate weight to organic solvent volume. Samples were homogenized and extracted using a Precellys 24 homogenizer (Bertin Corp., MD), 2 × 20 seconds, 5000 rpm, with a 20-second pause between intervals. The mixtures were then centrifuged at 10,000g, 4°C, for 10 minutes. An aliquot of 1.5 ml of the supernatant was transferred to a new sample tube and concentrated to near dryness under a gentle nitrogen flow. The sample residue was reconstituted in 200 μl of 40% acetonitrile aqueous solution, vortexed for 1 minute, and then centrifuged at 10,000g, 4°C, for 10 minutes. The supernatant was analyzed by LSC and LC-HRMS analysis and off-line radioactivity measurements.
Sample Analysis by LC-HRMS-RAD

For metabolite profiling of [14C]balcinrenone, plasma, urine, and fecal sample extracts were analyzed using a Waters Acquity UPLC system coupled with a Synapt G2-Si Q-TOF mass spectrometer (Waters, UK) and off-line radioactivity measurements. The software for instrument control and data acquisition was MassLynx (version 4.1).

LC System. Reversed-phase chromatography for separation of balcinrenone and its metabolites was performed using an Acquity UPLC HSS C18 SB column (100 A, 1.8 µm, 3 mm × 150 mm; Waters, Milford, MA) at 45°C with mobile phase A: 0.1% formic acid in water and mobile phase B: acetonitrile at a flow rate of 0.5 ml/min. A stepwise gradient was used from 14% to 90% mobile phase B over 54 minutes (14%–18% B from 0 to 35 minutes, 18%–45% B from 35 to 44 minutes, 45%–90% B from 44 to 49 minutes, held at 90% B for 5 minutes, then back to 14% B in 0.1 minute). The system was equilibrated at 14% B for 5 minutes before the injection of the next sample. The LC eluent was split, with approximately 0.4 ml/min transferred for fraction collection and approximately 0.1 ml/min directed for mass spectrometry (MS) detection. The recovery of total radioactivity from the LC column during a gradient run was determined in urine, feces, and plasma samples by comparison of the radioactivity in the eluent pre- and postcolumn following sample injections.

RAD. Plasma, urine, and feces homogenate extracts were injected in triplicate using the UPLC system described above. LC fractions were collected throughout the chromatographic run (every 7.2 seconds) into 96-well plates (LumaPlate, PerkinElmer, Waltham, MA). After drying the collected samples using an EZ2 dry down plate (Pall, Port Washington, NY), they were resuspended in 100 µl of formic acid-water (1:1). 10 µl of the sample was analyzed by liquid chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) using a Waters Acquity UPLC system coupled to a Synapt G2-Si Q-TOF mass spectrometer (Waters, Milford, MA). The MS/MS data were acquired on selected metabolites to produce fragment ions, used for metabolite identification are described in Supplemental Methods. The MassLynx software (version 4.1) was used for instrument control and data acquisition. MassLynx and Metablynx were used for data analysis and processing for metabolite identification.

Metabolite Characterization. Precursor ions ([M+H]+ or [M–H]−) of balcinrenone and metabolites in LC-HRMS chromatograms were identified at retention times that agreed with RAD peaks in the corresponding radio-chromatograms. Observed HRMS full-scan precursor ions were used to propose molecular compositions, and product ion spectra (MS/MS) were used to propose tentative metabolite structures. Some of the metabolites were available as synthesized standards, which were analyzed for unambiguous metabolite identification.

Determination of Blood-Plasma Partitioning

The extent of distribution of total [14C]radioactivity into blood cells was evaluated by assessing the total radioactivity in whole blood to plasma ratio in period 2 at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, and 24 hours post-100 mg [14C]balcinrenone dose.

PK Analysis

The PK parameters for balcinrenone, [14C]balcinrenone, and total radioactivity were estimated by noncompartmental analysis methods using Phoenix WinNonlin software (v8.0, Certara USA, Inc.). Data were summarized using descriptive statistics.

In Vitro Human Plasma Protein Binding

Equilibrium dialysis was used to assess the fraction unbound in human plasma as previously described (Wernvik et al., 2020). Details of experimental procedures are provided in Supplemental Methods.

In Vitro P450 Phenotyping

The experimental procedures for in vitro P450 phenotyping were performed by incubation of balcinrenone in human recombinant cytochrome P450s and in human hepatocytes in the absence and presence of the potent and selective CYP3A4/5 inhibitor ketoconazole as previously described (Lindmark et al., 2018). Details of experimental procedures are provided in Supplemental Methods.

In Vitro Drug Transporter Studies

The potential of balcinrenone to be a substrate of human efflux transporters P-glycoprotein 1 (P-gp) and breast cancer resistance protein (BCRP) was assessed in polarized Madin-Darby canine kidney (MDCK) cells transfected with multidrug resistance 1 gene (MDR1) and in polarized Caco-2 cell monolayers, respectively. Details of experimental procedures are provided in Supplemental Methods. Efflux ratios were determined for balcinrenone in the absence and presence of reference inhibitors for P-gp and BCRP (Supplemental Methods).

An efflux ratio >2, which is reduced toward unity by at least 50% in the presence of a reference inhibitor [with an accompanying decrease in B–A apparent permeability (Papp)], indicates whether the test compound is a substrate of the transporter being investigated (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in-vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions).

The potential for balcinrenone to be a substrate of renal solute carrier (SLC) transporters, organic anion transporters (OATs) (OAT1, OAT2, OAT3), organic cation transporter 2 (OCT2), and multirad and toxin effluxion (MATE) transporters (MATE1 and MATE2-K) was evaluated in human embryonic kidney 293 (HEK293) cells transiently transfected with the drug transporter of interest. Details of experimental procedures are provided in Supplemental Methods. Up-take ratios were determined in the absence and presence of reference inhibitors of the respective transporter. An uptake ratio >2, which is reduced toward unity by at least 50% in the presence of a reference inhibitor, indicates whether the test compound is a substrate of the transporter being investigated at the given conditions (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in-vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions).

Results

Study Population

Eight healthy male subjects were enrolled, with a mean age of 41.6 years and a mean body mass index of 25.5 kg/m². All eight subjects were White (Supplemental Table 1). Eight subjects received treatment in period 1, and seven subjects received treatment in period 2 and completed the study. One subject withdrew from the study prior to period 2 due to personal reasons.

Mass Balance and Excretion

Following a single oral dose of [14C]balcinrenone, 94.1% of the radioactivity administered was recovered by the end of the sampling period (168 hours); 45.2% was recovered from the urine, and 48.9% was recovered from the feces. Within the first 24 hours postdose, 42.7% and 4.65% of the total radioactivity was recovered in the urine and feces, respectively (Fig. 2; Supplemental Table 2).

PK Results

Table 1 summarizes the PK parameters following administration of a single oral 100-mg dose of balcinrenone in a capsule, followed by a 100-µg [14C]balcinrenone intravenous infusion of 15 minutes in period 1 and a 100-µg [14C]balcinrenone oral suspension in period 2.

In period 1, the maximum concentration of [14C]balcinrenone was reached at the end of the intravenous microtracer infusion as anticipated.
After infusion was stopped, concentrations showed a rapid distribution phase followed by an elimination phase with a geometric mean half-life of 4.2 hours. Geometric mean clearance, volume of distribution at steady state, and apparent volume of distribution of [14C]balcinrenone were determined to be 14.2 l/h, 37.8 l, and 86.2 l, respectively. The geometric mean renal clearance (CLR) of [14C]balcinrenone was estimated to be 5.6 l/h, which corresponds to approximately 40% of the total clearance. The geometric mean absolute bioavailability of the balcinrenone capsule was determined to be 52%. Exposure of total 14C radioactivity was approximately twofold higher compared with exposure of [14C]balcinrenone, with geometric mean AUC(0-inf) values of 36 and 17.7 nmol/h/l, respectively.

PK profiles of balcinrenone in plasma and total 14C radioactivity in plasma and whole blood following oral administration of [14C]balcinrenone in suspension (period 2) are displayed in Fig. 3. Maximum plasma concentrations of balcinrenone in period 2 were reached 0.5–1 hours postdose, suggesting rapid absorption of balcinrenone when formulated in a suspension. Rate of absorption was somewhat slower with balcinrenone formulated in a capsule (Period 1), with maximum plasma concentrations reached 0.75–2.5 hours postdose. The overall plasma exposures (Cmax, AUC0-24h) of balcinrenone given either as an oral suspension (geometric mean of 3210 nmol/l, 9060 nmol*h/l) or capsule (geometric mean of 2160 nmol/l, 9180 nmol*h/l) were comparable. The clearance/bioavailability (F) of balcinrenone following oral administration, either in capsule formulation or in a suspension, was similar (geometric mean of 27.3 and 28.6 l/h, respectively). The geometric mean terminal plasma half-life of balcinrenone when administered in a capsule was determined to be 12.3 hours, which was longer than that observed following the intravenous dose (4.2 hours). The geometric mean CLR of balcinrenone following oral administration in capsule was similar (5.4 l/h) to CLR estimated after intravenous administration (5.6 l/h). This corresponded to approximately 20% of total oral clearance. In period 2, balcinrenone exposure (AUC0-24h) accounted for 44% of circulating plasma total radioactivity, which is somewhat reduced compared with the ratio observed after intravenous administration of [14C]balcinrenone.

### Blood-Plasma Partitioning of 14C Total Radioactivity

Geometric mean whole blood-to-plasma concentration ratios of 14C total radioactivity following the oral dose of [14C]balcinrenone in period 2 ranged from 0.431 to 1.476, with ratios <1 for all time points except at 24 hours postdose.

### Metabolite Profiling

After a single oral dose of [14C]balcinrenone suspension, metabolite profiles in the pooled plasma, urine, and fecal samples were established using LC separation and fractionation, followed by HRMS and off-line RAD analysis. The recovery of total radioactivity following sample preparation was determined to be 92% in plasma, 105% in urine, and 94% in feces. Column recovery of total radioactivity was determined as complete (plasma, 101%; urine, 96%; and feces, 99%). Radio-chromatograms and percentages of unchanged balcinrenone and metabolites expressed as percent AUCO-24h in plasma and percent of dose in excreta are shown in Fig. 4 and Table 2, respectively. In the AUCO-24h pooled plasma, unchanged balcinrenone was identified as the primary circulating species, accounting for approximately 54.8% of the AUCO-24h. The radio-chromatogram indicated the formation of four quantifiable metabolites, M1, M2, M7, and M9, accounting for

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**Table 1**

Summary of PK parameters.

<table>
<thead>
<tr>
<th>PK Parameter, Geometric Mean (% CV)*</th>
<th>100 µg [14C]balcinrenone Microtracer Infusion, Period 1</th>
<th>100 µg Balcinrenone Capsule, Period 1</th>
<th>100 µg [14C]balcinrenone Oral Suspension, Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tlag (h)</td>
<td>0.3 (0.1–0.3)</td>
<td>0.25 (0.00–0.27)</td>
<td>0.5 (0.5–1.0)</td>
</tr>
<tr>
<td>Tm1/2 (h)</td>
<td>12.5 (23.3)</td>
<td>2160 (27.7)</td>
<td>3210 (32.2)</td>
</tr>
<tr>
<td>AUC0–inf (nmol/l)</td>
<td>NC</td>
<td>9000 (24.9)</td>
<td>3820 (27.8)</td>
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<tr>
<td>AUCO–as (nmol/ml)</td>
<td>17.6 (35.5)</td>
<td>9070 (25.9)</td>
<td>2610 (32.3)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>4.22 (101.6)</td>
<td>9180 (26.9)</td>
<td>9270 (22.9)</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>5.61 (40.0)</td>
<td>9070 (25.9)</td>
<td>9280 (22.9)</td>
</tr>
<tr>
<td>CL/F (l/h)</td>
<td>14.2 (35.5)</td>
<td>9180 (26.9)</td>
<td>19,400 (17.4)</td>
</tr>
<tr>
<td>Vplasma (l)</td>
<td>NC</td>
<td>2160 (27.7)</td>
<td>9060 (24.9)</td>
</tr>
<tr>
<td>Vss (l)</td>
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<td>9070 (25.9)</td>
<td>2160 (32.3)</td>
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<tr>
<td>TV (l)</td>
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<td>9070 (25.9)</td>
<td>2160 (32.3)</td>
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<tr>
<td>MRTV (h)</td>
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<td>9070 (25.9)</td>
<td>2160 (32.3)</td>
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<tr>
<td>F (%)</td>
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<td>9070 (25.9)</td>
<td>9270 (22.9)</td>
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<tr>
<td>AUC0–inf (n)</td>
<td>0.3 (0.1–0.3)</td>
<td>0.25 (0.00–0.27)</td>
<td>0.5 (0.5–1.0)</td>
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<tr>
<td>AUCO–as (n)</td>
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<td>2160 (27.7)</td>
<td>3210 (32.2)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
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<td>9280 (22.9)</td>
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<tr>
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<tr>
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<td>9070 (25.9)</td>
<td>19,400 (17.4)</td>
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<td>9270 (22.9)</td>
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</tbody>
</table>

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*Unless specified otherwise (see Tlag).

*Whole blood PK parameters derived based on reduced sampling schedule.

*Median (range).
5.1%, 2.4%, 3.7%, and 4.4%, respectively, of the AUC0-24h. The quantified radioactive peaks altogether accounted for 70.4% of total radioactivity in the chromatogram. The remaining radioactivity attributed to a number of lesser radioactive components, each with an area of <7 cpm (i.e., below 2% of total radioactivity or to chromatographic background noise). In the 0–24-hour urine pool, unchanged balcinrenone accounted for 22% of the dose. The radio-chromatograms indicated the formation of seven major metabolite fractions in urine, of which M9 was the most abundant minor metabolite (representing 3.8% of the dose). In the 0–120-hour fecal pool, unchanged balcinrenone accounted for 6.2% of the dose, with M6 being the major drug-related material (accounting for 8.5% of the dose). Several minor peaks corresponding to <3% or 5% of the dose in urine and feces, respectively, were attributed to other minor metabolites or to chromatographic background noise.

Metabolite Identification

In total, 12 metabolites were quantified by RAD and their structures characterized based on HRMS spectra. LC retention times of the metabolites identified by HRMS were in agreement with the corresponding radio-chromatographic peaks identified by RAD. HRMS data were used to propose a molecular composition, and product ion spectra were used to propose metabolite structures. The structures and presence of balcinrenone and six metabolites (M2 and M5–9) in samples were confirmed by comparing LC retention times and MS spectra with the synthesized standards. Tentative structures of the remaining six metabolites (M1, M3, M4, M10–12) were proposed based on their fragment ions and a comparison of the product ion spectra of synthesized standards of balcinrenone and metabolite analogs. Representative product ion spectra and diagnostic fragments of balcinrenone and its metabolites are shown in Supplemental Fig. 1 and Supplemental Table 3. Proposed biotransformation pathways of balcinrenone are shown in Fig. 5.

In summary, the major metabolic pathways after oral administration in humans were via oxidation of balcinrenone, which resulted in the formation of the majority of metabolites. Several hydroxylated metabolites

Human Plasma Protein Binding

Following method optimization, the plasma protein binding of balcinrenone was determined after an 18-hour equilibrium dialysis. The percentage unbound concentrations were 17.3%, 17.6%, 18.6%, and 20.5% at concentrations of 0.1, 1, 10, and 100 μM balcinrenone, respectively.

P450 Phenotyping in Vitro

Using human recombinant P450s, CYP3A4 was shown to be the P450 contributing the most to P450-mediated metabolism of balcinrenone. The fractions of total metabolism occurring via CYP3A4, CYP2D6, and
CYP3A5 were determined to be 91%, 3%, and 6%, respectively. In all other P450 isoforms, balcinrenone was metabolically stable, and CL\text{int} could not be determined. Following incubation of balcinrenone in human hepatocytes, in the presence and absence of ketoconazole, CL\text{int} values were determined to be 0.28 and 0.021 m\text{L} \cdot \text{min}^{-1} \cdot (10^6 \text{ cells})^{-1}, respectively, and the fraction of total hepatic metabolism occurring via CYP3A4/5 was determined to be 93%.

### In Vitro Drug Transporter Studies

The potential of balcinrenone to be a substrate of human efflux transporter P-gp was evaluated in MDCK-MDR1 cell monolayers. At a balcinrenone concentration of 1 mM, efflux ratios in the absence and presence of P-gp inhibitor cyclosporin A were determined to be 52 and 1.1, respectively, suggesting that balcinrenone is a substrate of P-gp.

The potential of balcinrenone to be a substrate of human efflux transporter BCRP was evaluated in Caco-2 cell monolayers. At a balcinrenone concentration of 1 mM, efflux ratios in the absence and presence of the BCRP inhibitor fumitremorgin C were determined to be 41 and 18, respectively. The accompanying B\text{–}\text{AP app values in the absence and presence of the inhibitor were comparable, suggesting that balcinrenone is unlikely to be a substrate of BCRP.}

The potential of balcinrenone to be a substrate of human renal SLC transporters OAT1, OAT2, OAT3, OCT2, MATE1, and MATE2-K was evaluated in HEK293 cells transfected with these transporters. Data generated on uptake ratios, at a 5 mM concentration, in the absence and presence of the inhibitors for the evaluated SLC transporters, suggested that balcinrenone is a potential substrate of OAT3, an unlikely substrate of OAT2, and not substrate of OAT1, OCT2, or MATE1. While data generated at a 5 mM concentration suggest that balcinrenone is a substrate of MATE2-K, taking the results of all concentrations evaluated into consideration, it was concluded that balcinrenone is unlikely to be a substrate of MATE2-K. Efflux and uptake ratios for balcinrenone in the evaluated cell lines expressing efflux and SLC transporters, respectively, are reported in Table 3 and Supplemental Tables 4–11.

### Tolerability

Single doses of 100 mg balcinrenone and 100 \mu g i.v. [14C]balcinrenone in period 1 and 100 mg oral [14C]balcinrenone in period 2 were well tolerated by the healthy subjects enrolled in this study. All adverse effects were mild and transient.

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**Fig. 5.** Proposed metabolic pathways of [14C]balcinrenone after a single oral dose. *Designates the site of 14C labeling. Structures of M2 and M5–9 were confirmed by comparison with synthetic standards.
events were mild, and none were considered to be related to \([14C]\)balcinrenone. Clinical laboratory evaluations, vital signs, electrocardiograms, and physical examinations revealed no clinically relevant findings.

### Discussion

This study had a 2-period design. In period 1, an intravenous microtracer of \([14C]\)balcinrenone was administered shortly after a single oral dose of unlabeled balcinrenone formulated in a capsule to estimate the absolute bioavailability and basic PK parameters of balcinrenone. In period 2, \([14C]\)balcinrenone was administered as an oral suspension to characterize its ADME properties, including mass balance. This study design allowed capsule and suspension formulations of balcinrenone to be compared in the same subjects, with reduced data variability despite the small number of subjects enrolled.

After single oral administration of \([14C]\)balcinrenone, 94.1% of the administered radioactivity was recovered in excreta over a 168-hour sampling period, in line with guidelines (www.emea.europa.eu/en/documents/newsletter/news-bulletin-small-medium-sized-enterprises-issue-23_en.pdf). Similar amounts of total radioactivity were recovered between the urine and feces (45% and 49% of the dose, respectively). In urine, approximately half of the drug-related material could be assigned to intact parent compound (22% of the radioactive dose). In feces, only a minor portion of the radioactivity was assigned to balcinrenone (6% of the dose), suggesting no effect or a minor effect of transporters on the absorption of balcinrenone. Renal clearance of balcinrenone was estimated to be 5.4 l/h, which is more than \(f_u\) multiplied by glomerular filtration rate (i.e., \(0.185 \times 7.5 = 1.4\) l/h), indicating excretion not only by glomerular filtration but also by involvement of active tubular secretion. The potential for balcinrenone to be a substrate of human efflux and SLC transporters was evaluated in vitro, with the results suggesting that P-gp and/or OAT3 contribute toward active renal elimination of balcinrenone. In two previous studies of single doses of balcinrenone and repeated twice-daily dosing in healthy subjects, renal clearance was consistent across the entire dose range evaluated (single dose, 5–1200 mg; repeated dosing, 50-300 mg bid) (Erlandsson et al., 2018; Whittaker et al., 2020). It can therefore be concluded that transporters involved in the renal clearance of balcinrenone are not saturated in this dose range. The low amounts of parent drug detected in feces could be explained by unabsorbed drug, and the fraction absorbed (\(f_{abs}\)) was estimated to be 94% following oral suspension administration. It is acknowledged that \(f_{abs}\) 94% might be somewhat underestimated because elimination via the bile cannot be ruled out. A quantitative mass balance diagram after oral administration of balcinrenone is given in Fig. 6.

The whole blood-to-plasma total radioactivity concentration ratios tended to indicate nonpreferential distribution of total radioactivity to the cellular components of whole blood. No or negligible saturation in the plasma protein binding (unbound percentage 17.3%–20.5%), in the concentration range evaluated (0.1–100 \(\mu M\)), suggests binding to plasma albumin rather than \(\alpha-1\)-acid glycoprotein. Results also suggest that plasma protein binding of balcinrenone is constant at anticipated therapeutic exposures, high nM or low \(\mu M\), provided albumin levels are normal.

To mitigate a biased disposition profile with a microdose, at exposure levels much lower than therapeutic levels, an intravenous microtracer was administered at anticipated \(C_{max}\) after an oral administration of 100 mg

### TABLE 3

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Without inhibitor</th>
<th>With inhibitor</th>
<th>Result/Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td>51.9</td>
<td>1.1</td>
<td>Is a substrate</td>
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<tr>
<td>BCRP</td>
<td>40.9</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
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<td>1.1</td>
<td>2.6</td>
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</tr>
<tr>
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<td>1.1</td>
<td>1.1</td>
<td>Not a substrate</td>
</tr>
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<td>Not a substrate</td>
</tr>
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</tr>
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<td>1.1</td>
<td>1.1</td>
<td>Not a substrate</td>
</tr>
<tr>
<td>MATE2-K</td>
<td>2.1</td>
<td>0.9</td>
<td>Likely a substrate</td>
</tr>
</tbody>
</table>

\(^{a}\) No accompanying change in \(P_{app} B\) to \(A\) when incubated in the presence of inhibitor.  
\(^{b}\) Uptake ratio at a 0.5 \(\mu M\) concentration of balcinrenone.  
\(^{c}\) Uptake ratio at a 30 \(\mu M\) concentration of balcinrenone.  
\(^{d}\) Uptake ratio at a 50 \(\mu M\) concentration of balcinrenone.

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**Fig. 6.** Balcinrenone: quantitative mass balance diagram. Accounting for 94% recovery determined in mass balance part of the study.
unlabeled balcinrenone. According to ICH guideline M3(R2), the maximum microdose allowed is 100 μg, and the selected dose must be ≤1/100th of the no observed adverse effect level (determined in nonclinical safety studies) and <1/100th of the pharmacologically active dose (www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-market-authorisation_en.pdf). These criteria were both met for the selected microtracer of [14C]balcinrenone in this study. A strength with using an intravenous microtracer rather than a high dose is that the study can be qualified by the existing oral toxicology studies, and no additional preclinical toxicology study with intravenous administration is required [ICH guideline M3(R2)]. To achieve the bioanalytical sensitivity needed, the low levels of radioactivity in plasma were determined using the ultrasensitive combustion AMS technique (van Duijn et al., 2014). The low radioactive dose (equivalent to 0.0207 millisievert) falls into the International Commission on Radiologic Protection category I, which is associated with a negligible risk to the dosed subjects (ICRP, 1991). Based on intravenous microtracer and coadministered therapeutic oral dose, the basic PK parameters of balcinrenone were estimated for the first time in humans. The short half-life of 4.2 hours, as determined after an intravenous dose, is likely to reflect the elimination half-life of balcinrenone. The longer terminal half-life of 12.3 hours, as determined following oral capsule administration, is likely affected by absorption rate limited elimination. Clearance and steady-state volume of distribution were estimated to 14.2 l/h and 37.8 l, respectively. Assuming the nonrenal clearance (8.8 l/h) is hepatic (CLh), the fraction escaping first-pass hepatic metabolism (Fh) was estimated to be 90% using a liver blood flow value of 84 l/h (Fh = 1 – CLh/84 l/h). A first-pass hepatic metabolism of only 10% is consistent with a marginal difference in AUC0-inf,total radioactivity/AUC0-inf,balcinrenone ratio after oral and intravenous administration, estimated as 2.3 and 2.0, respectively. The absolute F was determined to be 52%, and with knowledge of F and Fh, Fabs · Fgut was estimated to be 58% (F = Fabs · Fgut · Fh). Utilizing the estimated Fabs 94%, the fraction escaping gut metabolism (Fgut) was estimated to be 62%.

Metabolite profiling and identification showed that the metabolism of [14C]balcinrenone is mediated primarily by oxidative mechanisms (approximately 95%), with minor fractions metabolized via amide hydrolysis. This finding, considered alongside in vitro P450 phenotyping data, indicates that in vivo metabolism is primarily mediated by CYP3A4. Even though the fraction of balcinrenone eliminated via metabolism was estimated to be only 62% (renal 38%), the first-pass extraction in the intestine is high (fapp about 62%), which is likely due to CYP3A4 metabolism. Thus, overall, it could not be ruled out that exposure to balcinrenone would be influenced by inhibition of CYP3A4. Therefore, a clinical drug-drug interaction study with the strong CYP3A4 inhibitor irtraconazole was performed, demonstrating that balcinrenone is a moderately sensitive substrate of CYP3A4 (NCT03843060, data on file).

Following single oral administration of [14C]balcinrenone suspension, the half-life of both balcinrenone and total radioactivity plasma profiles were similar, suggesting formation rate limited kinetics of metabolites. Four metabolites (M1, M2, M7, and M9) of balcinrenone were quantified and identified in the plasma, with M1 the most abundant and accounting for 5.1% of drug-related exposure in pooled AUC0-24h plasma. In total, balcinrenone and quantified major metabolites accounted for 70.4% of the radioactivity in the pooled AUC0-24h plasma. Numerous minor metabolites (each accounting for <2% of total radioactivity) were also found in plasma; however, no detailed structure characterization was conducted. Due to the exposure of all circulating metabolites accounting for <10% of the total drug-related-exposure, with no indication of longer half-life than the parent compound, no further safety assessment of these circulating metabolites was warranted.

www.ema.europa.eu/en/documents/other/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en.pdf; www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation_en.pdf; www.fda.gov/media/72279/download. According to the US Food and Drug Administration in vitro drug-drug interaction guidelines, there is no need to evaluate if a metabolite is a substrate of enzymes or transporters if its contribution to the overall pharmacological effect is <50% (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in-vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions). Among the four circulating metabolites, three are less active than balcinrenone as determined in an MR reporter gene antagonist assay (data on file): M9 is 1.8-fold less active than balcinrenone, M2 is 7.3-fold less active than balcinrenone, and M7 is inactive. M1 is not available as a synthetic standard but is likely to be low active or inactive based on the structure/activity relationship established within this chemical series (Granberg et al., 2019). This, together with low exposure of the metabolites compared with balcinrenone, clearly indicated that their contribution to the overall pharmacological activity is negligible.

In summary, this human ADME study has provided invaluable insights on the disposition of balcinrenone. The absolute F, Fabs, fraction escaping first-pass hepatic metabolism (Fh), and fraction escaping gut metabolism (Fgut) were determined for the first time in humans. These data provided further insights into the potential risk for altered exposure of balcinrenone by inhibition of CYP3A4, both in the liver and intestine and in organ-impaired patients. There were no human circulating metabolites that warrant any further safety assessment nor exposures of active metabolites that could contribute to a meaningful effect on pharmacodynamic response identified. The low plasma exposure to metabolites suggests negligible risk of drug-drug interactions by any of the identified metabolites.

Acknowledgments

The authors thank the subjects who participated in the study, and the personnel at Pharmaron, Quotient Sciences, and Eurofins Selcia, for their contributions to the conduct of this study. The authors thank Constanze Hilgendorf who contributed with interpreting the in vitro transporter data.

Data Availability

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca’s data sharing policy described at https://astrazenecagrouptrials.pharmaccm.com/ST/Submission/Disclosure. Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli could be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/. AstraZeneca Vivli member page is also available outlining further details: https://vivli.org/o/members/member/astrazeneca/.

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Performed data analysis: Lindmark, Li, Bhattacharya, Housler, Pelay-Gimeno, Vaes, Ely Pizzato, Ericsson.
Wrote or contributed to the writing of the manuscript: Lindmark, Li, Bhattacharya, Heijer, Pelay-Gimeno, Ericsson, Johansson.

References
