Revised Clinical Study Protocol

Drug Substance	Ceftazidime Avibactam (CAZ-AVI)
Study Code	D4280C00020
Edition Number	1.0
Date	

A Phase I, Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability and Pharmacokinetics of Ceftazidime-Avibactam Administered as Single and Repeated Intravenous Doses in Healthy Chinese Subjects

Sponsor:

AstraZeneca Research and Development site representative

Date

The following Amendment(s) and Administrative Changes are included in this revised protocol:

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment
1.0			

Administrative change No.	Date of Administrative Change	Local Administrative change No.	Date of local Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

A Phase I, Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability and Pharmacokinetics of Ceftazidime-Avibactam Administered as Single and Repeated Intravenous Doses in Healthy Chinese Subjects

Principal Investigator:

Study centre(s) and number of subjects planned

This will be a single centre study. Approximately 16 healthy male and female (of non-child bearing potential) Chinese subjects, aged 18 to 45 years, inclusive, will be recruited. Subjects will be randomised to active or placebo treatment in a 3:1 ratio to obtain approximately 9 evaluable subjects on active treatment and 3 subjects on the placebo treatment. An evaluable subject is defined as a volunteer completing 90% of all study procedures with adequate plasma samples collected for PK analysis.

Study period	Phase of development
Estimated date of first subject enrolled	I
Estimated date of last subject completed	I

Objectives

Primary Objective

- To investigate the safety and tolerability of ceftazidime-avibactam (CAZ -AVI) administered in single and repeated intravenous (IV) infusions in healthy Chinese subjects.

Secondary Objective

- To investigate the pharmacokinetics (PK) of AVI administered in combination with CAZ.
- To investigate the PK of CAZ administered in combination with AVI.

Study design

This is a randomized, double-blind, placebo-controlled study to assess the safety, tolerability, and PK of ceftazidime-avibactam (CAZ-AVI) administered as single and repeated IV doses in healthy Chinese subjects.

Subjects will receive a single dose of CAZ-AVI or matched placebo as a 120 minute IV infusion on Day 1, followed by administration of CAZ-AVI or matched placebo as a 120 minute IV infusion every 8 hours (q8h) for 7 days (Day 2 to Day 8), and one single dose of CAZ-AVI or matched placebo as a 120 minute IV infusion on Day 9.

This study will be conducted in healthy volunteers. Subjects will be recruited and randomized to AVI 500 mg + CAZ 2000 mg or matched-placebo

In total, approximately 16 Chinese healthy subjects will be randomised to active and placebo treatment in a 3:1 ratio to obtain approximately 9 evaluable subjects on active treatment and 3 on the placebo treatment (allowing for the possibility that a small proportion of subjects may not complete the study).

Plasma and urine PK samples will be obtained at the times noted in Table 2

Plasma and urine samples for PK analysis will be collected following the single dose on Day 1 and Day 9. Plasma samples for PK analysis will be also collected pre-dose prior to the first dose on Days 6, 7 and Day 8 of the repeated dosing days. Safety monitoring will occur throughout the study via vital signs measurements, oral body temperature, electrocardiograms (ECGs), clinical laboratory measurements, physical examinations, and assessment of adverse events (AEs).

Target subject population

Healthy male and female (of non-child bearing potential) Chinese subjects, aged 18 to 45 years, inclusive.

Investigational product, dosage and mode of administration

Intravenous infusion of AVI 500 mg + CAZ 2000 mg. A single 120 minute IV infusion on Day 1, followed by three times daily (every 8 hours, q8h) as 120 minute IV infusions for 7 days (Day 2 to Day 8), and a single 120 minute IV infusion on Day 9.

Comparator, dosage and mode of administration

Single and repeated 120 minute IV infusions of placebo (normal saline, 0.9%).

Duration of treatment

The total length of the study for each volunteer is up to 42 days, including up to 28 days for screening, 9 days of in-house treatment, and the follow-up visit 3 to 5 days after the last dosing day.

Outcome variable(s):

- Pharmacokinetic

  The following plasma PK parameters will be calculated using non-compartmental analysis.

  After single IV infusion (Day 1):

  C_{max}, t_{max}, C_{last}, t_{last}, $AUC_{(0-t)}$, AUC, $AUC_{(0-8)}$, λ_z, $t_{1/2}$, V_{ss}, V_z, CL, MRT, etc.

  The trough concentration C_{min} (Days 6, 7, 8)

  After repeated infusion (Day 9):

  C_{min}, $t_{max,ss}$, $C_{max,ss}$, C_{av}, AUC_{ss}, CL_{ss}, V_{ss}, λ_z, $t_{1/2}$, V_z, MRT, accumulation ratio for C_{max} [RC_{max}], and AUC [RAUC],linearity factor, etc

  From urine data, the following parameters will be calculated:

  Cumulated urinary excretion (amount), percentage of cumulated urinary recovery (% dose) and renal clearance (CL_r). Non- renal clearance (CL_{nr}) will be calculated as the difference between total clearance (CL) and renal clearance (CL_r), if appropriate.

- Safety

  Adverse events, vital signs, ECGs, clinical laboratory measurements, physical examinations, oral body temperature.

Statistical methods

No formal statistical hypothesis testing will be performed.

Descriptive summary statistics will be provided for safety and PK variables. For safety variables, the number of subjects (n), minimum, first quartile, median, third quartile and maximum will be calculated. For PK variables, summaries will include arithmetic mean, standard deviation (SD), geometric mean (gmean), gmean +/- SD, coefficient of variation (CV), minimum, median and maximum as appropriate. All safety variables will be listed and, as applicable, presented graphically or summarized for the observed value at each scheduled assessment and for the corresponding change from baseline.

For categorical variables, frequency distributions, including percent values, will be provided. Graphical presentations will be given where appropriate.

In the event of treatment mis-assignment, subjects will be analyzed according to the treatment that they received, and not according to the treatment to which they were randomized.

No formal sample size calculations are performed. 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.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
λ_z	Elimination rate constant
AE	Adverse event (see definition in Section 6.4.1)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
aPTT	Activated Partial Thrombin Time
AST	Aspartate aminotransferase
AUC	Area under the curve extrapolated to infinity
$AUC_{(0-t)}$	Area under the curve from time 0 to the time corresponding to the last measurable concentration
$AUC_{(0-8)}$	Area under the curve from 0 to 8 hours post-dose
AUC_{ss}	Area under the curve during the dosing interval (0-8 hour post-dose) at steady state
AUMC	Area under the moment curve from time 0 to the time corresponding to the last measurable concentration
BLQ	Below the limit of quantification
BMI	Body Mass Index
BP	Blood pressure
BUN	Blood urea nitrogen
CAZ-AVI	Combination of AVI and CAZ
CL	Total clearance
CL_{ss}	Total plasma clearance at steady state
$C_{ss,max}$	Maximum plasma concentration at steady state
$C_{ss,min}$	Minimum plasma concentration at steady state
C_{last}	Last measurable plasma concentration
CL_{nr}	Non-renal clearance
CL_r	Renal clearance
C_{max}	Maximum plasma concentration
C_{min}	Minimum plasma concentration
CPU	Clinical Pharmacology Unit

Abbreviation or special term	Explanation
CRF	Case Report Form
CV	Coefficient of variation
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eCRF	Electronic CRF
ESBL	Extended spectrum β-lactamases
FSH	Follicle stimulating hormone
Gamma GT	Gamma glutamyl transferase
GCP	Good clinical practice
HBs	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IC_{50}	Half maximal inhibitory concentration
ICH	International Conference on the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IRB	Institutional Review Board
IV	Intravenous
KPC	Klebsiella pneumoniae carbapenemase
Linearity factor	AUC_{ss} on Day 9 compared with AUC on Day 1 to assess temporal changes in PK
MAD	Multiple ascending dose
MDMA	3,4-methylenedioxymethamphetamine
MDR	Multidrug resistant
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum Inhibition Concentration
MRT	Mean residence time
OAE	Other Significant Adverse Event
pECG	Paper print-out ECG
PK	Pharmacokinetics

Abbreviation or special term	Explanation
PR(PQ)	ECG interval measured from the onset of the P wave to the onset of the QRS complex.
PT	Prothrombin time
QDS	Quality Data Services, Inc.
QRS	ECG interval measured from the onset of the QRS complex to the J point.
QT	ECG interval measured from the onset of the QRS complex to the end of the T wave
QT_c	Corrected QT-interval
QT_cF	QT interval corrected for heart rate using Fridericia's formula
RBC	Red blood cell
RC_{max}	Accumulation ratio for C_{max} following multiple dosing
RR	The time between corresponding points on 2 consecutive R waves on ECG
RAUC	Accumulation ratio for AUC following multiple dosing
SAD	Single ascending dose
SAE	Serious adverse event (see definition in Section 6.4.2).
SBP	Systolic blood pressure
SD	Standard deviation
SOC	System organ class
ss	Steady state
SUSAR	Sudden unexpected serious adverse reaction
t_{ae}	The (assumed equal) dosing interval for steady-state data.
$t_{1/2}$	terminal half-life
$t_{ss, max}$	Time of peak drug concentration at steady state
TEAE	Treatment emergent adverse event
tid	Three daily
t_{last}	Time at last measurable plasma concentration
t_{max}	Time of peak drug concentration
ULN	Upper Limit of Normal
V_{ss}	Volume of distribution at steady state
WBC	White blood cells

1. INTRODUCTION

1.1 Background

1.1.1 Multidrug resistance

The prevalence of multidrug-resistance (resistance to at least 3 different antibiotic groups) strains among Gram-negative bacilli is increasing (Ambler et al 1991, Bush 2001, Cosgrove et al 2002, D'Agata 2004, Gales et al 2001, Karlowsky et al 2003a, Karlowsky et al 2003b). Compared with infections due to antimicrobial-susceptible Gram-negative bacilli, infections due to multidrug-resistant Gram-negative bacilli lead to longer hospital stays, increased mortality, and greater costs of hospitalization (Giske et al 2008).

Resistance to β-lactam drugs in Gram-negative bacteria is most commonly attributed to β-lactamase production, either chromosomally or plasmid borne. Chromosomally-mediated β-lactamase (Ambler Class C) production is mainly through expression of the *ampC* gene, which is either constitutive or inducible and is found among the Enterobacteriaceae and *Pseudomonas aeruginosa* (Jacoby 2009). Class C β-lactamases are resistant to marketed β-lactamase inhibitors (clavulanic acid, tazobactam, and sulbactam). In *Enterobacter*, the expression of the *ampC* gene is repressed, but genetically-stable derepressed variants can be selected by β-lactams, particularly third-generation cephalosporins. These mutants are resistant to most β-lactam antibiotics except carbapenems (Fraser et al 2010).

Serratia, *Morganella*, *Providencia*, *Enterobacter*, *Citrobacter freundii*, and *P. aeruginosa* have similar although not identical, chromosomal *ampC* β-lactamase genes that are inducible (Fraser et al 2010, Jacoby 1999). Plasmid-encoded AmpC enzymes have been reported from *Klebsiella* species and *Escherichia coli* isolates. Ampicillin, amoxicillin, first- and second-generation cephalosporins, and cephamycins are strong AmpC β-lactamase inducers. They are also rapidly inactivated by these β-lactamases; thus, resistance is readily documented in vitro (Fraser et al 2010).

1.1.2 Extended-spectrum β-lactamases

The most common of the β-lactamase-mediated mechanisms of resistance to β-lactam antibiotics among Gram-negative pathogens is that of extended-spectrum β-lactamases (ESBLs). These enzymes are plasmid-mediated β-lactamases of predominantly Ambler Class A. Extended-spectrum β–lactamases represent a major group of β-lactamases that are now found in a significant percentage of *E. coli*, *Klebsiella pneumoniae*, and other species of Enterobacteriaceae including *Enterobacter*, *Citrobacter*, *Proteus*, *Morganella morganii*, *Serratia marcescens*, and *Shigella dysenteriae*. They are also found in *P. aeruginosa* and *Burkholderia cepacia* (Bush 2001, Ambler et al 1991). Extended-spectrum β-lactamase-producing bacteria often show cross-resistance to other groups of antibiotics such as fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole.

Extended-spectrum β-lactamases are capable of efficiently hydrolyzing penicillins, narrow-spectrum cephalosporins, many extended-spectrum cephalosporins, cephalosporins

containing an oxyimino group (cefotaxime, ceftazidime), and monobactams (aztreonam). The majority of ESBL-producing organisms produce more than 1 β-lactamase and strains producing multiple ESBLs are being reported. Different strains vary in the actual amount of each β-lactamase produced (Go et al 2004).

Infections due to ESBL-producing organisms present a major therapeutic dilemma, as the choice of antibiotics is extremely limited. Clinical outcome is poor when third–generation cephalosporins are used to treat ESBL-producing organisms. Bacteria producing ESBLs should be considered resistant to all generations of cephalosporins, all penicillins, and to the monobactams (aztreonam). Even though cefepime (a fourth-generation cephalosporin) exhibits more stability to hydrolysis by ESBLs than the third–generation cephalosporins, a positive clinical outcome from treatment with this antibiotic has not been established. (Rodrigues et al 2004, Jacoby 1999, Rice et al 1996, Thauvin-Eliopoulos et al 1997)

Carbapenems are the drugs of choice for the serious infections caused by ESBL-producing organisms. Carbapenems are the only reliable β-lactam drugs for the treatment of severe *Enterobacter* infections. Resistance to carbapenems is rare but occurs in strains that produce serine-carbapenemases (*K. pneumoniae* carbapenemase [KPC] enzymes). Over the past decade a group of serine-carbapenemases has been increasingly reported from around the world (Hirsch et al 2010). As one example of this observation, resistance has been reported for imipenem in strains of *Enterobacter cloacae* (Fraser et al 2010). Hyper-production (stable derepression) of AmpC β-lactamases, in association with some decrease in permeability to the carbapenems, may also cause resistance to these agents. Carbapenems are strong AmpC β-lactamase inducers but, so far, are not degraded by the action of these β-lactamases. Widespread use of carbapenems may lead to the emergence of carbapenem-resistant *Acinetobacter baumannii*, *P. aeruginosa*, *Stenotrophomonas maltophilia*, and vancomycin-resistant enterococci (Rodrigues et al 2004).

1.1.3 Ceftazidime Avibactam

Avibactam is a novel, non-β-lactam, β-lactamase inhibitor with a spectrum of activity encompassing both Class A and Class C β-lactamases. Beta-lactamase inhibition is effected through the formation of a stable covalent carbamoyl linkage through the active site serine. AVI, when associated with CAZ, has also been shown to be active against strains that express a combination of β-lactamase types, as well as strains that are concomitantly resistant to other antibacterial classes such as fluoroquinolones.

Beta–lactamase inhibition by AVI is effected through the formation of a stable covalent carbamoyl linkage to the enzyme complex that is practically irreversible. It inhibited Class A and Class C β–lactamases by 50% at lower concentrations than did other currently marketed β–lactamase inhibitors such as clavulanic acid, tazobactam, and sulbactam. In addition, AVI is a potent inhibitor of Class C enzymes whereas clavulanic acid, tazobactam, and sulbactam lack any activity against this class of enzymes. Unlike currently available β-lactamase inhibitors, AVI does not induce β–lactamase production.

AVI inhibited KPC-2 β–lactamase in vitro and restored CAZ susceptibility to Enterobacteriaceae harboring KPC-2 or KPC-3 β–lactamase (Stachyra et al 2009). The potent in vitro activity of the CAZ and AVI combination against Enterobacteriaceae producing Class A, and more importantly Class C, β-lactamases has been confirmed in vivo in murine pneumonia, septicemia, and pyelonephritis models.

Currently the options for the treatment of Gram-negative infections, especially multidrug-resistant strains including ESBL producers, are extremely limited. Until recently, there have been no new investigational compounds under early or late development specifically targeted to combat these organisms. Hence availability and development of new agents to treat these infections will be a welcome addition to the existing treatments.

1.1.4 Human experience – Phase I

At the time of this protocol, 5 clinical pharmacology studies have been completed.

Two Phase I dosing studies were conducted in healthy male volunteers to assess safety, tolerability, and PK parameters. One single escalating dose study (Study NXL104/1001; n = 70) and 1 multiple ascending dose study (NXL104/1002; n = 49) with AVI (formerly referred to as NXL104) intravenous (IV) administration alone or in combination with CAZ. For these 2 studies, a total of 97 healthy volunteers received at least 1 IV administration of AVI with the dose ranging from 50 mg to 2000 mg and with the dose frequency ranging from a single dose to 3 times a day for 10 days (a total of up to 30 doses at 500 mg). For both studies, AVI was well tolerated at all doses regardless of administration frequency or the co-administration of CAZ. There was no evidence of local IV or injection site intolerance in either study. The incidence of treatment emergent adverse events (AEs) was relatively low (7.1% in the single dose study, 12.1% in the multiple dose study); AEs were of mild or moderate intensity and all healthy volunteers recovered without sequelae.

A clinical study to evaluate the effect of age and gender on the PK and safety of AVI (formerly known as NXL104) was performed in 33 healthy volunteers (Study NXL104/1004). Subjects were divided in to 4 cohorts of young (<45 years old) or elderly (≥ 65 years old) male or female and received a 500 mg single IV dose of AVI alone. Although there was a statistically significant gender effect for maximum concentration (C_{max}) (male subjects had 18% lower C_{max} values), there was no gender effect for area under the plasma concentration-time curve (AUC) parameters. In addition, young subjects had greater urinary excretion over a 24-hour period than elderly subjects regardless of gender (91% versus 68% for male and 86% versus 60% for female, respectively). A total of 18 AEs were reported in 10 subjects: 1 of 9 young men, 3 of 8 young women, 2 of 8 elderly men, and 4 of 8 elderly women. In view of the safety and tolerability of a 500 mg dose of AVI in young and elderly subjects with normal renal function, of both male and female gender, the differences in C_{max} and AUC parameters do not warrant any dose adjustment.

The effect of renal impairment on the safety and PK parameters of AVI (formerly known as NXL104) has been assessed in a study of 30 patients with renal impairment (Study NXL104/1003). Subjects were divided into 5 renal function groups from normal renal

function to end-stage renal failure requiring hemodialysis. Patients from the end-stage renal failure cohort participated in 2 randomized sessions (during and inter dialysis) separated by a washout period of 7 to 14 days. After a single 100 mg dose of AVI administered as a 30-minute infusion, mean clearance was about 14.5 L/hour in healthy subjects and fell to 5.8 L/hour in patients with mild to moderate renal impairment (–61%). The decrease was more pronounced in patients with moderate renal impairment and nondialyzed patients with severe renal impairment with mean clearance of 3.8 L/hour (–74%) and 2.2 L/hour (-85%), respectively. Clearance reached a residual value of about 1.0 L/hour (–93%) in patients with end-stage renal disease (off dialysis). These data were in agreement with the dominant urinary elimination of AVI. The relationship between AVI renal clearance and calculated creatinine clearance was found to be linear. Based on these data, dosage adjustments will be required in patients with moderate or severe renal impairment. Population PK and PK/pharmacodynamic (PD) modeling support adjustments in the dose amount and frequency of administration for CAZ-AVI that are consistent with those already recommended for CAZ (eg, in patients with a creatinine clearance level of 31 to 50 mL/min, a 50% reduction in dose, given every 12hours rather than every 8 hours).

A Phase I open-label, single dose study was conducted in healthy subjects (Study D4280C00008) to investigate the excretion and metabolism of [14C] AVI. The [14C] AVI ADME study showed that an average of 97 % (range 95 % to 98 %) of administered radioactivity was recovered from the urine, over 95 % within 12 hours of dosing. AVI is predominantly renally cleared and metabolism has little contribution to it's elimination. An average of 85 % (range 67 % to 101 %) of administered AVI was recovered from the urine during the study, with > 50 % being recovered within 2 hours of the start of the infusion. Renal clearance was 158 ml/min suggesting active tubular secretion.

Overall, preliminary data indicate that there were no major safety and tolerability concerns identified in this study. Additional details can be found in Section 5.1 of the CAZ-AVI Investigator Brochure.

In addition, 2 other Phase 1 studies have been conducted:

- A Phase I double-blind, randomized, placebo-controlled, 4-way crossover thorough QT study to assess PK and safety in healthy volunteers (Study D4280C00007)
- A Phase I single and multiple dose study in healthy male Japanese subjects (Study D4280C00010)

Data from Study D4280C00007 indicate that a single supratherapeutic IV dose of CAZ-AVI (3000 mg ceftazidime plus 2000 mg avibactam) does not prolong the QTc (corrected QT interval) corrected by Fridericia (QTcF) beyond 10 ms. There were no QTcF values greater than 450 ms nor were there any QTcF changes from Baseline greater than 30 ms after a single supratherapeutic IV dose of CAZ-AVI.

In Study D4280C00010, AVI alone and in combination with CAZ were well tolerated at the doses tested when administered as single and multiple doses to healthy male Japanese subjects. There were no clinically significant abnormal electrocardiogram (ECG) measurements, physical examination findings, or intestinal flora measurements following either treatment. For several individual subjects, vital sign findings and liver function parameter values were noteworthy but did not result in the identification of a new safety concern. One subject, a healthy 41-year-old Japanese male (randomized to AVI alone), had transaminase elevations that were classified as an other significant adverse event (OAE). After receiving multiple doses of AVI, his highest transaminase results were: alanine aminotransferase (ALT) 522 U/L (reference range: 17 to 63 U/L) and aspartate aminotransferase (AST) 246 U/L (reference range: 15 to 41 U/L). The transaminases decreased but had not normalized at the time of the last follow-up visit. The subject had no symptoms at the time of the transaminase elevations. The increase in the transaminase values was considered mild in severity and related to the investigational product (IP).

The PK of AVI alone or in combination with CAZ was similar in Japanese subjects to that observed in studies of Western subjects.

1.1.5 Human experience – Phase II

A prospective, multicenter, double-blind, randomized, 2 arms, parallel group (1:1) study in 203 patients aged between 18 and 88 years with complicated intra-abdominal infections (cIAI) has been completed (Study NXL104/2002; Lucasti et al 2011). This study was designed to assess safety, tolerability, and efficacy of CAZ-AVI (2000 mg CAZ plus 500 mg AVI IV every 8 hours over 30 minutes) plus metronidazole (500 mg IV every 8 hours over 1 hour) versus meropenem (1000 mg IV every 8 hours over 30 minutes) in the treatment of cIAI. The primary objective of the study was to estimate the efficacy of CAZ-AVI plus metronidazole with respect to the clinical response in Baseline microbiologically evaluable (ME) patients (ie, patients with at least 1 pathogen isolated that was susceptible to both study drugs) with cIAI at the Test of Cure (TOC) visit, 2 weeks post-treatment, compared with meropenem. Similar clinical response rates were seen in both treatment groups for the primary endpoint; 91.2% in the CAZ-AVI plus metronidazole group and 93.4% in the meropenem group. The most common AEs reported (>7.5% incidence overall) were nausea, vomiting, pyrexia, alanine aminotransferase (ALT) increased, aspartate aminotransferase (AST) increased, and alkaline phosphatase increased. Discontinuations due to AEs were infrequent (3.4% overall) in both groups. Five deaths were reported in the study (3 in the CAZ-AVI plus metronidazole group and 2 in the meropenem group); none were considered related to study drug. Clinically significant laboratory abnormalities occurred uncommonly, including abnormalities in liver enzymes.

A second Phase II study (Study NXL104/2001; Vasquez et al 2011) has been completed in patients with complicated urinary tract infections (cUTI). The study was a multicenter, investigator-blinded, randomized, 2 arms, parallel group (1:1) study to estimate the efficacy, safety, and tolerability of CAZ-AVI (500 mg CAZ/125 mg AVI IV every 8 hours over 30 minutes) versus imipenem (imipenem cilastatin 500 mg IV every 6 hours over 30 minutes) in

137 patients between the ages of 18 and 90 years with a cUTI. Twenty-seven patients (39.1%) in the CAZ-AVI group and 35 (51.5%) in the imipenem group were ME (ie, had at least 1 pathogen isolated that was susceptible to both study drugs). The primary objective of the study was to estimate the efficacy of CAZ-AVI with respect to microbiological response in ME patients with cUTI at the TOC visit, 5 to 9 days post-treatment, compared with imipenem cilastatin. Similar microbiological response rates were seen in both treatment groups; at the TOC visit, 19/27 patients (70.4%) in the CAZ-AVI group and 25/35 patients (71.4%) in the imipenem group had a favorable microbiological response (eradication). The most common AEs reported (overall incidence >7.5%) were headache, diarrhea, anxiety, and infusion site reaction. Discontinuations due to AEs were uncommon (2 patients in the CAZ-AVI group, 0 in the imipenem group). One death was reported in the study (in the imipenem group). Clinically significant laboratory abnormalities occurred uncommonly, including abnormalities in liver enzymes.

Additional details can be found in Section 5.2.2 of the CAZ-AVI Investigator Brochure.

1.2 Rationale for conducting this study

In order to facilitate development of CAZ-AVI in China, this study will investigate the safety and tolerability and the PK after single and repeated IV infused doses of CAZ-AVI, or placebo to healthy male and female (non-child bearing potential) Chinese subjects.

1.3 Benefit/risk and ethical assessment

There are no medical benefits for the subjects of this study.

The risks for CAZ-AVI have not been fully elucidated; however, it is assumed that known or potential risks for CAZ-AVI should include those identified in the clinical study experience with CAZ-AVI, AVI alone, and for CAZ alone.

The full risk profile for CAZ is described in the prescribing information for the product. Important risks as laid out in the warnings and precautions in product labelling for CAZ include:

- Hypersensitivity reactions. Though patients with hypersensitivity and serious allergic reactions to cephalosporins, carbapenem or other beta lactam antibiotics are excluded from the trial, first time episodes of such reactions could occur.
- Antibiotic-associated diarrhea, Clostridium difficile diarrhea, colitis, and pseudomembranous colitis.
- Bacterial overgrowth with non-susceptible organisms.
- Inadvertent intra-arterial administration of CAZ can result in distal necrosis.

- Use in patients with renal impairment - elevated levels of CAZ has been associated with neurological sequelae, such as tremor, myoclonus, seizures, encephalopathy, and coma.

Potential risks for CAZ-AVI include the occurrence of events seen with CAZ alone, but that go beyond the frequency and/or severity of those seen with CAZ. Local intolerance has been seen in the preclinical studies, and has been monitored in the clinical program. In the Phase 1 studies, erythema and hematoma at the administration site were reported. In the phase 2 study (NXL-2002) examining CAZ104 plus metronidazole versus meropenem as a comparator in complicated IAI infections, approximately 30% of participants in both the CAZ104 and meropenem comparator treatment group experienced at least 1 symptom of local intolerability, with pain, erythema, swelling, and tenderness reported most frequently across both groups. The majority of infusion site events were mild. There was a slightly greater percentage of patients with moderate/severe intensity in the CAZ104 group, who also received IV metronidazole (MTZ; 17/101, 16.8%) versus the meropenem group (11/102, 10.8%). Of note, patients in the NXL104/CAZ/MTZ group received infusion of 3 different agents per dose, while patients in the meropenem group received infusion with 1 study drug per dose.

In regard to hypersensitivity reactions, there was one report in the CAZ-AVI clinical trials, where the clinical investigator considered the events of skin rash and elevated liver function tests to be a possible hypersensitivity reaction because of the temporal relationship of the events to drug administration. Rashes have also been reported.

In summary, the known and potential risks of receiving the developmental antibiotic combination CAZ-AVI are expected to be similar to those seen with CAZ and cephalosporins in general. Thus far, no unique risks have been identified for the AVI component or the combination of CAZ and AVI (CAZ-AVI). Safety and tolerability will be continuously monitored in accordance with AstraZeneca procedure.

For further information regarding an overall risk benefit assessment, see the IB.

2. STUDY OBJECTIVES

2.1 Primary objective

- To investigate the safety and tolerability of Ceftazidime-Avibactam (CAZ-AVI) as single and repeated intravenous (IV) infusions in healthy Chinese subjects

2.2 Secondary objectives

- To investigate the PK of AVI administered in combination with CAZ
- To investigate the PK of CAZ administered in combination with AVI

3. STUDY PLAN AND PROCEDURES

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

3.1 Overall study design and flow chart

This is a randomized, double-blind, placebo-controlled study to assess the safety, tolerability, and PK of AVI in combination with CAZ (CAZ-AVI) Administered as Single and Repeated IV infusion of doses in healthy Chinese Subjects.

Subjects will receive a 120 minute IV infusion of CAZ-AVI or matched placebo as a single dose on Day 1, followed by IV infusions every 8 hours (q8h) for 7 days (Day 2 to Day 8), and a single dose on Day 9.

Plasma and urine sampling for PK analysis will be performed following the single dose on Day 1 and Day 9. Plasma sampling for PK analysis will be performed pre-dose prior to the first dose on Days 6, 7 and Day 8. Safety monitoring will occur throughout the study via vital sign measurements, oral body temperature, ECGs, clinical laboratory measurements, physical examinations, and assessment of AEs. Subjects will return 3 to 5 days following the last dose for a follow-up visit.

Plasma and urine PK samples will be obtained at the times noted in the PK Plasma and Urine Sampling schedule (Table 2). Throughout the residential period there will be safety monitoring.

Subjects will undergo screening assessment (Visit 1) during the 28-day period preceding administration days of the first dose. Following the screening visit, there will be one admission period (visit 2) to the Clinical Pharmacology Unit (CPU) from the day before dosing starts (Day -1) until discharge on Day 10, followed by a post study follow-up visit (visit 3), 3 to 5 days after the last dose administration (see Study Flowchart Table 1).

Subjects will be randomised to receive a 120 minute IV infusion of CAZ-AVI or match placebo. On Day 1 subjects will receive a single dose of CAZ-AVI alone or placebo. Repeated dosing will commence on Day 2, subjects will be given an IV infusion for 120 minutes every 8 hours (q8h) on Day 2-8. A final single dose will be given on Day 9.

Table 1 Study Flowchart

Visit	**1**			**2**								**3**
Study days	**Enrolment ≤ Day-28**	**Residential period Days**										**Follow-up :3 to 5 days after last dosing**
		-1	1	2	3	4	5	6	7	8	9	12-14
Informed consent	X											
Inclusion/exclusion criteria	X	X										
Demographics	X											
Medical/surgical history	X											
Drugs of abuse + Ethanol screenings	X	X										
HBV/HCV, HIV tests	X											
FSH and Serum Pregnancy Test [7] (females only)	X	X										
Physical examination[3]	X	X	X	X	X	X	X	X	X	X	X	X
Blood Pressure and Pulse	X	X	X	X	X	X	X	X	X	X	X	X
Oral Body Temperature		X	X				X				X	
Height	X											
Weight	X											
Randomization			X									
Safety labs(Clinical chemistry, Haematology, Urinalysis)[5]	X	X		X		X		X		X	X	X
12-lead pECG[6]	X	X	X		X		X		X		X	X

Table 1 Study Flowchart

Visit	**1**	**2**									**3**
Study days	**Enrolment ≤ Day-28**	**Residential period Days**									**Follow-up :3 to 5 days after last dosing**
		-1	1	2	3	4	5	6	7	8	9	12-14
PK blood sampling[2]			X					X	X	X	X	
PK Urine collection[2,4]			X								X	
Infusion of study drug[1]			X	X	X	X	X	X	X	X	X	
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X
AEs recording	X	X	X	X	X	X	X	X	X	X	X	X
SAEs recording	X	X	X	X	X	X	X	X	X	X	X	X

1 A single 120 minute infusion will be given on Day 1 in the morning, and every 8 hours (q8h) 2-hour infusions will be given on Days 2 to 8, and single 2-hour infusion will be given on Day 9, which starts on the same time as Day 1.

2 For the PK Plasma and Urine Sampling Schedule, refer to Table 2.

3 A complete physical examination will be performed at screening, Day-1 and at discharge from the unit. At admission on Day 1 to Day 9, and follow-up visit only a brief physical examination is required.

4 The exact collection times and weights of urine will be recorded in the CRF. Samples will be stored at approx -75 °C until shipment in dry ice.

5 Safety test should be done before the dosage in the morning.

6 ECG is performed before first PK sample. Detail refer to Section 6.4.7.1

7 The FSH and a serum β-HCG pregnancy test must be drawn for women of childbearing potential at the screening visit. If the results of the serum β-HCG cannot be obtained prior to dosing of the investigational product, a volunteer may be enrolled on the basis of a negative urine pregnancy test, though serum β-HCG must still be obtained. If either test is positive, the subject should be excluded. Since urine and serum tests may miss a pregnancy in the first days after conception, relevant sexual history, including methods of contraception, should be considered. Any volunteer whose sexual history suggests the possibility of early pregnancy should be excluded.

Table 2 PK Plasma and Urine Sampling Schedule

Sample	Schedule
Plasma samples	Four (4) mL samples for the determination of AVI and CAZ plasma concentrations will be obtained at the following times: Days 1 and Day 9: Pre-dose , 60min, 90 min, 120 min, 2.25h, 2.5h 3h, 3.5h, 4h, 5h, 6h, 8h, 12h, 18h, 24h(post the start of the IV infusion). Days 6, 7 and Day8: Pre-dose (the first dose of the day only).
Urine samples	10mL urine should be obtained before infusion = blank urine; then urine collection at [0-6h], [6-12h], [12-24h], post dosing, on days 1and 9 after start of single administration.

3.2 Rationale for study design, doses and control groups

The safety, tolerability and PK of AVI alone were investigated in non-Chinese healthy volunteers, single AVI dose range was up to 2000 mg and multiple AVI dose range was up to 1000 mg given every 8 hours for 5 days. The safety, tolerability and PK of 500 mg AVI in combination with 2000 mg CAZ given as a single and multiple dose given as every 8 hours were also studied in non-Chinese healthy volunteers and patients with infection. The dose range investigated in non-Chinese healthy volunteers and patients were well tolerated. The study is double blind and includes placebo with randomization between active treatment and placebo to minimize bias and to facilitate identification of effects related to administration of drug rather than the study procedures, etc.

The study will be performed in healthy male and female volunteers to aid compliance with complex study procedures and avoid interference with the results from disease processes and other drugs.

The dose levels chosen are expected to give adequate characterization of the pharmacokinetic profile around the expected exposures to CAZ-AVI from the anticipated therapeutic dose.

A multiple repeated dose of 7 days are selected to represent the approximate average treatment duration of CAZ-AVI for patients with infection in clinical practice.

The sample size is considered sufficient to characterize the PK characteristics of each dose based on previous experiences from single and repeated dosing studies, as well as the results in the global SAD (Single Ascending Dose) and MAD (Multiple Ascending Dose) studies.

4. SUBJECT SELECTION CRITERIA

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

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 (of non-child bearing potential) Chinese subjects, aged 18 to 45 years, with suitable veins for cannulation or repeated venipuncture.

3. Have a body mass index (BMI) between 19 and 24 kg/m^2 inclusive and weigh at least 50 kg and no more than 100 kg.

4. Females must have a negative pregnancy test at screening and on admission to the CPU, must not be lactating, and must be of non-childbearing potential, confirmed at screening by fulfilling 1 of the following criteria:

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

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

5. Male subjects should be willing to use condoms, in association with another method of contraception, from the day of the first dosing until 3 months after the last dosing with the investigational product.

6. As judged by the Investigator, 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. Any condition requiring the regular use of any medication.

2. Use within 14 days (or 5 half-lives, whichever is longer) prior to the first study dose or intended use of any over-the-counter (including St. John’s Wort or any herbal products) or prescription medication including those that are known to cause PK drug interaction.

3. Prolonged QTcF (QT interval corrected for heart rate using Fridericia's formula) >450 ms or shortened QTcF < 350 ms, family history of long QT syndrome, family history of unexplained sudden death, family history of sick sinus syndrome, or any clinically relevant cardiovascular disease.

4. PR interval > 250 ms, second or third degree atrioventricular block, delayed intraventricular conduction with QRS >120 ms, left bundle branch block, complete right bundle branch block, or Wolf-Parkinson-White syndrome.

5. 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 30 days (on the condition that 5 half-lives of the compound is < 30 days) of screening in this study. The period of exclusion begins at the time of the last follow-up visit of the prior study.

6. Has participated in a clinical study of a biologic within 6 months of screening.

7. Treatment in the previous 3 months with any drug with well defined toxicity potential to a major organ.

8. Symptoms of a clinically significant illness in the 3 months before the study.

9. Presence or sequelae of gastrointestinal (Known Clostridium difficile associated diarrhea), liver or kidney disease, or other conditions known to interfere with the absorption, distribution, metabolism, or excretion of drugs.

10. Subjects with on-going liver disease or inexplicably elevated liver chemistry values. If subject was excluded from study based on elevated liver chemistries; re-screening with subsequent enrolment is discouraged.

11. Estimated Creatinine clearance ≤ 80 mL/min calculated by Cockcroft-Gault equation.

$$\text{Males: } CL_{CR} = \frac{(140\text{-age}) \times (\text{kg body weight})}{(72 \times \text{mg/dl serum creatinine})}$$

$$\text{Females: } CL_{CR} = \frac{(140\text{-age}) \times (\text{kg body weight}) \times 0.85}{(72 \times \text{mg/dl serum creatinine})}$$

12. Abnormal vital signs at screening and at admission, after 10 minutes supine rest, defined as any of the following and confirmed on at least one repeat measurement:

   – Systolic BP > 140 mmHg;

   – Diastolic BP > 90 mmHg;

   – Resting heart rate < 40 or > 85 bpm

13. Positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody, or HIV antibodies.

14. History of hypersensitivity (eg, anaphylaxis), serious allergy, or any serious reaction to carbapenem, cephalosporin, or other β-lactam antibiotics.

15. History of significant allergic disease and acute phase of allergic rhinitis in the previous 14 days before randomization. History of food allergy.

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

17. Blood or plasma donation of more than 500 mL during the previous 1 month before randomization.

18. Smoker of more than 5 cigarettes/day or equivalent use of nicotine products during the previous 3 months.

19. Excessive intake of caffeine-, xanthine-containing foods or beverages within 48 hours preceding study drug administration.

20. Current evidence of drug abuse or history of drug abuse within one year of randomization, and/or positive results of urine drug and alcohol test at screening and at admission.

21. Subjects who have an average weekly alcohol intake that exceeds 21 units per week or subjects unwilling to stop alcohol consumption for the duration of the study (1 unit = 12 oz or 360 mL of beer; 5 oz or 150 mL of wine; 1.5 oz or 45 mL of distilled spirits).

22. Consumption of grapefruit or grapefruit-containing products within 7 days of the first study dose.

23. Subject is unlikely to survive the 6 to 8 week study period or have a rapidly progressive or terminal illness.

24. Clinically relevant abnormality in the opinion of the investigator from the following: medical and/or surgical history, physical examination, vital signs, 12-lead ECG, clinical chemistry, hematology, and urinalysis.

Procedures for withdrawal of incorrectly enrolled subjects see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

Subjects must:

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

2. Abstain from consuming any of the following:

   – Alcohol from 72 hours before admission, during the residential period 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 follow-up

- Grapefruit, grapefruit juice, or other products containing grapefruit from 7 days before the first dose until after the final medical examination at the study follow-up

3. Refrain from consumption of xanthine-containing foods or beverages (such as caffeine) within 48 hours preceding study drug infusion until after the final medical examination at the study follow-up.

4. Abstain from drugs of abuse from time of consent until after the final medical examination at the study follow-up.

5. Abstain from smoking and nicotine use from time of admission to the CPU until after discharge from the facility.

6. Abstain from taking any medication (prescribed or over the counter products), other than paracetamol/acetaminophen, from 14 days prior to the first administration of investigational product until after the final medical examination at the study follow-up. However, this should not obviate necessary medical treatment. If any medication is necessary during the residential period, it should be prescribed by the investigator and the AstraZeneca Study Team Physician should be informed (Section 5.6).

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

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

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

5.2 Subject enrolment and randomisation

The Principal Investigator will ensure:

- Signed informed consent is obtained from each potential subject before any study specific procedures are performed.

- Each potential subject is assigned a unique enrolment number, beginning with 'E0001001'.
- The eligibility of each subject is determined (See Sections 4.1 and 4.2).
- Each eligible subject is assigned a unique randomization code (subject number), beginning with '1001'.

Randomization will be performed on the morning of the first dose.

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

If a subject withdraws his/her participation in the study, then his/her enrolment/randomization code cannot be reused.

5.2.1 Procedures for randomisation

A randomization scheme will be produced by AstraZeneca R&D using the global randomization system (GRand). Subjects will be allocated to active or placebo treatment. The randomization will be done using consecutive randomization codes (subject numbers).

Randomisation codes will be assigned strictly sequentially as subjects become eligible for randomisation.

5.3 Procedures for handling subjects incorrectly randomised subjects

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

Where a subject, who does not meet the selection criteria, is randomized in error and this is identified before dosing, the subject should be withdrawn from the study. A discussion should occur between the AstraZeneca Study Team Physician and the investigator regarding whether a replacement may be considered.

Where a subject, who does not meet the selection criteria, is randomized in error and started on treatment, or where a subject subsequently fails to meet the study criteria post initiation, a discussion should occur between the AstraZeneca Study Team Physician and the investigator regarding whether to continue or discontinue the subject from treatment. If treatment is discontinued the subject should be advised to continue assessments to ensure their safety. In situations where an agreement cannot be reached, the subject should have their study treatment discontinued.

The AstraZeneca Study Team Physician is to ensure all decisions are appropriately documented.

5.4 Blinding and procedures for unblinding the study

5.4.1 Methods for ensuring blinding

This study is double-blind with regard to active or placebo treatment. The following personnel will have access to the randomization list:

- The AstraZeneca personnel carrying out the labeling and packaging of investigational product
- The pharmacy personnel preparing study drug at the CPU
- The personnel analyzing the PK samples

The randomization list should be kept in a secure location until the end of the study.

The pharmacist at the CPU will be unblinded to ensure the correct preparation of the treatments. All infusions prepared by the pharmacy will be provided to the clinic in a manner to ensure all infusions look identical, in order to maintain the blind.

5.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomization for each randomized subject, will be available to the investigators or pharmacists at the CPU.

The treatment code should not be broken except in medical emergencies when the appropriate management of the subject requires knowledge of the treatment randomisation. The investigator documents and reports the action to AstraZeneca, without revealing the treatment given to subject to the AstraZeneca staff.

AstraZeneca retains the right to break the code for SAEs that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

5.5 Treatments

5.5.1 Identity of investigational product(s)

Table 3 Investigational Products

Investigational product	Dosage form and strength
CAZ-AVI	Sterile crystalline powder, 2000 mg ceftazidime and 500 mg avibactam for solution for infusion

Normal saline (0.9%) Sodium Chloride will be used as the placebo infusion. This will be supplied by the study site.

CAZ-AVI will be supplied to the pharmacy at the study site as an open labelled bulk supply for further dispensing

Details for the preparation of the IV solutions are described in the handling instructions provided to the pharmacy.

5.5.2 Doses and treatment regimens

Subjects will receive a single administration 120 minute IV infusion on Day 1, followed by multiple administrations every 8 hours (q8h) 120 minute IV infusions for 7 days (Day 2 to Day 8), and one single administrations 120 minute IV infusion on Day 9.

Subjects may eat normally without a fasting period and can be fed before the infusions.

The unblinded pharmacist will prepare the treatments according to the randomization list. Handling instructions detailing how the infusions are to be prepared will be provided to the pharmacy.

5.5.3 Labelling

Labels will be prepared in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines.

5.5.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The investigational product label on the kits specifies the appropriate storage. The storage location will be locked and accessible to authorised personnel only.

5.6 Concomitant and post-study treatment(s)

Apart from paracetamol/acetaminophen, no concomitant medication or therapy will be allowed. The subjects should be instructed that no other medication is allowed, including herbal remedies, vitamin supplements, and over-the-counter products, without the consent of the investigator.

Medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the investigator during the residential period. When any medication is required, it should be prescribed by the investigator and recorded in the appropriate sections of the Case Report Form.

5.7 Treatment compliance

The administration of all study drugs (including investigational products) should be recorded in the appropriate sections of the Case Report Form.

Treatment compliance will be assured by supervised administration of the investigational product by the investigator or delegate. The dates and times of administration of the investigational product will be recorded and checked by the monitor at monitoring visits.

5.7.1 Accountability

The study drug provided for this study will be used only as directed in the study protocol.

It is the Investigator's responsibility to establish a system for handling study treatments, including investigational products, to ensure that:

- Deliveries of such products from AstraZeneca are correctly received by a responsible person (eg, pharmacist)
- Such deliveries are recorded
- Study treatments are handled and stored safely and properly
- The study drug provided for this study will be used only as directed in the study protocol
- The study personnel will account for all drugs received at the site, dispensed for the subject, and returned to the pharmacy. Any discrepancies should be documented, investigated, and appropriately resolved
- At the end of the study, site personnel will account for all unused drugs and for appropriate destruction/return of all unused drugs to a designated facility or AstraZeneca for destruction. Certificates of delivery and destruction/return should be signed preferably by the Investigator or pharmacist.

5.8 Discontinuation of investigational product

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

- Subject decision. The subject is at any time free to discontinue treatment, without prejudice to further treatment
- Positive pregnancy test at any time during the study
- Adverse Event
- Hepatic disorder:
  - ALT > 3x ULN
  - ALP > 2x ULN

- Total bilirubin > 2x ULN

- Severe non-compliance to study protocol
- Incorrect enrolment
- Subject lost to follow-up

5.8.1 Procedures for discontinuation of a subject from investigational product

A subject that decides to discontinue investigational product will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator(s). Adverse events will be followed up (See Sections 6.4.3 and 6.4.4).

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

5.9 Withdrawal from study

Subjects are at any time free to withdraw from study (investigational product and assessments), without prejudice to further treatment (withdrawal of consent). Such subjects will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator. Adverse events will be followed up (See Sections 6.4.3 and 6.4.4).

Subjects who withdraw from the study due to reasons other than an AE may be replaced at the discretion of the Sponsor and/or investigator. All replacements must be approved by AstraZeneca. Subjects who are withdrawn from the study by the investigator due to AEs after the start of dosing will not be replaced. If a subject withdraws his/her participation in the study, then his/her enrolment code cannot be reused.

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below, and the timing of these assessments are detailed in the Study Plans.

It is important that PK sampling occurs as close as possible to scheduled time. In order to achieve this, other assessments scheduled at the same time may be initiated prior to the time point. The sequence at a particular time point is:

1. ECG recordings
2. Vital signs measurement
3. Blood sampling for determination of PK of CAZ-AVI (performed at the precise protocol scheduled time)

4. Blood sampling for laboratory assessments

The actual time for all assessments will be recorded in the eCRF. Pre-dose assessments, may be done up to 30 minutes prior to dosing.

6.1 Recording of data

The Investigator will ensure that data are recorded on the electronic CRFs (eCRFs) as specified in the study protocol. He/she ensures the accuracy, completeness, and timeliness of the data recorded, for data queries and all required reports according to any instructions provided.

The Principal Investigator will sign the completed eCRFs. A copy of the completed eCRFs will be archived at the CPU.

6.2 Data collection at enrolment and follow-up

6.2.1 Enrolment procedures

At enrollment (Visit 1), each potential subject will provide informed consent prior to starting any study specific procedures.

Demographic data and other characteristics will be recorded and will include: date of birth, gender, race, alcohol consumption, and smoking history.

Each subject will undergo screening during the 28 days prior to admission to confirm eligibility. This will consist of:

1. A standard medical, medication, and surgical history with review of the inclusion and exclusion criteria with the subject

2. A complete physical examination

3. Height, weight and calculation of BMI

4. Vital signs – resting supine BP, pulse and oral temperature

5. 12-lead ECG (after resting in a supine position for at least 10 minutes prior to the evaluation)

6. A blood sample for clinical chemistry, hematology, serum pregnancy and FSH test for females with childbearing potential, and screen for hepatitis B surface antigen (HBs), antibodies to hepatitis C virus (HCV), and antibodies to HIV

7. A midstream urine sample for routine urinalysis, drugs of abuse screen, and ethanol screen

8. Adverse events

9. Concomitant medication

After admission on Day -1 and before randomization, the Investigator should reassess each subject to reconfirm eligibility.

6.2.2 Follow-up procedures

A post-study medical examination will be performed 3 to 5 days after the last dose. This will include a brief physical examination, vital signs, recording triplicate 12-lead paper ECGs, a blood sample for clinical chemistry and haematology, a urine sample for urinalysis, and assessment of any AEs, SAE, and concomitant medication.

6.3 Efficacy

Not applicable

6.4 Safety

The Principal 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 adverse event 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, electrocardiogram). 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 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 in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect

- Is an important medical event that may 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, see related Appendix B to the Clinical Study Protocol.

6.4.3 Recording of adverse events

Time period for collection of adverse events

Adverse Events will be collected from the time of signature of informed consent throughout the treatment period and including the follow-up period.

Follow-up of unresolved adverse events

Any 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 CRF. 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 collect for each AE;

- AE (verbatim)
- The date and time when the AE started and stopped
- Intensity
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study *(*yes or no*)*
- Outcome.

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

- 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
- Description of AE

Additional variables will be collected for all SAEs including treatment given for the event.

The following intensity ratings will be used:

1. mild (awareness of sign or symptom, but easily tolerated)
2. moderate (discomfort sufficient to cause interference with normal activities)
3. 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 a 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 a SAE.

Causality collection

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

For SAEs causal relationship will also be assessed for 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 to the Clinical Study Protocol.

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 CRF. 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 protocol mandated laboratory tests, vital signs, ECGs, and other safety assessments will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, ECGs, and other safety assessments 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 investigational product.

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 uses 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 investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

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 of when he or she becomes aware of it **and report to the Ethics Committee and Regulatory authority based on local drug administration laws and regulations.**

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

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

Once the Investigators or other site personnel indicate an AE is serious in the WBDC system, an automated email alert is sent to the designated AstraZeneca representative.

If the WBDC system is not available, then the Investigator or other study site personnel reports a SAE to the appropriate AstraZeneca representative by telephone.

The AstraZeneca representative will advise the Investigator/study site personnel how to proceed.

6.4.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, hematology and urinalysis will be taken at the times indicated in the Study Plan (Table 1). The date and time of collection of all laboratory tests will be recorded in the appropriate CRF.

The following laboratory variables will be measured:

Table 4 Laboratory variables

Clinical chemistry	**Haematology**
Alanine aminotransferase (ALT)	Haemoglobin
Aspartate aminotransferase (AST)	Hematocrit
Alkaline phosphatise (ALP)	Red blood cell (RBC)count
Bilirubin, total and direct	Leukocyte (WBC) count
Total protein	Absolute leukocyte differential count
Creatinine	Platelet count
Blood urea nitrogen (BUN)	
Calcium, total	**Urinalysis**
Phosphorus	pH
Fasting glucose	Glucose
Potassium	Ketones
Sodium	Leukocytes
Chloride	Blood
Triglycerides	Protein
Cholesterol, total	
Gamma-glutamyl transpeptidase	

Note: The administration of CAZ may result in a false-positive reaction for glucose in the urine when using Clinitest® tablets.

Additionally, at screening all subjects will be tested for HIV, hepatitis B surface antigen, and antibodies to hepatitis C. Urine will be tested for alcohol and the following drugs of abuse at

screening and admission: amphetamines, barbiturates, tricyclic antidepressants, cocaine, methadone, 3,4-Methylenedioxymethamphetamine (MDMA [ecstasy]), benzodiazapines, morphine, phencyclidine, tetrahydrocannabinol, and opiates. A FSH test will be performed at screening for subjects who report that they are post menopausal, and a serum pregnancy test will be performed for all female subjects at screening and admission (Day -1). The results of these tests will be used to determine if the subject meets criteria for exclusion from the study.

Detailed liver chemistry sampling aimed at identifying the cause of changes observed during routine monitoring should be done for all subjects fulfilling intensified monitoring criteria. Detailed liver chemistry sampling should also be made in subjects with liver clinical chemistry values that are repeatedly closed to fulfilling discontinuation criteria and for whom it is considered important to evaluate the cause of the observed changes.

Table 5 The handling of a potential DILI signal

Liver chemistry variable	Intensified Monitoring	Stopping Criterion
ALT*	>2x ULN, monitor at least q 48 hours until level returns to WNL or stable per investigator	>3x ULN
ALP*	Increase by 100%; check GGT level; monitor at least q 48 hours until return to WNL or stable per investigator	>2x ULN
Bilirubin	>1.5ULN, monitor at least q 48 hours until return WNL or stable per investigator	>2x ULN

* The ratio of ALT/ALP is used to classify a liver injury as hepatocellular, cholestatic or mixed

If any of the tests performed on the samples taken after investigational product administration show clinically significantly abnormal results as judged by the investigator, new blood samples will be obtained and tests repeated until the results return to baseline or the cause is assessed. The investigator will provide an evaluation of the clinical importance of the deviation. The development of any clinically relevant deterioration in any laboratory parameter may constitute an AE if it leads to discontinuation of the study drug or if it fulfills the criteria of seriousness. The investigator will record on the laboratory report whether the abnormality is Clinically Significant (CS) or Not Clinically Significant (NCS).

A potential Hy's Law(PHL) case is defined as any situation where a study subject has an increase in both AST **or** ALT ≥3x ULN **and** total bilirubin ≥ 2x ULN, irrespective of ALP, at any point during the study. The elevations do not have to occur at the same time or within a specified timeframe.

Hy's Law should be considered satisfied if ALT is increased 3x ULN and total bilirubin in increased 2x ULN in the absence of significant increase in ALP and in the absence of an alternative diagnosis that explains the increase in total bilirubin.

The process described in the AZ OPI" Handling of Potential Hy's Law Cases and Hy's Law Cases in Clinical Studies"(refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law') will be applied to any subject who meets the criteria for a potential Hy's Law case or the discontinuation criteria. A thorough investigation into other potential causes must be conducted and follow-up monitoring as described in the OPI must be completed. This monitoring includes the liver CRFs and must be filled out by the investigator.

For blood volume see Section 7.1.

6.4.6 Physical examination

Physical examinations will be conducted according to the schedule outlined in the Study Plan Table 1.

6.4.6.1 Complete physical examination

The complete physical examination will include an assessment of the following: general appearance, skin, head and neck (including eyes, ears and throat), lymph nodes, thyroid, musculoskeletal/extremities (including spine), cardiovascular, lungs, abdomen, and neurological systems.

Complete physical examination data to be recorded in the eCRF will include:

- Normal
- Abnormal with a description of any abnormalities
- Same as previous

6.4.6.2 Brief physical examination

The brief physical examination will include an assessment of the following items: general appearance, abdomen, lungs, and the cardiovascular system.

Brief physical examination data to be recorded in the eCRF will include:

- Normal
- Abnormal with a description of any abnormalities
- Same as previous

6.4.6.3 Height and weight measurements

Height (m) and weight (kg) will be measured without shoes. BMI will be calculated as weight (kg)/height^2 (m^2) and will be recorded in the eCRF.

6.4.7 ECG

For timing of assessments refer to the Study Plan Table 1.

6.4.7.1 12-lead ECG

12-lead pECGs will be obtained at the each time point presented in the Study Plan (Table 1) after the subject has been resting in supine position for at least 10 minutes in each case.

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

The ECGs will be captured as close to PK sampling time as possible and taken prior to PK sampling. The ECG recordings will be taken in triplicate 5 minutes prior to PK sampling, with each of the 3 recordings being of 10 second duration and separated by at least 1 minute interval. The subject should be in the same supine body position (maximum 30 degrees flexion in hip and feet not in contact with the footboard) at each recording time point during the study.

In order to collect blood samples for PK analysis at the precise scheduled time, 12-lead ECG may be initiated prior to their scheduled time point to ensure that the PK blood sample is collected on time. The precise time for 12-lead ECG recordings will be recorded in the eCRF. See Section 6.1.

All ECGs will be documented by recording date, time of collection, heart rate, PR, RR, QRS, and QT intervals, and the derived QTcF and Intervals (the QT correction factor will be based on the Fridericia's formula) from the 12-lead ECG.

The investigator will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided as to whether or not the abnormality is clinically significant or not clinically significant. The reason for the abnormality will be recorded in the eCRF. These ECGs will be documented in the eCRF by recording date, time, heart rate, and overall assessment as normal and abnormal.

All 12-lead ECGs will be recorded and evaluated by the investigator. If indicated additional 12-lead ECG assessments can be made at the discretion of the investigator. These assessments should be entered as an unscheduled assessment in the appropriate eCRF.

6.4.8 Vital signs

Vital sign measurement will be taken at the times indicated in The Study Plan (Table 1).

In order to collect blood samples for PK analysis at the precise scheduled time, vital signs measurement may be initiated prior to their scheduled time point to ensure that the PK blood sample is collected on time. The precise time for vital signs recordings will be recorded in the eCRF. See Section 6.1.

6.4.8.1 Pulse and blood pressure

For timing of individual measurements refer to the Study Plan (Table 1).

Screening day, Day-1, Day 1, Day 9 and Follow-up day: blood pressure and pulse will be measured once a day.

On Day 2 to Day 8: At pre−dose, 2 and 6 hours from the start of infusion (first dose only) 1 blood pressure and pulse will be measured. Blood pressure and heart rate will be measured as per site procedures with an appropriate cuff size. Volunteers will be resting in a supine position for at least 10 minutes prior to the assessments. As much as possible, blood pressure will be measured using the same arm per volunteer throughout the study.

6.4.8.2 Body temperature

Body temperature will be measured in degrees Celsius at the times indicated in the Study Plan (Table 1). Measure the temperature in the morning and again in the afternoon. The highest daily temperature will be recorded.

6.5 Patient reported outcomes (PRO)

Not applicable

6.6 Pharmacokinetics

6.6.1 Collection of blood &Urine samples

Blood samples (4 mL) for the determination of AVI and CAZ in plasma will be taken at the times presented in Table 2.

Plasma samples should be taken as close to the nominal sampling times as possible. However, minor deviation from the nominal sample time as follows:

- ± 5 minutes deviation for blood sampling at 60min, 90 min, 120 min (at the end of the infusion), 2.25h, 2.5h, 3h after the start of the iv infusion
- ± 15 minutes deviation for blood sampling at 3.5h, 4h, 5h, 6h after the start of the iv infusion

- ± 30 minutes deviation for blood sampling at 12h, 18h, 24h after the start of the iv infusion

are considered to be acceptable and should not treated as protocol deviation, as long as the exact time of the sample collection is noted on the source document and data collection tool (eg, eCRF).

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

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

For blood volume see Section 7.1.

6.6.2 Determination of drug concentration

Samples for determination of AVI and CAZ concentrations in plasma and urine will be analyzed by , AstraZeneca R&D, using an appropriate bioanalytical method. Full details of the bioanalytical method used will be described in a separate bioanalytical report.

All samples still within the known stability of the analytes of interest (ie, AVI and CAZ) at the time of receipt by the bioanalytical laboratory will be analysed.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 6 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	5	8	40
	Haematology	3	8	24
	Serology	5	1	5
	Follicle-stimulating hormone	3	2	6
	serum β-HCG	4	2	8
PK		4	33	132
Discard sample prior to PK sample*		2	33	66
Total				281

* This discarded blood from predraws used to remove fluid from flushed catheters.

7.2 Handling, storage and destruction of biological samples

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

7.2.1 Pharmacokinetic samples

Samples will be disposed of 6 months after the Clinical Study Report has been finalized, unless retained for future analyses, see below.

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

7.3 Labelling and shipment of biohazard samples

The Principal Investigator ensures that samples are labeled and shipped in accordance with the Laboratory Manual for this Clinical Study Protocol and the Biological Substance, Category B regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C of this Clinical Study Protocol 'IATA 6.2 Guidance Document'.

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

7.4 Chain of custody of biological samples

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

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

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

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

Samples retained for further use are registered in the AstraZeneca bio bank 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 of/destroyed, and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

The Principal Investigator:

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

AstraZeneca ensures the central laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/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 ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

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

8.3 Ethics and regulatory review

An Ethics Committee should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable Ethics Committee, and to the study site staff.

The opinion of the IRB should be given in writing. The investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study.

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

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

If required by local regulations, the protocol should be re-approved by the IRB annually.

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

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

AstraZeneca will provide the Regulatory Authority, IRB, and Principal Investigator with safety updates / reports according to local requirements, including Suspected Unexpected Serious Adverse Reactions (SUSARs), where relevant.

The Principal Investigator is also responsible for providing the IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Principal Investigator so he/she can meet these reporting requirements.

8.4 Informed consent

The Principal Investigator(s) at each 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 Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject

- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

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 study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Revised Clinical Study Protocol).

The amendment is to be approved by the relevant Ethics Committee and if applicable, also the national regulatory authority approval, before implementation. Local requirements are to be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to each Principal Investigator(s). For distribution to Ethics Committee see Section 8.3.

If a protocol amendment requires a change to a centre's Informed Consent Form, AstraZeneca and the centre's Ethics Committee are to approve the revised Informed Consent Form before the revised form is used.

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

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee 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 protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (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 BY ASTRAZENECA

9.1 Pre-study activities

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

- Determine the adequacy of the facilities

- Determine availability of appropriate subjects for the study

- Discuss with the investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the investigator.

9.2 Training of study site personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and system(s) utilized.

The Principal 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 Principal 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(s).

- Confirm that facilities remain acceptable.

- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in accordance with the Laboratory Manual and that study drug accountability checks are being performed.

- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (eg, clinic charts).

- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

The AstraZeneca representative will be available between visits if the investigator(s) or other staff at the centre needs information and advice about the study conduct.

9.3.1 Source data

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

9.4 Study agreements

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

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

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the Clinical Study Agreement (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 Q3 2013 and to end by Q4 2013.

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 CAZ-AVI.

10. DATA MANAGEMENT BY ASTRAZENECA

Data management will be performed by the AstraZeneca Data Management Centre (DMC).

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

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

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

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

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

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

11. EVALUATION AND CALCULATION OF VARIABLES BY ASTRAZENECA

11.1 Calculation or derivation of safety variable(s)

11.1.1 Demographics

Age will be determined at the time of enrolment.

Body Mass Index (kg/m^2) will be calculated at the time of enrolment as: weight (kg) / [height (m) x height (m)].

11.1.2 Physical examination

The complete physical examination will include an assessment of the following: general appearance, skin, head and neck (including eyes, ears and throat), lymph nodes, thyroid, musculoskeletal/extremities (including spine), cardiovascular, lungs, abdomen, and neurological systems.

The brief physical examination will include an assessment of the following items: general appearance, abdomen, lungs, and the cardiovascular system.

11.1.3 Vital signs and oral temperature

Change in BP, pulse rate and oral temperature at each post treatment time point will be calculated as the post treatment measurement value minus the baseline value observed on Day -1.

11.1.4 ECG

The following variables will be reported: heart rate, RR, PR, QRS, and QT intervals from the 12-lead ECG and the derived variable QTcF (the QT correction will be based on the Fridericia formula).

Three replicate 10-second ECGs will be recorded at each scheduled timepoint within a 5 minute interval and the measurements of each ECG variable will be recorded at each

scheduled time point. The average of these triplicate measurements for each variable will be used in all data presentations.

Baseline is defined (after taking the average of the triplicates) as the latest non-missing averaged triplicate prior to the first study drug infusion (Day-1).

11.1.5 Safety laboratory (hematology, chemistry and urinalysis)

Change in laboratory test value at each post treatment time point will be calculated as the post treatment value minus the baseline value observed on Day -1 . The recorded lower limit of detection (LLD) will be used in place of any non-missing zero or <LLD baseline values.

The units used for presentation of the data will follow AstraZeneca standards for the reporting of laboratory data.

11.1.6 Adverse events

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

The onset of an AE relative to treatment will be calculated as the time difference between the onset (date and time) of the AE and the start time of the last dose (date and time) prior to the AE.

The duration of a resolved AE will be calculated as the difference between the resolution (date and time) of the AE and the onset (date and time) of the AE.

11.1.7 Other significant adverse events (OAE)

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 DAEs. Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered OAEs and reported as such in the Clinical Study Report. A similar review of laboratory, vital signs and ECG data will be performed for identification of OAEs.

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.

11.2 Calculation or derivation of Pharmacokinetic variable(s)

PK analysis of the plasma and urine concentration data for AVI and CAZ will be performed at AstraZeneca R&D.

The actual sampling times will be used in the PK parameter calculations. PK parameters will be derived using non-compartmental methods with WinNonlin® Professional Version 5.2, or

higher, (Pharsight Corp., Mountain View, California), or SAS® Version 9.1, or higher (SAS Institute, Inc., Cary, North Carolina). All PK computations will be performed using WinNonlin® Professional 5.2, 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 (Systat Software, Inc., San Jose, California); Excel 2007, or higher (Microsoft Corp., Seattle, Washington); or WinNonlin® Professional 5.2, or higher.

Where possible, the following plasma PK parameters will be determined for CAZ and AVI, from plasma concentrations recorded on the single dosing days:

- Maximum plasma concentration (C_{max}, μg/mL), obtained directly from the observed concentration versus time data
- Minimum plasma concentration during a dosing interval (C_{min}, μg/mL), obtained directly from the observed concentration versus time data
- Time of maximum plasma concentration (t_{max}, hr), obtained directly from the observed concentration versus time data
- Last quantifiable plasma concentration (C_{last}, μg/mL), obtained directly from the observed concentration versus time data
- Time of last plasma concentration (t_{last}, hr), obtained directly from the observed concentration versus time data
- Area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration [$AUC_{(0-t)}$, μg.hr/mL], calculated by linear up/log down trapezoidal summation
- Area under the plasma concentration time curve from zero (pre-dose) extrapolated to infinity (AUC, μg.hr/mL), calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable concentration divided by the elimination rate constant: $AUC_{(0-t)} + C_{last}/\lambda_z$. If the extrapolated area (C_{last}/λ_z) is greater than 20% of AUC, then AUC and related parameters will not be reported
- $AUC_{(0-8)}$ (μg.hr/mL): AUC from time 0 to 8 hours postdose.
- Lambda z (λ_z): The terminal elimination rate constant determined by selection of at least three data points on the terminal phase of the concentration-time curve.
- Terminal half-life ($t_{1/2}$, hr). Visual assessment will be used to identify the terminal linear phase of the concentration-time profile. A minimum of 3 data points will be used for determination
- Systemic plasma clearance (CL, L/hr)

- Mean residence time (MRT, hr), calculated as ([AUMC/AUC] - [infusion time / 2]), where AUMC is area under the moment curve.
- Volume of distribution at steady-state (V_{ss}, L), calculated as Mean Residence Time×CL
- Volume of distribution at the terminal phase (V_z, L), calculated as CL/λ_z

For Days 6, 7 and 8: the trough concentration C_{min}(μg/mL).

For Day 9 of multiple dosing, the following steady-state PK parameters will be calculated:

- Minimum plasma concentration during a dosing interval (C_{min}, μg/mL), obtained directly from the observed concentration versus time data
- Time of maximum plasma concentration ($t_{max,ss,}$ hr), obtained directly from the observed concentration versus time data
- Maximum plasma concentration ($C_{max,ss,}$ μg/mL), obtained directly from the observed concentration versus time data
- Average concentration (C_{av}, μg/mL), calculated as $AUC_{(0-\tau)}/\tau$, where τ is the dosing interval (i.e. 8 hours).
- Linearity factor, the Day 9 AUC_{ss} / Day 1 AUC.
- Area under the plasma concentration-time curve during the dosing interval (0-8 hour post-dose) at steady state [AUC_{ss}, μg.hr/mL], calculated by linear up/log down trapezoidal summation
- Steady state clearance (CL_{ss}, L/hr) calculated as $Dose/AUC_{(0-\tau)}$
- Volume of distribution at steady-state (V_{ss}, L), calculated as Mean Residence Time×CL
- Accumulation ratio for C_{max} (RC_{max}): $C_{max\ (Day\ 9)}$ / $C_{max\ (Day\ 1)}$
- Accumulation ratio for $AUC_{ss(RAUC)}$ will be determined as $AUC_{ss(Day9)}/AUC_{(0-8)(Day\ 1)}$
- Lambda z (λ_z)
- Terminal half-life ($t_{1/2}$, hr).
- Volume of distribution at the terminal phase (V_z, L)
- Mean residence time (MRT, hr)

From urine concentration data, the following PK parameters will be calculated:

- Amount of drug excreted unchanged into urine ($Ae_{(0\text{-}t),\ mg}$), calculated as the product of the urine volume and the urine concentration. The amount will be calculated and reported for each collection interval and cumulatively
- Fraction of dose excreted as unchanged drug into urine (fe; % dose). The fraction will be calculated and reported for each collection interval and cumulatively
- Renal clearance (CL_r, L/hr), calculated as $Ae_{(0\text{-}t)}$ divided by $AUC_{(0\text{-}t)}$ for single dose or as $Ae_{(0\text{-}8)}$ divided by $AUC_{(0\text{-}8)}$ at steady-state on Day 9.
- Non renal clearance (CL_{nr}, L/hr) will be calculated as the difference between total clearance and renal clearance, if appropriate.

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 $t_{1/2}$ ($t_{1/2}$, Interval)
- Number of data points ($t_{1/2}$, N) included in the log-linear regression analysis to determine $t_{1/2}$
- Coefficient of determination (Rsq) for calculation of λ_z. If Rsq is less than or equal to 0.8, λ_z and related parameters will not be reported
- Percent of AUC extrapolated: Percentage of AUC that is due to extrapolation beyond t_{last} ($\%AUC_{ex}$)

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY ASTRAZENECA

12.1 Description of analysis sets

12.1.1 General principles

The analysis of data will be based on different subsets according to the purpose of analysis, ie, for safety and PK, respectively. The decision regarding validity of data for each of the analysis sets will be based on a blind review of data.

The as-treated principle will be applied to all evaluations; ie, subjects who received another treatment than the one assigned in the randomization list will be analyzed as belonging to the actual treatment assignment, and not that assigned by randomization.

12.1.2 Safety analysis set

All subjects who received at least one dose of randomized investigational product (CAZ-AVI, or placebo) and for whom any postdose data are available will be included in the safety population.

12.1.3 PK analysis set

The PK analysis population will include all subjects who satisfy all the following: (1) have received at least 1 dose of study compound to be measured; (2) complete the study without any major protocol deviation thought to interfere with the absorption, distribution, metabolism, and excretion (ADME) of the compound to be measured; (3) provide evaluable data in support of the PK analyses.

The assessment of (2) will be agreed by the study team prior to clean file and code break.

Subjects who receive placebo will not be part of the PK analysis set.

12.2 Methods of statistical analyses

12.2.1 General principles

Given the exploratory nature, no formal statistical hypothesis testing will be performed in this study. Consequently, no corrections for multiplicity will be used.

Data that are reported as missing will be excluded from all descriptive and non-descriptive data analysis. There will be no imputation of data. Observations that are spurious (extreme relative to the majority of the data) will not be altered or removed from any presentation of the data unless there is documented agreement that they are clearly erroneous (eg, values incompatible with life).

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

Subjects from different cohorts receiving placebo treatment will be pooled into a single group for data summaries, but presented alongside the corresponding active-treated subjects in any data listings.

12.2.2 Subject characteristics

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

12.2.3 Safety and tolerability

Data will be presented by treatment assignment for the purpose of summarizing the safety results.

All AEs, ECG outliers, and clinical laboratory outliers that occur following administration of investigational product will be included in the tabulations of AEs and outlier events, including episodes that occur at unscheduled evaluations.

Adverse events will be summarized by treatment assignment, Preferred Term and System Organ Class using Medical Dictionary for Regulatory Activities (MedDRA version 12.0 or higher). Furthermore, SAEs and AEs leading to withdrawal will be listed, and the number of subjects who had any AEs, SAEs, AEs that led to withdrawal, and AEs with severe intensity will be summarized.

Tabulations and listings of data for vital signs, clinical laboratory tests, ECGs, oral temperature, and physical examination findings will be presented. Where applicable, data will be presented for the observed value at each scheduled assessment and for the corresponding change from baseline. In addition, laboratory and other safety parameters will be reviewed via graphical presentations (eg, individual profiles, box plots) to explore the safety profile of AVI.

For clinical laboratory tests, listings of values for each subject will be presented with abnormal or out-of-range values flagged. Extra measurements (such as unscheduled or repeat assessments) will not be included in summary tables, but will be included in the subject listings.

Categorical variables (eg, urinalysis) will be summarized in frequency tables (frequency and percentage) by treatment assignment and scheduled assessment time.

Continuous variables (hematology, clinical chemistry, temperature, vital signs, ECG) will be summarized using descriptive statistics (minimum, first quartile, median, third quartile, maximum and n) by treatment assignment and scheduled assessment time, both as absolute values and as change from baseline. Shift tables of the number and percentage of subjects with normal, low or high results, as defined by the project standard ranges, will be presented from baseline to the most extreme post baseline value by treatment assignment. Similar shift tables will be presented for categorical urinalysis variables.

Further categorical summaries of observed QTcF values (> 450 msec, > 480 msec, > 500 msec) and change from predose values in QTcF values (> 30 msec, > 60 msec) will also be produced.

12.2.4 PK

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

PK variables (AVI and CAZ plasma concentrations, urine amounts, and PK parameters, when applicable) will be summarized by study day and measurement time using appropriate descriptive statistics (eg, n, mean, SD, min, median, max, geometric mean [gmean], coefficient of variation [CV]). The geometric mean is calculated as exp (u) where u is the

arithmetic mean calculated from data on a log(base e) scale. The CV is calculated as $100 \times \sqrt{\left(\exp\left(s^2\right)-1\right)}$ where s is the SD of the data on a log (base e) scale. Mean, SD, gmean and CV will not be calculated for t_{max}. PK concentration summaries will also include lower and upper SD bounds [gmean±SD] which are defined as exp (u ± s).

For descriptive statistics, non-quantifiable (NQ) values of plasma concentrations will be handled as follows:

- If, at a given time point, 50% or fewer of the plasma concentrations are NQ, the mean, SD, gmean, gmean±SD, and CV will be calculated by substituting the limit of quantification (LOQ) for values which are NQ. If the calculation of the geometric mean minus SD results in a value less than the LOQ, NQ will be displayed

- If more than 50%, but not all, of the concentrations are NQ, the mean, SD, gmean, gmean±SD, and CV will be reported as not calculable (NC). The max value will be reported from the individual data, and the min and median will be set to NQ.

- If all concentrations are NQ, the gmean and mean will be reported as NQ and the gmean±SD, SD and CV as NC.

- The number of quantifiable values (n above LOQ) will be reported for each time point

Individual plasma concentration profiles will be presented graphically on both linear and semi-logarithmic scales, showing all subjects on a single plot on each study day, and all study days (1 and 9) on a single plot for each subject/analyte.

Figures of geometric mean concentration-time profiles (with lower/upper SD bounds as defined above) will be presented on both linear and semi-logarithmic scales, including:

- gmean (±SD) AVI concentration-time data for study days 1 and 9 will be overlaid on the same plot. gmean (±SD) CAZ concentration-time data for study days 1 and 9 will be overlaid on the same plot.

- gmean (±SD) concentration-time data for CAZ-AVI, separated by study day.

An exploratory evaluation of achievement of steady-state will be performed graphically. Figures of individual subject and mean plasma trough concentrations versus study day following single and multiple dosing will be summarized and displayed graphically on a linear scale.

Additional graphical presentations of PK data may be added at the discretion of the PK scientist.

Individual and cumulative amounts of AVI and CAZ excreted in urine will be tabulated. Urinary excretion will be summarized as the fraction of the total dose excreted unchanged in urine, using descriptive statistics N, mean, SD, gmean, CV, minimum, median, and maximum. Renal clearance will be reported similarly.

12.3 Determination of sample size

No formal sample size calculations are performed. A sample size of 16 evaluable volunteers is considered sufficient to characterize the PK characteristics and provide safety and tolerability data in healthy subjects.

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 Study Delivery Team Leader. If the Study Delivery Team Leader is not available, contact the Study Delivery Team Physician at AstraZeneca Research and Development.

Table 7 Medical Emergency and AstraZeneca Contact Information

Name	Role in the study	Address & telephone number
	Study Delivery Team (SDT) Leader responsible for the protocol at central R&D site	
	SDT Physician responsible for the protocol	

13.2 Overdose

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

An overdose without associated symptoms is only reported on the Overdose CRF module.

If an overdose on an AstraZeneca study drug occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives **within one 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.

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 investigational product should be discontinued immediately and the pregnancy reported to AstraZeneca.

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product 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 was 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 one 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

Pregnancy of the subject's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented. The outcomes of any conception occurring from the date of the first dose until 3 months after the last dose of study treatment must be followed up and documented.

All outcomes of pregnancy must be reported to AstraZeneca on the pregnancy outcomes report form.

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