Clinical Study Protocol

Drug Substance	CAZ104
Study Code	D4280C00009
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A Phase I Open-Label, 2-Part, 3-Cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma Using at Least Two Different Dosing Regimens in Healthy Volunteers

Sponsor:

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

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

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

AstraZeneca

PROTOCOL SYNOPSIS

A Phase I Open-Label, 2-Part, 3-Cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma Using at Least Two Different Dosing Regimens in Healthy Volunteers

Principal Investigator

Study centre and number of subjects planned

This study will be conducted at 1 study centre. Up to 63 healthy subjects will be enroled; up to 3 subjects in a procedural pilot Part 1, and 60 subjects in Part 2.

Study period	Phase of development
Estimated date of first healthy volunteer enroled	Phase I
Estimated date of last healthy volunteer completed	

Objectives

Primary objective

The primary objective of this study is to measure and compare the concentration of avibactam and ceftazidime in bronchial epithelial lining fluid (ELF) and plasma following administration of at least 2 different dosing regimens in healthy subjects.

Secondary objective

The secondary objective is to assess the safety and tolerability of avibactam and ceftazidime when administered every 8 hours for 3 days via a 2-hour infusion (Cohorts A and B) or a 4-hour infusion (optional Cohort C).

Exploratory objective

The exploratory objective is to correlate the plasma and ELF concentration-time courses by a population pharmacokinetic (PK) modelling approach.

The results from the exploratory analyses will be reported separately outside of the Clinical Study Report (CSR).

Study design

This is an open-label, 3-cohort, single-centre study designed to assess the concentration of ceftazidime in combination with avibactam (ceftazidime avibactam [CAZ104]) in bronchial ELF (by bronchoalveolar lavage [BAL]) and plasma following a 3-day administration in healthy subjects.

The study will be split into 2 parts: a procedural pilot Part 1 (including up to 3 subjects) will be performed first with no administration of the investigational product (IP), and a Part 2 (including maximum 60 subjects) with administration of the IP will be performed thereafter. The key procedures and timings will be same in Parts 1 and 2 of the study. For Part 1, PK sample collection will not take place and the subjects will not have a 5-day residential period. The subjects who will participate in Part 1 can be included in Part 2 after 21 days.

Target subject population

Healthy male and female subjects aged 18 to 50 years with suitable veins for cannulation or repeated venepuncture; female subjects must be postmenopausal or surgically sterile or with a negative pregnancy test.

Investigational product, dosage and mode of administration

Avibactam 500 mg + ceftazidime 2000 mg (intravenous [IV])

Avibactam 1000 mg + ceftazidime 3000 mg (IV)

Comparator, dosage and mode of administration

None.

Duration of treatment

The study will comprise of 3 visits. Visit 1 (screening and enrolment) will take place within 28 days prior to Visit 2. The treatment visit will be Visit 2 which will have a duration of 5 days. Visit 3 (follow-up) will occur 7 to 10 days after Visit 2.

Outcome variables:

Pharmacokinetics:

Where the data allow, the following PK parameters will be calculated for avibactam and ceftazidime following the last dose on the last day: maximum concentration (C_{max}) in plasma and ELF, time to C_{max} (t_{max}) in plasma and ELF, area under the concentration-time curve during a dosing interval τ (AUC_{τ}) in plasma and ELF, terminal half-life ($t_{½\lambda z}$) in plasma and ELF, plasma clearance (CL), volume of distribution at steady state (V_{ss}) and at the terminal phase (V_z) in plasma, ratio of C_{max} in ELF over C_{max} in plasma and ratio of AUC_{τ} in ELF over AUC_{τ} in plasma. The PK parameters in ELF will be derived from the composite

concentration-time profile consisting of the median concentration at each scheduled time point.

Additional PK parameters may be determined if deemed appropriate.

Safety:

- Incidence and severity of adverse events (AEs)
- Physical examination findings
- Safety laboratory tests:
  - clinical chemistry
  - haematology
  - coagulation (activated partial thromboplastin time and prothrombin time)
  - urinalysis
- Vital sign measurements (blood pressure, heart rate, temperature, respiration rate and pulse oximetry)
- Electrocardiogram (ECG)
- Additional paper ECG and telemetry .

Statistical methods

No formal statistical hypothesis testing will be performed in this study.

The statistical analyses of safety data will be descriptive and consist of subject listings, graphs and summary statistics comprising coefficient of variation, arithmetic mean, standard deviation, median, minimum and maximum, as appropriate.

The PK variables (avibactam and ceftazidime concentrations in plasma and ELF and PK parameters in plasma) and ratios of ELF concentration relative to plasma concentration for avibactam and ceftazidime will be summarised using appropriate descriptive statistics.

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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
AE	Adverse event (see definition in Section 6.4.1)
ALT	Alanine aminotransferase
AmpC	Ambler Class C
aPTT	Activated plasma thromboplastin time
AST	Aspartate aminotransferase
AUC_{τ}	Area under the plasma concentration-time curve during a dosing interval
$AUC_{\tau,ELF}$	Area under the plasma concentration-time curve during a dosing interval in epithelial lining fluid
$AUC_{\tau,p}$	Area under the plasma concentration-time curve during a dosing interval in plasma
β	Beta
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BL-BLI	β-lactam–β-lactamase inhibitor
BMI	Body mass index
CAZ104	Ceftazidime avibactam
C_{BAL}	Concentration in bronchoalveolar lavage
C_{ELF}	Concentration in epithelial lining fluid
cIAI	Complicated intra-abdominal infection
CL	Plasma clearance
C_{last}	Last quantifiable concentration
C_{max}	Maximum concentration
$C_{max,p}$	Maximum concentration in plasma
COPD	Chronic obstructive pulmonary disease
C_p	Concentration in plasma
CPA	Clinical Pharmacology Alliance
CRF	Case Report Form
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol

Abbreviation or special term	Explanation
CSR	Clinical Study Report
CTX	Cefotaximase
$C_{urea,p}$	Urea concentration in plasma
$C_{urea,BAL}$	Urea concentration in bronchoalveolar lavage
cUTI	Complicated urinary tract infection
CV%	Geometric coefficient of variation (%)
CYP450	Cytochrome P450
DDI	Drug-drug interaction
EC	Ethics Committee
ECG	Electrocardiogram
ELF	Epithelial lining fluid
eCRF	Electronic CRF
ESBL	Extended spectrum β-lactamase
FSH	Follicular-stimulating hormone
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transpeptidase
GMP	Good Manufacturing Practices
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IATA	International Air Transport Association
IC_{50}	Half maximal inhibitory concentration
ICF	Informed consent form
ICH	International Conference on Harmonisation
INR	International normalised ratio
IP	Investigational product
IV	Intravenous(ly)
KPC	*Klebsiella pneumoniae* carbapenemase
LDH	Lactate dehydrogenase
LIMS	Laboratory information management system
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation or special term	Explanation
NA	Not applicable
NQ	Not quantifiable
PD	Pharmacodynamic
PK	Pharmacokinetics
PT	Prothrombin time
QTcF	QT interval corrected for heart rate using Fridericia's formula
Rsq	Regression coefficient
SAE	Serious adverse event (see definition in Section 6.4.2).
SD	Standard deviation
SDV	Source data verification
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reactions
$t_{1/2\lambda z}$	Terminal half-life
t_{max}	Time of maximum concentration
TSH	Thyroid-stimulating hormone
V_{ss}	Volume of distribution at steady state
V_z	Volume of distribution at terminal phase

1. INTRODUCTION

1.1 Background

1.1.1 Beta-lactamases and Beta-lactam-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 (Bush et al 2010). The most important β-lactamases of clinical relevance are enzymes that utilise 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 hydrolyse 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, localised, clinical isolate, with characterisation confined to a unique amino acid sequence and minimal, if any, functional information (Bush 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 pneumonia*e 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 beta-lactamase mediated resitance 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 septicaemia, bone and joint infections, gynaecological infections and central nervous system infections (including meningitis) caused by susceptible pathogens.

Over the past 15 years or so, resistance to ceftazidime has been increasing worldwide. The commonest 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 (CAZ104)

are developing ceftazidime in combination with avibactam (ceftazidime avibactam [CAZ104]), a β-lactam – β-lactamase inhibitor (BL-BLI), as an intravenously (IV) 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, when associated with ceftazidime, has also 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.

Avibactam is a novel non BL-BLI 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.

At present 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.

1.2 Summary of relevant preclinical/clinical information to date

1.2.1 Preclinical information

The preclinical safety evaluation program for avibactam alone includes toxicity studies 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 preclinical species and is not associated with target

organ toxicity with the exception of local tolerance issues when administered IV via a peripheral vein. These local tolerance issues are not seen when avibactam is administered IV via a central vein in surgically prepared animals.

The preclinical 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 injection 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 please refer to the CAZ104 Investigator's Brochure 2010.

1.2.2 Clinical information

Four clinical pharmacology studies have been completed, including:

- A Phase I double-blind, placebo-controlled, escalating single-dose study with and without ceftazidime in healthy young male subjects (avibactam/1001).

- A Phase I double-blind, placebo-controlled, multiple-dose study over 5 or 10 days with and without ceftazidime, IV and oral formulations, in healthy young male subjects (avibactam/1002).

- A Phase I open-label, single-dose study to assess the effect of renal impairment on pharmacokinetic (PK) parameters in subjects with varying degrees of renal insufficiency and in subjects with end-stage renal failure on haemodialysis (avibactam/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 (avibactam/1004).

The clinical pharmacology studies completed to date have demonstrated the PK and tolerability of avibactam alone or in combination with ceftazidime in healthy young and elderly male and female subjects. The PK and tolerability of avibactam have also been determined in subjects with different degrees of renal impairment. Since CAZ104 is administered by IV infusion, and both ceftazidime and avibactam are predominately excreted unchanged in urine, the drug-drug interaction (DDI) potential with cytochrome P450 (CYP450) 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, etc, were collected from the Phase II studies evaluating CAZ104 plus metronidazole versus meropenem in subjects with cIAI (Study avibactam/2002) and evaluating CAZ104 versus imipenem in the treatment of cUTI (Study avibactam/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 Phase II study in subjects with cIAI (Study avibactam/2002). A population PK/pharmacodynamic (PD) analysis has been

conducted using data from the Phase II study in subjects with cIAI; these data have supported dose selection for the Phase III clinical program and is found to be not associated with target organ toxicity with the exception of local tolerance issues, when administered IV. Population PK and PK/PD analysis of data from the Phase II study in subjects with cUTI are ongoing. Please see Section 3.2 for study rationale for the starting dose for Cohort A in this study including the change from 30-minute infusion in Phase II, to a proposal for 2-hour infusion.

For further details please refer to CAZ104 Investigator's Brochure 2010.

1.3 Research hypothesis

Not applicable.

1.4 Rationale for conducting this study

This study will evaluate the relationship between concentrations of ceftazidime and avibactam in bronchoalveolar lavage fluid (BALF)/bronchial epithelial lining fluid (ELF), and plasma in healthy subjects.

1.5 Benefit/risk and ethical assessment

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

In clinical studies containing avibactam only treatment arm, the adverse drug reactions observed to date include abdominal pain, anxiety, dizziness somnolence, sense of oppression, orthostatic hypotension, injection site erythema and injection site haematoma.

Ceftazidime has been licensed for use since 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 aetiology:

- Local effect (fewer than 2% of subjects): phlebitis, pain and inflammation at the site of injection.

- 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): diarrhoea, 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 paraesthesia. Seizures have also been reported with several cephalosporins, including ceftazidime.

- Less frequent AEs (fewer than 1% of subjects): candidiasis (including oral thrush) and vaginitis.

- Haematological: rare cases of haemolytic anaemia.

- Laboratory test changes noted during clinical studies were transient and included: eosinophilia, positive Coombs test without haemolysis, thrombocytosis, hyperbilirubinaemia, 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, 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 anaemia, haemorrhage and jaundice.

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

Theoretical, preclinical and clinical findings from the CAZ104 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 ongoing creation of a Patient Risk Management Plan.

In all of the Phase I studies, there were no serious or severe AEs, or AEs that led to premature withdrawal from the study. The safety profile of CAZ104 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 CAZ104 was generally well tolerated.

To ensure subject safety during the study, routine clinical monitoring will include AEs, vital signs, physical examination findings, routine safety laboratory assessments (haematology,

coagulation, clinical chemistry and urinalysis), clinical assessments and electrocardiograms (ECGs).

Bronchoscopy with bronchoalveolar lavage (BAL) is a standard diagnostic procedure usually performed in an outpatient setting. The commonest risks of the procedure which can occur in a minority of patients include transient hypoxaemia, cough, dyspnoea and fever. The safety and utility of bronchoscopy with BAL in healthy subjects has been previously evaluated (Gotfried et al 2008 and Furuie et al 2010). To further minimise the risks of the procedure and the co-administration of sedative and anaesthetic agents, an experienced bronchoscopist will perform the procedure in accordance with the guidelines published by the British Thoracic Society (British Thoracic Society 2001). Only healthy subjects with normal lung function and no history of respiratory disease will be enroled. Continuous monitoring of pulse rate and oxygen saturation will be employed during the procedure.

A potential future benefit may result from the development of CAZ104 in new indications. Although subjects will not gain therapeutic benefit from participation in the study, information on PK in plasma and ELF obtained in this study may support the development of CAZ104 for new therapeutic indications. Given the existing safety and tolerability data and design of this study, which include stopping criteria to minimise the risk to subjects (see Section 3.1), it is reasonable to progress with the investigation of PK of CAZ104 in ELF and plasma in this study.

For further details, please refer to CAZ104 Investigator's Brochure 2010.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of this study is to measure and compare the concentration of avibactam and ceftazidime in ELF and plasma, following administration of at least 2 different dosing regimens in healthy subjects.

2.2 Secondary objective

The secondary objective is to assess the safety and tolerability of avibactam and ceftazidime when administered every 8 hours for 3 days via a 2-hour infusion (Cohorts A and B) or a 4-hour infusion (optional Cohort C).

2.3 Exploratory objective

The exploratory objective of this study is to correlate the plasma and ELF concentration-time courses by a population PK modelling approach.

3. STUDY PLAN AND PROCEDURES

This Clinical Study Protocol (CSP) has been subject to a peer review according to AstraZeneca standard procedures.

3.1 Overall study design and flow chart

This is an open-label, 3-cohort, single-centre study designed to assess the concentration of CAZ104 in ELF and plasma following a 3-day administration in healthy subjects.

The study will be split into 2 parts. A procedural pilot Part 1 (including up to 3 subjects) to verify optimal execution of the procedures and acquisition of satisfactory samples, will be performed first with no administration of the IP. A Part 2 (including maximum 60 subjects) with administration of the IP will be performed thereafter. The key procedures and timings will be same in Parts 1 and 2 of the study. For Part 1, PK sample collection will not take place and the subjects will not have a 5-day residential period. The subjects who participate in Part 1 can be included in Part 2 after 21 days.

Part 1: The pilot part will include up to 3 subjects. The study days and procedures are as follows:

Screening: Procedures will be performed as per normal screening visit for Part 2 (see Table 1).

Day -1: Subjects will be admitted to the study unit. Procedures include safety laboratory tests (blood to including coagulation and urea); and measurement of vital signs (blood pressure, heart rate, temperature, respiration rate and pulse oximetry).

Day 1: Procedures include measurement of vital signs followed by a bronchoscopy for the subjects at one of the following time points: 2 hours, 4 hours, 6 hours, 8 hours or 12 hours. Bronchoscopy will only be performed once in each subject. Subjects will have only bronchoscopy or BAL.

Follow-up: Procedures will be performed as per Part 2, the main study part (see Table 1).

Part 2: Twenty healthy subjects will be assigned to Cohort A and 20 healthy subjects will be assigned to Cohort B.

Cohort A: Each subject will receive 500 mg avibactam + 2000 mg ceftazidime infused over 2 hours.

Cohort B: Each subject will receive 1000 mg avibactam + 3000 mg ceftazidime infused over 2 hours.

For both these cohorts, bronchoscopy with BAL will be performed once on each subject. After the last dose, BAL will be performed from the start of infusion at the following time points: 2 hours, 4 hours, 6 hours and 8 hours.

Following Cohorts A and B, samples will be analysed. If further information for future decision making for dose cannot be gained by prolonging the infusion time, then Cohort C will not proceed and the study will be stopped. Please see Section 3.2 for more information on the study design.

Optional Cohort C: Twenty healthy subjects will be assigned to this cohort. Each subject will receive 500 mg avibactam + 2000 mg ceftazidime or
1000 mg avibactam + 3000 mg ceftazidime, infused over 4 hours based on the decision made following Cohorts A and B. Bronchoscopy with BAL will be performed once on each subject. On the last dose, BAL will be performed at the following time points: 4 hours, 6 hours, 8 hours and 12 hours.

For all 3 cohorts, subjects will receive 1 dose every 8 hours for 3 days (9 doses in total). Infusion volume will be 100 mL. Bronchoscopy with BAL will be performed in 5 subjects per time point in all the cohorts. The BAL time points will be from the start of infusion. Due to the time required to prepare for these procedures, subjects will not be randomised. Plasma samples will be collected predose and at 1 hour, 2 hours, 2.5 hours, 3 hours, 4 hours, 6 hours, 8 hours, 12 hours, 16 hours and 24 hours relative to start of the last infusion.

Blood samples will be collected for complete laboratory safety assessments at screening, Day -1 (excluding thyroid-stimulating hormone; TSH test), at discharge on Day 5 (excluding TSH and coagulation) and follow-up; and reduced laboratory safety assessments on Days 3 and 4. A complete physical examination will be performed at screening and follow-up, while a brief physical examination on Day -1 and at discharge. Vital sign measurements will be recorded at screening, Day -1, predose on Day 1 and every 24 hours thereafter till discharge, and at follow-up. Vital signs will be measured before BAL on Day 4. The ECG will be performed at screening, Day -1, Day 2, Day 4, Day 5 (at discharge), and follow-up. An additional paper ECG will be done for each dose of the study drug. Paper ECG will be done at the following time points: 30 minutes before the start of the first infusion, and 1 hour, 2 hours, 3 hours and 6 hours after start of the first infusion. Telemetry will be conducted for each subject from 1 hour before to 24 hours after the first infusion (0-24 h) if there are any safety concerns telemetry will be continued. The period of telemetry may be be prolonged up to 48 hours after the first infusion if deemed necessary by the Investigator. Lung function test will only be performed at screening.

It is expected that dose administration will commence in the afternoon so that bronchoscopy is performed during the day. Subjects will eat normally without any required fasting period and can be fed prior to infusion, with the exception of the time prior to the bronchoscopy on Day 4 which requires a 6-hour fast prior to the procedure (6 hours for solids and 4 hours for liquids).

The Part 2 of the study will comprise of 3 visits:

- Visit 1: enrolment and screening will take place up to 28 days prior to admission in the study unit (Day -1). Screening procedures will only be performed for subjects who provide voluntary written informed consent.

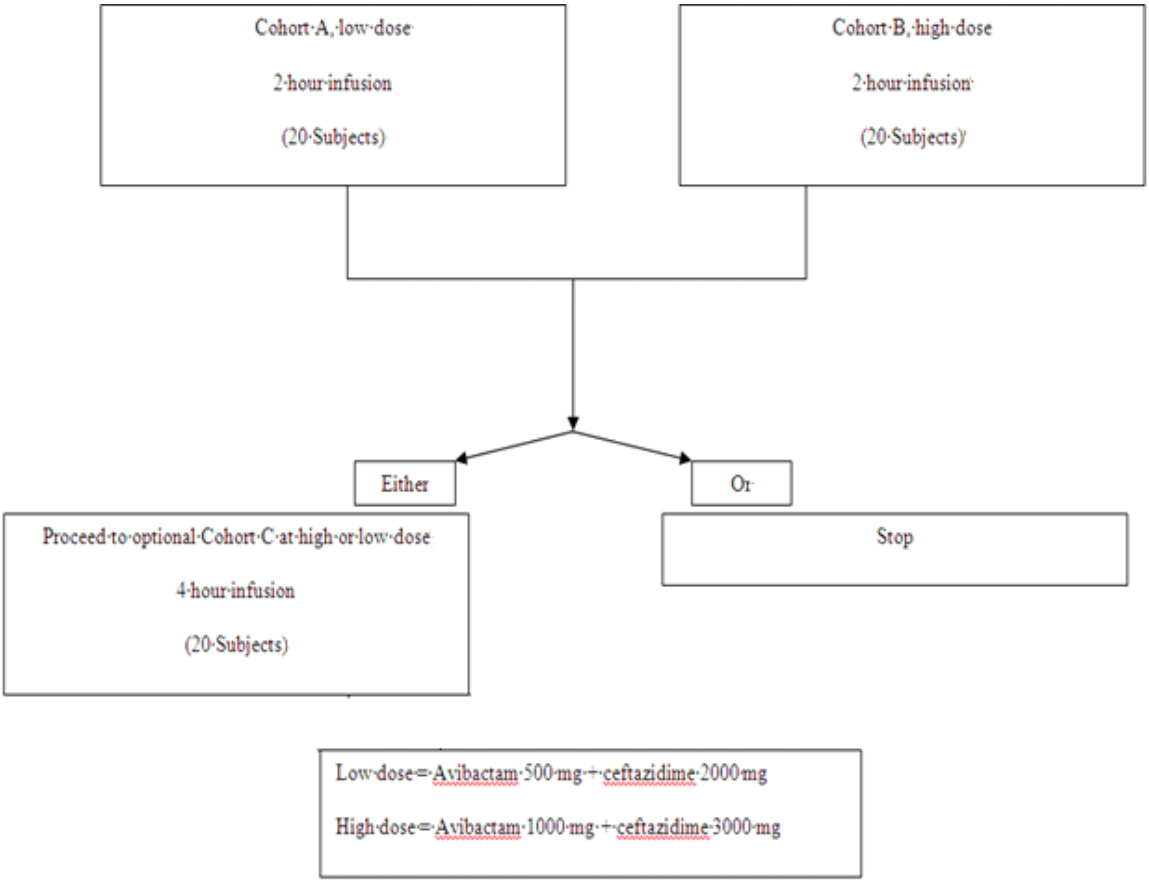
- Visit 2: comprises the residential treatment period in which each of the healthy subjects will receive CAZ104 every 8 hours for 3 days (ie, 9 doses in total). Bronchoscopy with BAL will be performed once on each subject.

- Visit 3: post study follow-up examination 7 to 10 days after the final dose of IP.

Up to 3 subjects will be enroled in Part 1. In Part 2, a minimum of 40 (if the study comprises of 2 cohorts) to a maximum of 60 (if the study comprises of all 3 cohorts in Part 2) subjects will be enroled. Up to 63 subjects in total will be enroled at 1 study centre.

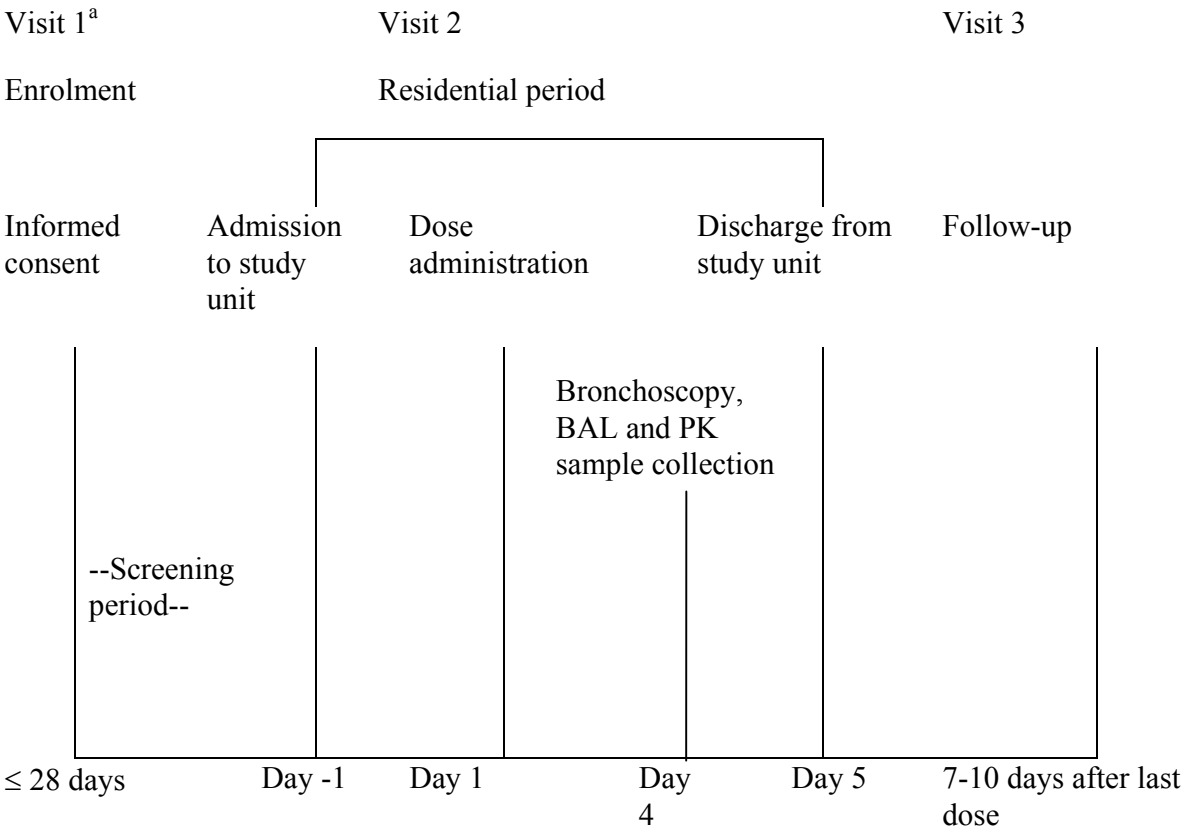
The study flow chart and visit flow chart for Part 2 are presented in Figure 1 and Figure 2, respectively, the overall study plan for Part 2 in Table 1 and the study time schedule for Part 2 in Table 2.

Figure 1 Study flow chart (Part 2)



Note: All the procedures, visits and timings of Part 1 (up to 3 subjects) will be same as Part 2; investigational product will not be administered in Part 1. For Part 1, pharmacokinetic sample collection will not take place and the subjects will not have a 5-day residential period.

Figure 2 Visit flow chart (Part 2)



[a] Visit 1 may be conducted over 1 or more days during the screening period.

Note: All the procedures, visits and timings of Part 1 will be same as Part 2; investigational product will not be administered in Part 1. For Part 1, pharmacokinetic sample collection will not take place and the subjects will not have a 5-day residential period.

Table 1 Overall study plan (Part 2)

Study assessments	Visit 1[a] (Screening) ≤28 days prior to Visit 2	Visit 2 (Treatment) Days -1 to 5 (Residential)	Visit 3 (Follow-up) 7 to 10 days after the last dose
Informed consent	X		
Demographics	X		
Medical/surgical history	X		
Physical examination[b]	X	X	X
Inclusion/exclusion criteria	X	X	
Weight and height	X		
Vital signs[c] (blood pressure, heart rate, temperature, respiration rate and pulse oximetry)	X	X	X
Lung function test	X		
Safety paper ECG[d]	X	X	X
Safety laboratory assessments[e] (haematology[f], clinical chemistry, coagulation and urinalysis)	X	X	X
Urine for creatinine, drug, and alcohol screen	X	X	
HIV antibody/hepatitis B and C virus screen	X		
Administration of dose		X	
Blood sampling for analysis of avibactam, ceftazidime and urea		X	
Bronchoscopy with BAL fluid collection for analysis of avibactam, ceftazidime and urea		X	
Concomitant medications	X	X	X
AE questioning[g]	X	X	X

AE = adverse event, BAL = bronchoalveolar lavage, ECG = electrocardiogram, HIV = human immunodeficiency virus, SAE = serious adverse event, TSH = thyroid-stimulating hormone.

[a] Screening visit can occur up to 28 days prior to Visit 2 and may be divided into 2 separate visits.

[b] A complete physical examination will be performed at screening and follow-up, while a brief physical examination on Day -1 and at discharge. Brief physical examination will include assessment of respiratory and cardiovascular systems, and general inspection.

[c] Vital sign measurements will be recorded at screening, Day -1, predose on Day 1 and every 24 hours thereafter till discharge, and at follow-up. Vital signs will be measured before BAL on Day 4. Vital signs will be recorded after ECG.

[d] ECG will be performed at screening, Day -1, Day 2, Day 4 (prior to BAL), Day 5 (prior to discharge from the study unit), and follow-up. Additional paper ECG for safety review by the Investigator will be performed as required. An additional paper ECG will be done at the following time points: 30 minutes before the start of the first infusion, and 1 hour, 2 hours, 3 hours and 6 hours after start of the first infusion. Telemetry will be conducted for each subject from 1 hour before to 24 hours after the first infusion (0-24 h) if there are any safety concerns telemetry will be continued. The period of telemetry may be be prolonged up to 48 hours after the first infusion if deemed necessary by the Investigator.

.[e] Blood samples will be collected for complete laboratory safety assessments (see Table 4) at screening, Day -1 (excluding TSH test), at discharge on Day 5 (excluding TSH and coagulation) and follow-up; and reduced laboratory safety assessments including total bilirubin on Days 3 and 4.

[f] Also includes follicle-stimulating hormone test, in female subjects.

[g] All AEs and SAEs will be collected from the time of obtaining informed consent through the follow-up visit.

Note: All the procedures, visits and timings of Part 1 will be same as Part 2; investigational product will not be administered in Part 1. For Part 1, pharmacokinetic sample collection will not take place and the subjects will not have a 5-day residential period.

Table 2 Study time schedule (Part 2)

Assessment	Predose	Hours postdose: 0	8	16	24	32	40	48	56	64	65	66[d]	67	68	70	72	76	80	88	Follow-up
Drug administration		X	X	X	X	X	X	X	X	X										
Blood sampling for analysis of avibactam, ceftazidime and urea in plasma	X									X	X	X	X	X	X	X	X	X	X	
Bronchoscopy for analysis of avibactam, ceftazidime and urea in BALF[a]												X[b]		X	X	X	X[c]			
Adverse event questioning	X →																		X	X

BALF = Bronchoalveolar lavage fluid.

[a] For each cohort, bronchoscopy with BAL will be performed once on each subject following the last dose, at one of 4 scheduled time points (Note: BAL will be performed in 5 subjects at each time point). Subjects will require a 6-hour fast prior to the procedure (6 hours for solids and 4 hours for liquids).

[b] Only to be performed for the 2-hour infusion.

[c] Only to be performed for the 4-hour infusion.

[d] An additional plasma sample will be analysed at 66.5 hours.

Note: All the procedures, visits and timings of Part 1 will be same as Part 2; investigational product will not be administered in Part 1. For Part 1, pharmacokinetic sample collection will not take place and the subjects will not have a 5-day residential period.

3.2 Rationale for study design and doses

In order to support the use of antimicrobial therapy for clinical indications such as respiratory tract infections, the BAL technique can be utilised to investigate the distribution of the drug in the lungs of humans. The BAL technique allows measurement of the concentration of drug in the ELF. Measuring the concentration of antimicrobial therapy in ELF is considered as a reliable marker of the concentration of antibiotics into lung tissue. This can give an indication of a drug's utility and potential effectiveness in respiratory tract infections.

This study will evaluate the relationship between concentrations of ceftazidime and avibactam in BALF and plasma in healthy subjects.

Dose and infusion duration are based on emerging data from the preclinical ELF study of avibactam (Investigator's Brochure 2010) and a clinical ELF study of ceftazidime (Bush et al 2010), which showed that the concentrations of avibactam or ceftazidime are lower in lung compared to those in plasma.

The study will be split into a procedural pilot component Part 1 with no IP administration and Part 2 with IP administration. During Part 1, all key procedures and timings will be similar to those in Part 2. The purpose of Part 1 is to verify optimal execution of the procedures and acquisition of satisfactory samples prior to entry of the first subject into the main part of the study (Part 2).

The maximum CAZ104 dose, 2000 mg ceftazidime/500 mg avibactam, is selected as the starting dose for Cohort A. To increase the potential of achieving greater concentration in ELF while decreasing the potential side effects associating with maximum concentration (C_{max}), the infusion duration is increased to 2 hours. A higher dose of CAZ104 (3000 mg ceftazidime/1000 mg avibactam) is also proposed to explore the relationship between plasma and ELF concentrations at this higher dose. Following completion of Cohorts A and B, samples will be analysed.

The decision to proceed to Cohort C will be based on several parameters including safety and tolerability of the IP and the BAL procedure, the PK of CAZ104 in plasma and ELF from Cohorts A and B as well as emerging data from preclinical studies. If the data suggests that adequate lung penetration cannot be gained by prolonging the infusion time, then Cohort C may not proceed. For Cohort C, the dose will not be higher than the dose used in Cohort B, but will be given over 4 hours.

4. SUBJECT SELECTION CRITERIA

The Investigator will keep a record, the subject screening log, of all the healthy subjects entering the screening before the study.

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

4.1 Inclusion criteria

For inclusion in the study subjects should fulfil 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 with veins suitable for cannulation or repeated venepuncture; female subjects must be postmenopausal or surgically sterile. Female subjects must have a negative pregnancy test at screening and on admission to the unit, must not be lactating and must be of non-child-bearing potential, confirmed at screening by fulfilling one of the following criteria:

- Post-menopausal defined as amenorrhoea for at least 12 months following cessation of all exogenous hormonal treatments and with follicle-stimulating hormone (FSH) levels in the laboratory defined post-menopausal range.

- Documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation.

3. Male subjects should be willing to use barrier contraception ie, condoms, from dosing to 3 months after dosing with the IP.

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

5. As judged by the Investigator, all the subjects must be able to understand and be willing to comply with study procedures, restrictions and requirements.

4.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

1. History or presence of any clinically significant disease or disorder (including a history of chronic respiratory disease eg, asthma, chronic obstructive pulmonary disease [COPD], cystic fibrosis or interstitial lung disease) 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. Any clinically significant illness, medical/surgical procedure or trauma within 4 weeks of the first administration of IP.

4. Any clinically significant abnormalities in physical examination, lung function test, ECG, clinical chemistry, haematology, coagulation screen, or urinalysis results as judged by the Investigator.

5. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and human immunodeficiency virus (HIV).

6. Prolonged QT interval corrected for heart rate using Fridericia's formula (QTcF) >450 ms or shortened QTcF<350 ms or family history of prolonged QT interval syndrome.

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

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

9. Positive screen for drugs of abuse, nicotine or alcohol.

10. Current smokers, ex-smokers who have smoked or used nicotine products within the previous 6 months and/or have smoked more than 10 pack years (no of pack years = [number of cigarettes smoked x number of years]/20).

11. 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 IP or longer if the medication has a long half-life. Occasional use of paracetamol/acetaminophen is allowed for minor pains and headache. Subjects may also take any local anaesthetic or sedation that is prescribed for the BAL procedure.

12. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Investigator or history of hypersensitivity to drugs with a similar chemical structure or class to avibactam, ceftazidime and/or excipients.

13. Known allergy to lidocaine/lignocaine, midazolam, alfentanyl or other anaesthetics/sedatives in similar classes to these agents.

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

15. Involvement in the planning and/or conduct of the study (applies to staff).

16. 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 3 months of the first administration of IP 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 administered with IP dose in this study or a previous Phase I study, are not excluded.

17. Judgement by the Investigator that the subjects should not participate in the study if they are considered unlikely to comply with study procedures, restrictions and requirements.

18. Individuals who are unsuitable for bronchoscopy.

19. Female subjects of child-bearing potential.

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

For procedures for withdrawal of incorrectly enroled 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. Fasting for at least 6 hours before planned start of bronchoscopy. A moderate amount of water is allowed up to 4 hours prior to bronchoscopy and may be resumed 2 hours after bronchoscopy. A meal can be given 2 hours after bronchoscopy.

2. Diet comprising of only standardised meals and drinks provided (apart from water) during the residential period in the unit.

3. Abstinence from consuming any of the following:

- Alcohol from 72 hours before admission, during the residential periods and for 72 hours before the study 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 study follow-up visit.

- Poppy seeds found in speciality bread from time of consent until after the final medical examination at the study follow-up.

- Grapefruit, grapefruit juice, Seville oranges, bitter oranges, all types of marmalade or other products containing grapefruit or Seville oranges from 7 days before admission until after the final medical examination at the study follow-up.

4. Limitation of caffeine intake to 5 cups per day from enrolment and limiting to 3 cups per day at meal times from admission to the clinic and during the study visits.

5. Abstinence from nicotine use, smoking and drugs of abuse from time of consent until after the final medical examination at the study follow-up.

6. Abstinence 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 IP or longer if the medication has a long half-life until after the final medical examination at the study follow-up. Occasional use of paracetamol/acetaminophen is allowed for minor pains and headache. However, this should not obviate necessary medical treatment. Subjects may also take any local anaesthetic or sedation that is prescribed for the BAL procedure.

7. Subjects should refrain from strenuous physical activity, which is not within their normal daily routine, from 7 days prior to admission to the unit until after the final medical examination at the study follow-up.

8. Abstinence from blood or plasma donation until 3 months after the final medical examination at the study follow-up.

9. Abstinence from scheduled inpatient surgery or hospitalisation during the course of the study.

10. 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 date of first dose administration until 3 months after the last dose administration with the IP.

5.2 Subject enrolment, randomisation and initiation of investigational product

The Investigator will:

- Obtain signed informed consent from the potential subjects before any study-specific procedure is performed.
- Assign potential subject a unique enrolment number, beginning with ‘E0001001’.
- Determine subject eligibility. See Sections 4.1 and 4.2.
- Assign eligible subject a unique subject number, beginning with ‘1001’.

If a subject withdraws participation in the study, then his/her enrolment code cannot be re-used.

If subjects have withdrawn their participation in the study after dosing they cannot re-enter into the study.

5.2.1 Procedures for randomisation

No randomisation will be performed. Bronchoscopy/BAL procedures will be performed once on each subject at one of the following time points: 2, 4, 6, and 8 hours after start of administration of the IP (Cohorts A and B) or 4, 6, 8 and 12 hours after start of administration of the IP (Cohort C). BAL will be performed in 5 subjects per time point in all the cohorts. Due to the time required to prepare for these procedures, subjects will not be randomised.

5.3 Procedures for handling subjects incorrectly enroled or randomised or initiated on investigational product

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

Where subjects who do not meet the selection criteria are enroled in error or incorrectly started on treatment they will be discontinued. Where subjects subsequently fail to meet the study criteria post initiation of the IP, a discussion should occur between the AstraZeneca Clinical Pharmacology Alliance (CPA) Physician and the Investigator regarding whether to continue or discontinue the subject from the study.

The AstraZeneca CPA Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the subjects should have their study therapy stopped.

5.4 Blinding and procedures for unblinding the study

This is an open-label study.

5.5 Treatments

5.5.1 Identity of investigational products

The IPs used in this study are presented in Table 3.

Table 3 Identity of the investigational products

Investigational product	Dosage form and strength	Manufacturer
Avibactam	Lyophilisate for concentrate for solution for infusion, 600 mg vial	
Ceftazidime	Sterile crystalline powder, 2 g vial	

Avibactam will be packaged and labelled in accordance with current Good Manufacturing Practice (GMP) and supplied to the clinical site as an open-labelled bulk supply for reconstitution and dilution.

Commercially available ceftazidime will be sourced and supplied to the clinical site for reconstitution and dilution.

5.5.2 Doses and treatment regimens

Please see Section 3.1 for doses and treatment regimen information.

The IP will be administered at Visit 2 and subjects are not required to fast prior to infusion. However, subjects will undergo a fasting period prior to the bronchoscopy (see Section 5.1 for more detail).

The total infusion time for Cohorts A and B will be 2 hours per dose. If the decision is made to proceed to Cohort C, the total infusion time for this cohort will be 4 hours per dose.

Avibactam and ceftazidime will be reconstituted and diluted when added to the saline bag for infusion.

5.5.3 Additional study drug

None.

5.5.4 Labelling

Labels will be prepared in accordance with GMP and local Regulatory guidelines. The labels will fulfil GMP Annex 13 requirements for labelling.

5.5.5 Storage

All IPs must be kept in a secure place under appropriate storage conditions. The labelling on the IP specifies the appropriate storage.

5.6 Concomitant and post study treatment

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 IP or longer if the medication has a long half-life will not be allowed in this study. Occasional use of paracetamol/acetaminophen is allowed for minor pains and headache. Subjects may also take any local anaesthetic or sedation that is prescribed for the BAL procedure.

Other medication, which is 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 Case Report Form (CRF).

5.7 Treatment compliance

The administration of all study drugs (including IPs) should be recorded in the appropriate sections of the CRF.

Treatment compliance will be assured by supervised administration of the IP by the Investigator or delegate. The dose, date and time of administration of the IP will be recorded and checked by the Medical Monitor at monitoring visits.

5.7.1 Accountability

The IP provided for this study will be used only as directed in the CSP.

The study site personnel will account for all IPs dispensed to the healthy subjects participating in this study.

The study site personnel will account for all IPs received at the site, unused IPs and for appropriate destruction. Destruction must not take place unless AstraZeneca has approved it. Certificates of delivery and destruction must be signed.

5.8 Discontinuation of investigational product

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons that may cause the subjects to discontinue are as follows:

- A subject is free to discontinue his/her participation in the study at any time, without prejudice to further treatment.
- Severe non-compliance to CSP as judged by the Investigator and/or AstraZeneca.
- AEs.

5.8.1 Procedures for discontinuation of a subject from investigational product

A subject who decides to discontinue the IP will always be asked about the reason(s) and the presence of any AE. If possible, they will be seen and assessed by the Investigator. AEs will be followed up (see Sections 6.4.3 and 6.4.4).

For more information on the withdrawal criteria, see Section 5.9.

5.9 Withdrawal from study

Subjects are free to withdraw from study at any time (IP and assessments), without prejudice to further treatment (withdrawal of consent). Please see Section 5.8.1 for details of the procedure to be followed if this happens. Subjects who do not complete critical study procedures may be replaced after discussion between the Principal Investigator and the Sponsor.

Specific criteria that if fulfilled, the subjects must be discontinued from treatment, are as follows:

- Risk to subjects as judged by the Investigator and/or AstraZeneca,
- Eligibility criteria not fulfilled,
- Subject lost to follow-up,
- Death,

- Any significant and clinically relevant changes in the safety parameters (eg, ECG parameters, additional paper ECG and telemetry findings ,vital signs, laboratory assessments and AEs) making the continuation of dose administration unjustified.

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below. The study plan and timing of these assessments are detailed in Section 3.1, Table 1 and Table 2, respectively.

6.1 Recording of data

The Investigator will ensure that data are recorded on electronic case report forms (eCRFs) as specified in the CSP and in accordance with the instructions provided.

The Investigator will ensure the accuracy, completeness, legibility (applicable for paper source documents) and timelines of the data recorded and of the provision of answers to data queries according to the Project Agreement. The Investigator will sign the completed eCRFs and a copy of the completed eCRF will be archived at the study site.

Procedures for data editing, entry and handling of the data query process will be described in the Data Management Plan (see Section 10).

6.2 Data collection and enrolment

At screening (Visit 1), each potential subject will provide written informed consent prior to starting any study-specific procedures. Please see Table 1.

Each subject will undergo screening during the 28 days prior to Visit 2 to confirm eligibility (please see Table 1). Subjects will be will be administered IP during Visit 2. Please see Table 1 for procedures to be performed during Visit 2.

A post study medical examination (follow-up visit) will be performed 7 to 10 days after the last dose. Please see Table 1 for procedures to be performed at Visit 3.

6.3 Efficacy

Not applicable.

6.4 Safety

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

6.4.1 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, pulse oximetry). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

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

6.4.2 Definitions of serious adverse event

A serious adverse event (SAE) is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death,
- Is immediately life-threatening,
- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability or incapacity,
- Is a congenital abnormality or birth defect,
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of a SAE, please refer to Appendix B of the CSP.

6.4.3 Recording of adverse events

Time period for collection of adverse events:

All AEs and SAEs will be collected from the time of obtaining informed consent throughout the treatment period and including the follow-up period.

Follow-up of unresolved AEs:

All AEs that are unresolved at the subject's last visit in the study are followed up 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),
- The date and time when the AE started and stopped,

- Maximum intensity,
- Whether the AE is serious or not,
- Investigator causality rating against the IP (yes or no),
- Action taken with regard to IP,
- AE caused subject withdrawal from study (yes or no),
- Outcome.

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

- Date AE met criteria for serious AE,
- Date Investigator became aware of serious AE,
- AE is serious due to,
- Date of hospitalisation,
- Date of discharge,
- Probable cause of death,
- Date of death,
- Autopsy performed,
- Causality assessment in relation to study procedure(s),
- Causality assessment in relation to other medication,
- Causality assessment in relation to additional IP,
- Description of AE.

The following intensity ratings will be used:

- 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).

- It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.4.2. 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 causal relationship between IP 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 IP?'

For SAEs causal relationship will be assessed for study treatment, other medication and study procedures. 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 for the 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 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 CSP mandated laboratory tests and vital signs will be summarised in the Clinical Study Report (CSR). Deterioration as compared to baseline in CSP-mandated laboratory values or vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the IP.

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

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.

6.4.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the IP, or to the study procedures. All SAEs will be recorded in the eCRF.

If any SAE occurs in the course of the study, then Investigators or other site personnel inform appropriate AstraZeneca representatives within 1 day ie, immediately but **not later than the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative will work with the Investigator to ensure that all the necessary information is provided to the AstraZeneca subject 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 to be undertaken immediately. Investigators or other site personnel will inform AstraZeneca representatives of any follow-up information on a previously reported SAE within 1 calendar day ie, immediately but **not later than the end of the next business day** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the Investigator's Brochure for the AstraZeneca drug CAZ104 and the European Union Summary of Product Characteristics (SPC) for avibactam and ceftazidime.

6.4.5 Laboratory safety assessments

Blood samples will be collected for complete laboratory safety assessments on at screening, Day -1 (excluding thyroid-stimulating hormone [TSH] test), at discharge on Day 5 (excluding TSH and coagulation) and follow-up; and reduced laboratory safety assessments on Days 3 and 4.

All laboratory parameters are listed in Table 4.

Table 4 Laboratory safety assessments

Clinical chemistry (plasma)	Haematology (blood)
Albumin	Haemoglobin[b]
Alanine aminotransferase (ALT)[b]	Neutrophils
Aspartate aminotransferase (AST)[b]	Eosinophils
Alkaline phosphatase	Leucocyte count[b]
Bilirubin (total)[b]	Leucocyte differential count[b]
Calcium (total)	Platelet count
Creatinine	Basophils
Glucose (fasting)	Lymphocytes
Potassium	Monocytes
Sodium	Other
Free thyroxine (T4)	Follicle-stimulating hormone (female subjects only)[a]
Thyroid-stimulating hormone (TSH)	
Urea	

Virology	Urinalysis	Coagulation
HIV[a]	Blood	Activated partial thromboplastin time (aPTT)
Hepatitis B and C virus[a]	Glucose	Prothrombin time (PT)
	Protein	International normalised ratio (INR)

[a] To be done at the screening visit only.
[b] Reduced safety laboratory test to be done at Days 3 and 4 only.

Urine will be tested for alcohol and the following drugs of abuse at screening and admission: nicotine and its metabolites, amphetamines, ecstasy, barbiturates, benzodiazepines, tricyclic antidepressants, cocaine, methadone, morphine, phencyclidine, tetrahydrocannabinol and opiates. If a subject tests positive to any of these screening tests he or she will be excluded from the study. Female subjects will have a urinary pregnancy test at screening and admission.

For blood volume, see Section 7.1, Table 5.

6.4.6 Physical examination

A complete physical examination, according to normal clinical routines, will be performed for all subjects at screening and follow-up. This will include examination of general appearance, skin, lymph nodes, thyroid, musculoskeletal/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. A brief physical examination will be performed on Day -1 and prior to discharge from the unit. This will include assessment of respiratory and cardiovascular systems, and general inspection.

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.

In the study database only information whether the assessment was performed or not is to be recorded as well as any AE.

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

6.4.7 Electrocardiogram

6.4.7.1 Resting 12-lead ECG

A 12-lead paper ECG after 10 minutes' rest in the supine position, will be recorded at screening, Day -1, Day 2, Day 4, Day 5 (at discharge), and follow-up.. The paper ECG for safety review by the Investigator will be performed at additional intervals if required. Paper ECG will be done at the following time points: 30 minutes before the start of infusion, and 1 hour, 2 hours, 3 hours and 6 hours after start of the infusion. The outcome of the overall evaluation is to be recorded as normal/abnormal in the eCRF, with any abnormalities specified. The printout of the paper ECG is to be signed, dated and filed in the information security form along with a signed and dated copy (if the printouts are not on archive-quality paper). For timing of assessments see Table 1 and Table 2.

6.4.7.2 Real time display (telemetry)

Telemetry will be conducted for each subject from 1 hour before to 24 hours after the first infusion (0-24h) if there are any safety concerns telemetry will be continued. The period of telemetry may be be prolonged up to 48 hours after the first infusion if deemed necessary by the Investigator.

6.4.8 Vital signs

Vital signs (blood pressure, heart rate, temperature, respiration rate and pulse oximetry) will be measured at screening, Day -1, predose on Day 1 and every 24 hours thereafter till discharge, and at follow-up. Vital signs will be measured before BAL on Day 4. Measurements will be performed according to local procedures, subsequent to 10 minutes' rest. Systolic and diastolic blood pressure will be measured using the same cuff size, appropriate for arm circumference, throughout the study. Vital signs will be recorded after ECG.

6.4.9 Lung Function Test

Lung function test will be performed for each subject at the screening visit.

6.5 Pharmacokinetics

6.5.1 Collection of samples

Venous blood samples (approximately 4 mL) for the determination of concentrations of avibactam and ceftazidime will be taken at times presented in Table 2. An additional 2 mL of blood will be collected at the bronchoscopy time point for the determination of concentrations of urea in plasma. The timing and number of PK samples may change based on emerging data. For blood volumes, see Section 7.1.

The results of the exploratory objective will be analysed and reported separately.

In each cohort, standardised bronchoscopy with BAL will be performed once for each subject at one of 4 scheduled time points following the start of the last dose infusion on Day 3. The bronchoscopy with BAL will be performed at one of the following time points based on start of the last infusion: at 2 hours, 4 hours, 6 hours, and 8 hours for the 2-hour infusion (Cohorts A and B); and at 4 hours, 6 hours, 8 hours and 12 hours for the 4-hour infusion (Cohort C). Five subjects will be assessed per BAL time point.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

Note: The time points should correspond with the approximate timing of the saline installation/aspiration process.

Bronchoscopy with BAL procedure and collection of samples:

The following bronchoscopy with BAL procedures must be performed: (Gotfried et al 2008)

- Subjects will fast for a minimum of 6 hours prior to the bronchoscopy with BAL procedure.
- Subjects will receive lidocaine spray to the oropharynx and/or lidocaine jelly to the nasal passageway. Four percent lidocaine will be instilled on the vocal cords and 2% lidocaine in the lower airways.
- Note: Any single subject will have the minimum possible amount of lidocaine applied. All of the lidocaine administrations must be recorded in the concomitant medication form.
- A 50 mL aliquot of sterile normal saline (0.9% weight/volume) will be instilled through the bronchoscope, aspirated, and then discarded to prevent contamination of the BAL specimens from larger airway secretions and from pre-medications. This procedure will be repeated 3 times and each instillation will be retained for analysis after collection.
- Immediately after completion of lavage, BAL aspirates will be pooled and the volume will be recorded.

- 4 mL of BALF will be separated into 2 aliquots, which will be processed in duplicate by the local clinical laboratory for total cell count and differential analysis.

- The remaining pooled BALF will be placed immediately on ice. The pooled BALF will be centrifuged at 400 x g for 5 minutes.

- BAL supernatant will be collected without disturbing the pellet. Two 3 mL aliquots of BALF supernatant will be prepared in separate tubes for bioanalysis of avibactam and ceftazidime, and two 3 mL aliquots of BALF supernatant for analysis of urea.

- The BAL pellet samples will be flash frozen and stored at -70ºC until they can be analysed by Quotient Bioresearch.

6.5.2 Determination of drug concentration

Samples for determination of avibactam and ceftazidime concentrations in plasma and BALF will be analysed by , on behalf of AstraZeneca using an appropriate bioanalytical method. Full details of the analytical methods used will be described in a separate bioanalytical report.

All samples will be analysed within a timeframe for which the stability of avibactam and ceftazidime in the samples has been validated and shown to be acceptable.

In addition, urea concentration in plasma and BALF will be analysed.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be collected from each subject in this study is presented in Table 5.

Table 5 Volume of blood to be drawn from each subject

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
Safety			
Clinical chemistry	5.0	6	30.0
Haematology	2.0	6	12.0
Virology	3.5	1	3.5
Coagulation	1.8	2	3.6
Pharmacokinetic	4.0	11	44.0
Urea	2.0	1	2.0
FSH (female subjects only) and TSH[a]	3.5	2	7

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
Safety			
Clinical chemistry	5.0	6	30.0
Haematology	2.0	6	12.0
Virology	3.5	1	3.5
Coagulation	1.8	2	3.6
Total	21.8		102.1

[a] Blood sample will be collected through serum separator tube for FSH and TSH test.

Note: an additional 1 mL of blood may be withdrawn to flush the cannula when blood samples are withdrawn through a cannula.

The maximum blood volume to be collected from each subject will not exceed 450 mL.

7.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed off after analyses or retained for further use as described here.

7.2.1 Pharmacokinetic and/or pharmacodynamic samples

After the analysis of avibactam and ceftazidime concentrations, the plasma and BALF samples will be retained by . These samples will be disposed off, on instruction from AstraZeneca on finalisation of the CSR. Data will be reported in the Bioanalysis Report. Additional avibactam and ceftazidime analysis may be conducted on the biological samples to investigate the reproducibility of the analytical results in incurred samples. Any results from such analysis will only be used to confirm the reproducibility of the method and the results will be reported in a separate table within the bioanalytical study contribution report.

7.3 Labelling and shipment of biohazard samples

The Investigator ensures that samples are labelled 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), please refer to Appendix C ‘International Air Transport Association (IATA) 6.2 Guidance Document’.

Any samples identified as infectious Category A materials will not be shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labelling, 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 lifecycle.

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

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

AstraZeneca will keep oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use will be registered in the AstraZeneca biobank system during the entire life cycle.

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 off/destroyed, and the action documented. If samples are already analysed, 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 be withdrawn from further study participation.

The Investigator will:

- Ensure subjects' 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 site, are immediately identified, disposed off or destroyed, and the action documented;
- Ensure that the laboratory holding the samples are informed about the withdrawn consent immediately and that samples are disposed off/destroyed, the action documented and the signed document returned to the study site;
- Ensure that the subject and AstraZeneca are informed about the sample disposal.

AstraZeneca will ensure that the central laboratory holding the samples is informed about the withdrawn consent immediately and that samples are disposed off/destroyed and the action documented and returned to the study site.

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 International Conference on Harmonisation (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 (ICF) 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 ICF 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 site staff.

The opinion of the EC should be given 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 ICF that are 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 ICF, is to be 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, EC and Principal Investigator with safety updates/reports according to local requirements, including Suspected Unexpected Serious Adverse Reactions (SUSAR), where relevant.

8.4 Informed consent

The Investigator at the centre 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 ICF(s) is/are stored in the Investigator's Study File.
- Ensure a copy of the signed ICF 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 ICF 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 a CSP amendment and where required in a new version of the CSP (revised CSP).

The amendment is to be approved by the relevant EC and if applicable, also the National Regulatory Authority, before implementation. Local requirements are to be followed for revised CSP.

AstraZeneca will distribute any subsequent amendments and new versions of the CSP to the Investigator. For distribution to EC, see Section 8.3.

If a CSP amendment requires a change to ICF, AstraZeneca and EC are to approve the revised ICF before the revised form is used.

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

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a Regulatory Authority, or an EC may perform audits or inspections at the centre, 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, analysed, and accurately reported according to the CSP, GCP guidelines of the ICH, and any

applicable Regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a Regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT

will manage the study on behalf of AstraZeneca.

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for a representative of AstraZeneca 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.
- Determine study personnel appropriately trained and experienced in performing bronchoscopy.
- Determine appropriate after-care for subjects after bronchoscopy.

9.2 Training of study site personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of the CSP and related documents with the investigational staff and also train them in any study specific procedures and systems utilised.

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

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

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the Investigator.
- Confirm that facilities remain acceptable.

- Confirm that the investigational team 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 IP accountability checks are being performed.

- Perform source data verification (a comparison of the data in the eCRFs 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 off/destroyed accordingly, and the action is documented, and reported to the subject.

The AstraZeneca representative will be available between visits if the Investigators or other staff at the centre need information and advice about the study conduct.

9.3.1 Source data

The location of data identified as source will be provided in a source data identification document provided by .

9.4 Study agreements

should comply with all the terms, conditions, and obligations of the Clinical Study Agreement (CSA), or equivalent, for this study. In the event of any inconsistency between this CSP and the CSA, the terms of 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 CSA shall prevail.

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

The Investigator should comply with all the terms, conditions and obligations of this CSP.

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the CSA.

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 3rd Quarter and end by 1st Quarter.

The study may be terminated 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 CAZ104 or avibactam.

10. DATA MANAGEMENT

Data management will be performed by .

A 21 Code of Federal Regulations Part 11 compliant eCRF will be used for this study. eCRFs will be produced by for each subject. The majority of study data collected will be either directly entered by clinical research staff or directly captured from devices onto the eCRF. Data will be available for AstraZeneca review via pre-defined reports extracted from the database at agreed intervals. The eCRFs must be kept in order and up-to-date so that they reflect the latest observations on the enroled healthy subjects.

When direct data entry onto the eCRF is inappropriate or impractical, data will be collected on paper source documents and subsequently transcribed, where necessary, onto the eCRFs by the clinical research staff of. All source documents will be retained by. Photocopies of completed source documents will be provided only if essential (ie, for Regulatory purposes) at the request of AstraZeneca.

Laboratory data are managed within the laboratory information management system (LIMS) and only the date and time of sampling will be recorded in the eCRF. Data that is not directly captured eg, safety laboratory results and AE coding, will be managed externally from the main study database. These data will be merged with the data from the main study database in post production. Datasets supplied to the Sponsor will contain all study data.

The informed consent will be kept with a copy of the completed source documents in the appropriate file folder provided, or a note to indicate where the records can be located. All records should be kept in conformance to applicable national laws and regulations.

All eCRF entries, corrections, and alterations must be made by the Investigator or other, authorised, study-site personnel and only by individuals who have received training on the electronic data capture system. Site staff may be allowed access to the system only after training is completed. Training must be documented and a log of all electronic data capture users and their rights within the system be maintained.

The Data Management Plan will describe the methods used to collect, check and process clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process.

Validity and consistency of data will be checked by employing pre-programmed data validation rules that will be applied to the data extracted from the eCRF during the course of the study. The data management team will raise queries in the eCRF to resolve discrepancies. The Investigator must verify that all data entries in the eCRFs are accurate and correct. After completion of the study and when all collected data are validated, the database will be locked. Final data will be extracted from the eCRF and delivered to AstraZeneca in the form of SAS® datasets in accordance with defined project standards. A pdf copy of the eCRF will be produced for each study healthy subject and included in the final delivery.

The eCRF will keep track of all data entry, alterations and query resolution in an audit trail. The audit trail will form an integral part of the database and will be archived alongside with the dictionary coding. Medical coding will be done using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA) and AstraZeneca Drug Dictionary.

Management of external data

Data Management determines the format of the data to be received from external vendors and coordinates the flow of data to an external environment or clinical database. External data reconciliation will be done with the clinical database as applicable.

SAE/AE reconciliation

SAE Reconciliation Reports will be produced and reconciled with the Subject Safety database and/or the Investigational Site.

Data verification and validation

The source data verification (SDV) will be carried out by an AstraZeneca representative comparing database entered data to source documents (ie, ECG print-outs, laboratory results and other health records at the study site). Questions and corrections will be noted and verified by the Investigator.

Some or all of the clinical datasets from the main study may be merged with the genetic data in a suitable secure environment separate from the clinical database.

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of efficacy variable(s)

Not applicable.

11.2 Calculation or derivation of safety variables

Change-from-baseline variables will be calculated for the safety variables listed below, as the post treatment value minus the value at baseline. The baseline values will be as follows:

- Clinical laboratory tests: Day -1
- Vital signs: Day -1
- ECG: Day -1

11.2.1 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 other AEs. Based on the expert's

judgement, significant AEs of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered other AEs and reported as such in the CSR. A similar review of laboratory/vital signs/ECG/additional paper ECG and telemetry data will be performed for identification of other AEs.

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

11.3 Calculation or derivation of patient reported outcome variables

Not applicable.

11.4 Calculation or derivation of pharmacokinetic variables

The PK analyses of the plasma and ELF 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 individual avibactam and ceftazidime concentrations in ELF will be derived from avibactam and ceftazidime concentrations in the BALF (supernatant) using the urea dilution method (Rennard et al 1986, Furuie et al 2010). Urea, a small and nonpolar molecule that can diffuse across the membranes freely, is used as an endogenous dilution marker as the concentration of urea in the ELF is assumed to be the same as that in the plasma. Avibactam and ceftazidime concentrations in ELF (C_{ELF}) will be calculated as:
$C_{ELF} = C_{BAL}*(C_{urea,p}/C_{urea,BAL})$
where C_{BAL} is concentration in BALF, and $C_{urea,p}$ and $C_{urea,BAL}$ are urea concentrations in plasma and BALF, respectively. The individual ratios of C_{ELF} relative to plasma concentration (C_{ELF}/C_p) for avibactam and ceftazidime will also be calculated at each shared time point when applicable.

The actual sampling times will be used in the individual PK parameter calculations in plasma. The PK parameters in ELF will be derived from the composite concentration-time profile consisting of the median concentration at each scheduled time point. For area under the concentration-time curve calculations, the predose concentrations in ELF for the last dose on the last day will be estimated from the mean predose concentration in plasma using the ratio of C_{ELF}/C_p at 8 hours postdose. Other approaches such as imputing predose concentrations from trough values at 8 hours or setting to lower limit of quantification (LLOQ) may be used as appropriate.

The PK parameters will be derived using noncompartmental methods. All PK computations will be performed using WinNonlin Professional 5.2 or higher ( ); Excel 2007 or higher ( ); or SAS® Version 9.1, or higher ( ). Graphics may be prepared with SAS Version 9.1, or higher; SigmaPlot® 9.0, or higher ( ); Excel 2007, or higher; or WinNonlin Professional 5.2, or higher.

Due to collection of a blood sample at the end of the infusion, no additional calculation of a concentration at the end of infusion will be performed unless the sample is reported as missing. The decision to impute a missing end-of-infusion value or in situations where a sample is collected close to but not exactly at the end of infusion will be made on a case-by-case basis per Standard Operating Procedures (SOP) and Work Instructions. The approach applied in the analyses will be documented in the CSR.

Where possible, the following PK parameters will be determined for avibactam and ceftazidime following the last dose on the last day:

- The C_{max} (µg/mL) in plasma and ELF, obtained directly from the observed concentration versus time data
- Time to maximum concentration (t_{max}, hr) in plasma and ELF, obtained directly from the observed concentration versus time data.
- Area under the plasma concentration-time curve during a dosing interval τ during a dosing interval τ (AUC_{τ}, µg.hr/mL) in plasma and ELF, calculated by linear up/log down trapezoidal summation.
- Terminal half-life ($t_{1/2\lambda z}$, hr) in plasma and ELF. 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.
- Plasma clearance (CL, L/hr)
- Volume of distribution at steady state (V_{ss}, L) in plasma
- Volume of distribution at terminal phase (V_z, L) in plasma
- Ratio of C_{max} in ELF over C_{max} in plasma ($C_{max,ELF}/C_{max,p}$)
- Ratio of AUC_{τ} in ELF over AUC_{τ} in plasma ($AUC_{\tau,ELF}/AUC_{\tau,p}$)

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

- The time interval (hr) of the log-linear regression to determine $t_{1/2\lambda z}$ ($t_{1/2\lambda z}$, interval)
- Number of data points ($t_{1/2\lambda z}$, N) included in the log-linear regression analysis
- Goodness-of-fit statistic (Rsq) for calculation of λ_z (regression coefficient)

Additional PK parameters may be determined if deemed appropriate.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

12.1.1 Efficacy analysis set

Not applicable.

12.1.2 Safety analysis set

All subjects who received at least 1 dose of IP, and for whom any postdose data are available will be included in the safety population.

12.1.3 Pharmacokinetic analysis set

The PK analysis set will include all subjects who receive 9 doses of avibactam and ceftazidime and have at least 1 postdose PK measurement without important CSP deviations or violations thought to significantly affect the PK of the drug.

12.2 Methods of statistical analyses

12.2.1 General principles

Data will be presented by cohorts for the purposes of summarising the safety results. Categorical variables (eg, sex, AEs) will be summarised in frequency tables (frequency and proportion of subjects in analysis set).

A subject who withdraws prior to the last planned observation will be included in the analyses up to the time of discontinuation.

12.2.2 Subject characteristics

Continuous variables will be summarised using descriptive statistics (n, mean, standard deviation [SD], minimum, median, maximum) by cohort. Categorical variables will be summarised in frequency tables (frequency and proportion) by cohort.

12.2.3 Safety and tolerability

Continuous variables (haematology, clinical chemistry, thyroid function and vital signs) will be summarised using descriptive statistics (n, mean, SD, minimum, median, maximum) for each cohort and each scheduled assessment point, both as absolute values and as change from the baseline. Categorical variables (eg, urinalysis) will be summarised in frequency tables (frequency and proportion) by cohort and scheduled assessment point. Further, safety data will be judged regarding low/high values or huge changes from predose using the AstraZeneca extended reference limits.

Abnormalities found on physical examination should be listed.

All AEs will be collected for each subject from the time when informed consent is obtained (Visit 1) until the follow-up visit. AEs that occur before dosing will be reported separately.

AEs will be summarised by preferred term and system organ class using MedDRA. SAEs, AEs leading to discontinuation of IP, AEs with severe intensity and AEs causally related to IP, as judged by the Investigator, will be listed by preferred term.

The number of subjects who had any AEs, SAEs, AEs that led to discontinuation of IP, AEs of different intensity, and AEs judged causally related to IP by the Investigator will be summarised.

12.2.4 Interim analyses

Not applicable.

12.2.5 Pharmacokinetics

A listing of PK, blood and BALF sample collection times as well as derived sampling time deviations will be provided. A subject listing of all concentration-time data for each dose/study cohort will be presented.

The PK variables (avibactam and ceftazidime concentrations in plasma and ELF, and PK parameters in plasma) and ratios of ELF concentration relative to plasma concentration for avibactam and ceftazidime will be summarised using appropriate descriptive statistics (eg, n, arithmetic mean, SD, minimum, median, maximum, geometric mean, and geometric coefficient of variation [CV%]). 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. Mean, SD, geometric mean, and CV% will not be calculated for t_{max}.

For descriptive statistics, concentrations below the LLOQ values will be handled as follows:

- At a time point where at least 1 value is above LLOQ but less than or equal to 50% values are below LLOQ, all values below LLOQ will be set to LLOQ and a mean (arithmetic and geometric) value and SD and CV% will be calculated.

- At a time point where more than half of the observations are below LLOQ only individual values are reported; mean, SD, geometric mean and CV% will be set to not quantifiable (NQ). The minimum value and the median will be set to less than LLOQ.

- If all values are below LLOQ at any time point no descriptive statistics will be calculated for that time point. Not applicable will be written in the field for SD and CV% and less than LLOQ will be written in fields for mean, geometric mean, minimum, median and maximum in the table.

- The number of observations greater than LLOQ [number of observations above LLOQ (N>LLOQ)] will be reported in the table.

Figures of mean (SD) concentration-time data will be presented on linear and semi-logarithmic scales by study cohort. Individual subject concentration-time data will be graphically presented on linear and semi-logarithmic scales. Graphical presentations of PK data may be added at the discretion of the PK scientist.

12.3 Determination of sample size

The sample size is based on the desire to obtain adequate safety, tolerability, and PK data to achieve the objectives of the study while exposing as few subjects as possible to study medication and procedures. The sample size for this study is selected to be consistent with the research hypothesis as described in Section 1.3. Five evaluable subjects are required at each BAL time point. Therefore, subjects who do not complete critical study procedures may be replaced after discussion between the Principal Investigator and the Sponsor.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Principal 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 6.4.4.** In the case of a medical emergency the Investigator may contact the CPA Physician. If the CPA Physician is not available, contact the CPA Programme Director as detailed below.

Table 6 Medical emergencies and AstraZeneca contacts

Name	Role in the study	Address and telephone number

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 IP occurs in the course of the study, then Investigator or other site personnel will inform appropriate AstraZeneca representatives within 1 day, ie, immediately but not later than the end of the next **business day** of when he or 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 6.4.4. 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 IP should be discontinued immediately and the pregnancy reported to AstraZeneca.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up and documented even if the subject is discontinued from the study.

If any pregnancy occurs in the course of the study, then Investigators or other site personnel inform appropriate AstraZeneca representatives within 1 day ie, immediately but no later than the **end of the next business day** of when he or 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 within 1 or 5 days for SAEs, see Section 6.4.4 and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

13.3.2 Paternal exposure

Male subjects must refrain from fathering a child, including sperm donation, during the study and 3 months following the last dose, since the potential for chromosomal aberrations in male gametes, and possible teratogenic effects thereof, has not yet been thoroughly investigated.

Pregnancy of the subject's partner is not considered to be an AE. If the Investigator receives information that a pregnancy has occurred for the subject's partner during the study, despite the study restrictions, the Investigator should ask the subject whether this information can be forwarded to AstraZeneca. If this permission is granted, the Investigator should inform the AstraZeneca representative. The Investigator will follow up and document the outcome of the pregnancy.

14. LIST OF REFERENCES

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Clinical Study Protocol Appendix A

Drug Substance	CAZ104
Study Code	D4280C00009
Edition Number	1
Date	
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Phase I Open-Label, 2-Part, 3-Cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma Using at Least Two Different Dosing Regimens in Healthy Volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol/amendment.

AstraZeneca Research and Development site representative

_____ _____

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A Phase I Open-Label, 2-Part, 3-Cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma Using at Least Two Different Dosing Regimens in Healthy Volunteers

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I agree to the terms of this study protocol/amendment.

AstraZeneca Research and Development site representative

_____ _____

Date
(Day Month Year)

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Phase I Open-Label, 2-Part, 3-Cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma Using at Least Two Different Dosing Regimens in Healthy Volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations and I ensure that all relevant site staff follows the instructions given in the latest version of the Laboratory Manual for Investigators.

Centre No.: 1

Signature:

_____ _____

Date
(Day Month Year)

Clinical Study Protocol Appendix B

Drug Substance	CAZ104
Study Code	D4280C00009
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 recur 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.

Clinical Study Protocol Appendix C	
Drug Substance	CAZ104
Study Code	D4280C00009
Edition Number	1
Date	

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.

AstraZeneca

Clinical Study Protocol Amendment

Amendment Number	1
Drug Substance	CAZ104
Study Code	D4280C00009
Date	
Protocol Dated	

A Phase I Open-Label, 2-Part, 3-Cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma Using at Least Two Different Dosing Regimens in Healthy Volunteers

Sponsor:

Centres affected by the Amendment:

The study will be conducted at a single centre:

The protocol for the study is to be amended as follows:

The estimated date of last healthy volunteer completed has been changed from to as appropriate in the revised protocol.

At the time of this protocol amendment 2 subjects have completed the pilot part (Part1) of the study and in Part 2 of the study 20 subjects in Cohort A have completed and Cohort B (Part 2) is in progress.

The purpose of this amendment to study D4280C00009 is to:

- align it with study D4280C00011 by introducing the same standard subject monitoring, subject withdrawal, and study stopping criteria related to elevated hepatic enzyme levels;

- Increase the subjects numbers to account for lost PK samples due to a freezer failure;

- Change the timing of SAE reporting to within 24 hours of the investigator being aware of the SAE.

Subject withdrawal, and study stopping criteria

These criteria are being added following an observation in another study involving CAZ-AVI (study D4280C00011, "A Phase I, 2-Part, Open-label, Pharmacokinetic and Drug-Drug Interaction Study of CAZ104") in which two of eight healthy volunteers were reported to have alanine aminotransferase (ALT) levels above 2 x upper limit of normal (ULN) (2.5 x and 2.6 x ULN, respectively) at the end of dosing. According to pre-set study protocol criteria study D4280C00011 was halted based on these laboratory findings and the Medicines and Healthcare products Regulatory Agency (MHRA) were notified of the temporary halt on

AstraZeneca has assessed the data within study D4280C00011 and concluded that the observed rises of ALT greater than 2 ULN but less than 3 ULN were reversible and did not result in any clinical events. Adverse drug reactions (ADRs) for ceftazidime are considered to be ADRs for ceftazidime-avibactam. The European Union (EU) Summary of Product Characteristics (SmPC) for ceftazidime notes that transient elevations in one or more of the hepatic enzymes -- ALT, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT) and alkaline phosphatise (ALP) -- are common. These events are described as expected adverse events in the investigator's brochure for the ceftazidime-avibactam development programme.

To better understand the observation of increased ALT observed in study D4280C00011, AstraZeneca is planning to introduce enhanced monitoring and withdrawal criteria to all Phase I protocols. These criteria are provided in the table below.

Liver chemistry variable	Intensified Monitoring criteria	Individual subject withdrawal criteria
ALT	Level >2 upper limit of normal (ULN), monitor at least every 48 h until return to within normal limits or stable per investigator	Level >3 ULN
ALP	Increase by >100%: check GGT, monitor at least every 48 h until return to within normal limits or stable per investigator	> 2 ULN

Bilirubin	>1.5 ULN, monitor at least every 48 h until return to within normal limits or stable per investigator	> 2 ULN

A priori study-stopping criteria will also be added to all protocols involving healthy volunteers. The stopping criteria will take into consideration the known safety profile of ceftazidime, which includes elevations of liver tests. Any study will be halted if 3 or more subjects experience either ALT >3 ULN or 3 subjects experience ALP >2 ULN, or 3 subjects experience bilirubin >2 ULN. In addition, if one or more subjects meet Hy's Law criteria, the study will be stopped. All protocols will require that a prompt study review take place if 2 or more subjects meet any individual subject withdrawal criteria related to liver tests.

The observation of reversible elevations of ALT in study D4280C00011 does not alter the risk benefit profile for ceftazidime avibactam. As a consequence, the protocol changes outlined above are not being introduced as urgent safety amendments and will be implemented in study D4280C00009 following Regulatory and Ethics approval of the changes. The planned revisions to the protocol allow intensified monitoring, as well as protection of individual subjects.

Failure of a freezer

A failure of the freezer where the samples of two subjects (1012 and 1013) were stored following collection had occurred. The failure of the freezer resulted in the thawing of Blood Pharmacokinetic samples taken in these two subjects during and following the fibre-optic bronchoscopic procedure for the collection of the Epithelial Lining Fluid Pharmacokinetic sample. These samples included the primary objective pharmacokinetic samples i.e. Blood Pharmacokinetic samples taken at the time point of the corresponding Epithelial Lining Fluid Pharmacokinetic samples.

There are 4 data points (n = 5) for each of these paired Blood and Epithelial Lining Fluid Pharmacokinetic samples in the study which are critical data points for the primary objective of the study. Due to the freezer failure and resultant thawing, these samples are no longer within the stability period for bioanalysis rendering the results unviable.

The protocol was designed to ensure that the minimum number of subjects would undertake a fibre-optic bronchoscopy to obtain matched Blood and Epithelial Lining Fluid Pharmacokinetic samples, and any replacement of subjects who had undergone fibre-optic bronchoscopy would be evaluated on a case by case basis to ensure that the absolute minimum number of subjects would undertake a fibre-optic bronchoscopy.

As we have lost critical primary end-point data in these two subjects, the Pharmacokinetic data for these two subjects is not evaluable with regards to the primary objective of the study. The primary objective for the study is thus now underpowered, and these two subjects would need to be replaced to maintain the powering of the primary objective of the study.

Throughout the revised protocol the number of subjects to be enrolled in the study has been increased to 65 (total number of subjects and 62 subjects (number of subjects enrolled in Part 2).

Section of protocol affected:

Study centre and number of subjects planned (page 2)

Previous text:

This study will be conducted at 1 study centre. Up to 63 healthy subjects will be enrolled; up to 3 subjects in a procedural pilot Part 1, and 60 subjects in Part 2.

Revised text

This study will be conducted at 1 study centre. Up to 65 healthy subjects will be enrolled; up to 3 subjects in a procedural pilot Part 1, and 62 subjects in Part 2.

Section of protocol affected:

Study design (page 3)

Previous text:

The study will be split into 2 parts: a procedural pilot Part 1 (including up to 3 subjects) will be performed first with no administration of the investigational product (IP), and a Part 2 (including maximum 60 subjects) with administration of the IP will be performed thereafter. The key procedures and timings will be same in Parts 1 and 2 of the study. For Part 1, PK sample collection will not take place and the subjects will not have a 5-day residential period. The subjects who will participate in Part 1 can be included in Part 2 after 21 days.

Revised text

The study will be split into 2 parts: a procedural pilot Part 1 (including up to 3 subjects) will be performed first with no administration of the investigational product (IP), and a Part 2 (including maximum 62 subjects) with administration of the IP will be performed thereafter. The key procedures and timings will be same in Parts 1 and 2 of the study. For Part 1, PK sample collection will not take place and the subjects will not have a 5-day residential period. The subjects who will participate in Part 1 can be included in Part 2 after 21 days.

Section of protocol affected:

Table of Contents (page 9)

Previous text:

LIST OF APPENDICES

Appendix A Signatures (Not Applicable)

Appendix B Additional Safety Information

Appendix C International Airline Transportation Association (IATA) 6.2 Guidance Document

Revised text:

LIST OF APPENDICES

Appendix A Signatures (Not Applicable)

Appendix B Additional Safety Information

Appendix C International Airline Transportation Association (IATA) 6.2 Guidance Document

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

Section of protocol affected:

3.1 Overall study design and flow chart (page 19)

Previous text:

The study will be split into 2 parts. A procedural pilot Part 1 (including up to 3 subjects) to verify optimal execution of the procedures and acquisition of satisfactory samples, will be performed first with no administration of the IP. A Part 2 (including maximum 60 subjects) with administration of the IP will be performed thereafter. The key procedures and timings will be same in Parts 1 and 2 of the study. For Part 1, PK sample collection will not take place and the subjects will not have a 5-day residential period. The subjects who participate in Part 1 can be included in Part 2 after 21 days.

Revised text

The study will be split into 2 parts. A procedural pilot Part 1 (including up to 3 subjects) to verify optimal execution of the procedures and acquisition of satisfactory samples, will be performed first with no administration of the IP. A Part 2 (including maximum 62 subjects) with administration of the IP will be performed thereafter. The key procedures and timings will be same in Parts 1 and 2 of the study. For Part 1, PK sample collection will not take place and the subjects will not have a 5-day residential period. The subjects who participate in Part 1 can be included in Part 2 after 21 days.

Section of protocol affected:

3.1 Overall study design and flow chart (page 21)

Previous text:

Up to 3 subjects will be enroled in Part 1. In Part 2, a minimum of 40 (if the study comprises of 2 cohorts) to a maximum of 60 (if the study comprises of all 3 cohorts in Part 2) subjects will be enroled. Up to 63 subjects in total will be enroled at 1 study centre.

Revised text

Up to 3 subjects will be enroled in Part 1. In Part 2, a minimum of 42 (if the study comprises of 2 cohorts) to a maximum of 62 (if the study comprises of all 3 cohorts in Part 2) subjects will be enroled. Up to 65 subjects in total will be enroled at 1 study centre.

Section of protocol affected:

5.8 Discontinuation of investigational product (page 34 and 35)

Previous text:

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons that may cause the subjects to discontinue are as follows:

- A subject is free to discontinue his/her participation in the study at any time, without prejudice to further treatment.
- Severe non-compliance to CSP as judged by the Investigator and/or AstraZeneca.
- AEs.

Revised text:

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons that may cause the subjects to discontinue are as follows.

Individual subject stopping criteria:

- A subject is free to discontinue his/her participation in the study at any time, without prejudice to further treatment.
- Severe non-compliance to CSP as judged by the Investigator and/or AstraZeneca.
- AEs
- Individual subject withdrawal and intensified monitoring criteria:

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

Study stopping criteria:

- 3 or more subjects have either >3x ULN of ALT or 3 subjects have >2x ULN ALP or 3 subjects have elevations of total bilirubin >2x 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).

Study review criteria

- If any 2 subjects meet any of the individual subject withdrawal criteria related to liver tests for the study, the investigator will contact the AZ CPA physician to initiate a review process that will, at minimum, include the MSD and the GSP. The review process will consider study and subject information, and determine the subsequent necessary actions.

Section of protocol affected:

6.4.4 Reporting of serious adverse events (page 40)

Previous text:

If any SAE occurs in the course of the study, then Investigators or other site personnel inform appropriate AstraZeneca representatives within 1 day ie, immediately but **not later than the end of the next business day** of when he or she becomes aware of it.

Revised text:

If any SAE occurs in the course of the study, then Investigators or other site personnel inform appropriate AstraZeneca representatives immediately, **or no later than 24 hours** ~~1 day ie, immediately but **not later than the end of the next business day**~~ of when he or she becomes aware of it.

Section of protocol affected:

Table 6 Medical emergencies and AstraZeneca contacts (page 56)

Previous text:

Name	Role in the study	Address and telephone number

Revised text:

Name	Role in the study	Address and telephone number
_____		

_____		

Persons who initiated the Amendment:

Clinical Project Team

AstraZeneca

Clinical Study Protocol Amendment 1: Appendix A

Drug Substance	CAZ104
Study Code	D4280C00009
Edition Number	1
Date	
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Phase I Open Label, Randomised, 2-part, 3-cohort, Single-Centre Study to Assess the Concentration of NXL104 and Ceftazidime in Epithelial Lining Fluid and Plasma in at Least Two Different Dosing Regimens in Healthy Volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol/amendment.

AstraZeneca Research and Development site representative

_____ _____

Date
(Day Month Year)

Signing on behalf of

ASTRAZENECA SIGNATURE(S)

A Phase I Open Label, Randomised, 2-part, 3-cohort, Single-Centre Study to Assess the Concentration of NXL104 and Ceftazidime in Epithelial Lining Fluid and Plasma in at Least Two Different Dosing Regimens in Healthy Volunteers

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AstraZeneca Research and Development site representative

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Phase I Open Label, Randomised, 2-part, 3-cohort, Single-Centre Study to Assess the Concentration of Avibactam and Ceftazidime in Epithelial Lining Fluid and Plasma in at Least Two Different Dosing Regimens in Healthy Volunteers

---

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I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations and I ensure that all relevant site staff follows the instructions given in the latest version of the Laboratory Manual for Investigators.

Centre No.:

Signature:

Date
(Day Month Year)