Assessment of the Mass Balance Recovery and Metabolite Profile of Avibactam in Humans and In Vitro Drug-Drug Interaction Potential

Karthick Vishwanathan, Stuart Mair, Anshul Gupta, James Atherton, Jacqueline Clarkson-Jones, Timi Edeki, and Shampa Das

Quantitative Clinical Pharmacology, AstraZeneca R&D, Waltham, Massachusetts (K.V.); Drug Metabolism and Pharmacokinetics, Infection Innovative Medicines Unit, AstraZeneca R&D, Waltham, Massachusetts (A.G., J.A.); Quotient Clinical Ltd, Nottingham, United Kingdom (S.M.); Global Medicines Development, AstraZeneca, Wilmington, Delaware (T.E.); and AstraZeneca, Macclesfield, United Kingdom (J.C.-J., S.D.)

Received October 29, 2013; accepted March 7, 2014

ABSTRACT

Avibactam, a novel non-β-lactam β-lactamase inhibitor with activity against Ambler class A, class C, and some class D enzymes is being evaluated in combination with various β-lactam antibiotics to treat serious bacterial infections. The in vivo mass balance recovery and metabolite profile of [14C] avibactam (500 mg/1-h infusion) was assessed in six healthy male subjects, and a series of in vitro experiments evaluated the metabolism and drug-drug interaction potential of avibactam. In the mass balance study, measurement of plasma avibactam (using a validated liquid chromatography-tandem mass spectrometry method) and total radioactivity in plasma, whole blood, urine, and feces (using liquid scintillation counting) indicated that most of the avibactam was excreted unchanged in urine within 12 hours, with recovery complete (>97% of the administered dose) within 96 hours. Geometric mean avibactam renal clearance (158 ml/min) was greater than the product of unbound fraction of drug and glomerular filtration rate (109.5 ml/min), suggesting that active tubular secretion accounted for some renal elimination. There was no evidence of metabolism in plasma and urine, with unchanged avibactam the major component in both matrices. Avibactam demonstrated in vitro substrate potential for organic anion transporters 1 and 3 (OAT1/OAT3) inhibitors expressed in human embryonic kidney 293 cells (Km > 1000 μM; >10-fold the Cmax of a therapeutic dose), which could account for the active tubular secretion observed in vivo. Avibactam uptake by OAT1 and OAT3 was inhibited by probenecid, a potent OAT1/OAT3 inhibitor. Avibactam did not interact with various other membrane transport proteins or cytochrome P450 enzymes in vitro, suggesting it has limited propensity for drug-drug interactions involving cytochrome P450 enzymes.

Introduction

Infections caused by Gram-negative pathogens are becoming increasingly challenging to treat as a result of the global spread of multidrug resistance among many clinically important species (Peleg and Hooper, 2010) and a limited armamentarium of new agents with intrinsic Gram-negative activity (Boucher et al., 2009). A key facet of bacterial drug resistance, particularly among the Enterobacteriaceae, is the expression of β-lactamases, a large family of hydrolytic enzymes that confer reduced susceptibility to β-lactam antibiotics (Kanj and Kanafani, 2011). Extended spectrum β-lactamases inactivate extended-spectrum cephalosporins, as well as penicillins, and typically require treatment with a carbapenem or β-lactam/β-lactamase inhibitor combination. Recently, the emergence of molecular class A β-lactamases with carbapenemase activity (e.g., Klebsiella pneumoniae carbapenemase [KPC]) has been reported; treatment options for such infections are severely limited (Hirsch and Tam, 2010; Nordmann et al., 2011).

Avibactam, formerly known as NXL104, is a novel non-β-lactam inhibitor of Ambler class A and C (and some class D) β-lactamases, including KPC (Stachyra et al., 2009; Shlaes 2013; Zhan et al., 2013) (Fig. 1). Avibactam has a unique mechanism of action involving covalent, slow, reversible β-lactamase inhibition (Ehmann et al., 2012), which restores the in vitro activity of β-lactam antibiotics, including ceftazidime, ceftaroline, and aztreonam against extended-spectrum β-lactamases–producing pathogens (Livermore

**ABBREVIATIONS:** Aexp, amount of total radioactivity or parent drug eliminated; AE, adverse event; AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CYP450, cytochrome P450; ECG, electrocardiogram; HEK293, human embryonic kidney 293 cells; HPLC, high-performance liquid chromatography; IDA, interaction potential; KPC, Klebsiella pneumoniae carbapenemase; LC, liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry detection; LSC; liquid scintillation counting; MDCKII, Madin–Darby canine kidney II cells; MDR1, multidrug resistance 1 protein; MRP4, multidrug resistance-associated protein; OAT1/OAT3, organic anion transporters 1/3; OATP1B1/OATP1B3, organic anion transporting polypeptide proteins 1 and 3; OCT1/OCT2; organic cation transporters 1 and 2; PK, pharmacokinetic.
et al., 2011). Previous investigations have demonstrated that the pharmacokinetic (PK) profile of i.v. avibactam is dose-proportional up to 2000 mg (the highest dose tested), that elimination is largely by the renal route (Felices et al., 2010; Merdjan et al., 2007, 2010), and that there are no drug-drug interactions between avibactam and cefazidime (Edeki et al., 2013). In these studies, the amount of unchanged avibactam eliminated in urine varied between 80% and 100%. To fully understand and characterize the elimination pathways of avibactam and to understand the impact of covalent, reversible tissue binding, a [14C] avibactam mass balance study was undertaken in healthy human subjects.

This report describes the results of the mass balance study as well as a series of additional in vitro studies undertaken to further characterize the disposition of avibactam, including assessments of the potential of avibactam as a substrate or inhibitor of human hepatic and renal transporter proteins and cytochrome P450 enzymes.

Materials and Methods

Overall Study Design and Objectives

The mass balance recovery study (ClinicalTrials.gov identifier NCT01448395) was an open-label, single-center phase I clinical trial conducted at Quintiles Clinical, Nottingham, UK (with metabolite profiling of study samples conducted at AstraZeneca R&D, Waltham, MA). The study was performed in accordance with the Declaration of Helsinki and International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) and applicable regulatory requirements, with protocol approval by an independent ethics committee; all participants provided written informed consent. The UK Department of Health Administration of Radioactive Substances Advisory Committee provided prior approval of the chosen dose of radioactivity.

The primary objectives were to determine the mass balance and routes of [14C] avibactam metabolism and excretion after a single 500-mg i.v. dose of [14C] avibactam (this dose of avibactam is currently being investigated in combination with cefazidime in phase III trials), to estimate the whole blood and plasma partitioning of total radioactivity, and to determine the urinary and fecal recovery of radioactivity. Secondary objectives were to assess the PK of avibactam; identify and characterize avibactam metabolites in plasma, whole blood, urine, and feces; and obtain additional safety and tolerability information for avibactam.

The in vitro studies comprised a human membrane transporter study (conducted at Netherlands Organisation for Applied Scientific Research, Zeist, Netherlands), two human cytochrome inhibition studies (respectively conducted at Biopredic, Saint Grégoire, France, and GenPharmTox Biotech AG, Planegg, Germany); a human cytochrome induction study (conducted at GenPharmTox Biotech AG), and a hepatic metabolism study using microsomes from various species (conducted at Biopredic). The studies were designed with reference to industry guidelines and carried out in accordance with relevant standards of the U.S. Food and Drug Administration (www.fda.gov/downloads/Drugs/Guidance/ComplianceRegulatoryInformation/Guidances/UCM292362.pdf), the Organisation for Economic Co-Operation and Development (http://search.oecd.org/officialdocuments/publicdisplaydocumentpdf/?ote=ENV/OCHEM/98/16&Language=En), and the International Transporter Consortium (Giacomini et al., 2010).

The objective of the membrane transporter study was to evaluate the substrate and inhibitor potential of avibactam for a range of human transporter proteins expressed in cultured human and canine cells or human vesicles. The objectives of the cytochrome inhibition and induction and microsomal metabolism studies were to evaluate the possible hepatic metabolism and drug interaction potential of avibactam on hepatic drug-metabolizing enzymes.

Mass Balance Study

Subjects and Treatments. Healthy male subjects aged 30–65 years, with body mass index between 18 and 32 kg/m² and body weight between 50 and 100 kg, were eligible to participate; volunteers were required to have a clinically normal physical examination (including negative test results for drugs of abuse, alcohol, carbon monoxide breath test, hepatitis B surface antigen, antibodies to hepatitis C virus, and antibodies to HIV at the screening visit) and had to be willing to use an adequate method of contraception for 3 months from the day of dosing with the study drug. Exclusion criteria included clinically significant disease or clinically relevant abnormal findings in physical examination, vital signs, clinical chemistry, hematometry, or urinalysis that, in the investigator’s opinion, could put the volunteer at risk by participation in the study; QT interval corrected for heart rate >450 ms or QT > 500 ms or other electrocardiogram (ECG) abnormality; and history of drug or alcohol abuse or smoking. After an overnight fast of ≥10 hours, subjects received a single clinically relevant dose of [14C] avibactam (Quotient Bioresearch Ltd, Nottingham, UK) of approximately 500 μCi diluted in 100 ml of 0.9% saline by 60 minutes of i.v. infusion. The target dose of [14C] was more than 300 μCi (11.1 MBq).

Assessments. Venous blood samples for analysis of whole blood and plasma radioactivity and plasma avibactam concentrations were collected via venipuncture/indwelling catheter at predose and at 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hours postdose. Urine and feces samples for analysis of total radioactivity and avibactam were collected at predefined intervals (urine: −12–0 hours predose and 0–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, and 72–96 hours postdose; feces: −24–0 hours predose and 0–24, 24–48, 48–72, and 72–96 hours postdose). Subjects were discharged from the study unit at 96 hours, with scheduled assessments planned to continue to up to 168 hours (240 hours for feces) if required. Concentrations of avibactam in plasma and urine were measured using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, for which the limits of quantification were 10.0 ng/ml (plasma) and 500 ng/ml (urine). Whole blood, plasma, and urine total radioactivity concentrations were measured using a liquid scintillation counting (LSC) method (feces samples were first combusted and the resultant CO2 collected into scintillation fluid before counting). The limits of detection for total radioactivity were 0.028 μg equiv/ml (plasma), 0.089 μg equiv/g (whole blood), 0.012 μg equiv/g (urine), and 0.07 μg equiv/g (feces).

Safety assessments included analysis of adverse events (AEs; coded using MedDRA version 14.1), laboratory assessments of blood and urine, vital signs, ECG, and physical examinations.

Metabolite Profiling. Plasma samples obtained during the first 4 hours postdose were pooled across subjects in a time-interval proportional fashion for Economic Co-Operation and Development (http://search.oecd.org/officialdocuments/publicdisplaydocumentpdf/?ote=ENV/OCHEM/98/16&Language=En), and the International Transporter Consortium (Giacomini et al., 2010).

The objective of the membrane transporter study was to evaluate the substrate and inhibitor potential of avibactam for a range of human transporter proteins expressed in cultured human and canine cells or human vesicles. The objectives of the cytochrome inhibition and induction and microsomal metabolism studies were to evaluate the possible hepatic metabolism and drug interaction potential of avibactam on hepatic drug-metabolizing enzymes.

Mass Balance Study

Subjects and Treatments. Healthy male subjects aged 30–65 years, with body mass index between 18 and 32 kg/m² and body weight between 50 and 100 kg, were eligible to participate; volunteers were required to have a clinically normal physical examination (including negative test results for drugs of abuse, alcohol, carbon monoxide breath test, hepatitis B surface antigen, antibodies to hepatitis C virus, and antibodies to HIV at the screening visit) and had to be willing to use an adequate method of contraception for 3 months from the day of dosing with the study drug. Exclusion criteria included clinically significant disease or clinically relevant abnormal findings in physical examination, vital signs, clinical chemistry, hematometry, or urinalysis that, in the investigator’s opinion, could put the volunteer at risk by participation in the study; QT interval corrected for heart rate >450 ms or QT > 500 ms or other electrocardiogram (ECG) abnormality; and history of drug or alcohol abuse or smoking. After an overnight fast of ≥10 hours, subjects received a single clinically relevant dose of [14C] avibactam (Quotient Bioresearch Ltd, Nottingham, UK) of approximately 500 μCi diluted in 100 ml of 0.9% saline by 60 minutes of i.v. infusion. The target dose of [14C] was more than 300 μCi (11.1 MBq).

Assessments. Venous blood samples for analysis of whole blood and plasma radioactivity and plasma avibactam concentrations were collected via venipuncture/indwelling catheter at predose and at 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hours postdose. Urine and feces samples for analysis of total radioactivity and avibactam were collected at predefined intervals (urine: −12–0 hours predose and 0–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, and 72–96 hours postdose; feces: −24–0 hours predose and 0–24, 24–48, 48–72, and 72–96 hours postdose). Subjects were discharged from the study unit at 96 hours, with scheduled assessments planned to continue to up to 168 hours (240 hours for feces) if required. Concentrations of avibactam in plasma and urine were measured using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, for which the limits of quantification were 10.0 ng/ml (plasma) and 500 ng/ml (urine). Whole blood, plasma, and urine total radioactivity concentrations were measured using a liquid scintillation counting (LSC) method (feces samples were first combusted and the resultant CO2 collected into scintillation fluid before counting). The limits of detection for total radioactivity were 0.028 μg equiv/ml (plasma), 0.089 μg equiv/g (whole blood), 0.012 μg equiv/g (urine), and 0.07 μg equiv/g (feces).

Safety assessments included analysis of adverse events (AEs; coded using MedDRA version 14.1), laboratory assessments of blood and urine, vital signs, ECG, and physical examinations.

Metabolite Profiling. Plasma samples obtained during the first 4 hours postdose were pooled across subjects in a time-interval proportional fashion (Hamilton et al., 1981) such that the final pool represented the area under the curve (AUC) over the 4-hour postdose period. Pooled plasma was denatured and precipitated with a 2-fold excess volume of acetonitrile to extract drug-related material. The supernatant obtained after centrifugation was evaporated and reconstituted in water before analysis. Similarly, urine samples obtained during the 0– to 24-hour collection interval were pooled across subjects. The volume of a given sample represented in the pool was weighted based on the net weight of urine collected for the time interval. The pooled urine was analyzed without further processing.

LC-MS using LTQ-Orbitrap was used to analyze avibactam and its related components in pooled plasma and urine samples (see Supplemental Methods).
In Vitro Studies

Membrane Transporter Study. Assay systems to evaluate the inhibition and substrate (uptake) potential of avibactam for human membrane transporters were developed using transporter protein cDNA stably transfected into Madin-Darby canine kidney II (MDCKII) cells, human embryonic kidney (HEK293) cells, or human membrane vesicles expressing native transporter protein. Avibactam substrate and inhibition potential for breast cancer resistance protein (BCRP) and multidrug resistance 1 protein (MDR1) were assessed with transporters expressed in MDCKII cells. Human multidrug resistance-associated protein (MRP4), organic anion transporting polypeptide proteins 1 and 3 (OATP1B1 and OATP1B3), organic anion transporters 1 and 3 (OAT1 and OAT3), and organic cation transporters 1 and 2 (OCT1 and OCT2) expressed in HEK293 cells were assessed for avibactam inhibition potential. In addition, MRP4, OAT1, OAT3, and OCT2 expressed in HEK293 cells were assessed for avibactam substrate potential. Inhibition potential of avibactam for human bile salt export pump (BSEP) was assessed in membrane vesicles isolated from cells overexpressing BSEP and control membrane vesicles (see Supplemental Methods). Inhibition potential of avibactam was assayed by measurement of radiolabeled model substrate uptake in the presence of avibactam (AstraZeneca, Macclesfield, UK). Substrate potential of avibactam was assessed by measuring the bidirectional transport (MDCKII cells) or uptake (HEK293 cells) of [14C] avibactam in the absence or presence of the following model inhibitors: MDR1, ketocazole (Sigma-Aldrich, Zwijndrecht, Netherlands); BCRP, Ko143 (Allen et al., 2002; synthesized by Prof. G. J. Koomen, Van’t Hoff Institute for Molecular Sciences, Netherlands/Tocris Bioscience, Bristol, UK); MRP4, dipyridamole; OAT1 and OAT3, probenecid; OCT2, quinidine (all from Sigma-Aldrich). Empty vectors treated with test substances were used as negative controls. Effects of avibactam at concentrations of 0.5–300 μM on cytotoxicity or monolayer cell viability were assessed for the MDCKII and HEK293 control cells using a neutral red uptake assay (Borenfreund and Puerner, 1985) with Triton-X100 (1%) as positive control.

Cytchrome P450 Inhibition Studies. The in vitro potential of avibactam to inhibit the activity of human cytochrome P450 isoforms 1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5 was investigated in human liver microsomes (see Supplemental Table 1 and Supplemental Methods). In one study, microsomes (individual and pooled samples from 15–30 donors) were preincubated with avibactam with and without NADPH (2 mM) for 20 minutes to evaluate time-dependent inhibition of cytochrome enzymes. Parallel incubations were done with reference inhibitors as positive controls. Since a minor inhibition of CYP2C9 was observed in the first study, a further study was conducted using a similar method to evaluate the potential of avibactam to inhibit CYP2C9 activity at concentrations up to 5000 μM in pooled microsomes from 50 donors with preincubation with NADPH (2 mM) for 10 minutes. Enzymatic activities were determined by measuring the concentration of formed metabolite using high-performance liquid chromatography (HPLC) with ultraviolet detection method for all enzymes except CYP2A6, which was determined by fluorimetry.

Cytchrome P450 Induction Study. The in vitro induction potential of avibactam on the catalytic activity of the human hepatic cytochrome isoforms 1A2, 2B6, 2C9, 2E1, and 3A4 was investigated in freshly isolated human hepatocytes from three donors (see Supplemental Table 2 and Supplemental Methods). The induction potential of avibactam was tested at 200–5000 μM,

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Radioactivity in Plasma</th>
<th>Total Radioactivity in Whole Blood</th>
<th>Avibactam in Plasma ( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (μg/ml)</td>
<td>22.4 (17.4)</td>
<td>14.6 (15.2)</td>
<td>23.1 (11.1)</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
</tr>
<tr>
<td>( AUC_{0-\infty} ) (μg.h/ml)</td>
<td>47.6 (22.7)</td>
<td>36.2 (24.0)</td>
<td>47.10 (20.0)</td>
</tr>
<tr>
<td>( \text{Fe%} )</td>
<td>NC</td>
<td>NC</td>
<td>47.20 (20.0)</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>NC</td>
<td>NC</td>
<td>2.778 (0.6)</td>
</tr>
<tr>
<td>( \text{MRT} ) (h)</td>
<td>NC</td>
<td>NC</td>
<td>1.887 (16.0)</td>
</tr>
<tr>
<td>( \text{Vss} ) (ml)</td>
<td>NC</td>
<td>NC</td>
<td>21.200 (10.7)</td>
</tr>
<tr>
<td>( \text{CLR} ) (ml/min)</td>
<td>NC</td>
<td>NC</td>
<td>1.1800 (18.6)</td>
</tr>
<tr>
<td>( \text{CLR} ) (ml/min)</td>
<td>NC</td>
<td>NC</td>
<td>158.0 (21.2)</td>
</tr>
<tr>
<td>( \text{Fe%} )</td>
<td>97.0 (1.0)</td>
<td>0.2 (0.03)</td>
<td>84.9 (11.8)</td>
</tr>
</tbody>
</table>

**Supplemental Methods** on radiochromatographic monitoring). Total radioactivity of plasma samples before extraction or analysis, and of column effluent in recovery experiments, was measured using LSC. Recovery of radioactivity from the LC column during a gradient run was determined using the same LSC method. An aliquot of sample before injection was analyzed by LSC. Another aliquot of the same sample was then injected onto the LC system, and the column effluent was collected quantitatively into a volumetric cylinder. Aliquots of the column eluate were likewise analyzed by LSC and compared with preinjection samples.

Control human plasma, which was fortified with [14C] avibactam after extraction, and [14C] avibactam dose solution were also analyzed to assess possible degradation during sample processing.

**Statistical Analysis.** No formal sample size calculation was performed. A planned sample size of six subjects was chosen based on previous experience with mass balance studies. PK parameter estimates and analyses of whole blood and plasma radioactivity were performed for all subjects who received the complete dose of [14C] avibactam and who had sufficient concentration data (plasma, whole blood, urinary, and fecal) for mass balance determination and PK parameter estimation (PK population).

Safety data were summarized for all enrolled subjects who received any fraction of the dose of [14C] avibactam (safety population). Summary statistics were prepared using Statistical Analysis System software (v. 8.2; SAS Institute Inc., Cary, NC); however, no formal statistical analysis was planned. PK parameters were estimated using WinNonlin (v. 6.1, Pharsight Corporation, St. Louis, MO) except for the amount of total radioactivity or parent drug eliminated (\( A_e \)), which was calculated using Statistical Analysis System software.

### Key pharmacokinetic (PK) parameters for total radioactivity and avibactam after a single intravenous infusion of [14C] avibactam (PK population; \( n = 6 \))

- Fe: fraction of the dose administered as [14C] avibactam that was excreted in urine
- AUC0–\( t \): area under the concentration-time curve from dosing to the last measurable time point
- AUC0–\( \infty \): area under the concentration-time curve from dosing to infinity
- MRT: mean residence time
- Vss: volume of distribution at steady state
- CLR: clearance
- \( t_{1/2} \): terminal half-life
- \( t_{max} \): time to reach \( C_{\text{max}} \)

Values shown are geometric mean (%CV) except for \( t_{max} \), which are median (range), and \( t_{1/2} \) and Fe%, which are arithmetic mean (S.D.)

*Units are μg equiv/g for total radioactivity in plasma and μg equiv h/g for total radioactivity in whole blood.

**CYP2C9**

Inhibition of CYP2C9 was observed in the first study, a further study was conducted using a similar method to evaluate the potential of avibactam to inhibit CYP2C9 activity at concentrations up to 5000 μM in pooled microsomes from 50 donors with preincubation with NADPH (2 mM) for 10 minutes. Enzymatic activities were determined by measuring the concentration of formed metabolite using high-performance liquid chromatography (HPLC) with ultraviolet detection method for all enzymes except CYP2A6, which was determined by fluorimetry.

**CYP3A4**

The induction potential of avibactam was tested at 200–5000 μM,
expected to cover therapeutic concentrations and span a safety margin >50-fold the $C_{\text{max}}$ of a 500-mg dose. Hepatocytes were incubated in 0.5 ml of hepatocyte incubation medium at 37 ± 2°C for 72 ± 4 hours (induction phase) with avibactam, a fixed concentration of reference inducer (positive controls), or hepatocyte incubation medium only (negative controls). After completion of the induction phase, supernatants were removed and hepatocytes were incubated in Krebs’ Henseleit buffer containing 3 mM salicylamide and marker substrate for 3 hours ± 15 minutes (reaction phase). Proteins were then precipitated in acetonitrile, and substrate metabolites (markers of enzyme activity) were analyzed by assay-specific HPLC and fluorimetry methods.

![Graph](image)

**Fig. 2.** Geometric mean concentrations of avibactam in plasma (determined using LC-MS/MS detection) and total $^{14}$C radioactivity in plasma and whole blood after a single i.v. infusion of 500 mg of $[^{14}C]$ avibactam in healthy male subjects (PK population; $n = 6$). Error bars represent the interval of geometric mean/ geometric S.D., geometric mean × geometric mean S.D.

![Graph](image)

**Fig. 3.** Mean cumulative recovery of avibactam and total radioactivity in urine as a percentage of the dose administered (Fe%) after a single i.v. infusion of 500 mg of $[^{14}C]$ avibactam in healthy male subjects (PK population; $n = 6$). Error bars represent S.D.

![Graph](image)

**Fig. 4.** Representative LC radiochromatograms (A) 0- to 4-hour pooled human plasma after a single i.v. infusion of 500 mg of $[^{14}C]$ avibactam in healthy male subjects (PK population; $n = 6$) and (B) control human plasma fortified with $[^{14}C]$ avibactam.
Microsomal Metabolism Study. The in vitro metabolism of avibactam was evaluated in pooled microsomes (all supplied by Biopredic, Saint Grégoire, France) isolated from liver samples from humans \((n = 15)\), CD1 mice \((n = 9)\), Sprague-Dawley rats \((n = 10)\), New Zealand rabbits \((n = 10)\), and beagle dogs \((n = 5)\). Microsomes were incubated with avibactam and relevant cofactors at nominal concentrations of 3.125 and 200 \(\mu M\) for up to 90 minutes (each experiment was performed in duplicate). At the end of the incubation, reactions were terminated by the addition of 0.5 ml of ice-cold acetonitrile and analyzed.

![Representative LC radiochromatograms (A) 0- to 24-hour pooled urine after a single i.v. infusion of 500 mg of \(^{14}\)C avibactam in healthy male subjects (PK population; \(n = 6\)) and (B) control \(^{14}\)C avibactam dose solution.](image)

**TABLE 2**

Summary of results from the in vitro evaluation of avibactam as a substrate for membrane transporter proteins

<table>
<thead>
<tr>
<th>Transporter Assay</th>
<th>Test System</th>
<th>[^{14}\text{C}]\text{-Avibactam Concentration} (\mu M)</th>
<th>Transport Ratio(^a)</th>
<th>Reference Inhibitor</th>
<th>Inhibition(^b)</th>
<th>Substrate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>MDCKII-MDR1 cells</td>
<td>0.58</td>
<td>1.39</td>
<td>Ketoconazole 25 ((\mu M))</td>
<td>17.3</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.16</td>
<td>1.11</td>
<td></td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.5</td>
<td>0.90</td>
<td></td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>BCRP</td>
<td>MDCKII-BCRP cells</td>
<td>0.58</td>
<td>1.35</td>
<td>Ko143 (1 (\mu M))</td>
<td>35.6</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.16</td>
<td>1.31</td>
<td></td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.5</td>
<td>1.04</td>
<td></td>
<td>27.4</td>
<td></td>
</tr>
<tr>
<td>MRP4</td>
<td>HEK-MRP4 cells</td>
<td>1.23</td>
<td>0.81</td>
<td>Dipyridamole (100 (\mu M))</td>
<td>NC</td>
<td>No(^c)</td>
</tr>
<tr>
<td>OAT1</td>
<td>HEK-OAT1 cells</td>
<td>1.38</td>
<td>6.45</td>
<td>Probenecid (100 (\mu M))</td>
<td>70.4</td>
<td>Yes ((K_m &gt; 1000 \mu M))</td>
</tr>
<tr>
<td>OAT3</td>
<td>HEK-OAT3 cells</td>
<td>1.38</td>
<td>4.34</td>
<td>Probenecid (100 (\mu M))</td>
<td>56.1</td>
<td>Yes ((K_m &gt; 1000 \mu M))</td>
</tr>
<tr>
<td>OCT2</td>
<td>HEK-OCT2 cells</td>
<td>1.38</td>
<td>0.88</td>
<td>Quinidine (100 (\mu M))</td>
<td>NI</td>
<td>No</td>
</tr>
</tbody>
</table>

**BCRP**, breast cancer resistance protein; **HEK**, human embryonic kidney; **MDCKII**, Madin-Darby canine kidney II; **MDR1**, multidrug resistance 1 protein; **MRP4**, multidrug resistance-associated protein 4; **OAT1** and **OAT3** organic anion transporters 1 and 3; **OCT2**, organic cation transporter 2; **NC**, not calculated; **NI**, no significant inhibition observed (<10%)

\(^a\)MDCKII cells: Corrected efflux ratio = mean \((P_{a\rightarrow b})\/a\/a\/b\) from MDCKII-MDR1 or MDCKII-BCRP)/mean \((P_{a\rightarrow b})\/a\/a\/b\) from MDCKII-control; HEK cells: Uptake ratio = mean \((P_{a\rightarrow b})\/b\/b\/a\) from HEK-OAT1, HEK-OAT3, or HEK-OCT2)/mean uptake \((\mu M)\) in HEK-control.

\(^b\)MDCKII cells: % Inhibition = 100 – ([mean \((P_{a\rightarrow b})\/a\/a\/b\) from MDCKII-MDR1 or MDCKII-BCRP)/mean \((P_{a\rightarrow b})\/a\/a\/b\) from MDCKII-control] \* 100); HEK cells: % inhibition = 100 – ([mean uptake (+ inhibitor)/mean uptake (- inhibitor)]) \*100%)

\(^c\)As the inhibitor had no effect (increase) on the accumulation of \(^{14}\text{C}\) avibactam, it was concluded that avibactam is not a potential substrate for MRP4 transporter proteins when expressed in HEK cells.
by LC-MS/MS. Positive control incubations were conducted to evaluate the metabolic competency of the microsomal preparations.

Results

Mass Balance Study

Subject Characteristics. Six healthy male subjects were enrolled and completed the study between October 17 and November 11, 2011. Subjects' age ranged from 36 to 63 years (mean ± S.D. 48.0 ± 10.6 years). Five subjects were white, and one subject was English-Caribbean. Mean ± S.D. body mass index was 25.82 ± 2.82 kg/m². The mean ± S.D. creatinine clearance was 119 ± 48 ml/min. All six subjects received a single dose of 500 mg of [14C] avibactam and were included in the PK and safety analysis populations.

Pharmacokinetic and Total Radioactivity Assessments. Key PK parameters for avibactam in plasma and total radioactivity in plasma and whole blood are shown in Table 1. Plasma avibactam concentrations peaked at the end of the infusion and declined in a biphasic manner thereafter (Fig. 2). Quantifiable concentrations of avibactam in plasma were recorded for up to 16 to 24 hours after the start of the infusion. Similarly, maximum concentrations of radioactivity in plasma and whole blood were observed at 1 hour after the start of infusion (i.e., at the end of the infusion) and declined in a biphasic manner after the end of the infusion (Fig. 2). Most of the total radioactivity in plasma was gone by 12 hours, but a small fraction of total radioactivity remained detectable for 24–72 hours after start of the infusion.

Geometric mean avibactam and total radioactivity concentrations in plasma were similar up to 8 hours after start of the infusion. From 12 hours after the start of the infusion until concentrations approached the lower limits of quantification, concentrations of avibactam in plasma appeared to decline more quickly than those for total radioactivity (Fig. 2). Geometric mean plasma and whole blood concentrations of total radioactivity were similar up to 6 hours after start of the infusion (the geometric mean ratio was 1.204 at 6 hours), although after 8 hours, the ratio decreased (geometric mean ratio was 0.317 at 24 hours after start of the infusion), suggesting that there was limited binding of total radioactivity to the cellular components of whole blood during the first 8 hours. Since the total radioactivity measurements at later time points were near the limits of quantification, terminal PK parameters could not be reliably estimated.

Based on LC-MS/MS quantitation using the validated bioanalytical method, a mean of 84.9% (range, 66.6%–101.3%) of unchanged avibactam was recovered from urine within 96 hours, with 40% to 70% recovered within the first 2 hours after start of the infusion. The geometric mean avibactam renal clearance rate was 84.6% of the total clearance rate, consistent with the predominantly renal route of avibactam excretion (Table 1).

Cumulative recovery (Fe%) of avibactam and total radioactivity from urine from −12 hour predose to 96 hours postdose are shown in Fig. 3. A mean of 97.2% (range, 95.6%–98.3%) of administered radioactivity was recovered within 96 hours: 97.0% (range, 95.3%–98.1%) from urine; 0.2% (range, 0.17%–0.23%) from feces (Table 1). Subjects were discharged from the clinical unit at 96 hours postdose as mass balance recovery was complete. Most of the administered radioactivity (mean, 95.6%) was recovered from urine within 12 hours of dosing, with 62.4% recovered in the first 2 hours (Fig. 3).

Safety. A single AE of headache occurring approximately 4 days after discharge from the clinic (reported as mild and considered unrelated to study treatment) was reported by one subject. No other AEs were experienced by any other subject, and there were no discontinuations because of AEs. Mean hematology and clinical chemistry values were within normal reference ranges at admission and discharge, and there were no clinically significant changes from baseline for any parameter. There were no notable mean changes from baseline for any vital signs or ECG parameters or abnormal physical examination findings postdose that were not present at screening.

Metabolite Profiling. The recovery of radioactivity after extraction, evaporation, and reconstitution of the pooled human plasma sample was 98.6%. The recoveries of radioactivity from the LC column during a gradient run, determined from an injection of processed pooled plasma or urine were 88% and 93%, respectively. Representative LC chromatograms for pooled plasma and urine are shown in Figs. 4 and 5, respectively.

Parent avibactam and one related product (an uncharacterized degradant) were observed in the pooled plasma sample, with parent avibactam accounting for 72.5% of total radioactivity and the unknown degradant accounting for 27.5% (Fig. 3). This unknown peak was also observed in

<table>
<thead>
<tr>
<th>Transporter Assay</th>
<th>Test System</th>
<th>Reference Substrate</th>
<th>Avibactam Concentration</th>
<th>Inhibition at Maximum Concentration</th>
<th>Inhibition Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>MDCKII-MDR1 cells</td>
<td>[3H]-diganoxin (0.05 μM)</td>
<td>100</td>
<td>NI</td>
<td>No</td>
</tr>
<tr>
<td>BCRP</td>
<td>MDCKII-BCRP cells</td>
<td>[14C]-lactate (1 μM)</td>
<td>100</td>
<td>14.4</td>
<td>No</td>
</tr>
<tr>
<td>MRP4</td>
<td>HEK-MRP4 cells</td>
<td>[14C]-bis-POM-PMEA (1 μM)</td>
<td>100</td>
<td>NC</td>
<td>Noa</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>HEK-OATP1B1 cells</td>
<td>[3H]-estradiol-17β-D-glucuronide (1 μM)</td>
<td>100, 500, 1000</td>
<td>NI</td>
<td>No</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>HEK-OATP1B3 cells</td>
<td>[3H]-estradiol-17β-D-glucuronide (1 μM)</td>
<td>100, 500, 1000</td>
<td>NI</td>
<td>No</td>
</tr>
<tr>
<td>OAT1</td>
<td>HEK-OAT1 cells</td>
<td>[3H]-tyramine (2 μM)</td>
<td>100, 500, 1000</td>
<td>32.5</td>
<td>Yes (IC50 &gt; 1000 μM)</td>
</tr>
<tr>
<td>OAT3</td>
<td>HEK-OAT3 cells</td>
<td>[3H]-estrone-3-sulfate (2 μM)</td>
<td>10–1000</td>
<td>46.7–53.2</td>
<td>Yes (IC50 &gt; 1000 μM)</td>
</tr>
<tr>
<td>OCT1</td>
<td>HEK-OCT1 cells</td>
<td>[3H]-estrone-3-sulfate (2 μM)</td>
<td>10–1000</td>
<td>16.0</td>
<td>No</td>
</tr>
<tr>
<td>OCT2</td>
<td>HEK-OCT2 cells</td>
<td>[3H]-estrone-3-sulfate (2 μM)</td>
<td>10–1000</td>
<td>16.0</td>
<td>No</td>
</tr>
<tr>
<td>BSEP</td>
<td>HEK-OAT1 cells</td>
<td>[3H]-tyramine (2 μM)</td>
<td>100, 500, 1000</td>
<td>16.0</td>
<td>No</td>
</tr>
</tbody>
</table>

aMDCKII cells: % inhibition = 100 − (mean (Papp b / a) (inhibitor)/mean (Papp b / a) (no inhibitor))×100%; HEK cells: % inhibition = 100 − (mean uptake (+ inhibitor) / mean uptake (− inhibitor))×100%; membrane vesicles: % inhibition = 100 − (mean ATP-dependent uptake (+ inhibitor)/mean ATP-dependent uptake (− inhibitor))×100%.

bBecause the test substances had no effect (increase) on the accumulation of [14C]-bis-POM-PMEA, it was concluded that avibactam did not inhibit MRP4 transporter proteins in this assay.

cData from two independent experiments.
control human plasma when fortified with [14C] avibactam undergoing the sample processing steps. As a result of the very low signal-to-noise ratio on the plasma radiochromatogram (Fig. 4), significant sample concentration procedures were undertaken but proved unsuccessful.

Parent avibactam and decarboxylated avibactam (metabolite M1) were observed in pooled urine. Parent avibactam was the major component, accounting for 93.0% of the radioactivity, and M1 accounting for 7.0% (Fig. 5). A small amount of M1 was also observed in the dose solution, indicating potential degradation either on storage or through sample processing. This metabolite was also previously observed in the rat and dog metabolism studies (data not shown). The full-scan MS and MS/MS and fragmentation details of avibactam and M1 are shown in Supplemental Fig. 1 and Supplemental Fig. 2, respectively.

In Vitro Studies

Membrane Transporter Study. Results of the transporter substrate and inhibition studies are shown in Tables 3 and 4. Avibactam was not a substrate of MDR1 or BCRP expressed in MDCKII cells (Table 2) and did not demonstrate inhibitory activity in these assay systems (Table 3). Similarly, avibactam did not inhibit MRP4 or exhibit substrate or inhibitory activity for human OCT2 proteins expressed in HEK293 cells.

Avibactam did show potential substrate activity for the human OAT1 and OAT3 proteins expressed in the HEK293 cell system (Km > 1000 μM, equivalent to 265.2 μg/ml). The reference OAT1/OAT3 inhibitor probenecid (100 μM) inhibited the uptake of avibactam by 70.4% in HEK293-OAT1 cells and by 56.1% in HEK293-OAT3 cells (Table 2). In addition, avibactam demonstrated the potential to inhibit human OAT1 and OAT3 transporters in the HEK293 cell system (IC50 values > 1000 μM; Table 3), inhibiting uptake of the reference substrate by 32.5% (OAT1) and 46.7% (OAT3) at 1000 μM. Avibactam at concentrations up to 1000 μM did not inhibit OATP1B1, OATP1B3, or OCT1 in the HEK293 cell system or the BSEP transporter protein in the vesicle system (Table 3).

Cytochrome P450 Inhibition Studies. Incubation of microscope preparations with reference inhibitors demonstrated the viability of the assay systems. In the initial study, nominal avibactam concentrations up to 200 μM showed minimal or no inhibition of all the cytochrome enzymes evaluated except CYP2C9 (Table 4). For CYP2C9, a mean of 29% inhibition versus controls was observed at 200 μM avibactam (equivalent to 53.0 μg/ml). To fully characterize this inhibition, a higher concentration range of avibactam was evaluated in a further study using microsomes from a different set of donors. A mean of 36% inhibition of CYP2C9 was observed at 5000 μM avibactam (the highest concentration tested), and no inhibition was observed at lower avibactam concentrations. In both studies, at the lower avibactam concentrations assessed (≤100 and ≤1000 μM, respectively), CYP2C9 inhibition was ≤10% and not concentration dependent. The reason for the apparent inhibition of CYP2C9 in the first study is not known. Since the plasma Cmax of a therapeutic dose of avibactam is expected to be 26 μg/ml, the potential for clinically relevant CYP2C9 interactions is considered unlikely.

Cytochrome P450 Induction Study. Avibactam showed no induction potential on the activity of CYP1A2, CYP2B6, CYP2C9, and CYP3A4 in human hepatocytes over a concentration range of 200–5000 μM (Table 5). A slight induction of CYP2E1 was observed in two of three donors (1.4- and 1.5-fold) at 5000 μM (not considered clinically relevant). Positive controls demonstrated a more than 2-fold induction of the cytochrome isofoms evaluated (1.5-fold for CY2E1 in two of three donors), confirming the functionality of the assay systems used.

### Table 4

**Summary of results from the in vitro evaluation of avibactam inhibition potential for human cytochrome P450 enzymes**

<table>
<thead>
<tr>
<th>Enzyme Assay</th>
<th>Control</th>
<th>Avibactam (200 μM)</th>
<th>Avibactam (5000 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A2</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Avibactam (200 μM)</th>
<th>Avibactam (5000 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A2</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
</tbody>
</table>

Values show mean percentage (CV%) enzyme activity vs. controls. Microsomes isolated from pooled liver samples from 15 to 30 male and female donors and two individual samples from separate male donors were preincubated with and without NADPH or the reference enzyme inhibitors, respectively. **Values show mean percentage (CV%) enzyme activity vs. controls. Microsomes isolated from pooled liver samples from 15 to 30 male and female donors and two individual samples from separate male donors were preincubated with and without NADPH or the reference enzyme inhibitors, respectively.**
Microsomal Metabolism Study. The positive control agents used in the study were metabolized to their respective metabolites, indicating the viability of the microsomes. Results of the incubations with avibactam are shown Fig. 6. In mouse, rabbit, dog, and human microsomes, no avibactam metabolism was observed, and no metabolic products were detected at either 3.125 or 200 μM. However, in rabbit and rat microsomes at 200 μM, a mean of 12% to 13% depletion of the initial avibactam concentrations at time 0 was seen after incubation for 90 minutes, but no metabolites were detected.

Discussion

The studies presented here provide additional information on the disposition of avibactam in healthy volunteers and highlight the limited propensity for avibactam to elicit drug-drug interactions through assessment of in vitro interaction with human membrane transporters and hepatic cytochrome enzymes. In the mass balance study, after a 1-hour i.v. infusion of 500 mg of [14C] avibactam, a mean of >93% of the administered dose was recovered in urine within 12 hours; >60% was recovered in the first 2 hours. This finding is consistent with previously reported PK studies, which showed that avibactam is predominantly cleared unchanged by renal elimination (Felices et al., 2010; Merdjan et al., 2010). With 97.2% of the administered radioactivity recovered overall, the study achieved mass balance by 96 hours, with urine accounting for 97% of the dose and feces 0.2% of the dose. Most of the urinary radioactivity was unchanged by avibactam (84.9% via LC-MS/MS and 93% via metabolite profiling). Avibactam was generally well tolerated in this small sample of healthy male volunteers, with only one AE of headache in one subject reported 4 days after completing the study treatment.

The similarity in plasma avibactam concentrations determined by the validated LC-MS/MS method and total radioactivity concentrations up to 8 hours suggests that avibactam accounted for all or most of the circulating component in plasma. This finding is also consistent with the high proportion of urine total radioactivity that could be attributed to unchanged avibactam (>93% of the urine radiocromatogram). The early eluting radiocromatographic peaks for pooled plasma (unknown; an as yet uncharacterized peak) and urine (M1; descarbonyl avibactam) were also observed at the same retention times in control samples undergoing the same sample processing steps, suggesting that these components are not products of in vivo metabolism but appear to arise during sample processing and analysis. As a result of the low signal-to-noise ratio of the LC-radiocromatogram, various sample concentration procedures were undertaken. Several different conditions, including acidified or basified conditions, were used in attempts to concentrate the sample by approximately 10-fold to get a good signal while minimizing degradation. Without sample processing, it was not possible to get good chromatography, and the more processing steps taken to concentrate the sample, the more degradation seemed to occur, especially to this unknown peak. The unknown peak elutes in 100% aqueous conditions at the solvent front and could not be retained under several evaluated conditions (HPLC column and/or mobile phase). Several different chromatography conditions were tried to elute this unknown peak with more retention on column, but these proved unsuccessful. Under both positive and negative ionization conditions, no ion transitions were observed that could be related to the parent compound. Avibactam is a polar, small molecule with a low molecular weight and is designed to covalently modify and thereby deactivate bacterial enzymes. These characteristics make the compound very reactive, light-sensitive, and prone to degradation. Even slight changes in mobile phase or reconstitution solvent had a profound impact on the retention, chromatography, and ionization of the parent compound.

In previous metabolism studies conducted in rats and dogs, M1 was observed as a metabolite and could also be formed via degradation. In the metabolite profiling of mass balance samples, although all precautionary steps were taken to minimize the formation of M1 via degradation, it is unclear whether the observed formation of M1 in urine was due to degradation or metabolism. However, since it can be formed via both processes, it is considered here as a metabolite. Avibactam binds covalently to bacterial enzymes and undergoes a slow reversible conversion back to parent avibactam. The longer elimination half-life of total radioactivity compared with that of plasma avibactam half-life may be due to covalent binding and/or distribution of avibactam to tissue compartments, which slowly reverses back to parent avibactam over time and is unlikely to be due to metabolism of avibactam or retention of any metabolite.

A proposed metabolic pathway for avibactam in human in vivo samples is shown in Fig. 7 and is consistent with findings from the in vitro metabolism study, in which metabolic turnover of avibactam (3.125 and 200 μM) in microsomal preparations from various species, including humans, mice, rats, rabbits, and dogs, was absent or minimal. Given this lack of evidence of metabolism in human liver microsomes, the PK profile of avibactam is unlikely to be affected by coadministered compounds that are cytochrome inhibitors or inducers. Since avibactam did not undergo any metabolism and no metabolites were detected during incubation with human liver microsomes, no evaluations of avibactam metabolism by cytochrome isoforms have been undertaken.

Notably, the geometric mean renal clearance rate of avibactam (158 ml/min) in the mass balance study was greater than the glomerular

---

**TABLE 5**

<table>
<thead>
<tr>
<th>Donor</th>
<th>CYP1A2</th>
<th>CYP2B6</th>
<th>CYP2C9</th>
<th>CYP3A4</th>
<th>CYP2E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1 D2 D4 D6</td>
<td>D1 D2 D4 D6</td>
<td>D1 D2 D3</td>
<td>D1 D2 D4 D6</td>
<td>D1 D2 D4 D6</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>14.8 11.9 27.3</td>
<td>24.4 9.5 19.3</td>
<td>2.3 2.1 5.2</td>
<td>13.0 12.6 12.6</td>
<td>1.3 1.7 1.6</td>
</tr>
<tr>
<td>Negative control</td>
<td>1.0 1.0 1.0</td>
<td>1.0 1.0 1.0</td>
<td>1.0 1.0 1.0</td>
<td>1.0 1.0 1.0</td>
<td>1.0 1.0 1.0</td>
</tr>
<tr>
<td>Avibactam (5 mM)</td>
<td>0.9 1.0 1.2</td>
<td>1.1 0.7 0.5</td>
<td>1.3 1.0 1.3</td>
<td>0.9 1.0 1.0</td>
<td>1.5 0.8 1.4</td>
</tr>
<tr>
<td>Avibactam (1 mM)</td>
<td>0.9 0.8 1.0</td>
<td>0.6 0.6 0.5</td>
<td>1.1 1.0 1.1</td>
<td>1.1 1.0 1.1</td>
<td>0.8 0.9 1.0</td>
</tr>
<tr>
<td>Avibactam (0.2 mM)</td>
<td>0.9 0.8 1.0</td>
<td>0.9 0.7 0.6</td>
<td>1.1 1.0 1.1</td>
<td>0.9 0.9 1.1</td>
<td>0.8 0.9 1.1</td>
</tr>
</tbody>
</table>

---

*In incubations with hepatocytes from donor 3 were not evaluated because the plated hepatocytes lacked the confluency to produce a reliable turnover of the marker substrate; hepatocytes from a fourth donor were assessed instead.
filtration rate for unbound drug (0.92 \times 119 = 109.5 \text{ ml/min}), suggestive of active tubular secretion of avibactam in addition to glomerular filtration. In the in vitro membrane transporter study, avibactam was both a substrate and potential inhibitor of human OAT1 and OAT3 transporters ($K_m$ and $IC_{50} > 1000 \text{ M}$, equivalent to 265.2 $\mu$g/ml), suggesting that these transporters might be responsible for the active secretion of avibactam observed in vivo. Probenecid (a potent OAT inhibitor), at a concentration of 100 $\mu$M, inhibited the uptake of avibactam by OAT1 and OAT3 in the HEK293 assay system.

OAT1 and OAT3 transporters are expressed on the basolateral membrane of the renal proximal tubule and are considered the rate-limiting step in the renal clearance of organic anion drugs, metabolites, and toxins in vivo (Gallegos et al., 2012). In addition to their role in renal drug clearance, both these transporters have potential roles in clinical drug-drug interactions (Giacomini et al., 2010). Avibactam IC$_{50}$ values were more than 20-fold higher than the mean $C_{\text{max}}$ of a 500-mg dose (23.1 $\mu$g/ml), and with the rapid elimination of avibactam, the potential for drug-drug interactions involving other substrates of OAT1 and OAT3 is thought to be low. Nonetheless, potent inhibitors of OAT1 and OAT3 (such as probenecid) have the potential to alter the elimination of avibactam when dosed. Since a clinical interaction study of avibactam and probenecid has not been conducted, coadministration of avibactam with probenecid is not recommended.

Avibactam had no significant effects on the other human renal and hepatic transporters evaluated (BRCP, MDRP, OCT2, OATP1B1, OATP1B3 and BSEP). Hence, drug-drug interactions involving codosing of avibactam with substrates or inhibitors of these transporters are considered unlikely.

At avibactam concentrations up to 200 $\mu$M (53.1 $\mu$g/ml or approximately 2-fold the mean $C_{\text{max}}$ of a 500-mg dose), inhibition of human CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5 was mostly absent or negligible and $\leq50\%$ in all cases, indicating IC$_{50}$ values $>200$ $\mu$M for all cytochrome P450 enzymes assessed. A 29$\%$ inhibition of CYP2C9 activity was observed at 200 $\mu$M avibactam, and
36% inhibition was observed at 5000 μM. However, inhibition of CYP2C9 by avibactam was determined to be unspecific and negligible at concentrations ≤1000 μM (265.2 μg/ml), which greatly exceed any clinically relevant exposure. Avibactam showed no significant induction of cytochrome enzymes in freshly isolated hepatocytes at therapeutic concentrations. However, a slight induction of CYP2E1 was observed in two of three donors at the highest concentration tested (5000 μM; equivalent to 1326 μg/ml or >50-fold the plasma Cmax of a 500-mg dose). Hence, the potential for cytochrome P450 mediated drug-drug interactions with codosing of avibactam appears unlikely. In summary, as well as supporting previous findings indicating that avibactam is predominantly eliminated unchanged by the renal route, these data suggest there is a limited role of OAT1 and OAT3 renal drug transporters in avibactam secretion and also highlight the low likelihood of interaction with a range of cytochrome enzymes. Active secretion via OAT1 and OAT3 transporters may account for a small proportion of overall avibactam clearance (indicating a potential for clinical drug-drug interactions when codosing avibactam with other compounds that inhibit OAT1 and/or OAT3). Extensive in vitro investigations found no evidence of potential for avibactam drug-drug interactions involving other renal or hepatic transporters or hepatic cytochrome P450 enzymes.

Acknowledgments
Medical writing support was provided by Mark Waterlow of Prime Medica Ltd, Knutsford, Cheshire, UK, funded by AstraZeneca. The design and conduct of the studies, as well as analysis of the study data and opinions, conclusions, and interpretation of the data, are the responsibility of the authors. The authors thank Fanny Boislevé and Béatrice Lopez (Biopredic, France), Friedrich Krätzer and Katrinina Fuchs (GenPharmTox BioTech AG, Germany), Paul Newell (AstraZeneca, UK), and Rianne AF de Ligt (Netherlands Organisation for Applied Scientific Research, Netherlands) for their involvement in these studies.

Authorship Contributions
Participated in research design: Vishwanathan, Mair, Gupta, Clarkson-Jones.
Conducted experiments: Vishwanathan, Mair, Atherton.

Contributed new reagents or analytic tools: Vishwanathan.
Performed data analysis: Vishwanathan, Gupta, Atherton, Clarkson-Jones.
Wrote or contributed to the writing of the manuscript: Vishwanathan, Mair, Gupta, Atherton, Clarkson-Jones, Edeki, Das.

References
Edeki T, Armstrong J, and Li J. Pharmacokinetics of avibactam (AVI) and ceftazidime (CAZ) following separate or combined administration in healthy volunteers. Poster A-1019 presented at 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 10–13 September, 2013, Denver, CO.
Fig. 7. Proposed metabolic pathway for avibactam in humans.
Fig. 7. Proposed metabolic pathway for avibactam in humans.

Peleg AY and Hooper DC (2010) Hospital-acquired infections due to gram-negative bacteria. 


Stachyra T, Levasseur P, Pechereau MC, Girard AM, Claudon M, Miossec C, and Black MT 
and Enterobacteriaceae expressing KPC carbapenemases. *J Antimicrob Chemother* **64**: 
326–329.

Zhanel GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagacé-Wiens PR, Denisuk A, 
Rubinstein E, Gin AS, and Hoban DJ, et al. (2013) Ceftazidime-avibactam: a novel cephalo-

Address correspondence to: Karthick Vishwanathan, AstraZeneca, 35 Gate-
house Drive, Waltham, MA 02451. E-mail: karthick.vishwanathan@astrazeneca.com
Online Supplemental Material

Assessment of the mass balance recovery and metabolite profile of avibactam in humans and in vitro drug–drug interaction potential

Karthick Vishwanathan, Stuart Mair, Anshul Gupta, James Atherton,
Jacqueline Clarkson-Jones, Timi Edeki and Shampa Das

Drug Metabolism and Pharmacokinetics, Infection Innovative Medicines Unit,
AstraZeneca R&D, Boston, MA, USA (KV, AG, JA, TE); Quotient Clinical Ltd,
Nottingham, UK (SM); AstraZeneca, Macclesfield, UK (JC-J, SD)

Supplemental Methods

Metabolite profiling methods

Liquid scintillation counting

Total radioactivity of samples prior to extraction and/or analysis and of column effluent in recovery experiments was measured by LSC using a Packard Tricarb 2200C (Packard Instrument Co., Downers Grove, IL).

Liquid chromatography-mass spectrometry (LC-MS)

The LC-MS system consisted of a Acquity™ gradient pump and autosampler (Waters Inc, Milford, MA) and LTQ Orbirtap mass spectrometer with electrospray interface (Thermo Electron Corporation, Bremen, Germany). Xcalibur software (v2.0.7)
(Thermo Fisher Scientific Inc., Waltham, MA) was used for instrument control (LC system and mass spectrometer), and for acquisition and evaluation of data.

The electrospray ionization source of the mass spectrometer was operated in the negative ion mode, with a source voltage of 3.5 kV and capillary temperature setting of 275°C. The nitrogen sheath gas and auxiliary gas flows were 40 and 10 (arbitrary units), respectively. Negative ion spectra were acquired in the full scan FT (fourier transform, ie, accurate mass) mode (m/z range of 125 – 800 Da) using a mass resolution of 15000. The mass spectrometer was operated in the data-dependent scan mode with a mass resolution of 15000 and a 2Da isolation width. Collision-induced dissociation product ion spectra were acquired for the most intense full-scan FT parent molecular ion. Helium served as the collision gas to produce collision-induced dissociation CID spectra using a collision energy setting of 30%.

Liquid chromatographic separation of avibactam and its related components in human plasma and urine was conducted at ambient temperature. A binary solvent system was employed with a mobile phase A of water and mobile phase B of 9 parts acetonitrile to 1 part water containing 10 mM ammonium acetate. Separations were performed with an Inertsil® ODS-3 column (250 x 4.6 mm id, 5 μm particle size, GL Sciences, Inc., Tokyo, Japan) at a flow rate of 0.8 mL/min throughout the gradient run. The first 4 minutes of the flow to the mass spectrometer was diverted to waste. The initial mobile phase composition of 100% A was held for 24 min, after which mobile Phase B was increased linearly to 50% over the next 0.5 min. At 24.5 min, mobile Phase B was again increased to 90% in a linear fashion for 5.5 min and held for an additional 4 minutes. At 34.1 min, the initial gradient conditions (0% B) were established and held for 31 minutes before commencement of the next injection.
Radioactivity monitoring

In-line radio-chromatographic data were collected using an IN/US β-RAM model 5 controlled with Laura (v 4.1) software (LabLogics Systems Ltd., Sheffield, UK). The LC flow was split at a ratio of approximately 1:9 between the LTQ-Orbitrap mass spectrometer and the β-RAM. The LC flow to the β-RAM was complemented with IN-FLOW™ scintillant (IN/US Systems, Tampa, FL) at a rate of 1.6 mL/min throughout the first 40 minutes of the chromatographic analysis.

Membrane transporter study methods

Madin-Darby Canine Kidney II (MDCKII) cell lines

MDCKII cell lines, transfected with either human multi-drug resistance 1 protein (MDR1) cDNA (MDCKII-MDR1), human breast cancer resistance protein (BCRP) cDNA (MDCKII-BCRP), or with an empty vector (MDCKII-control) were obtained from The Netherlands Cancer Institute (Amsterdam, Netherlands). The MDCKII-MDR1 cell line was prepared according to similar methods to those described for the MDCKII-MRP1 cell line (Bakos et al 1998). The methods for preparation of the MDCKII-BCRP cell line were as described by Pavek et al (2005). Each cell line was stored as frozen stock cultures in liquid nitrogen. Subcultures were prepared from these stocks for experimental use.

Human Embryonic Kidney (HEK293) cell lines

HEK293 cell lines, stably transfected with human cDNA for organic anion transporting polypeptide protein 1 (OATP1B1; HEK-OATP1B1), organic anion transporting polypeptide protein 3 (OATP1B3; HEK-OATP1B3), organic cation transporter 1 (OCT1; HEK-OCT1), organic cation transporter 2 (OCT2; HEK-OCT2), organic anion transporter 1 (OAT1; HEK-OAT1), organic anion transporter 3 (OAT3;
HEK-OAT3) or with an empty vector (HEK-control), were developed at TNO (Zeist, Netherlands). Stable HEK293 cells transfected with human multidrug resistance-associated protein 4 (MRP4; HEK-MRP4) were obtained from The Netherlands Cancer Institute (Amsterdam, Netherlands). Each cell line was stored as frozen stock cultures in liquid nitrogen. Subcultures were prepared from these stocks for experimental use.

**Cell culture**

All transfected MDCKII and HEK293 cells were grown in culture medium consisting of Dulbecco's modified Eagle medium (DMEM) with L-GlutaMax (4.5 g of glucose per liter), supplemented with heat-inactivated fetal calf serum (10% v/v) and 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were cultured by seeding approximately 2 million cells in 75 cm$^2$ tissue culture flasks containing culture medium. Near confluent cell cultures were harvested by trypsinization, re-suspended in culture medium and the process repeated once or twice weekly to provide sufficient cells for use. The cells were cultured in a humidified incubator at approximately 37°C in approximately 95% air/5% CO$_2$.

**Membrane vesicles**

Membrane vesicles isolated from cells overexpressing human bile salt export pump (BSEP), as well as control membrane vesicles, were obtained from Solvo Biotechnology (Budaörs, Hungary). Storage of membrane vesicles was below –70°C.

**Substrate and inhibition studies in MDCKII cells**

Interactions of avibactam with MDR1 and BCRP were assessed by measuring the bidirectional transport of a range of concentrations of [¹⁴C] avibactam across
MDCKII-control, MDCKII-MDR1 and MDCKII-BCRP cells in the absence and presence of ketoconazole (MDR1 inhibitor; Sigma-Aldrich, Zwijndrecht, Netherlands) and Ko143 (BCRP inhibitor; synthesized by Prof. G.J. Koomen, Van’t Hoff Institute for Molecular Sciences, University of Amsterdam, Netherlands/Tocris Bioscience, Bristol, UK). In a pilot bidirectional transport study the optimal incubation time with avibactam was assessed.

The integrity of transfected MDCKII cells was determined with the reference substances $[^3]H$ mannitol (Perkin Elmer, Waltham, MA; paracellular transport marker) and $[^14]C$ caffeine (Perkin Elmer, Waltham, MA; transcellular transport marker), and functionally assessed using the reference substrates $[^3]H$ digoxin (Perkin Elmer, Waltham, MA) and $[^3]H$ quinidine (American Radiolabeled Chemicals, Inc., St Louis, MO) for MDCKII-MDR1 and $[^14]C$ PhIP (Toronto Research Chemicals, Toronto, Canada) for MDCKII-BCRP. Corrected efflux ratios were determined.

The potential inhibitory effect of avibactam and ceftazidime on MDR1 and BCRP was assessed by measuring the transport of the radiolabelled substrates $[^3]H$ digoxin and $[^14]C$ PhIP across transfected MDCKII cell monolayers in the presence of the unlabelled test substance. Inhibition of transport of radiolabelled reference substrates was also determined in MDCKII-MDR1 cells in the presence of ketoconazole, and in MDCKII-BCRP cells in the presence of Ko143.

Appropriate controls in all MDCKII studies were included throughout.

**Substrate and inhibition studies in HEK293 cells**

The interactions of avibactam with human organic anion transporting polypeptide proteins OATP1B1 and OATP1B3 were assessed by measuring the active uptake of
a single concentration of $[^{14}C]$ avibactam into HEK-control, HEK-OATP1B1 and HEK-OATP1B3 cells in the absence and presence of a known drug transporter inhibitor (cyclosporin A; Sigma-Aldrich, Zwijndrecht, Netherlands). The potential inhibitory effect of avibactam and ceftazidime on OATP1B1 and OATP1B3 was assessed by measuring the active uptake of the radiolabelled substrate $[^{3}H]$-estradiol-17β-D-glucuronide ($[^{3}H]$-EG; Perkin Elmer, Waltham, MA) in HEK-OATP1B1 and HEK-OATP1B3 cells in the presence of a range of concentrations of avibactam or ceftazidime.

The interactions of avibactam with OAT1 and OAT3 were assessed by measuring the active uptake of a single concentration of $[^{14}C]$ avibactam into HEK-control, HEK-OAT1 and HEK-OAT3 cells in the absence and presence of a known drug transporter inhibitor (probenecid; Sigma-Aldrich, Zwijndrecht, Netherlands). The potential inhibitory effect of avibactam and ceftazidime on OAT1 and OAT3 was assessed by measuring the active uptake of $[^{3}H]$-para-amino hippuric acid ($[^{3}H]$-PAH; American Radiolabeled Chemicals, Inc., St Louis, MO; OAT1 substrate) and $[^{3}H]$-estrone-3-sulphate (Perkin Elmer, Waltham, MA; OAT3 substrate) in HEK-OAT1 and HEK-OAT3 cells respectively in the presence of a range of concentrations of avibactam or ceftazidime.

The interactions of avibactam with human organic anion transporter proteins OCT1 and OCT2 were assessed by measuring the active uptake of a single concentration of $[^{14}C]$ avibactam into HEK-control, HEK-OCT1 and HEK-OCT2 in the absence and presence of a known drug transporter inhibitor (quinidine; Sigma-Aldrich, Zwijndrecht, Netherlands). The potential inhibitory effect of avibactam and ceftazidime on OCT1 and OCT2 was assessed by measuring the active uptake $[^{14}C]$-
tetraethyl ammonium ([14C]-TEA, American Radiolabeled Chemicals, Inc., St Louis, MO; OCT1 substrate) and [14C]-metformin (Moravek Biochemicals Inc., Brea, CA; OCT2 substrate) in HEK-OCT1 and HEK-OCT2 cells, respectively, in the presence of a range of concentrations of avibactam or ceftazidime.

The interactions of avibactam with MRP4 were assessed by measuring the active uptake of a single concentration of [14C] avibactam into HEK-control and HEK-MRP4 cells after 60 min incubation in the absence and presence of a known drug transporter inhibitor (dipyridamole; Sigma-Aldrich, Zwijndrecht, Netherlands). The potential inhibitory effect of avibactam and ceftazidime on MRP4 was assessed by measuring the active uptake of the radiolabelled substrate [14C]-bis-POM-PMEA (Moravek Biochemicals Inc., Brea, CA) in HEK-MRP4 cells in the presence of a range of concentrations of avibactam or ceftazidime.

Appropriate controls in all HEK studies were included throughout.

**Inhibition studies in membrane vesicles**

The potential inhibitory effect of avibactam and ceftazidime on BSEP was assessed through measuring the active uptake of the radiolabelled substrate [3H]-taurocholate (Perkin Elmer, Waltham, MA) in inside-out membranes overexpressing human BSEP transporters in the absence and presence of a range of concentrations of avibactam or ceftazidime. Appropriate controls were included in the vesicle experiment.

**CYP inhibition studies methods**

Inhibition of the biotransformation of CYP-specific substrates by avibactam was assessed in human liver microsomes. Ten series of incubations were run to test 8 CYP activities: phenacetin O-deethylase (CYP1A2), coumarin 7-hydroxylase
(CYP2A6), paclitaxel 6α-hydroxylase (CYP2C8), tolbutamide methylhydroxylase (mainly CYP2C9), S-mephenytoin 4'-hydroxylase (CYP2C19), dextromethorphan O-demethylase (CYP2D6), chlorzoxazone 6-hydroxylase (CYP2E1), midazolam 1’-hydroxylase (CYP3A4/5), nifedipine oxidase (CYP3A4/5) and testosterone 6β-hydroxylase (CYP3A4/5). Pooled microsomes isolated from liver samples from 15 to 30 male and female donors and two individual samples from separate male donors (Biopredic International, Rennes, France) were incubated at 37°C with reference test substrates and varying concentrations of avibactam (0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM) selected to span the expected range of therapeutic exposure (see Table 1 for details of reference substrates, incubation times and microsomal suspensions). Microsomes were pre-incubated with avibactam with and without NADPH (2 mM) for 20 min to evaluate time-dependent inhibition of CYP enzymes. Parallel incubations were done with reference inhibitors as positive controls.

For CYP3A4/5 assays, inhibition potential was studied using three substrates (midazolam, testosterone and nifedipine), which interact with three different binding sites of CYP3A4/5. To overcome limitations of the assay sensitivity, for CYP2C19 and CYP2E1, individual microsome preparations from separate male donors were evaluated because of their high enzymatic activity of the CYP being tested.

The potential of avibactam at concentrations up to 5000 µM to inhibit CYP2C9 activity was evaluated in a further study in pooled human microsomes from 50 donors (mixed gender; XenoTech LLC, Lenexa, KS) using three concentrations of diclofenac as reference substrate and pre-incubation with NADPH (2 mM) for 10 min. Enzymatic activities were determined by measuring the concentration of formed metabolite using a high performance liquid chromatography with ultraviolet detection.
(HPLC-UV) method for all enzymes except CYP2A6, which was determined by fluorimetry.

**CYP induction study methods**

Freshly isolated, pre-plated human primary hepatocytes (four individual donors) in collagen-coated 24-well plates were used in this study. The hepatocytes were equilibrated after transport for approximately 48 h at 37°C before starting the induction phase. Avibactam (200 μM, 1 mM and 5 mM; equivalent to 53.1 μg/mL, 265 μg/mL, and 1326 μg/mL, respectively) or reference inducers were applied in 500 μL medium to the cells. Solutions containing avibactam or reference inducers were renewed every 24 h. Incubations containing reference inducers for CYP1A2 (omeprazole), CYP2B6 (phenobarbital), CYP2C9 (rifampicin), CYP2E1 (ethanol) and CYP3A4 (rifampicin), respectively, served as positive controls. Three replicates per positive control were tested. Hepatocyte incubations containing incubation medium only were employed as negative controls.

**Microsomal metabolism study methods**

Pooled microsomal suspensions (0.5 mg of protein in 0.5 mL of 0.1M sodium phosphate buffer containing 5 mM mgCl2 (pH 7.4) were prepared for incubation in polypropylene tubes. Avibactam at nominal concentrations of 3.125 and 200 μM was added and pre-incubated for 1 min, at which point NADPH 1 mM was added. Preparations were then incubated at 37°C for 0, 5, 10, 30, 60 and 90 min with NADPH 1 mM supplementation at 30 and 60 min. After terminating the incubation by addition of ice-cold acetonitrile, preparations were centrifuged at 1500 g for 4 min at 4°C, and stored at –80°C until analysis. All incubations were performed in duplicate.
Supplemental Table 1. Overview of *in vitro* CYP inhibition studies in human liver microsomes

<table>
<thead>
<tr>
<th>Enzyme assay</th>
<th>Reference substrate</th>
<th>Reference inhibitor</th>
<th>Microsome origin</th>
<th>Microsomal suspension, mg/mL</th>
<th>Total incubation volume, µL</th>
<th>Incubation duration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Phenacetin 40 µM†</td>
<td>Furafylline 10 µM†</td>
<td>Pool of 15 liver samples (mixed gender)</td>
<td>1.6</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin 20 µM†</td>
<td>Methoxsalen 1 µM†</td>
<td>Pool of 15 liver samples (mixed gender)</td>
<td>0.5</td>
<td>50</td>
<td>7</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Paclitaxel 20 µM†</td>
<td>Quercetin 30 µM†</td>
<td>Pool of 15 liver samples (mixed gender)</td>
<td>1.0</td>
<td>300</td>
<td>20</td>
</tr>
<tr>
<td>CYP2C9 (Study DM0002)</td>
<td>Tolbutamide 200 µM†</td>
<td>Sulfaphenazole 5 µM†</td>
<td>Pool of 30 liver samples (mixed gender)</td>
<td>0.8</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>CYP2C9 (Study DM0007)</td>
<td>Diclofenac 10, 30 and 100 µM†</td>
<td>Sulfaphenazole 2 µM†</td>
<td>Pool of 50 liver samples (mixed gender)</td>
<td>0.2</td>
<td>200</td>
<td>10</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-mephenytoin 60 µM‡</td>
<td>Tranylcypromine 40 µM†</td>
<td>Single donor (male; liver metastasis)</td>
<td>1.6</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan 20 µM†</td>
<td>Quinidine 2 µM†</td>
<td>Pool of 15 liver samples (mixed gender)</td>
<td>1.6</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Chlorzoxazone 200 µM†</td>
<td>Disulfiram 200 µM†</td>
<td>Single donor (male; steatosis)</td>
<td>0.5</td>
<td>300</td>
<td>15</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>Nifedipine 20 µM†</td>
<td>Ketoconazole 0.5 µM†</td>
<td>Pool of 15 liver samples (mixed gender)</td>
<td>1.0</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>---------</td>
<td>------------------</td>
<td>-------------------</td>
<td>-------------------------------------</td>
<td>-----</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Midazolam 10 µM†</td>
<td>0.3</td>
<td>250</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone 20 µM†</td>
<td>0.5</td>
<td>250</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The *in vitro* inhibitory potential of avibactam towards human CYP enzymes was investigated in human liver microsomes (studies DM0002 and DM0007). In DM0002, inhibition of reference test substrates was measured following incubation of microsomes with avibactam at varying concentrations selected to span the expected range of therapeutic exposure (0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM). For CYP3A4/5, inhibition was studied using three substrates, midazolam, testosterone and nifedipine, which interact with three different enzyme binding sites. Inhibition was assessed in microsomes preincubated with and without NADPH (2 mM) for 20 min to evaluate time-dependent inhibition of CYP enzymes. Enzymatic activities were determined by measuring the concentration of formed metabolite using a high performance liquid chromatography with ultraviolet detection (HPLC-UV) method for all enzymes except CYP2A6, which was determined by fluorimetry. In study DM0007, the potential of avibactam to inhibit CYP2C9 was evaluated using three concentrations of diclofenac as reference substrate and avibactam concentrations of 1, 10, 30, 100, 300, 1000, and 5000 µM. All preparations were pre-incubated with NADPH (2 mM) for 10 min prior to addition of reference substrate.

†Supplied by Sigma-Aldrich (Zwijndrecht, Netherlands)

‡Supplied by tebu-bio (Le Perray-en-Yvelines, France)
Supplemental Table 2. Overview of in vitro CYP induction studies in freshly isolated human hepatocytes

<table>
<thead>
<tr>
<th>Enzyme assay</th>
<th>Reference inducer†</th>
<th>Reference substrate‡</th>
<th>Detection method (metabolite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Omeprazole 50 µM§</td>
<td>7-Ethoxyresorufin 2 µM§</td>
<td>Fluorimetry (resorufin)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Phenobarbital 1000 µM§</td>
<td>Bupropion 100 µM§</td>
<td>HPLC (hydroxybupropion)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Rifampicin 10 µM§</td>
<td>Diclofenac sodium salt 50 µM§</td>
<td>HPLC (4´-hydroxydiclofenac)</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Ethanol 100,000 µM¶</td>
<td>Chlorzoxazone 200 µM§</td>
<td>HPLC (6-hydroxychlorzoxazone)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Rifampicin 25 µM§</td>
<td>Testosterone 200 µM§</td>
<td>HPLC (6β-hydroxytestosterone)</td>
</tr>
</tbody>
</table>

The in vitro induction potential of avibactam on the catalytic activity of human hepatic CYP enzymes was investigated in freshly isolated human hepatocytes from three of four donors. The induction potential of avibactam was tested at three concentrations, 200 µM (53.1 µg/mL), 1 mM (265 µg/mL), and 5 mM (1326 µg/mL), and compared to reference inducers (positive controls) and plain incubation medium (negative controls) for effects on metabolism of reference substrates.

†Induction phase (72 ± 4 h incubation)
‡Reaction phase (3 h ± 15 min incubation)
§Supplied by Sigma-Aldrich (St Louis, MO)
¶Supplied by neoLab (Heidelberg, Germany)
Supplemental Figure 1. Full scan and MS/MS spectra of avibactam
Supplemental Figure 2. Full Scan and MS/MS spectra of metabolite M1

A

NL: 6.07E5
OR2_02232012_AVIBACTAM
.DOSE_OT_001#244  RT: 3.92  AV: 1 F: FTMS - c ESI
Full ms [125.00-800.00]

B

NL: 5.87E4
OR2_02232012_AVIBACTAM
.DOSE_OT_001#239  RT: 3.84  AV: 1 T: FTMS - c ESI d
Full ms2 238.05@cid30.00 [55.00-250.00]
Supplemental references
