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A Phase I, Open-Label, 3-way Crossover, Pharmacokinetic and Drug-Drug Interaction Study of Ceftazidime Avibactam (CAZ-AVI [formerly CAZ104]) and Metronidazole when Administered Alone and in Combination in Healthy Subjects

Sponsor:

AstraZeneca Research and Development site representative

_____ _____

Date

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment
1			

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change
1			

AstraZeneca

PROTOCOL SYNOPSIS

A Phase I, Open-Label, 3-way Crossover, Pharmacokinetic and Drug-Drug Interaction Study of Ceftazidime Avibactam (CAZ-AVI [formerly CAZ104]) and Metronidazole when Administered Alone and in Combination in Healthy Subjects

Principal Investigator

Study center and number of subjects planned

This study will be conducted at a single study center, . At least 28 subjects will be enrolled to ensure 24 evaluable subjects.

Study period	Phase of development
Estimated date of first subject enrolled	Clinical Pharmacology (Phase I)
Estimated date of last subject completed	

Objectives

Primary objectives

To investigate the effect on the pharmacokinetics of ceftazidime, avibactam, and metronidazole when administering ceftazidime avibactam (CAZ-AVI [formerly CAZ104]) plus metronidazole in combination compared to administration of the individual components (CAZ-AVI and metronidazole).

Secondary objective

To assess safety and tolerability of CAZ-AVI and metronidazole when administered as a 2- and 1-hour infusion, respectively, every 8 hours.

Exploratory objective

To collect and store plasma samples from healthy volunteers for possible biomarker research.

The exploratory results will not be reported in the Clinical Study Report.

Study design

This study is an open-label, randomized, 3-way crossover study in at least 28 healthy male and female subjects. Subjects will be randomized to 1 of 3 treatment sequences and all subjects will receive all treatments (Treatment A, Treatment B, and Treatment C). Treatment A will be a 2-hour infusion of CAZ-AVI, Treatment B will be a 1-hour infusion of metronidazole, and Treatment C will be a 1-hour infusion of metronidazole followed by a 2-hour infusion of CAZ-AVI after flushing the intravenous line with saline solution. In each treatment period subjects will receive a single infusion on the morning of Day 1 and every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day). Subjects will receive a single infusion on Day 4. Serial blood and urine samples for pharmacokinetic assessments will be collected on Day 1 and Day 4 of each treatment period. The treatment periods will be separated by a wash-out period of at least 48 hours.

Target subject population

Healthy male and post-menopausal or surgically sterile female subjects aged 18 to 50 years (inclusive) with a body mass index between 19 and 30 kg/m^2.

Investigational product, dosage, and mode of administration

Treatment A: 2-hour infusion of CAZ-AVI (2000 mg ceftazidime and 500 mg avibactam) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day), and another single infusion on Day 4, total 8 infusions.

Treatment B: 1-hour infusion of metronidazole (500 mg) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day), and another single infusion on Day 4, total 8 infusions.

Treatment C: 1-hour infusion of metronidazole (500 mg) followed by a 2-hour infusion of CAZ-AVI (2000 mg ceftazidime and 500 mg avibactam) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions of each investigational product per day), and another single infusion of each investigational product on Day 4, total 16 infusions (8 infusions of each investigational product). The intravenous line will be flushed with saline solution between administration of metronidazole and CAZ-AVI.

Comparator, dosage, and mode of administration

None

Duration of treatment

This study will comprise 5 visits: Visit 1 (screening), Visit 2 (treatment period 1), Visit 3 (treatment period 2), Visit 4 (treatment period 3), and Visit 5 (follow-up). Subjects will be screened for eligibility within 28 days of Visit 2, when subjects will be admitted to the study center on Day -1. Subjects will be residential in the study center until Day 5. After a wash-out period of at least 48 hours, subjects will return to the study center on Day -1 of Visit 3 and Visit 4 for treatment period 2 and treatment period 3, respectively, separated by a

wash-out period of at least 48 hours. Then 7 to 10 days after the last investigational product administration at Visit 4 subjects will return for a follow-up visit.

Outcome variables:

- Pharmacokinetic (primary)

  On serial pharmacokinetic sampling days, where the data allow, the following pharmacokinetic parameters will be calculated when applicable:

  Plasma: maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), minimum plasma concentration (C_{min}), time to C_{min} (t_{min}), last quantifiable plasma concentration (C_{last}), time of C_{last} (t_{last}), area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration ($AUC_{(0-t)}$), from zero extrapolated to infinity (AUC, Day 1 only), at steady state during the dosing interval ($AUC_{(0-\tau)}$), half-life ($t_{1/2}$), systemic plasma clearance (CL), volume of distribution at steady-state (V_{ss}), volume of distribution at the terminal phase (V_z), accumulation ratio for C_{max} and $AUC_{(0-\tau)}$ ($RAUC_{(0-\tau)}$), and linearity index

  Urine: amount of drug excreted unchanged in urine from zero to time t ($A_{e(0-t)}$), fraction of dose excreted unchanged in urine (f_e; % dose), and renal clearance (CL_R)

  Additional pharmacokinetic parameters may be determined if deemed appropriate

- Safety

  Adverse events, clinical laboratory assessments, vital signs, safety and digital electrocardiograms, physical examination, and withdrawals

Statistical methods

Pharmacokinetic variables (ceftazidime, avibactam, and metronidazole plasma concentrations, urine amounts, and pharmacokinetic parameters, when applicable) will be summarized using appropriate descriptive statistics.

An exploratory evaluation of achievement of steady-state will be performed graphically when applicable.

Treatments will be compared between test (Treatment C) and reference (Treatment A for CAZ-AVI and Treatment B for metronidazole). Analyses will be performed by day (1 and 4) with a linear mixed-effects model using the logarithm of AUC (Day 1 only), $AUC_{(0-\tau)}$ (Day 4 only), and C_{max}. Geometric least-squares means together with 95% confidence intervals will be estimated and presented. Also, ratios of geometric least-squares means together with 90% confidence intervals will be estimated and presented.

Safety variables will be summarized categorically with incidence of occurrence or with descriptive statistics as appropriate.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
β	Beta
$\%AUC_{ex}$	The percent of area under the concentration-time curve which is extrapolated to infinity
AE	Adverse event (see definition in Section 6.3.1)
$A_{e(0-t)}$	Amount of drug excreted unchanged in urine from zero to time t
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AmpC	Ambler Class C
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve from zero to infinity
$AUC_{(0-t)}$	Area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration
$AUC_{(0-\tau)}$	Area under the plasma concentration-time curve during the dosing interval
BLQ	Below the limit of quantification
BMI	Body mass index
CAZ-AVI	Ceftazidime avibactam
CI	Confidence interval
cIAI	Complicated intra-abdominal infections
CL	Systemic plasma clearance
C_{last}	Last quantifiable plasma concentration
CL_R	Renal clearance
C_{max}	Maximum plasma concentration
C_{min}	Minimum plasma concentration
CPA	Clinical Pharmacology Alliance
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CTX	Cefotaximase
cUTI	Complicated urinary tract infections
CV%	Geometric coefficient of variation (%)

Abbreviation or special term	Explanation
CYP	Cytochrome P450
DBP	Diastolic blood pressure
dECG	Digital electrocardiogram
EC	Ethics Committee
ECG	Electrocardiogram
EClysis©	User-interactive, modular computer-based system for dECG data processing, analysis and measurement of ECG intervals and wave amplitudes, exports and reports, used by the AstraZeneca ECG Centre
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ESBL	Extended-spectrum β-lactamases
f_e	Fraction of dose excreted unchanged in urine
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transpeptidase
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate
IATA	International Air Transport Association
IB	Investigator's Brochure
IC_{50}	Half-maximal inhibitory concentration
ICH	International Conference on Harmonization
KPC	A type of Class A β-lactamase (*Klebsiella pneumoniae* carbapenemase)
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantification
max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
min	Minimum
NA	Not applicable

Abbreviation or special term	Explanation
ND	Not determined
OAE	Other significant adverse event (see definition in Section 11.1.1)
PD	Pharmacodynamic(s)
pECG	Paper print-out electrocardiogram
PK	Pharmacokinetic(s)
PR(PQ)	ECG interval measured from the onset of the P wave to the onset of the QRS complex
QRS	ECG interval measured from the onset of the QRS complex to the J point
QT	ECG interval measured from the onset of the QRS complex to the end of the T wave
QTcF	QT interval corrected for heart rate using Fridericia's formula
$RAUC_{(0-\tau)}$	Accumulation ratio for $AUC_{(0-\tau)}$
RC_{max}	Accumulation ratio for C_{max}
RR	The time between corresponding points on 2 consecutive R waves on ECG
Rsq	Coefficient of determination
SAE	Serious adverse event (see definition in Section 6.3.2)
SBP	Systolic blood pressure
SD	Standard deviation
SHV	A type of Class A β-lactamase
$t_{1/2}$	Half-life
TEM	A type of Class A β-lactamase (Temoniera)
t_{last}	Time of last quantifiable plasma concentration
t_{max}	Time to maximum plasma concentration
t_{min}	Time to minimum plasma concentration
ULN	Upper limit of normal
USA	United States of America
V_{ss}	Volume of distribution at steady-state
V_z	Volume of distribution at the terminal phase
WNL	Within normal limits

1. INTRODUCTION

1.1 Background

1.1.1 β-lactam and β-lactamases resistant Gram-negative bacteria

Beta (β)-lactamases are enzymes that are a major contributing factor to β-lactam resistance among gram-negative bacteria. Although over 890 individual enzymes have been described, only a small number of these are associated with the majority of penicillin, cephalosporin, and carbapenem resistance in gram-negative pathogens (Louie et al 2010). The most important β-lactamases of clinical relevance are enzymes that utilize serine at their active site to facilitate β-lactam hydrolysis (Rossolini and Docquier 2006), these include: 1) the Ambler Class C (AmpC)-type cephalosporinases in the *Enterobacteriaceae* and *Pseudomonas aeruginosa,* enzymes that may be produced either constitutively (chromosomally encoded) or via plasmids; 2) the common TEM and SHV β-lactamases with hydrolytic activity against first and second generation cephalosporins; 3) the extended-spectrum β-lactamases (ESBLs) that hydrolyze later generation cephalosporins and monobactams; and 4) the carbapenemases that confer resistance to most β-lactams, including carbapenems and the monobactams.

Among each of these groups of enzymes, only a few β-lactamases have become prominent; the majority of reports are of a single enzyme from a single, localized, clinical isolate, with characterization confined to a unique amino acid sequence and minimal, if any, functional information (Louie et al 2010). The AmpC β-lactamases differ slightly in structural properties, but all tend to have similar cephalosporinase activities. TEM-1 and SHV-1 β-lactamases remain important, as they continue to be identified in many clinical isolates. Within the ESBL family, the cefotaximase (CTX)-M enzymes have become well established worldwide, with CTX-M-15 most frequently identified globally, followed by CTX-M-1, CTX-M-3, and CTX-M-14. Occasional ESBLs in the TEM and SHV families are still identified, but are found less often than CTX-M enzymes. The serine carbapenemases, especially *Klebsiella pneumoniae* carbapenemase (KPC)-2 and KPC-3, produced in many species of the *Enterobacteriaceae* and also in non-fermentative bacteria, have become threats to the use of β-lactams in virtually all parts of the world (Schwaber and Carmeli 2008).

β-lactam-resistant infections are appearing in both the hospital and community settings. Since many β-lactamases are now carried on transferable elements, they can be expected to proliferate throughout gram-negative pathogens, with multiple β-lactamases produced in each organism. One therapeutic approach that would allow the continued use of β-lactam antibiotics would be the introduction of a broad-spectrum β-lactamase inhibitor that inactivates multiple groups of enzymes.

1.1.2 Ceftazidime and β-lactamase mediated resistance to ceftazidime

Ceftazidime is an injectable third generation cephalosporin that has been in clinical use worldwide for more than 20 years. Its spectrum of antibacterial activity includes gram-negative organisms (including *Pseudomonas aeruginosa*), and with lower potency, some gram-positive organisms. It is approved for the treatment of lower respiratory tract infections,

skin and skin-structure infections, urinary tract infections, bacterial septicemia, bone, and joint infections, gynecological infections, and central nervous system infections (including meningitis) caused by susceptible pathogens.

Over the past 15 years, resistance to ceftazidime has been increasing worldwide. The most common mechanism of that resistance is bacterial production of β-lactamases, in particular the so-called ESBLs, which are molecular Class A enzymes. The range of approved dosage for ceftazidime is from 1 g 8-hourly to 2 g 12-hourly in healthy adults.

1.1.3 Ceftazidime avibactam (CAZ-AVI)

AstraZeneca-Forest/Cerexa are developing ceftazidime in combination with avibactam (ceftazidime avibactam [CAZ-AVI] [formerly CAZ104]), a β-lactam – β-lactamase inhibitor, as an intravenously administered compound for parenteral treatment of patients with infections caused by gram-negative pathogens, including pathogens that are resistant to ceftazidime. Clinical development will initially be focused on patients with complicated urinary tract infections (cUTI), including acute pyelonephritis, and patients with complicated intra-abdominal infections (cIAI).

Avibactam is a novel non-β-lactam – β-lactamase inhibitor with a spectrum of activity encompassing both Ambler Class A ESBLs and AmpC enzymes (Livermore 2008). Avibactam binds to these enzymes with a lower inhibition half-maximal inhibitory concentration (IC_{50}) as compared to currently marketed β-lactamase inhibitors clavulanic acid, tazobactam, and sulbactam. In addition, avibactam is a potent inhibitor of AmpC enzymes whereas clavulanic acid, tazobactam, and sulbactam lack any clinically useful activity. Unlike currently available β-lactamase inhibitors, avibactam does not induce β-lactamase production.

the concomitant administration of ceftazidime and avibactam has been shown to be active against strains which express a combination of β-lactamase types, as well as strains which are concomitantly resistant to other antibacterial classes such as fluoroquinolones.

At the time of this Clinical Study Protocol (CSP), the options for the treatment of gram-negative infections, especially multi-drug resistant strains including ESBL producers, are extremely limited. Hence development and availability of new agents to treat these infections are seen as important additions to the existing treatment options.

The half-life ($t_{1/2}$) of avibactam, determined up to the intended administration interval for avibactam in clinical practice (8 hours) ranged from 1.4 to 1.7 hours and resulted in little or no accumulation upon repeated administration. Pharmacokinetic (PK) parameters for ceftazidime when administered in combination with avibactam were similar to those for ceftazidime alone reported in the literature (Rains et al 1995; Richards and Brogden 1985).

1.1.4 Metronidazole

Metronidazole is a synthetic antiprotozoal and antibacterial compound used to prevent or treat infections caused by anaerobic bacteria (bacteria that can live without oxygen).

Metronidazole is active in vitro against most obligate anaerobes, but does not appear to possess any clinically relevant activity against facultative anaerobes or obligate aerobes. Against susceptible organisms, metronidazole is generally bactericidal at concentrations equal to or slightly higher than the minimal inhibitory concentrations.

Metronidazole as an infusion is used when metronidazole cannot be taken by mouth may also be used in combination with other antibiotics.

Disposition of metronidazole in the body is similar for both oral and intravenous dosage forms, with an average elimination $t_{1/2}$ in healthy humans of 8 hours ranging from 6 to 8 hours (Flagyl Product Monograph and Siegmund et al 1992).

1.2 Summary of relevant pre-clinical/clinical information to date

1.2.1 Pre-clinical information

The pre-clinical safety evaluation program for avibactam alone includes toxicity studies of up to 3 months duration in rats and dogs, safety pharmacology, genetic toxicology, reproductive toxicology (male and female fertility in rats, embryofoetal development in the rat and rabbit), immunotoxicology, local tolerance studies, and an in vitro phototoxicity study. These studies show that avibactam is well tolerated in pre-clinical species and is not associated with target organ toxicity with the exception of local tolerance issues when administered intravenously via a peripheral vein. These local tolerance issues are not seen when avibactam is administered intravenously via a central vein in surgically prepared animals.

The pre-clinical safety evaluation program also includes combination toxicity studies of 1 month duration in rats and dogs with avibactam and ceftazidime (ratio at dosing 1:4 avibactam:ceftazidime). Local tolerance issues were observed at the infusion site in both species (dosing via a peripheral vein). The remaining toxicities observed in animals receiving the combination were considered to be related to the administration of ceftazidime.

For further information refer to the Investigator's Brochure (IB).

1.2.2 Clinical information

At the time of this CSP, 6 clinical pharmacology studies have been completed:

- A Phase I double-blind, placebo-controlled, escalating single dose study with and without ceftazidime in healthy young male subjects (NXL104/1001)
- A Phase I double-blind, placebo-controlled, multiple dose study over 5 or 10 days with and without ceftazidime, intravenous and oral formulations, in healthy young male subjects (NXL104/1002)
- A Phase I open-label, single dose study to assess the effect of renal impairment on PK parameters in patients with varying degrees of renal insufficiency and in patients with end-stage renal failure on hemodialysis (NXL104/1003)

- A Phase I open-label, single dose study to assess effect of age and gender in healthy young and elderly male and female subjects (NXL104/1004)

- A single-center, randomized, double-blind, placebo-controlled, four-way crossover Phase I study to investigate the effect on QT/QTc interval of a single dose of intravenous ceftazidime avibactam (3000/2000 mg) or ceftaroline fosamil avibactam (1500/2000 mg), compared with placebo, using moxifloxacin (Avelox®) as a positive control, in healthy male subjects (D4280C00007)

- A Phase I, randomized, double-blind, placebo-controlled, parallel-group study to assess the safety, tolerability and PK of avibactam alone and in combination with ceftazidime administered as single and repeated intravenous doses in healthy Japanese subjects (D4280C00010)

The completed clinical pharmacology studies at the time of this CSP have demonstrated the PK and tolerability of avibactam alone or in combination with ceftazidime in healthy young and elderly, male and female, and Japanese subjects. The PK and tolerability of avibactam have also been determined in subjects with different degrees of renal impairment. Since CAZ-AVI is administered by intravenous infusion and both ceftazidime and avibactam are predominately excreted unchanged in urine, the drug-drug interaction potential with cytochrome P450 (CYP) inducers or inhibitors is unlikely. Furthermore, avibactam exhibited very little metabolism either in vitro or in vivo, and the inhibition or induction potential was determined to be minimal.

In addition, plasma samples and other disease status and demographic factors, were collected from the Phase II studies evaluating CAZ-AVI plus metronidazole versus meropenem in subjects with cIAI (Study NXL104/2002) and evaluating CAZ-AVI versus imipenem in the treatment of cUTI (Study NXL104/2001). These studies showed both efficacy and safety of avibactam. A population PK analysis has been conducted using the combined data from Phase I studies in healthy subjects and the Phase II study in subjects with cIAI (Study NXL104/2002), and a population PK/pharmacodynamics (PD) analysis has been conducted using data from the Phase II study in patients with cIAI. This data has supported dose selection for the Phase III clinical program.

For further information refer to the IB.

1.3 Rationale for conducting this study

One of the indications that CAZ-AVI is currently being developed for, is the treatment of cIAI. In this setting it is necessary to administer CAZ-AVI with metronidazole to cover anaerobic infections. The objective of this study is to assess whether there is any PK interaction between CAZ-AVI and metronidazole when dosed to steady state.

This study will further establish the safety and tolerability of CAZ-AVI.

1.4 Benefit/risk and ethical assessment

There will be no benefit for subjects participating in this study.

The major risk for healthy subjects who participate in the study is from adverse events (AEs) induced by the investigational products. There are no direct benefits for healthy subjects participating in the study.

Expected adverse reactions for avibactam include infusion site erythema and infusion site hematoma.

Ceftazidime has been licensed for use since 1983 and has been widely used since then, either alone or in combination with other treatments. The safety profile of ceftazidime is well established due to the large database of clinical study safety information and post-marketing experience with this compound. According to the label, the following adverse effects from clinical studies were considered to be either related to ceftazidime therapy or were of uncertain etiology:

- Local effect (fewer than 2% of subjects): phlebitis, pain, and inflammation at the site of the infusion
- Hypersensitivity reactions (in 2% of subjects): pruritus, rash, and fever. Toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme have also been reported with cephalosporin antibiotics, including ceftazidime. Angioedema, urticaria, anaphylaxis (bronchospasm and/or hypotension), and allergic reactions, which, in rare instances, are severe (eg, cardiopulmonary arrest)
- Gastrointestinal symptoms (fewer than 2% of subjects): diarrhea, nausea, vomiting, and abdominal pain. The onset of pseudomembranous colitis symptoms may occur during or after treatment
- Central nervous system reactions (fewer than 1% of subjects): headache, dizziness, and paresthesia. Seizures have also been reported with several cephalosporins, including ceftazidime
- Less frequent AEs (fewer than 1% of subjects): candidiasis (including oral thrush) and vaginitis
- Hematological: rare cases of hemolytic anemia
- Laboratory test changes noted during clinical studies were transient and included: eosinophilia, positive Coombs test without hemolysis, thrombocytosis, hyperbilirubinemia, and slight elevations in one or more of the hepatic enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH], gamma-glutamyl transpeptidase [GGT], and alkaline phosphatase)

- Transient elevations of blood urea nitrogen, and/or serum creatinine

- Transient leucopenia, neutropenia, agranulocytosis, thrombocytopenia, and lymphocytosis

Cephalosporin-class adverse reactions: in addition to the adverse reactions listed above that have been observed in subjects treated with ceftazidime, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics:

- Adverse reactions: colitis, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, and jaundice

- Altered laboratory tests: prolonged prothrombin time, false-positive test for urinary glucose, and pancytopenia

Theoretical, pre-clinical, and clinical findings from the CAZ-AVI development programs as well as known effects of ceftazidime and the cephalosporin class of antibiotics have been considered from a safety perspective and are the basis for the ongoing creation of a Patient Risk Management Plan.

At the time of this CSP preparation, no serious AEs (SAEs) or severe AEs were reported in any of the completed Phase I studies.

In the D4280C0007 study, 1 subject who received a supratherapeutic dose of CAZ-AVI prematurely discontinued from the study due to an AE of urticaria.

In the D4280C00010 study, 1 subject, a healthy 41 year-old randomized male to avibactam alone, had transaminase elevations that were classified as an other significant AE (OAE). After receiving multiple administrations of avibactam, the highest transaminase results were: ALT 522 μ/L (reference range; 17 to 63 μ/L) and AST 246 μ/L (reference range; 15 to 41 μ/L). The transaminases decreased but had not normalized at the time of the last follow-up visit. The subject had no symptoms at the time of the transaminase elevations. The transaminase elevations were considered mild in severity and related to the investigational product.

The safety profile of CAZ-AVI appeared to be similar to those following the individual avibactam dose. The safety data from the Phase II study in subjects with cIAI showed that CAZ-AVI was generally well tolerated.

Metronidazole is an antimicrobial drug with high activity against anaerobic bacteria and protozoa and is available in oral, intravenous, and rectal formulations. Metronidazole is active against a wide range of pathogenic microorganisms, notably species of *Bacteroides*, *Fusobacteria*, *Clostridia*, *Eubacteria*, anaerobic cocci and *Gardnerella vaginalis*, as well as protozoa. It has been proven useful in the prophylaxis and treatment of infections in which anaerobic bacteria have been identified or are suspected to be the cause of infection. The PK

and PD characteristics of metronidazole have been well studied in the past and there are no particular concerns for the generic formulation.

The following adverse/undesirable effects have been reported by subjects receiving metronidazole:

- Common undesirable effects (between 1 and 10% of subjects): diffuse symptoms of intolerance (like nausea, vomiting), metallic taste, stomatitis and glossitis, dry mouth, and myalgia
- Uncommon undesirable effects (between 0.1 and 1% of subjects): leukopenia, headaches, and weakness
- Rare undesirable effects (less than 0.1% of subjects): fever, skin rashes, urticaria, erythema multiforme anaphylactic shock, Quincke edema, and pustolosis
- Neurology: drowsiness, dizziness, ataxia, peripheral neuropathy or transient epileptiform seizures, and hallucinations
- Blood: agranulocytosis, neutropenia, thrombocytopenia, and pancytopenia. Blood dyscrasia is generally reversible but fatal cases have been reported
- Liver: abnormal function tests, cholestatic hepatitis jaundice, and pancreatitis; rare and reversible cases of pancreatitis are reported
- Gastrointestinal: mucositis, epigastralgia, nausea, vomiting, diarrhea, and anorexia
- Urine: darkening of urine
- Eyes: diplopia and myopia
- Herxheimer reaction

Changes in the blood picture as well as peripheral neuropathy observed after prolonged treatment or high dosages generally abate after treatment withdrawal.

Alcoholic beverages and medications containing alcohol should be avoided during treatment with metronidazole and at least 48 hours afterwards because of disulfram-like (antabuse effect) reactions such as warmth, flushing, vomiting, and tachycardia.

Increased effects of oral anticoagulants and a risk of hemorrhage (decrease in liver catabolism) are possible. A large number of subjects have been reported showing an increase in oral anticoagulant activity while receiving concomitant antibiotic therapy. The infectious and inflammatory status of the subject, together with their age and general well-being are all risk factors in this context. However, in these circumstances it is not clear as to the part played by the disease itself or its treatment in the occurrence of prothrombin time disorders.

Some classes of antibiotics are more likely to result in this interaction, notably fluoroquinolones, macrolides, cyclines, cotrimoxazole and some cephalosporins.

To ensure subject safety during the study, routine clinical monitoring will include AEs, vital signs, physical examination findings, routine safety laboratory assessments (hematology, coagulation, clinical chemistry, and urinalysis), clinical assessments, and electrocardiogram (ECG). Standard definitions of AEs will be used and all AEs will be assessed for seriousness, severity and relatedness. Laboratory values and vital signs will be monitored for abnormalities, shifts and changes from baseline.

2. STUDY OBJECTIVES

2.1 Primary objective

To investigate the effect on the PK of ceftazidime, avibactam, and metronidazole when administering CAZ-AVI plus metronidazole in combination compared to administration of the individual components (CAZ-AVI and metronidazole).

2.2 Secondary objectives

To assess safety and tolerability of CAZ-AVI and metronidazole when administered as a 2- and 1-hour infusion, respectively, every 8 hours.

2.3 Exploratory objective

To collect and store plasma samples from healthy volunteers for possible biomarker research.

The exploratory results will not be reported in the Clinical Study Report (CSR).

3. STUDY PLAN AND PROCEDURES

This CSP has been subject to a peer review according to the AstraZeneca standard procedures.

3.1 Overall study design and flow chart

This is an open-label, randomized, 3-way crossover study designed to investigate the effect on the PK of CAZ-AVI and metronidazole, both alone and in combination, following single and multiple administrations in healthy male and female subjects. At least 28 subjects will be enrolled to ensure 24 evaluable subjects. The study duration will be approximately 9 weeks including the follow-up visit.

Subjects will be randomly assigned to 1 of 3 treatment sequences in a 1:1:1 ratio on the morning of Day 1 of Visit 2 as presented in Table 1.

- Treatment A: 2-hour infusion of CAZ-AVI (2000 mg ceftazidime and 500 mg avibactam) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day), and another single infusion on Day 4, total 8 infusions

- Treatment B: 1-hour infusion of metronidazole (500 mg) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day), and another single infusion on Day 4, total 8 infusions

- Treatment C: 1-hour infusion of metronidazole (500 mg) followed by a 2-hour infusion of CAZ-AVI (2000 mg ceftazidime and 500 mg avibactam) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions of each investigational product per day), and another single infusion of each investigational product on Day 4, total 16 infusions (8 infusions of each investigational product). The intravenous line will be flushed with saline solution between administration of metronidazole and CAZ-AVI

Table 1 Treatment periods and sequences

	Period 1	Period 2	Period 3
Sequence ABC	A	B	C
Sequence BCA	B	C	A
Sequence CAB	C	A	B

The study will comprise 5 visits:

Visit 1: subjects will be screened for eligibility within 28 days of Visit 2.

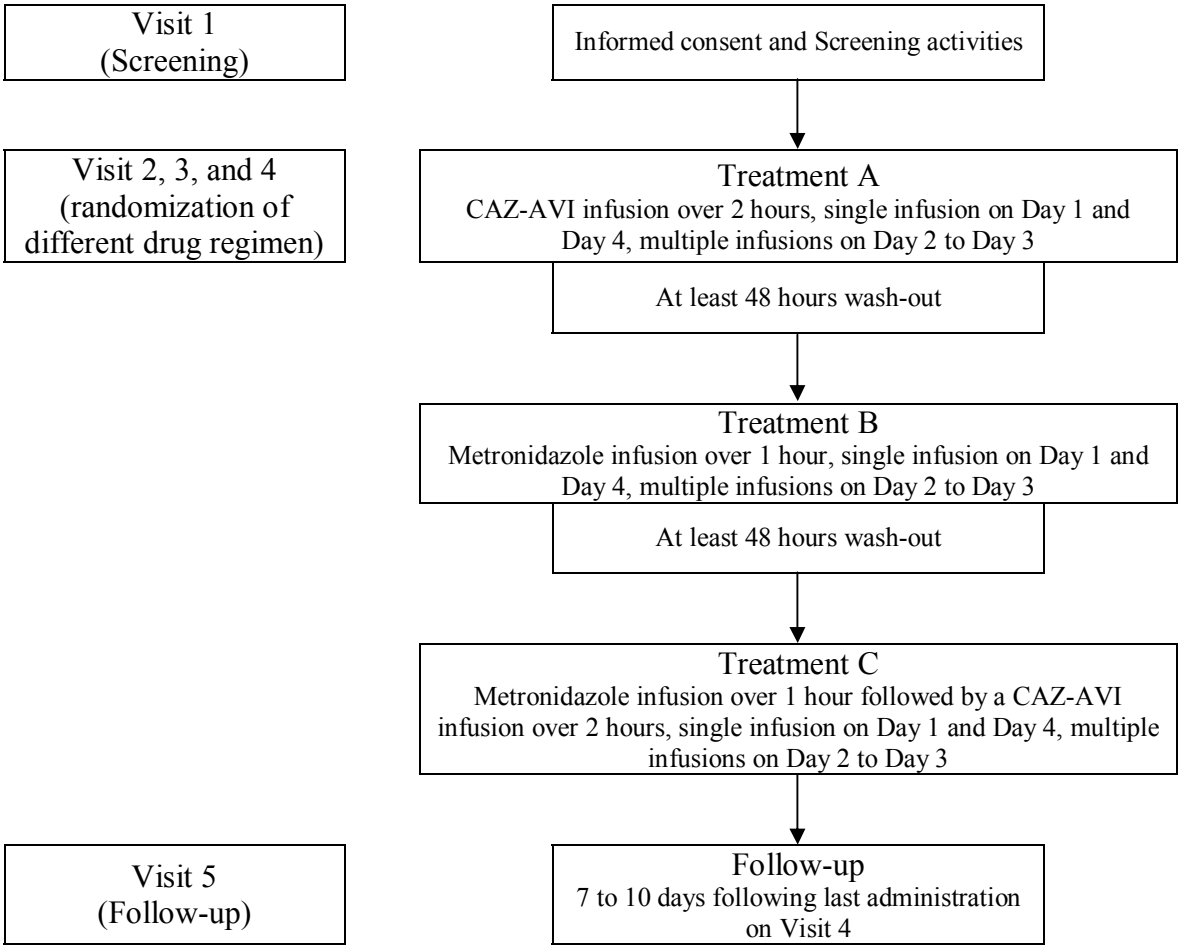
Visits 2, 3, and 4: subjects will be admitted to the study center on Day -1 of each visit and receive treatment in their randomized treatment sequence from Day 1 to Day 4. All subjects will receive a total of 8 infusions during Treatment A and Treatment B and a total of 16 infusions during Treatment C (8 infusions of each investigational product) with a volume of 100 mL per infusion. Subjects will be discharged on Day 5 after the last PK blood sampling, 24 hours after the last infusion of each treatment period. Each visit will be separated by a wash-out period of at least 48 hours.

Visit 5: follow-up assessments will be performed 7 to 10 days after the last investigational product administration at Visit 4.

Subjects may eat normally without any required fasting periods and can have meals before the infusion. Specific food restrictions are listed in Section 5.1.

A flow chart is presented in Figure 1 showing the overall study design diagrammatically.

Figure 1 Flow chart (treatment sequence ABC is illustrated)



The study plan to be followed is presented in Table 2.

Table 2 Study plan

Study Assessments	**Visit 1**[a] Screening ≤28 days prior to Visit 2	**Visit 2, 3, and 4** Randomized Treatment Days -1 to 5 (residential)	**Visit 5** Follow-up 7 to 10 days after Visit 4
Informed consent (including optional biomarker research)	X		
Demographics	X		
Medical/surgical history	X		
Physical examination	X	X[b]	X[n]
Inclusion/exclusion criteria	X	X[c]	
Pregnancy test (female subjects only)	X	X[d]	
Weight and height	X		
Vital signs[e]	X	X[b]	X
Safety paper ECG[f]	X	X[b]	X
Safety laboratory assessments (clinical chemistry, hematology, and urinalysis)	X	X[b]	X
Follicle-stimulating hormone (female subjects only)	X		
Luteinizing hormone (female subjects only)	X		
Urine drugs of abuse, cotinine, and alcohol screen	X	X[g]	
HIV antibody/hepatitis B and C screen	X		
Randomization[h]		X	
Investigational product administration		X	
Blood sampling for PK analysis		X[i]	
Urine sampling for PK analysis		X[j]	
Optional biomarker sampling		X[k]	
Digital ECG		X[l]	
Concomitant medication	X	X	X
Adverse event questioning[m]	X	X	X

[a] Screening can occur up to 28 days prior to Visit 2 and may be divided into 2 separate occasions.
[b] Safety laboratory assessments, vital signs, physical examination, and paper ECG will be performed at Day -1 (admission) and Day 5 before discharge from the study center of each treatment period.
[c] Only on Day -1 and on Day 1 before administration of the investigational product of Visit 2.
[d] Only on Day -1 of each visit.
[e] Vital signs will include resting supine blood pressure and pulse rate. Blood pressure will be measured after the paper ECG.
[f] A paper ECG for safety review by the Investigator will be performed as required.
[g] Day -1 of each treatment period.

[h] Subjects will be randomized to a treatment sequence on Day 1 of Visit 2.

[i] Time points for the measurement of CAZ-AVI and metronidazole concentrations are presented in Table 6, Table 7, and Table 8.

[j] Urine will be collected for 24 hours on Days 1 and 4 to estimate renal clearance of CAZ-AVI at pre-dose and during the following intervals: 0 to 2 hours, 2 to 4 hours, 4 to 8 hours, 8 to 12 hours, and 12 to 24 hours post-dose for Treatments A and Treatment B; 0 to 4 hours, 4 to 8 hours, 8 to 12 hours, and 12 to 24 hours post-dose for Treatment C. The exact collection times and weights of urine will be recorded in the Case Report Form.

[k] The sample can be collected at any time prior to administration of the investigational product.

[l] Time points for digital ECGs are presented in Table 3, Table 4, and Table 5.

[m] All adverse events and serious adverse events will be collected daily from the time of obtaining informed consent through follow-up.

[n] Abbreviated physical examination

ECG: electrocardiogram; HIV: human immunodeficiency virus; PK: pharmacokinetics.

The time points for collection of the digital electrocardiograms (dECG) are presented in Table 3, Table 4, and Table 5 for Treatment A, Treatment B, and Treatment C, respectively.

Table 3 Time schedule for digital electrocardiogram assessments on Treatment A: CAZ-AVI

Study day	ECG number	Adjusted ECG number[a]	Investigational product administration	Time Start hour:min[e]	Stop time	dECG cont.[b,d,e]	Other
1				-01:00			Apply the electrodes[c]
1	1	3		-00:30	-00:20	10 minutes	Pre-dose ECG
1				-00:20	-00:10		Toilet use recommended
1			CAZ-AVI	00:00	02:00		
1	2	5		00:25	00:30	5 minutes	
1	3	6		00:55	01:00	5 minutes	
1	4	7		01:25	01:30	5 minutes	
1	5	8		01:55	02:00	5 minutes	End of CAZ-AVI administration
1	6	10		02:25	02:30	5 minutes	
1	7	12		02:55	03:00[f]	5 minutes	
1	8	14		04:55	05:00	5 minutes	
1	9	15		06:55	07:00	5 minutes	
1	10	16		10:55	11:00	5 minutes	
2	11	17		23:55	24:00	5 minutes	
4				-01:00			Apply the electrodes[c]
4	12	20		-00:30	-00:20	10 minutes	Pre-dose ECG
4				-00:20	-00:10		Toilet use recommended
4			CAZ-AVI	00:00	02:00		
4	13	22		00:25	00:30	5 minutes	
4	14	23		00:55	01:00	5 minutes	

Table 3 Time schedule for digital electrocardiogram assessments on Treatment A: CAZ-AVI

Study day	ECG number	Adjusted ECG number[a]	Investigational product administration	Time Start hour:min[e]	Stop time	dECG cont.[b,d,e]	Other
4	15	24		01:25	01:30	5 minutes	
4	16	25		01:55	02:00	5 minutes	End of CAZ-AVI administration
4	17	27		02:25	02:30	5 minutes	
4	18	29		02:55	03:00[f]	5 minutes	
4	19	31		04:55	05:00	5 minutes	
4	20	32		06:55	07:00	5 minutes	
4	21	33		10:55	11:00	5 minutes	
5	22	34		23:55	24:00	5 minutes	

[a] The "ECG numbers" are adjusted so that all PK/dECG sample pairs in Treatments A and B, respectively, have the same ECG number, at the corresponding time points compared to those for each investigational product in Treatment C. The purpose of this is to facilitate data management at the end of the study.
[b] The subject must be in same supine (maximum 30 degrees flexion in the hip) body position at each time point and at all visits with feet not in contact with footboard.
[c] Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied 30 minutes before first recording.
[d] Time points for dECG may be adjusted according to emerging PK data.
[e] All ECG recordings will be preceded by a 10 minute controlled rest period.
[f] Lunch to be served after the dECG has been taken.
dECG: digital electrocardiogram; ECG: electrocardiogram; PK: pharmacokinetics.

Table 4 Time schedule for digital electrocardiogram assessments on Treatment B: metronidazole

Study day	ECG number	Adjusted ECG number[a]	Investigational product administration	Time Start hour:min[e]	Stop time	dECG cont.[b,d,e]	Other
1				-01:00			Apply the electrodes[c]
1	1	1		-00:30	-00:20	10 minutes	Pre-dose ECG
1				-00:20	-00:10		Toilet use recommended
1			Metronidazole	00:00	01:00		
1	2	2		00:25	00:30	5 minutes	
1	3	3		00:55	01:00	5 minutes	End of metronidazole administration
1	4	5		01:25	01:30	5 minutes	
1	5	6		01:55	02:00	5 minutes	
1	6	8		02:55	03:00	5 minutes	
1	7	12		03:55	04:00[f]	5 minutes	
1	8	14		05:55	06:00	5 minutes	

Table 4 Time schedule for digital electrocardiogram assessments on Treatment B: metronidazole

Study day	ECG number	Adjusted ECG number[a]	Investigational product administration	Time Start hour:min[e]	Stop time	dECG cont.[b,d,e]	Other
1	9	15		07:55	08:00	5 minutes	
1	10	16		11:55	12:00	5 minutes	
2	11	17		23:55	24:00	5 minutes	
4				-01:00			Apply the electrodes[c]
4	12	18		-00:30	-00:20	10 minutes	Pre-dose ECG
4				-00:20	-00:10		Toilet use recommended
4			Metronidazole	00:00	01:00		
4	13	19		00:25	00:30	5 minutes	
4	14	20		00:55	01:00	5 minutes	End of metronidazole administration
4	15	22		01:25	01:30	5 minutes	
4	16	23		01:55	02:00	5 minutes	
4	17	25		02:55	03:00	5 minutes	
4	18	29		03:55	04:00[f]	5 minutes	
4	19	31		05:55	06:00	5 minutes	
4	20	32		07:55	08:00	5 minutes	
4	21	33		11:55	12:00	5 minutes	
5	22	34		23:55	24:00	5 minutes	

[a] The "ECG numbers" are adjusted so that all PK/dECG sample pairs in Treatments A and B, respectively, have the same ECG number, at the corresponding time points compared to those for each investigational product in Treatment C. The purpose of this is to facilitate data management at the end of the study.

[b] The subject must be in same supine (maximum 30 degrees flexion in the hip) body position at each time point and at all visits with feet not in contact with footboard.

[c] Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied 30 minutes before first recording.

[d] Time points for dECG may be adjusted according to emerging PK data.

[e] All ECG recordings will be preceded by a 10 minute controlled rest period.

[f] Lunch to be served after the dECG has been taken.

dECG: digital electrocardiogram; ECG: electrocardiogram; PK: pharmacokinetics.

Table 5 Time schedule for digital electrocardiogram assessments on Treatment C: metronidazole in combination with CAZ-AVI

Study day	ECG number	Adjusted ECG number[a]	Investigational product administration	Time Start hour:min[e]	Stop time	dECG cont.[b,d,e]	Other
1				-01:00			Apply the electrodes[c]
1	1	1		-00:30	-00:20	10 minutes	Pre-dose ECG
1				-00:20	-00:10		Toilet use recommended
1			Metronidazole	00:00	01:00		
1	2	2		00:25	00:30	5 minutes	
1	3	3		00:55	01:00	5 minutes	End of metronidazole administration and pre-dose of CAZ-AVI administration
1			CAZ-AVI	01:00	03:00		
1	4	5		01:25	01:30	5 minutes	
1	5	6		01:55	02:00	5 minutes	
1	6	7		02:25	02:30	5 minutes	
1	7	8		02:55	03:00	5 minutes	End of CAZ-AVI administration
1	8	10		03:25	03:30	5 minutes	
1	9	12		03:55	04:00[f]	5 minutes	
1	10	14		05:55	06:00	5 minutes	
1	11	15		07:55	08:00	5 minutes	
1	12	16		11:55	12:00	5 minutes	
2	13	17		23:55	24:00	5 minutes	
4				-01:00			Apply the electrodes[c]
4	14	18		-00:30	-00:20	10 minutes	Pre-dose ECG
4				-00:20	-00:10		Toilet use recommended
4			Metronidazole	00:00	01:00		
4	15	19		00:25	00:30	5 minutes	
4	16	20		00:55	01:00	5 minutes	End of metronidazole administration and pre-dose of CAZ-AVI administration
4			CAZ-AVI	01:00	03:00		
4	17	22		01:25	01:30	5 minutes	
4	18	23		01:55	02:00	5 minutes	
4	19	24		02:25	02:30	5 minutes	
4	20	25		02:55	03:00	5 minutes	End of CAZ-AVI administration
4	21	27		03:25	03:30	5 minutes	
4	22	29		03:55	04:00[f]	5 minutes	

Table 5 Time schedule for digital electrocardiogram assessments on Treatment C: metronidazole in combination with CAZ-AVI

Study day	ECG number	Adjusted ECG number[a]	Investigational product administration	Time Start hour:min[c]	Stop time	dECG cont.[b,d,e]	Other
4	23	31		05:55	06:00	5 minutes	
4	24	32		07:55	08:00	5 minutes	
4	25	33		11:55	12:00	5 minutes	
5	26	34		23:55	24:00	5 minutes	

[a] The "ECG numbers" are adjusted so that all PK/dECG sample pairs in Treatments A and B, respectively, have the same ECG number, at the corresponding time points compared to those for each investigational product in Treatment C. The purpose of this is to facilitate data management at the end of the study.
[b] The subject must be in same supine (maximum 30 degrees flexion in the hip) body position at each time point and at all visits with feet not in contact with footboard.
[c] Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied 30 minutes before first recording.
[d] Time points for dECG may be adjusted according to emerging PK data.
[e] All ECG recordings will be preceded by a 10 minute controlled rest period.
[f] Lunch to be served after the dECG has been taken.
dECG: digital electrocardiogram; ECG: electrocardiogram; PK: pharmacokinetics.

The time points for collection of the PK blood samples are presented in Table 6, Table 7, and Table 8 for Treatment A, Treatment B, and Treatment C, respectively.

Table 6 Pharmacokinetic blood sampling time points for Treatment A: CAZ-AVI

Sample number	Adjusted dECG number[a]	Study day	Protocol time (hours after start of the first administration on Day 1)	Protocol time (days and hours)[b]
1	3	1	0 (pre-dose)	Day 1, 0 (pre-dose)
2	5	1	0.5	Day 1, 0.5
3	6	1	1	Day 1, 1
4	7	1	1.5	Day 1, 1.5
5	8	1	2	Day 1, 2 (end of CAZ-AVI infusion)
6		1	2.25	Day 1, 2.25
7	10	1	2.5	Day 1, 2.5
8		1	2.75	Day 1, 2.75
9	12	1	3	Day 1, 3[c]
10		1	4	Day 1, 4
11	14	1	5	Day 1, 5
12	15	1	7	Day 1, 7
13	16	1	11	Day 1, 11
14	17	2	23	Day 1, 23 (pre-dose on Day 2)[d]
15	20	4	73	Day 4, 0 (pre-dose)
16	22	4	73.5	Day 4, 0.5
17	23	4	74	Day 4, 1
18	24	4	74.5	Day 4, 1.5
19	25	4	75	Day 4, 2 (end of CAZ-AVI infusion)
20		4	75.25	Day 4, 2.25
21	27	4	75.5	Day 4, 2.5
22		4	75.75	Day 4, 2.75
23	29	4	76	Day 4, 3[c]
24		4	77	Day 4, 4
25	31	4	78	Day 4, 5
26	32	4	80	Day 4, 7
27	33	4	84	Day 4, 11
28	34	5	95	Day 4, 23 (Day 5)

[a] The "ECG numbers" are adjusted so that all PK/dECG sample pairs in Treatments A and B, respectively, have the same ECG number, at the corresponding time points compared to those for each investigational product in Treatment C. The purpose of this is to facilitate data management at the end of the study.
[b] Hours indicated are relative to the time of the start of the infusion of the most recent CAZ-AVI dose.
[c] Lunch to be served after the dECG has been taken.
[d] Pre-dose on Day 2 refers to the morning infusion.
dECG: digital electrocardiogram.

Table 7 Pharmacokinetic blood sampling time points for Treatment B: metronidazole

Sample number	Adjusted dECG number[a]	Study day	Protocol time (hours after start of the first administration on Day 1)	Protocol time (days and hours)[b]
1	1	1	0 (pre-dose)	Day 1, 0 (pre-dose)
2	2	1	0.5	Day 1, 0.5
3	3	1	1	Day 1, 1 (end of metronidazole infusion)
4		1	1.25	Day 1, 1.25
5	5	1	1.5	Day 1, 1.5
6	6	1	2	Day 1, 2
7	8	1	3	Day 1, 3
8	12	1	4	Day 1, 4[c]
9	14	1	6	Day 1, 6
10	15	1	8	Day 1, 8
11	16	1	12	Day 1, 12
12	17	2	24	Day 1, 24 (pre-dose on Day 2)[d]
13	18	4	72	Day 4, 0 (pre-dose)
14	19	4	72.5	Day 4, 0.5
15	20	4	73	Day 4, 1 (end of metronidazole infusion)
16		4	73.25	Day 4, 1.25
17	22	4	73.5	Day 4, 1.5
18	23	4	74	Day 4, 2
19	25	4	75	Day 4, 3
20	29	4	76	Day 4, 4[c]
21	31	4	78	Day 4, 6
22	32	4	80	Day 4, 8
23	33	4	84	Day 4, 12
24	34	5	96	Day 4, 24 (Day 5)

[a] The "ECG numbers" are adjusted so that all PK/dECG sample pairs in Treatments A and B, respectively, have the same ECG number, at the corresponding time points compared to those for each investigational product in Treatment C. The purpose of this is to facilitate data management at the end of the study.
[b] Hours indicated are relative to the time of the start of the infusion of the most recent metronidazole dose.
[c] Lunch to be served after the dECG has been taken.
[d] Pre-dose on Day 2 refers to the morning infusion.
dECG: digital electrocardiogram.

Table 8 Pharmacokinetic blood sampling time points for Treatment C: metronidazole and CAZ-AVI

Sample number	Adjusted dECG number[a]	Study day	Protocol time (hours after start of the first administration on Day 1)	Protocol time (days and hours)[b]
1	1	1	0 (pre-dose)	Day 1, 0 (pre-dose metronidazole infusion)
2	2	1	0.5	Day 1, 0.5
3	3	1	1	Day 1, 1 (end of metronidazole infusion, prior to CAZ-AVI infusion)
4		1	1.25	Day 1, 1.25
5	5	1	1.5	Day 1, 1.5
6	6	1	2	Day 1, 2
7	7	1	2.5	Day 1, 2.5
8	8	1	3	Day 1, 3 (end of CAZ-AVI infusion)
9		1	3.25	Day 1, 3.25
10	10	1	3.5	Day 1, 3.5
11			3.75	Day 1, 3.75
12	12	1	4	Day 1, 4[c]
13		1	5	Day 1, 5
14	14	1	6	Day 1, 6
15	15	1	8	Day 1, 8
16	16	1	12	Day 1, 12
17	17	2	24	Day 1, 24 (pre-dose on Day 2)[d]
18	18	4	72	Day 4, 0 (pre-dose metronidazole infusion)
19	19	4	72.5	Day 4, 0.5
20	20	4	73	Day 4, 1 (end of metronidazole infusion, prior to CAZ-AVI infusion)
21		4	73.25	Day 4, 1.25
22	22	4	73.5	Day 4, 1.5
23	23	4	74	Day 4, 2
24	24	4	74.5	Day 4, 2.5
25	25	4	75	Day 4, 3 (end of CAZ-AVI infusion)
26		4	75.25	Day 4, 3.25
27	27	4	75.5	Day 4, 3.5

Table 8 Pharmacokinetic blood sampling time points for Treatment C: metronidazole and CAZ-AVI

Sample number	Adjusted dECG number[a]	Study day	Protocol time (hours after start of the first administration on Day 1)	Protocol time (days and hours)[b]
28		4	75.75	Day 4, 3.75
29	29	4	76	Day 4, 4[c]
30		4	77	Day 4, 5
31	31	4	78	Day 4, 6
32	32	4	80	Day 4, 8
33	33	4	84	Day 4, 12
34	34	5	96	Day 4, 24 (Day 5)

[a] The "ECG numbers" are adjusted so that all PK/dECG sample pairs in Treatments A and B, respectively, have the same ECG number, at the corresponding time points compared to those for each investigational product in Treatment C. The purpose of this is to facilitate data management at the end of the study.
[b] Hours indicated are relative to the time of the start of the infusion of the most recent metronidazole dose.
[c] Lunch to be served after the dECG has been taken.
[d] Pre-dose on Day 2 refers to the morning infusion.
dECG: digital electrocardiogram.

3.2 Rationale for study design, doses and control groups

The dose levels administered in this study (2000 mg ceftazidime and 500 mg avibactam) are the same dose levels that will be used in the Phase III program. Subjects will receive a single administration on Day 1 and on Day 4 and multiple administrations every 8 hours for 2 days (Day 2 to Day 3). Steady state for all investigational products should be reached by Day 4.

This study will be conducted in healthy subjects in order to avoid interference with the study results from disease processes and other drugs. The inclusion and exclusion criteria are chosen in order to select healthy subjects who are known to be free from any significant illness relevant to the proposed study. For safety reasons, only post-menopausal or surgically sterile women will be included.

A crossover design where each subject acts as his/her own control is used. Inter-individual variability is thereby eliminated. The risk of carry-over has been addressed by a wash-out period between the administrations of the investigational product.

The primary objectives of this study are concentration-time profiles and the resulting PK parameters, which are objective measurements. The risk of bias in this study is therefore minimal and can be conducted open-label.

4. SUBJECT SELECTION CRITERIA

The Investigator should keep a record, the subject screening log, of subjects who entered pre-study screening.

Each subject must meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be any exceptions to this rule.

4.1 Inclusion criteria

For inclusion in the study subjects must fulfill the following criteria:

1. Provision of signed and dated, written informed consent prior to any study-specific procedures

2. Healthy male and female subjects aged 18 to 50 years (inclusive) with suitable veins for cannulation or repeated venipuncture.

3. Female subjects must have a negative pregnancy test at screening and on admission to the study center, must not be lactating, and must be of non-childbearing potential as evidenced by either surgical sterilization or being post-menopausal, defined as:

   - Women under 50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and with luteinizing hormone and follicle-stimulating hormone (FSH) levels in the post-menopausal range

   - Women over 50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments

   If the above criteria are not met, the subject should be regarded as having childbearing potential.

4. Male subjects should be willing to use barrier contraception ie, condoms, from the first day of the investigational product administration until 3 months after the last administration of the investigational product

5. Have a body mass index (BMI) between 19 and 30 kg/m^2

6. Be able to understand and willing to comply with study procedures, restrictions, and requirements, as judged by the Investigator

In addition, for inclusion in the optional biomarker research, subjects should fulfill the following criterion:

7. Provision of signed and dated, written informed consent for the optional biomarker research

   Subjects who refuse to provide written informed consent for the biomarker research will not be excluded from other aspects of the study described in the CSP, provided they give written informed consent

4.2 Exclusion criteria

Subjects must not be randomized in the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study

2. History or presence of gastrointestinal, hepatic or renal disease, or any other condition known to interfere with absorption, distribution, metabolism or excretion of drugs

3. Known history of serious allergy, hypersensitivity (eg, anaphylaxis), or any serious reaction to carbapenem or cephalosporin antibiotics or other β-lactam antibiotics or any other investigational product to be administered as part of the study

4. Any clinically significant illness, medical/surgical procedure or trauma within 4 weeks prior to the first administration of investigational product

5. Any clinically significant abnormalities in physical examination, ECG, clinical chemistry, hematology or urinalysis results, as judged by the Investigator

6. Abnormal vital signs, after 10 minutes supine rest, defined as any of the following:

   - Systolic blood pressure (SBP) >140 mmHg
   - Diastolic blood pressure (DBP) >90 mmHg
   - Heart rate <40 or >85 beats per minute

7. Prolonged QTcF (>450 ms) or shortened QTcF (<350 ms) or a family history of long QT syndrome

8. Any positive result on screening for serum hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody or the human immunodeficiency virus (HIV)

9. Known or suspected history of drug abuse, as judged by the Investigator

10. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator

11. Positive screen for drugs of abuse or cotinine (nicotine) at screening or on admission to the study center or positive screen for alcohol on admission to the study center prior to the first administration of the investigational product

12. Current smokers or ex-smokers who have smoked or used nicotine products within the previous 3 months and/or have smoked more than 10 pack years [number of pack years = (number of cigarettes smoked x number of years)/20]

13. Use of any prescribed or non-prescribed medication including antacids, analgesics other than paracetamol/acetaminophen, herbal remedies, vitamins, and minerals during the 2 weeks prior to the first administration of investigational product or longer if the medication has a long half-life. Occasional use of paracetamol/acetaminophen is allowed for minor pains and headache

14. Plasma donation within 1 month of screening or any blood donation/blood loss during the 3 months prior to screening

15. Has received another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment within 1 month of the first administration of investigational product in this study. The period of exclusion begins at the time of the last visit of the prior study. Note: subjects consented and screened, but not dosed in this study or a previous Phase I study are not excluded

16. Involvement in the planning and/or conduct of the study (applies to and personnel, and any other personnel involved in the study)

17. Any intake of grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges within 7 days of the first administration of the investigational product

18. Excessive intake of caffeine-containing drinks eg, coffee (more than 5 cups of coffee or the equivalent per day), tea, caffeine-containing energy drinks, and cola

19. Judgment by the Investigator that the subject should not participate in the study if he/she is considered unlikely to comply with study procedures, restrictions, and requirements

20. A serum β-human chorionic gonadotropin (HCG) pregnancy test must be drawn for women of childbearing potential at the screening visit. If the results of the serum

β-HCG cannot be obtained prior to administration of the investigational product, a subject may be enrolled on the basis of a negative urine pregnancy test, though serum β-HCG must still be obtained. If either test is positive, the subject should be excluded. Since urine and serum tests may miss a pregnancy in the first days after conception, relevant sexual history, including methods of contraception, should be considered. Any subject whose sexual history suggests the possibility of early pregnancy should be excluded

21. Known history of past or current epilepsy or seizure disorders, excluding febrile seizures of childhood

22. Subject is unlikely to survive the 6- to 8-week study period or has a rapidly progressive or terminal illness

Procedures for withdrawal of incorrectly enrolled subjects see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

The following restrictions apply for the specified times during the study period:

1. Eat and drink only the standardized meals and drinks provided (apart from water) during the residential period in the study center

2. Abstain from consuming any of the following:

   - Alcohol from 72 hours before admission, during the residential periods, for an additional 24 hours after discharge, and for 72 hours before the follow-up visit

   - Energy drinks containing taurine or glucuronolactone eg, Red Bull from 72 hours before admission, during the residential periods, and for 72 hours before the follow-up visit

   - Poppy seeds found in specialty bread from the time of consent until after the final assessment at the follow-up visit

   - Grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade, or other products containing grapefruit or Seville oranges from 7 days before admission until after the final assessment at the follow-up visit

3. Limit caffeine intake to 5 cups per day from enrolment and limit to 3 cups per day at meal times from admission to the study center and during the study visits

4. Abstain from nicotine use, smoking, and drugs of abuse from the time of consent until after the final assessment at the follow-up visit

5. Abstain from taking any prescribed or non-prescribed medication including antacids, analgesics other than paracetamol/acetaminophen, herbal remedies, vitamins, and minerals during the 2 weeks prior to the first administration of investigational product or longer if the medication has a long half-life until after the final assessment at the follow-up visit. Occasional use of paracetamol/acetaminophen is allowed for minor pains and headache. However, this should not obviate necessary medical treatment

6. Refrain from strenuous physical activity, which is not within the subject's normal daily routine, from 7 days prior to admission to the study center until after the final assessment at the follow-up visit

7. Abstain from blood or plasma donation until 3 months after the final assessment at the follow-up visit

8. Abstain from scheduled in-patient surgery or hospitalization during the course of the study

9. Male subjects should use a condom to prevent pregnancy and drug exposure of a partner, and refrain from donating sperm or fathering a child from the first investigational product administration until 3 months after the last administration of the investigational product

5.2 Subject enrolment and randomization and initiation of investigational product

The Investigator will:

1. Obtain signed and dated informed consent from the potential subject before any study-specific procedures are performed

2. Assign the potential subject a unique enrolment number, beginning with 'E#'

3. Determine subject eligibility. See Sections 4.1 and 4.2

4. Assign each eligible subject a unique subject number and a unique randomization code

If a subject withdraws from participation in the study, then his/her enrolment/randomization code cannot be re-used. Additional randomization numbers will be included in the randomization schedule to use for replacement subjects. Subject numbers and replacement subject numbers will have the same number of digits.

5.2.1 Procedures for randomization

A randomization scheme will be produced by using the AstraZeneca global randomization system (GRAND). Subjects will be randomized to 1 of 3 treatment sequences in a 1:1:1 ratio on the morning of Day 1 (Visit 2).

Randomization codes will be assigned strictly sequentially as subjects become eligible for randomization.

5.3 Procedures for handling subjects incorrectly enrolled or randomized or initiated on investigational product

Subjects who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be enrolled or randomized. There can be no exceptions to this rule.

Where subjects that do not meet the selection criteria and are incorrectly started on treatment, randomized, or where subjects subsequently fail to meet the study criteria after initiation, a discussion should occur between the AstraZeneca Clinical Pharmacology Alliance (CPA) Physician and the Investigator regarding whether to continue or discontinue the subject from treatment.

The AstraZeneca CPA Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, further administration of the investigational product should be stopped.

5.4 Blinding and procedures for unblinding the study

This study will be open-label.

5.5 Treatments

5.5.1 Identity of investigational products

AstraZeneca, or a company acting on its behalf, will supply the investigational products to the study center. The investigational products used in this study are presented in Table 9.

Table 9 Identity of the investigational products

Investigational product	Dosage form (strength)	Manufacturer
Ceftazidime avibactam	Powder for concentrate solution for infusion, 2000 mg/500 mg per vial	
Metronidazole	Solution for infusion 500 mg/100 ml	

The CAZ-AVI will be packaged and labeled in accordance with current Good Manufacturing Practice (GMP) and supplied to the study center. Commercially available metronidazole will be sourced and supplied to the study center.

The investigational products will be administered intravenously. The CAZ-AVI will be reconstituted and transferred to the infusion bag. The metronidazole is provided as a ready to use solution.

Handling instructions detailing how the infusions are to be prepared will be provided to the study center by

5.5.2 Doses and treatment regimens

Subjects will each receive the following:

- Treatment A: 2-hour infusion of CAZ-AVI (2000 mg ceftazidime and 500 mg avibactam) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day), and another single infusion on Day 4, total 8 infusions

- Treatment B: 1-hour infusion of metronidazole (500 mg) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions per day), and another single infusion on Day 4, total 8 infusions

- Treatment C: 1-hour infusion of metronidazole (500 mg) followed by a 2-hour infusion of CAZ-AVI (2000 mg ceftazidime and 500 mg avibactam) on the morning of Day 1, every 8 hours from Day 2 to Day 3 (inclusive) (3 infusions of each investigational product per day), and another single infusion of each investigational product on Day 4, total 16 infusions (8 infusions of each investigational product). The intravenous line will be flushed with saline solution between administration of metronidazole and CAZ-AVI

A wash-out period of at least 48 hours will separate the treatment periods.

An appropriate flush with 0.9% sodium chloride infusion solution should be administered at the end of the infusion to ensure the patient receives the whole dose. The flush should be administered according to local procedures and be appropriate for the infusion lines used by the study center.

Subjects may eat normally without any required fasting periods and can have meals before the infusion. Specific food restrictions are listed in Section 5.1.

5.5.3 Labeling

Labels will be prepared in accordance with GMP and local regulatory guidelines. Label text will be in English.

5.5.4 Storage

All investigational products should be kept in a secure place under appropriate storage conditions in the pack provided. Descriptions of the appropriate storage are specified on the investigational product label.

5.6 Concomitant and post-study treatment(s)

Subjects must abstain from taking any prescribed or non-prescribed medication including antacids, analgesics other than paracetamol/acetaminophen, herbal remedies, vitamins, and minerals during the 2 weeks prior to the first administration of investigational product or longer if the medication has a long half-life until after the final assessment at the follow-up visit. Occasional use of paracetamol/acetaminophen is allowed for minor pains and headache. However, this should not obviate necessary medical treatment.

Medication, considered necessary for the subject's safety and well-being, may be given at the discretion of the Investigator and recorded in the appropriate sections of the electronic Case Report Form (eCRF).

5.7 Treatment compliance

The administration of all investigational products should be recorded in the appropriate sections of the eCRF.

Treatment compliance will be assured by supervised administration of the investigational product by the Investigator or a qualified representative. The dose, date, and time of administration of the investigational product will be recorded by the Investigator and checked by the monitor at monitoring visits.

5.7.1 Accountability

The investigational product provided for this study will be used only as directed in this CSP.

The study center personnel will account for all investigational products administered to the subject.

The study center personnel will account for all investigational products received at the study center, unused investigational product, and for appropriate destruction. Certificates of delivery and destruction should be signed.

5.8 Discontinuation of investigational product

Subjects must be discontinued from the investigational product in the following situations:

- Subject is considered to be at increased risk, as judged by the Investigator and/or
- Eligibility criteria not fulfilled
- Subject lost to follow-up
- Death

- Any significant and clinically relevant changes in the safety parameters (eg, ECG, blood pressure, pulse rate, laboratory measurements, and AEs) causing continuation of the investigational product administration to be unjustified

- A QTc prolongation defined as QTcF >500 ms or an increase in QTcF >60 ms above baseline confirmed (persistent for ≥5 minutes) and determined post-dose either during continuous 12-lead ECG monitoring or on a repeat 12-lead ECG

- Positive pregnancy test at any time during the study

Individual subject discontinuation and intensified monitoring criteria:

Liver chemistry variable	**Intensified Monitoring criteria**	**Individual subject withdrawal criteria**
ALT	Level >2 x ULN, monitor at least every 48 hours until return to within normal limits (WNL) or stable per the Investigator	Level >3 x ULN
ALP	Increase by >100%: check GGT, monitor at least every 48 hours until return to WNL or stable per the Investigator	>2 x ULN
Bilirubin	>1.5 x ULN, monitor at least every 48 hours until return to WNL or stable per the Investigator	>2 x ULN

Any subject who meets the individual subject withdrawal criteria in the table above will be withdrawn from the study and advised to continue assessments to ensure his/her safety.

Subjects may be discontinued from the investigational product in the following situations:

- Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment

- Severe non-compliance to the CSP, as judged by the Investigator and/or AstraZeneca

- AEs

<u>Study review criteria</u>

- If any **2 subjects** meet any of the individual discontinuation criteria for the study, the Investigator will contact the AstraZeneca study physician to initiate a review process that will, at minimum, include the Medical Science Director and the Global

Safety Physician. The review process will consider study and subject information, and determine the subsequent necessary actions

Study stopping criteria

The study should be stopped in the following situations:

- 3 or more subjects have either >3 x the ULN of ALT or 3 subjects have >2 x ULN ALP or 3 subjects have elevations of total bilirubin >2 x ULN
- 1 or more subjects, who fulfill Hy's law defined as ALT >3 x ULN and total bilirubin >2 x ULN in the absence of significant increase in ALP and in the absence of an alternative diagnosis that explains the increase in total bilirubin (see Appendix D for follow-up procedures)
- 2 or more subjects have a QTc prolongation defined as QTcF >500 ms or an increase in QTcF >60 ms above baseline confirmed (persistent for ≥5 minutes) and determined post-dose either during continuous 12-lead ECG monitoring or on a repeat 12-lead ECG

5.8.1 Procedures for discontinuation of a subject from investigational product

A subject who decides to discontinue the investigational product administration will always be asked about the reason(s) and the presence of any AEs. If possible, he/she will be seen and assessed by the Investigator. Adverse events will be followed up (see Sections 6.3.3 and 6.3.4).

If a subject is withdrawn from the study, see Section 5.9.

5.9 Withdrawal from study

Subjects are at any time free to withdraw from the study (investigational product and study assessments), without prejudice to further treatment. Such subjects will always be asked about the reason(s) and the presence of any AEs. If possible, he/she will be seen and assessed by the Investigator. Withdrawn subjects will undergo follow-up assessments 7 to 10 days after the last investigational product administration. Adverse events will be followed up (see Sections 6.3.3 and 6.3.4).

Withdrawn subjects may be replaced to ensure 24 evaluable subjects.

When a subject withdraws from the study, he/she should be specifically asked whether he/she also withdraws consent for the optional safety biomarker research.

6. COLLECTION OF STUDY VARIABLES

See Table 2 for the study assessments to be performed.

When more than 1 assessment is required at a particular time point, PK samples should take precedence.

6.1 Recording of data

Subject data will be collected by using the Phase I electronic data capture (EDC) system. The Investigator will ensure that data are recorded in the eCRF as specified in the CSP and in accordance with the instructions provided.

The Investigator ensures the accuracy, completeness, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. The Investigator will sign the completed eCRFs. A copy of the completed eCRFs will be archived at the study center.

Procedures for data editing, entry, and handling of the data query process will be described in the Data Management Plan.

6.2 Data collection at enrolment and follow-up

6.2.1 Enrolment procedures

Each subject will undergo screening to confirm eligibility. This will consist of the following:

- Obtaining informed consent (and optional informed consent for biomarker sample collection) before starting any study-specific procedures
- Review of the inclusion/exclusion criteria with the subject
- Recording of demographic data (date of birth, gender, and race)
- A standard recording of medical and surgical history
- A complete physical examination
- Height, weight, and calculation of BMI
- Vital signs (resting supine SBP, DBP, and pulse rate) (after ECG)
- Blood sampling for routine clinical chemistry and hematology measurements, a HBsAg, HCV antibody, and HIV screen, and FSH (female subjects only)
- Pregnancy test (female subjects only)
- Urine sampling for routine urinalysis
- Drugs of abuse, cotinine, and alcohol screen in urine
- Paper ECG

- AE questioning
- Concomitant medication recording

6.2.2 Follow-up procedures

Follow-up procedures will be performed 7 to 10 days after the last investigational product administration at Visit 4. These assessments will include an abbreviated physical examination, vital signs, paper ECG, safety laboratory assessments, AE questioning, and concomitant medication recording.

6.3 Safety

The Investigator is responsible for ensuring that all personnel involved in the study are familiar with the content of this section.

6.3.1 Definition of adverse events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver), or the abnormal results of an investigation (eg, laboratory findings, ECG). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or wash-out periods, even if no investigational product has been administered.

The term AE is used to include both serious and non-serious AEs.

6.3.2 Definitions of serious adverse event

An SAE is an AE or suspected adverse reaction occurring during any study phase (ie, run-in, treatment, wash-out, follow-up), which in the view of either the Investigator or AstraZeneca, results in any of the following:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent 1 of the outcomes listed above

For further guidance on the definition of an SAE, see Appendix B to this CSP

6.3.3 Recording of adverse events

Time period for collection of adverse events

All AEs (including SAEs) will be collected from the time of signing the informed consent and throughout the study, including the follow-up period.

Follow-up of unresolved adverse events

Any AEs that are unresolved at follow-up will be monitored by the Investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE:

- AE (verbatim)
- Date and time when the AE started and stopped
- Intensity, rated according to the following scale:
  - Mild (awareness of sign or symptom, but easily tolerated)
  - Moderate (discomfort sufficient to cause interference with normal activities)
  - Severe (incapacitating, with inability to perform normal activities)
- Whether the AE is serious or not
- Investigator causality rating against the investigational product (yes or no)
- Action taken with regard to the investigational product
- Whether the AE caused the subject's withdrawal from study (yes or no)
- Outcome

In addition, the following variables will be collected for SAEs:

- Date the AE met the SAE criteria
- Date the Investigator became aware of the SAE
- AE is serious due to
- Date of hospitalization

- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to the study procedure(s)
- Causality assessment in relation to other medication
- Description of the AE

It is important to distinguish between SAEs and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

Causality collection

The Investigator will assess the causal relationship between the investigational product and each AE, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAEs, the causal relationship will also be assessed for other medication and study procedures and any additional investigational product. Note that for SAEs that could be associated with any study procedure, the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix B to this CSP.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study center personnel: 'Have you had any health problems since the previous visit/you were last asked?' or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from the CSP-mandated laboratory tests and vital signs will be summarized in the CSR. Deterioration as compared to baseline in the CSP-mandated laboratory values, vital

signs, or ECG should therefore only be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory/vital signs value is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory/vital signs result will be considered as additional information. Wherever possible the Investigator uses the clinical, rather than the laboratory term (eg, anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Subjects with ALT >2 x ULN or with an ALP increase of 100% or with total bilirubin >1.5 x ULN will qualify for intensified monitoring. Detailed liver chemistry (including GGT) sampling aimed at identifying the cause of changes observed during routine monitoring should be done for all subjects fulfilling intensified monitoring criteria. Monitor liver enzymes at least 48 hours until the elevated levels return to within the normal limits or are stable, as judged by the Investigator. See Section 5.8 for individual subject investigational product discontinuation criteria.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination, as compared with the baseline assessment, will be reported as an AE.

NB. Cases where a subject shows an AST **or** ALT value >3 x ULN **or** total bilirubin >2 x ULN may need to be reported as SAEs, please refer to Appendix E 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the eCRF.

If any SAE occurs during the course of the study, then Investigators or other site personnel inform the appropriate AstraZeneca representatives immediately or **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within 1 calendar day** of initial receipt for fatal and life-threatening events **and within 3 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. The Investigator or other study center personnel will inform the representatives of any follow-up information on a previously reported SAE within 1 calendar day, ie, immediately but **no later than the end of the next business day** of when he/she becomes aware of it.

The reference document for definition of expectedness/listedness is the CAZ-AVI IB.

6.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, hematology, and urinalysis will be collected at the time points specified in the study plan (Table 2).

The safety laboratory variables are presented in Table 10.

Table 10 Safety laboratory variables

Clinical chemistry (serum)	**Hematology (blood)**
Alkaline phosphatase	Hemoglobin
Aspartate aminotransferase	Platelet count
Alanine aminotransferase	Leukocyte count[a]
Albumin	Leukocyte differential count[a]
Total bilirubin	Neutrophils
Creatinine	Eosinophils
Total calcium	Basophils
Potassium	Lymphocytes
Sodium	Monocytes
Glucose (preferably fasting)	
Thyroid stimulating hormone[a]	**Urinalysis**
Urea	Blood
	Protein
Endocrinology	Glucose
Free thyroxine[a]	Creatinine

Serology	**Other**
HIV and Hepatitis B and C[a]	Follicle-stimulating hormone (female subjects only)[a]
	β-HCG pregnancy test
	Luteinizing hormone (female subjects only)

[a] At screening only.
β: beta, HCG: human chorionic gonadotropin, HIV: human immunodeficiency virus.

NB. In case a subject shows an AST **or** ALT of >3 x ULN **or** total bilirubin >2 x ULN please refer to Appendix E 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

Urine will be tested for the following drugs of abuse, cotinine, and alcohol at screening and Day -1 of each visit: amphetamines, barbiturates, tricyclic anti-depressants, cocaine, methadone, morphine, phencyclidine, tetrahydrocannabinol, and opiates.

Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated. Subjects in whom suspected clinical significance is confirmed, will either not be included in the study or if already enrolled, will be monitored until normalization or for as long as the Investigator considers necessary. Additional laboratory assessments may be performed for safety reasons if judged necessary by the Investigator.

The safety laboratory samples will be analyzed using routine methods at the Pathology Laboratory of in the United Kingdom.

For blood volume see Section 7.1.

6.3.6 Physical examination

A full physical examination, according to normal clinical routines, will be performed on all subjects at the time points specified in the study plan (Table 2) and will include an examination of general appearance, skin, lymph nodes, thyroid, musculo-skeletal/extremities, neurological condition, mouth, teeth, throat, cardiovascular system, lungs, and abdomen. The outcome of the examination is to be recorded as normal/abnormal in the eCRF, with any abnormalities specified. Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Only a brief physical examination is required at follow-up. Only information on whether the assessment was performed or not and any AEs are to be recorded in the study database.

Height will be measured in centimeters and weight in kilograms. Measurements should be taken without shoes and the same scale used for all measurements. The BMI will be calculated from the height and weight measurements.

6.3.7 ECG

6.3.7.1 Resting 12-lead ECG

12-lead ECG recordings will be obtained at the time points specified in the study plan (Table 2), after 10 minutes' rest in the supine position. Paper ECGs for safety review by the Investigator will be performed at additional intervals if required. The outcome of the overall evaluation is to be recorded as normal/abnormal in the eCRF, with any abnormalities specified. The print-out of the ECG is to be signed, dated, and filed in the Investigator's Study File, along with a signed and dated copy (if the print-outs are not on archive-quality paper).

6.3.7.2 Digital ECG

will perform the dECG analysis in this study, using the EClysis© system, Version 3.3, or higher.

At protocol-indicated time points (Table 3, Table 4, and Table 5), 12-lead continuous dECG will be recorded over at least 5 minutes with the Schiller Cardiovit CS-200 recorder (Schiller AG, Baar, Switzerland) and transmitted to the AZ central dECG repository, according to AstraZeneca ECG Center's standard procedures for settings, recording, and transmission of dECGs.

The same recorder will be used for each subject at all time points, when possible. Date and time settings must be checked at the start of each study day and aligned with an official timekeeper for all machines used in the study.

Skin preparation must be thorough and electrode positions must be according to standard 12-lead ECG placement. Electrode positions for dECG take precedence over telemetry electrodes. Electrode positions will be marked with an indelible pen at the start of each study day to ensure exact reposition. Permanent electrodes will be applied at least 30 minutes before the first study recording and left in place for the duration of each relevant study day.

Subjects will rest in a supine position for at least 10 minutes before the start of each recording. The subject should be in the same supine body position (maximum 30 degrees flexion of the hip and feet not in contact with the footboard) at each recording time point during the study.

The metadata for all dECG files will be checked and approved by the responsible personnel at the study center to ensure that the files transferred to the AstraZeneca central dECG files repository are made accessible to the ECG Scientific Advisors for analysis.

As standard, 10-second ECGs are extracted by the system twice per minute from the continuous recording and initially automatically analyzed by the software.

Lead V2 will be analyzed and reported as primary. Lead V5 will be analyzed, for all visits, as backup for the individual where analysis in lead V2 is not deemed possible for pre-dose, for significant parts of whole visits or for whole visits. The analysis is performed blinded to treatment.

The ECG Scientific Advisor will perform a preliminary analysis of the first 24 hours (as a minimum) of dECG recordings, in lead V2, with main focus on QT changes, wave morphology changes, and dysrhythmia. The ECG Center Cardiologist will review the data, perform an evaluation and interpretation of findings, and will provide a safety report.

The ECG Scientific Advisor will perform all required manual adjustments to the ECG annotations provided automatically by EClysis©. Finally, an external expert cardiologist will review the totality of data and perform all necessary adjustments before locking the EClysis© data into a read-only state.

The numerical values for ECG intervals and amplitudes will be exported and made accessible on the AstraZeneca central dECG repository to accredited data management specialists for conversion into SAS files. The following variables will be reported by the AstraZeneca ECG

Center: RR, PR, QRS, and QT intervals from the lead defined as primary in the protocol. Derived parameters (QTcF, heart rate (HR), and others, as applicable) are calculated by the study statistician or designee.

6.3.8 Vital signs

6.3.8.1 Pulse rate and blood pressure

Supine pulse rate (bpm) and blood pressure (mmHg) will be measured at the time points specified in the study plan (Table 2). Measurements will be performed according to local procedures after 10 minutes' rest. Blood pressure, SBP and DBP, will be measured using the same cuff size, appropriate for arm circumference, throughout the study.

6.4 Pharmacokinetics

6.4.1 Collection of samples

Blood samples (4 mL) for the determination of avibactam, ceftazidime and metronidazole in plasma will be taken at the times presented in Table 6, Table 7, and Table 8.

The volumes of urine collected over the time intervals presented in Table 2 will be recorded, and 10 mL aliquots from each collection interval will be stored for the determination of avibactam and ceftazidime in urine.

Blood and urine samples will be collected, labeled, stored, and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

6.4.2 Determination of drug concentration

Samples for determination of avibactam, ceftazidime and metronidazole concentrations in plasma and avibactam and ceftazidime concentrations in urine will be analyzed by on behalf of the AstraZeneca.

6.5 Collection of biomarker samples

Subjects **not on any concomitant medications** will be offered the option to participate in biomarker research. After giving written consent for optional biomarker research, a plasma sample will be collected in accordance with the inclusion criteria and study plan (Table 2).

The plasma sample must be collected before administration of any investigational product. Only 1 sample should be collected per subject for biomarker research during the study. A 10 mL plasma sample will be collected into a lithium heparin tube. Samples will be collected, labeled, stored, and shipped as detailed in the Safety Biomarker Laboratory Manual.

Some of the dataset (eg, age, gender, race, body weight, height, BMI, and fasting state) from the main study may be duplicated within AstraZeneca for exploratory analyses in combination with the optional biomarker data. This information will be provided to AstraZeneca with the

biomarker samples shipment. Neither the subjects' name nor any other personal identifiers will be part of this dataset. Optional biomarker data will not be reported in the CSR.

For blood volume see Section 7.1.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be collected from each subject is presented in Table 11.

Table 11 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)[a]	No. of samples	Total volume (mL)
Safety	Clinical chemistry	8.5	8	68
	Hematology	4	8	32
	Serology	8.5	1	8.5
Pharmacokinetics		4	86	344
Follicle-stimulating hormone/luteinizing hormone[b]		3.5	1	3.5
Biomarker[c]		10	1	10
Total				**466**

[a] If a cannula is used, an additional 1 mL of blood will be collected to flush the cannula at each sample time point.
[b] Female subjects only.
[c] Optional.

The number of samples collected and the volume required for each analysis may be changed during the study (ie, if additional samples are collected for repeated safety assessments). However, the maximum volume to be collected from each subject will not exceed 600 mL (samples total volume together with cannula flushing volume).

7.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed of after analyses or retained for further use as described in this section.

7.2.1 Safety samples

Safety samples will be disposed of after analysis.

7.2.2 Pharmacokinetic samples

Samples will be disposed of after the CSR has been finalized, unless retained for future analyses.

Selected PK samples may be used for metabolite identification and/or quantification. These samples will be retained by, or on behalf of, AstraZeneca for a maximum of 5 years following the finalization of the CSR. The results from any metabolite investigation will not be reported in the CSR, but separately in a metabolism report.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples and reported in a separate bioanalytical report.

7.2.3 Biomarker samples

Biological samples for biomarker research can be retained on behalf of AstraZeneca for a maximum of 15 years following the last subject's last visit in the study. The results from future analysis will not be reported in the CSR, but separately in a Scientific Report.

7.3 Labeling and shipment of biohazard samples

The Investigator ensures that samples are labeled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B Regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C International Air Transport Association (IATA) 6.2 Guidance Document.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labeling, shipment, and containment provisions are approved.

7.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their life cycle.

The Investigator keeps full traceability of collected biological samples from the subjects while in storage at the study center until shipment or disposal (where appropriate) and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps documentation of receipt of arrival.

AstraZeneca oversees the entire life cycle through internal procedures, monitoring of the study center, and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca biobank system during the entire life cycle of the sample.

7.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of or destroyed, and the action documented. If samples are already analyzed, AstraZeneca is not obliged to destroy the results of this research.

As collection of the biological samples is an integral part of the study, the subject will then be withdrawn from further study participation. However, as collection of the biomarker sample is an optional part of the study, the subject may continue in the study.

The Investigator will:

- Ensure that the subject's withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca
- Ensure that biological samples from that subject, if stored at the study center, are immediately identified, disposed of or destroyed, and the action documented
- Ensure that the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of or destroyed, the action documented, and the signed document returned to the study center
- Ensure that the subject and AstraZeneca are informed about the sample disposal

AstraZeneca ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of or destroyed and the action documented and returned to the study center.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with the International Conference on Harmonization (ICH)/Good Clinical Practice (GCP), applicable regulatory requirements, and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

8.3 Ethics and regulatory review

An Ethics Committee (EC) should approve the final CSP, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to

the subjects. The Investigator will ensure the distribution of these documents to the applicable EC and to the study center personnel.

The opinion of the EC should be provided in writing. The Investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study.

The EC should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form needed to meet local requirements.

If required by local regulations, the CSP should be re-approved by the EC annually.

Before enrolment of any subject into the study, the final CSP, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide regulatory authorities, the EC, and the Investigator with safety updates/reports according to local requirements, including suspected unexpected serious adverse reactions, where relevant.

8.4 Informed consent

The Investigator will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject

- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the Informed Consent Form that is approved by an EC

8.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Investigator and AstraZeneca.

If there are any substantial changes to the CSP, then these changes will be documented in an Amendment and where required in a new version of the CSP (Revised CSP).

The Amendment should be approved by an EC and if applicable, also the national regulatory authority, before implementation. Local requirements should be followed for Revised CSPs.

AstraZeneca will distribute any subsequent Amendments and Revised CSPs to the Investigator. For distribution to the EC see Section 8.3.

If an Amendment requires a change to the Informed Consent Form, AstraZeneca and the EC should approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the EC.

8.6 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority or an EC may perform audits or inspections at the study center, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted and data were recorded, analyzed and accurately reported according to the CSP, GCP, ICH guidelines, and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the study center.

9. STUDY MANAGEMENT BY ASTRAZENECA

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for a representative of AstraZeneca to visit the study center to:

- Determine the adequacy of the facilities
- Determine availability of appropriate subjects for the study
- Discuss with the Investigator (and other personnel involved with the study) their responsibilities with regard to CSP adherence and the responsibilities of

AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the Investigator

9.2 Training of study center personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of this CSP and related documents with the study center personnel and also train them in any study-specific procedures and system(s) utilized.

The Investigator will ensure that appropriate training relevant to the study is provided to all of these personnel and that any new information relevant to the performance of this study is forwarded to all the personnel involved.

The Investigator will maintain a record of all individuals involved in the study (medical, nursing, and other personnel).

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contact with the study center, including visits to:

- Provide information and support to the Investigator
- Confirm that facilities remain acceptable
- Confirm that the study center personnel is adhering to the CSP, that data are being accurately and timely recorded in the eCRFs, that biological samples are handled in accordance with the Laboratory Manual and that the investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of or destroyed accordingly and the action is documented and reported to the subject

The AstraZeneca representative will be available between visits if the Investigator or other personnel at the study center needs information and advice about the study conduct.

9.3.1 Source data

Refer to the Clinical Study Agreement for location of source data.

9.4 Study agreements

The Investigator at the study center should comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this CSP and the Clinical Study Agreement, the terms of the CSP shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the Clinical Study Agreement shall prevail.

Agreements between AstraZeneca and the Investigator should be in place before any study-related procedures can take place or subjects are enrolled.

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the Clinical Study Agreement.

9.5 Study timetable and end of study

The end of the study is defined as 'the last visit of the last subject undergoing the study'.

The study is expected to start in Q4 2011 and to end by Q1 2012.

The study may be terminated at the study center if the study procedures are not being performed according to GCP or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with CAZ-AVI or avibactam and ceftazidime alone.

10. DATA MANAGEMENT BY

Data management will be performed by .

Data will be entered in the EDC system at the study center. Trained personnel at the study center will be responsible for entering data on the observations, tests, and assessments specified in this CSP into the EDC system and according to the eCRF instructions. If direct entry is not practical, data may be recorded on paper source documents and subsequently entered into the EDC system. Data entered in the EDC system will be immediately saved to a central database and changes tracked to provide an audit trail. The data will then be Source Data Verified, reviewed or queried and updated as needed.

The data collected through third party sources will be obtained and reconciled against study data.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by .

Data queries will be raised for inconsistent, impossible, or missing data. All entries to the study database will be available in an audit trail.

The data will be validated as defined in the Data Management Plan. Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

When all data have been coded, validated, and signed, the database will be locked. Any treatment revealing data may thereafter be added and the final database will be locked.

Data associated with biological samples will be transferred to laboratories internal or external to AstraZeneca.

11. EVALUATION AND CALCULATION OF VARIABLES BY

11.1 Calculation or derivation of safety variable(s)

11.1.1 Other significant adverse events

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs and AEs leading to premature discontinuation. Based on the expert's judgment, significant AEs of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered OAEs and reported as such in the CSR. A similar review of laboratory, vital signs, and ECG data will be performed for identification of OAEs.

Examples of these are marked hematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction, or significant additional treatment.

11.2 Calculation or derivation of pharmacokinetic variables

The PK analyses of the plasma concentration data for avibactam, ceftazidime, and metronidazole and urine concentration data for avibactam and ceftazidime will be performed at . Standard Operation Procedures and Work Instructions will be used as the default methodology, unless otherwise specified.

The actual sampling times will be used in the plasma PK parameter calculations. Pharmacokinetic parameters will be derived using non-compartmental methods with WinNonlin® Professional Version 5.2, or higher, or SAS® Version 9.2, or higher. All PK computations will be performed using WinNonlin® Professional 5.2, or higher; or SAS® Version 9.2, or higher. Graphics may be prepared with SAS® Version 9.2, or higher;

SigmaPlot® 9.0, or higher; Excel 2007, or higher
; or WinNonlin® Professional 5.2, or higher.

Where possible, the following PK parameters will be determined for avibactam, ceftazidime, and metronidazole when applicable:

- Maximum plasma concentration (C_{max}, μg/mL), obtained directly from the observed concentration versus time data
- Time of maximum plasma concentration (t_{max}, hr), obtained directly from the observed concentration versus time data
- Minimum plasma concentration during a dosing interval (C_{min}, μg/mL) on Day 4 only, obtained directly from the observed concentration versus time data
- Time of minimum plasma concentration during a dosing interval (t_{min}, hr), obtained directly from the observed concentration versus time data
- Last quantifiable plasma concentration (C_{last}, μg/mL), obtained directly from the observed concentration versus time data
- Time of last quantifiable plasma concentration (t_{last}, hr), obtained directly from the observed concentration versus time data
- Area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration [$AUC_{(0-t)}$, μg.hr/mL], calculated by linear up/log down trapezoidal summation
- Area under the plasma concentration time curve from zero (pre-dose) extrapolated to infinity (AUC, μg.hr/mL) on Day 1 only, calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable concentration divided by the elimination rate constant: $AUC_{(0-t)} + C_{last}/\lambda_z$. If the extrapolated area (C_{last}/λ_z) is greater than 20% of AUC, then AUC and related parameters will not be reported
- Area under the plasma concentration-time curve at steady state during the dosing interval [$AUC_{(0-\tau)}$, μg.hr/mL], calculated by linear up/log down trapezoidal summation
- Half-life ($t_{1/2}$, hr). Visual assessment will be used to identify the terminal linear phase of the concentration-time profile. A minimum of 3 data points will be used for determination
- Systemic plasma clearance (CL, L/hr)

- Volume of distribution at steady-state (V_{ss}, L), calculated as Mean Residence Time*CL
- Volume of distribution at the terminal phase (V_z, L), calculated as CL/λ_z
- Accumulation ratio for C_{max} (RC_{max})
- Accumulation ratio for $AUC_{(0-\tau)}$ [$RAUC_{(0-\tau)}$]
- Linearity index will be determined as the ratio of Day 4 $AUC_{(0-\tau)}$ to Day 1 AUC
- Amount of drug excreted unchanged in urine, calculated as the product of the urine volume and the urine concentration. The amount will be calculated and reported for each collection interval and cumulatively ($A_{e(0-t)}$, mg)
- Fraction of dose excreted as unchanged drug in urine (f_e; % dose). The fraction will be calculated and reported for each collection interval and cumulatively
- Renal clearance (CL_R, L/hr), calculated as $A_{e(0-t)}$ divided by $AUC_{(0-t)}$ on Day 1 or as $A_{e(0-\tau)}$ divided by $AUC_{(0-\tau)}$ at steady-state

The following PK parameters will be calculated for diagnostic purposes and listed, but will not be summarized:

- The time interval (hr) of the log-linear regression to determine $t_{1/2}$ ($t_{1/2}$, Interval)
- Number of data points ($t_{1/2}$, N) included in the log-linear regression analysis to determine $t_{1/2}$
- Coefficient of determination (Rsq) for calculation of λ_z. If Rsq is less than or equal to 0.800, λ_z and related parameters will not be reported
- Percentage of AUC obtained by extrapolation (%AUCex)

Additional PK parameters may be determined if deemed appropriate.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY

12.1 Description of analysis sets

12.1.1 Safety analysis set

All subjects who receive at least 1 dose of investigational product and for whom any post-dose data are available will be included in the safety analysis set.

12.1.2 PK analysis set

The PK analysis set will include all subjects who receive at least 1 administration of CAZ-AVI or metronidazole and have at least 1 scheduled post-dose PK measurement without important protocol deviations, violations, or events thought to significantly affect the PK of the investigational product. Subjects will be analyzed according to the treatment they actually received.

12.2 Methods of statistical analyses

12.2.1 General principles

The PK, PD, and safety summaries, individual figures and data listings as well as the statistical analysis of the PK variables will be the responsibility of the study biostatistician at (using SAS® Version 9.2 or higher and, where appropriate, additional validated software).

In general, descriptive statistics will follow the rounding convention in the

12.2.2 Subject characteristics

Continuous variables will be summarized using descriptive statistics (n, mean, standard deviation [SD], minimum [min], median, and maximum [max]) in total. Categorical variables will be summarized in frequency tables (frequency and proportion) in total.

12.2.3 Safety analyses

All safety data will be listed in subject listings. Continuous variables (hematology, clinical chemistry, and vital signs) will be summarized using descriptive statistics (n, mean, SD, min, median, and max), treatment and/or each scheduled assessment point, both as absolute values and as change from baseline. Categorical variables (urinalysis, ECG interpretation) will be summarized in frequency tables (frequency and proportion) by scheduled assessment point. Laboratory values and vital signs outside reference limits will be marked high and low where appropriate.

Adverse events will be summarized by Preferred Term and System Organ Class using the MedDRA.

The number of subjects who had any AEs, SAEs, discontinuation due to AEs, OAEs, AEs with severe intensity, and AEs judged causally related to the investigational product by the Investigator will be summarized. Any SAEs and DAEs will be listed separately.

12.2.4 Pharmacokinetic analyses

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided. A subject listing of all concentration-time data by study day for each treatment will be presented.

Pharmacokinetic variables (CAZ-AVI [avibactam and ceftazidime] and metronidazole plasma concentrations, urine amounts, and PK parameters, when applicable) will be summarized by treatment and study day/measurement time using appropriate descriptive statistics (eg, n, arithmetic mean, SD, min, median, max, geometric mean, geometric coefficient of variation [CV%], and geometric mean ±SD). The geometric mean is calculated as the exponential of the arithmetic mean calculated from data on a log scale. The CV% is calculated as $100 \cdot \sqrt{\left(\exp\left(s^2\right)-1\right)}$ where *s* is the SD of the data on a log scale. The geometric mean ±SD are calculated as $exp(u \pm s)$ where *u* and *s* are the mean and SD of the data on a log scale. Mean, SD, geometric mean, CV%, and geometric mean ±SD will not be calculated for t_{max}.

For descriptive statistics, concentrations below lower limit of quantification (LLOQ) values will be handled as follows:

- At a time point where less than or equal to 50% of the values are below the LLOQ (BLQ), all BLQ values will be set to LLOQ, and all descriptive statistics will be calculated

- At a time point where more than half of the values are BLQ, the mean, SD, geometric mean, CV%, and geometric mean ±SD will be set to not determined (ND). The max value will be reported from the individual data, and the min and median will be set to BLQ

- If all values are BLQ at a time point, no descriptive statistics will be calculated for that time point. Not applicable (NA) will be written in the field for SD, CV%, and geometric mean ±SD, and BLQ will be written in fields for mean, geometric mean, min, median, and max

- The number of BLQ values (n below LLOQ) will be reported for each time point

Figures of arithmetic mean (±SD) concentration-time data will be presented on linear and semi-logarithmic scales by treatment and study day. Individual subject concentration-time data will be graphically presented on linear and semi-logarithmic scales. Figures of individual and geometric mean AUC (Day 1), $AUC_{(0-\tau)}$ (Day 4), and C_{max} by treatment will be presented. Additional graphical presentations of PK data may be added at the discretion of the PK scientist.

An exploratory evaluation of achievement of steady-state will be performed graphically when applicable. Figures of arithmetic mean (±SD) plasma trough concentrations of avibactam, ceftazidime and metronidazole versus study days will be presented on linear scale by treatment.

Treatments will be compared between test (Treatment C) and reference (Treatment A for CAZ-AVI and Treatment B for metronidazole). Analyses will be performed by day (1 and 4) with a linear mixed-effects model using the logarithm of AUC (Day 1 only), $AUC_{(0-\tau)}$, (Day 4 only), and C_{max} (both Day 1 and Day 4) as the response variables, sequence, treatment period,

and treatment as fixed effects, and subject nested within sequence as random effect. Transformed back from the logarithmic scale, geometric least-squares means together with 95% confidence intervals (CIs) will be estimated and presented. Also, ratios of geometric least-squares means together with 90% CIs will be estimated and presented.

12.3 Determination of sample size

Twenty-four (24) evaluable subjects will provide approximately 90% power to demonstrate that the combined treatment has no effect on the PK of each of the individual treatment components.

The power calculation was based on equivalence testing using standard equivalence limits of (0.8, 1.25), to compare:

- the C_{max} of avibactam after Treatment A versus C_{max} of avibactam after Treatment C
- the C_{max} of ceftazidime after Treatment A versus C_{max} of ceftazidime after Treatment C
- the C_{max} of metronidazole after Treatment B versus C_{max} of metronidazole after Treatment C

The within-subject SD of log(avibactam C_{max}) and log(ceftazidime C_{max}) were estimated as 0.2088 and 0.2171, respectively, using data from study CAZ104/1002, and assuming that no PK interaction exists between ceftazidime and avibactam. The within-subject variability of log(metronidazole C_{max}) is assumed to be no greater in magnitude.

The estimates of within-subject variability for the AUC parameters were lower than for C_{max}, therefore the sample size will also provide sufficient power for comparisons involving AUC. Furthermore, in another recent study in Japanese healthy subjects (D4280C00010), the observed within-subject variability was considerably lower.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 6.3.4.**

In the case of a medical emergency the Investigator may contact the CPA Physician. If the CPA Physician is not available, contact the CPA Program Director at AstraZeneca.

Name	Role in the study	Address & telephone number
	AstraZeneca CPA Program Director	
	AstraZeneca CPA Physician	
Serious adverse event reporting	24-hour emergency cover at central R&D site	
	Principal Investigator	
	Project Manager	

13.2 Overdose

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the eCRF and on the Overdose eCRF module

- An overdose without associated symptoms is only reported on the Overdose eCRF module

If an overdose on an AstraZeneca investigational product occurs during the course of the study, then the Investigator or other study center personnel will inform the appropriate AstraZeneca representatives **within 1 day**, ie, immediately but no later than **the end of the next business day** of when he/she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

For overdoses associated with SAE, standard reporting timelines apply, see Section 6.3.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca.

13.3.1 Maternal exposure

Women of childbearing potential are not allowed to be included in this study. Should a pregnancy still occur, the investigational product should be discontinued immediately and the pregnancy reported to AstraZeneca.

13.3.2 Paternal exposure

Male subjects should refrain from fathering a child or donating sperm during the study and for 3 months following the last administration of the investigational product.

Pregnancy of a subject's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should if possible be monitored and documented from the first administration until 3 months after the last administration of the investigational product.

14. LIST OF REFERENCES

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Clinical Study Protocol Appendix B

Drug Substance	CAZ-AVI
Study Code	D4280C00012
Edition Number	1
Date	

Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse.

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

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Clinical Study Protocol Appendix C	
Drug Substance	CAZ-AVI
Study Code	D4280C00012
Edition Number	1
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Appendix C
International Airline Transportation Association (IATA) 6.2 Guidance Document

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are eg, Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

Clinical Study Protocol Appendix D	
Drug Substance	CAZ-AVI
Study Code	D4280C00012
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Appendix D
Optional Biomarker Research Samples

OPTIONAL BIOMARKER RESEARCH SYNOPSIS

A Phase I, Open-Label, 3-way Crossover, Pharmacokinetic and Drug-Drug Interaction Study of Ceftazidime Avibactam (CAZ-AVI [formerly CAZ104]) and Metronidazole when Administered Alone and in Combination in Healthy Subjects

The research activities described in this appendix (including the collection and storage of body fluid samples), are optional for study centres as well as for individual healthy volunteers. These research activities will hereafter be referred to as "this research." The clinical study protocol to which this document is appended will be referred to as "the main study." The term "sample" means:

Plasma, Serum or Urine

This research will be performed only after the appropriate Ethics Committee/IRB has approved it. Informed consent for this research will be obtained using a separate Informed Consent Form from that used for the main study. All sections of the protocol for the main study also apply to this research.

Study centre(s) and number of patients who may be enrolled in this biomarker research

It is the intent of AstraZeneca to collect serum, plasma or urine samples from all Clinical Pharmacology studies conducted by the Clinical Pharmacology Alliance (CPA) to further the goal of improving biochemical markers that can be used to monitor or predict drug-induced organ damage. The goal will be to collect approximately 3000 such samples.

Objectives

Objective	Outcome variables
To analyse biological samples (eg, human plasma) for circulating biomarkers from consenting volunteers prior to drug treatment.	

Study design

It is proposed to collect a single serum, plasma or urine from each subject/healthy volunteer enrolled in the trial as optional samples for biomarker analysis. The type of sample to be collected will be determined at the outset of the trial. Provision of these samples for analysis will be optional for all healthy volunteers entering the study, and acceptance of this procedure will not be a requirement for participation in the main study.

The samples and data for optional biomarker analysis in this research will be coded. Each sample will be labelled with the study number and patient enrolment number (E-code). Only the investigator will be able to link the sample to the individual healthy volunteer. The samples and data will not be labelled with personal details.

Target population

All consenting volunteers in all centres participating in the main study.

Statistical methods

The number of volunteers who will agree to participate in this research is unknown. It is therefore not possible to establish whether sufficient data will be generated. A statistical analysis plan will be prepared where appropriate.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
eCRF	electronic Case Report Form
CSR	Clinical study report
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
ICH	International Conference on Harmonisation
pCRF	paper Case Record Form
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
RNA	Ribonucleic acid
UK	United Kingdom

1. BACKGROUND

As part of collaborative efforts with other pharmaceutical companies, diagnostic companies and academic institutions) AstraZeneca is collecting samples to perform general research for variations in "safety" biomarker profiles. These biomarkers may be derived from proteins and/or metabolites. By using this information, the aim is to better understand drug effect on major organs in the human body and how circulating biomarkers can be used to better monitor organ function and thus improve safety of drugs.

To achieve this goal, a systematic collection of biological samples (urine, serum and/or blood plasma) will be undertaken as specified where appropriate.

1.1 Rationale for research

AstraZeneca may perform optional sampling determination for biomarker research in some of the studies for the clinical programs of new chemical entities under development. The objective of this research is to explore normal variations in biomarkers (protein or small molecule based) that occur in individuals enrolled in this trial **prior to drug treatment**. In particular, developing better biochemical markers to help assess potential deleterious drug is the primary goal of this research. A key aspect to understanding how to use these new markers is to assess the normal variation in these markers in healthy individuals so we will appropriately interpret how changes in these new biochemical markers are affected by drug treatment. Understanding this normal variation is part of a process known as qualification which attempts to establish sufficient evidence of changes in these biomarkers in relationship to organ damage that they are suitable for monitoring safety for clinical trials. Other recent studies have suggested that using proteomic and metabolomic platforms may help identify other new predictive biomarkers that help explain ALT elevation (Andersson et al 2009 and Ozer et al 2008).

The ability to acquire appropriate consent to collect biological samples to establish an archive and allow future meta-analysis of data derived from a number of studies is of the utmost importance. This research forms part of this strategy.

The benefits of being able to explore associations between biomarker variations and clinical outcomes are potentially many including the possibility to identify volunteers early who may be at risk of adverse drug reaction or to explain potential adverse reactions related to drug exposure.

2. RESEARCH OBJECTIVES

Biomarker technologies enable the measurement of many different molecules, including proteins and metabolites, within a sample. The objective of this research is to determine if correlations exist between traditional biomarkers used to monitor organ function (such as

ALT and bilirubin for liver) and new biomarkers that may be more sensitive and/or specific indicators of drug induced organ damage Research plan and procedures

2.1 Research plan

The healthy volunteer will be asked to participate in this optional biomarker research during their enrolment or screening visit. If the volunteer agrees to participate the following samples will be requested:

A single 10 mL blood sample or a single urine sample of up to 15 mL.

2.2 Selection of optional biomarker research population

2.2.1 Study selection record

All healthy volunteers who take part in the study will be asked to participate in this optional biomarker research. Participation is voluntary and if a volunteer declines to participate in this optional biomarker research they will not be excluded from any aspect of the main study.

2.2.2 Withdrawal of healthy volunteers from this optional biomarker research

2.2.2.1 Criteria for withdrawal

Specific reasons for withdrawing a volunteer from this optional biomarker research are:

- Withdrawal of consent for optional biomarker research. Volunteers may withdraw from this optional biomarker research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary withdrawal will not prejudice further treatment.

2.2.2.2 Procedures for withdrawal

Volunteers who withdraw from the main study should always be asked specifically whether they are withdrawing or continuing their consent for this optional biomarker research. It must be established whether the volunteer:

- Agrees to the optional biomarker samples and any preparations derived from the sample being kept for research in the future

- Withdraws consent for the samples to be kept for optional biomarker research in the future and wishes the samples to be destroyed. Destruction of the samples (or the preparations derived from the samples) will only be possible so long as the particular samples are traceable. In the event that optional biomarker research has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

The principal investigator is responsible for providing written notification to AstraZeneca of any volunteer who has withdrawn consent for the use of the sample taken for optional

biomarker research. AstraZeneca will provide written confirmation to the investigator of the actions taken with the sample, which must be filed in the investigator study file.

3. MEASUREMENTS AND CO-VARIABLES

3.1 Summary of objectives and analysis

The purpose of this research is to generate data that will help interpret results from future clinical trials. The results of this research will not form part of the clinical study report (CSR) for the main study. The results may be pooled with data from other studies generate hypotheses to be tested in future studies.

3.2 Collection of samples for Optional Biomarker Research

AstraZeneca or its designee will act as the central laboratory for sample logistics. Details of sample collection, processing, shipping and storage will be described in the laboratory manual.

The samples and data for analysis in this research will be coded and will not be labelled with any personal details. Each sample will be identified with the study number and patient enrolment number. In this way biomarker data may be correlated with clinical data, samples destroyed in the case of withdrawal of consent and regulatory audit enabled. However, only the investigator will be able to link the biomarker sample to the individual volunteer.

The coded samples may be made available to groups or organisations working with AstraZeneca on this research or as part of the development drug project. However, the samples and any results will remain the property of AstraZeneca at all times. AstraZeneca will not give samples, sample derivatives or data derived from the samples to any other parties, except as required by law.

3.2.1 Biomarker Analysis

The precise details of the biomarker analysis will be established by AstraZeneca scientists. However, in some cases, samples may be sent to commercial or academic partners for specialized analyses.

In addition to studies to identify new candidate biomarkers, samples may also be used to measure existing candidate biomarkers by methods that will depend on the specific biomarker.

4. MANAGEMENT OF RESEARCH DATA

Some of the dataset from the main study may be duplicated within AstraZeneca for exploratory analyses in combination with the optional biomarker data. Neither the volunteer's name nor any other personal identifiers will be part of this dataset. Optional biomarker data will not be reported in the CSR. Only the date the volunteer gave consent to participation in

the research and the date and time the biological sample(s) (if applicable) was taken from the patient will be recorded in the electronic Case Record Form (eCRF) and database.

AstraZeneca will not provide optional biomarker research results to volunteers, their family members, any insurance company, an employer, clinical study investigator, general physician or any other third party, unless required to do so by law. The volunteer's samples will not be used for any purpose other than optional biomarker research.

Individual volunteers will not be identified in any report or publication resulting from this work. The data and results of this research may be reviewed with collaborators and published, but neither the volunteer's name nor any other personal identifiers will appear in any publication or report.

5. STATISTICAL METHODS

One of the primary goals of these exploratory studies is to establish a large sample cohort that will help us understand the variability of new biomarkers in a general population. The samples that compose this cohort will come from multiple different studies and a statistical analysis plan will be prepared for analyses of each new biomarker. This analysis will help us determine how many subjects will be needed for future trials. However, neither the results of this biomarker work nor the statistical analysis will be included in CSR for the trials from which these samples have been collected.

5.1 Monitoring

During the study, monitors will have regular contacts with the investigational centres. One of the purposes of these visits will be to perform source verification of the informed consent of participating volunteers and to ensure that the investigational team are adhering to the specific requirements of this optional biomarker research.

5.2 Training of staff

Before the first volunteer is entered into the study the investigational staff will have an opportunity to discuss the procedures associated with the collection of samples and optional biomarker research with a representative of AstraZeneca. The requirements for the collections of the volunteers' sample will also be made clear.

5.3 Changes to the protocol

Any changes to the optional biomarker research will comply with the principles described in Section 8.5 of the main body of the protocol.

5.4 Study agreements

The principal investigator at each study centre must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this research. In the event of any inconsistency between this Clinical Study Protocol (CSP) and the Clinical Study Agreement,

the CSP shall prevail. Specific reference to requirements relating to this optional biomarker research will be included in the study agreement(s).

6. ETHICS

6.1 Ethics review

In addition to documenting Ethics Committee/IRB approval of the main study, approval must be obtained for this optional biomarker research and the associated informed consent from the relevant Ethics Committee. It must be clearly stated in the approval that this optional biomarker research is approved. The investigator must submit written approval to AstraZeneca before any patient participates in this optional biomarker research.

6.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

6.3 Informed consent

The biomarker component of this research is optional and the volunteer may participate in other components of the study without participating in the optional biomarker component. To participate in the optional biomarker component of the study, the volunteer must sign and date both the consent form for the main study and the consent form for the optional biomarker component of the study. Copies of both signed and dated consent forms must be given to the volunteer and the originals filed at the study centre in the investigator's study file. The principal investigator is responsible for ensuring that consent is given freely and that the volunteer understands that they may freely discontinue from the optional biomarker aspect of the study at any time.

6.4 Volunteer data protection

All data protection and confidentiality principles, described in the main study protocol, are applicable to this optional biomarker research.

Due to the exploratory nature of this optional biomarker research, there will be no routine communication of results to volunteers. AstraZeneca will not provide individual results to volunteers, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

7. REFERENCES

Andersson et al 2009
Andersson U, Lindberg J, Wang S, Balasubramanian R, Marcusson-Ståhl M, Hannula M. A systems biology approach to understanding elevated serum alanine transaminase levels in a clinical trial with ximelagatran. Biomarkers. 2009 Sep 25. Biomarkers 2009;14(8):572-586

Ozer et al 2008
Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S The current state of serum biomarkers of hepatotoxicity . Toxicology. 2008 Mar 20;245(3):194-205.

AstraZeneca

Clinical Study Protocol Appendix E	
Drug Substance	CAZ-AVI
Study Code	D4280C00012
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Appendix E

Actions Required in Cases of Combined Increase of Aminotransferase and Total Bilirubin - Hy's Law

1. ACTIONS REQUIRED IN CASES OF AST OR ALT >3 X ULN OR TBL >2 X ULN

The Investigator is responsible for, without delay, determining whether the subject meets potential Hy's law (PHL) criteria; Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) >3 x Upper Limit of Normal (ULN) **and** Total Bilirubin (TBL) >2 x ULN at any point during the study, irrespective of Alkaline Phosphatase (ALP). The AST or ALT and total bilirubin values do not have to be elevated at the same visit or within any specified timeframe.

1.1 Identification

In cases of AST or ALT >3 x ULN **or** TBL >2 x ULN, please follow the instructions below.

- Review each laboratory report and if a subject has an increase in AST or ALT >3 x ULN **or** TBL >2 x ULN at any visit:
  - Notify the AstraZeneca representative
  - Promptly enter the laboratory date into the laboratory CRF.

1.2 Determination and Follow-up

1.2.1 Potential Hy's Law Criteria not met

If the subject **has not** had AST or ALT >3 x ULN **and TBL** >2 x ULN at any point in the study even if on different visits, irrespective of ALP

- Inform the AZ representative that the subject has not met PHL criteria
- Perform follow-up on subsequent laboratory results according to the guidance provided in the CSP.

1.2.2 Potential Hy's Law Criteria met

If the subject **has** had AST or ALT >3 x ULN **and TBL** >2 x ULN at any point in the study even if on different visits, irrespective of ALP:

- Notify the AZ representative who will then inform the central ST

The Study Physician (SP) contacts the Investigator, to provide guidance, discuss and agree an approach for the study subject's follow-up and the continuous review of data.

The Investigator:

- Follows the subject until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated.
- Investigates the etiology of the event and perform diagnostic investigations as discussed with the SP
- Completes the Liver CRF Modules.
- If at any time (in consultation with the SP) the PHL case meets serious criteria, it should be reported as an SAE using standard reporting procedures.

1.3 Review and Assessment

No later than 3 weeks after the biochemistry abnormality was initially detected, the Study Physician contacts the Investigator in order to review available data and agree on whether there is an alternative explanation for the elevations in liver biochemistry other than Drug Induced Liver Injury (DILI) caused by the Investigational Medicinal Product (IMP).

For the purpose of this process a Hy's Law case is defined as:

Any subject with an increase in both Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) >3 x Upper Limit of Normal (ULN) and Total Bilirubin (TBL) >2 x ULN, where no other reason can be found to explain the combination of increases, eg, elevated serum Alkaline Phosphatase (ALP) indicating cholestasis, viral hepatitis, another drug

If there **is** an agreed alternative explanation for the AST or ALT **and TBL** elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for a SAE.

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate CRF.
- If the alternative explanation is an AE/SAE, record the AE /SAE in the CRF accordingly and follow the AZ standard processes.

If it is agreed that there is **no** other explanation that would explain the AST or ALT and TBL elevations:

- Report an SAE (report term Hy's Law') according to AZ standard processes.
  - The 'Medically Important' serious criterion should be used if no other serious criteria apply

- As there is no alternative explanation for the HL case, a causality assessment of related should be assigned.

If, there is an unavoidable delay, of over 3 weeks, in obtaining the information necessary to assess whether or not the case meets the criteria for a HL case, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Report an SAE (report term 'Potential Hy's Law') applying serious criteria and causality assessment as per above

2. REFERENCES

FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation':

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064993.htm