UNIKLINIK KÖLN | Institut für Medizinische Statistik, Informatik und Epidemiologie

IMSIE

PPD

Prof. Dr. PPD

Statistical Analysis Plan

Study title:	A Phase IIa prospective, open-label, multicenter study to determine the pharmacokinetics (PK) and safety and tolerability of aztreonam-avibactam (ATM-AVI) for the treatment of complicated Intra-Abdominal Infections (cIAIs) in hospitalized adults (REJUVENATE)
Study code:	D4910C00009
	EudraCT-No.: 2015-002726-39
Investigational medicinal product:	aztreonam-avibactam (ATM-AVI), metronidazole
Comparator:	None
Indication:	complicated intra-abdominal infection (cIAI)
Sponsor:	AstraZeneca AB, 151 85 Södertälje, Sweden
Financial support:	
Protocol identification:	CSP 1.0, 03.11.2015
Development phase:	Phase IIa
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1   Background

1.1   Trial objective

The objective of this Phase IIa, patient pharmacokinetics (PK) study is to investigate the PK, tolerability and safety of aztreonam-avibactam (ATM-AVI) in patients with a serious bacterial infection and to provide dose confirmation for Phase III clinical trials. The Pharmacokinetic/Pharmacodynamic (PK/PD) data generated will contribute to a robust package of data, supported by prior experience with ATM and AVI administered separately, and validated by qualitative data for the combination from a proposed randomized Phase III clinical trial in patients with infections suspected to be caused by these specific but rare pathogens. [CSP section 2, p.28]

1.2   Trial design

This is a prospective, open-label, single-arm, dose-confirming multicenter Phase IIa study to determine the PK, safety and tolerability of ATM-AVI in the treatment of hospitalized patients with a complicated intra-abdominal infection (cIAI). Forty adult patients with a diagnosis of cIAI and the need for a surgical intervention will be enrolled. The surgical intervention may take place within 24 hours before or after administration of the first dose of study drug. After obtaining written informed consent and confirming eligibility, patients will be assigned the following treatment:

From the study start, only patients with normal renal function or mild renal impairment (i.e. creatinine clearance (CrCl) >50 mL/min) will be eligible. The proposed administration of ATM-AVI for these patients is a loading dose (500 mg ATM plus 137 mg AVI by intravenous [IV] infusion over a 30 minute period), immediately followed by a dose of 1500 mg ATM plus 410 mg AVI by IV infusion over a 3 hour period every 6 hours (maintenance infusion). The targeted total dose on Day 1 will be 6500 mg ATM and 1777 mg AVI. From Day 2 onwards, this will be 6000 mg ATM / 1640 mg AVI. For anaerobic coverage, patients will also receive 500 mg metronidazole infused over 1 hour every 8 hours (q8h), starting after the first ATM-AVI maintenance infusion.

The PK, safety and tolerability of the dosing regimen described above will be assessed in a first cohort of 10 patients (see section 6.8).

Patients with moderate renal impairment (CrCl of 31 to 50 mL/min) must not be included prior to the completion of the 10 patient safety review and subsequent confirmation of an appropriate dosing regimen. Patients with a creatinine clearance of 31 – 50 mL/min will be eligible if the results of the early PK and safety review (along with respective PK modelling)

- support inclusion of such patients and

- confirm a specific dosing regimen to be amended to the protocol.

Intravenous study therapy (ATM-AVI) plus metronidazole will be continued for a period of time (5 to 14 full days, where a full day is defined as a 24-hour period) deemed appropriate by the investigator based on the patient's clinical response. After at least 5 full days of IV study therapy and at the discretion of the investigator, all study therapies may be discontinued if the patient has shown significant clinical improvement.

It is anticipated that each patient will complete the study, including the Late Follow-up (LFU) visit. In the event that the patient is discharged from the hospital before the End of Treatment (EOT), Test of Cure (TOC), or LFU visit, they will return to the study centre for their scheduled assessment. If treatment with antibiotics is required beyond 14 days, the

designated COMBACTE-CARE national coordinator or other designated study personnel should be contacted.

All patients will undergo sparse pharmacokinetic sampling on Day 1 of treatment. On Day 4, the first twenty five (25) patients will undergo intensive pharmacokinetic sampling, whereas the following fifteen (15) patients will undergo sparse sampling.

A flow-chart of the overall study design is shown in Figure 1. The detailed study plan and timing of procedures is presented in Table 1.

Figure 1   Study Outline
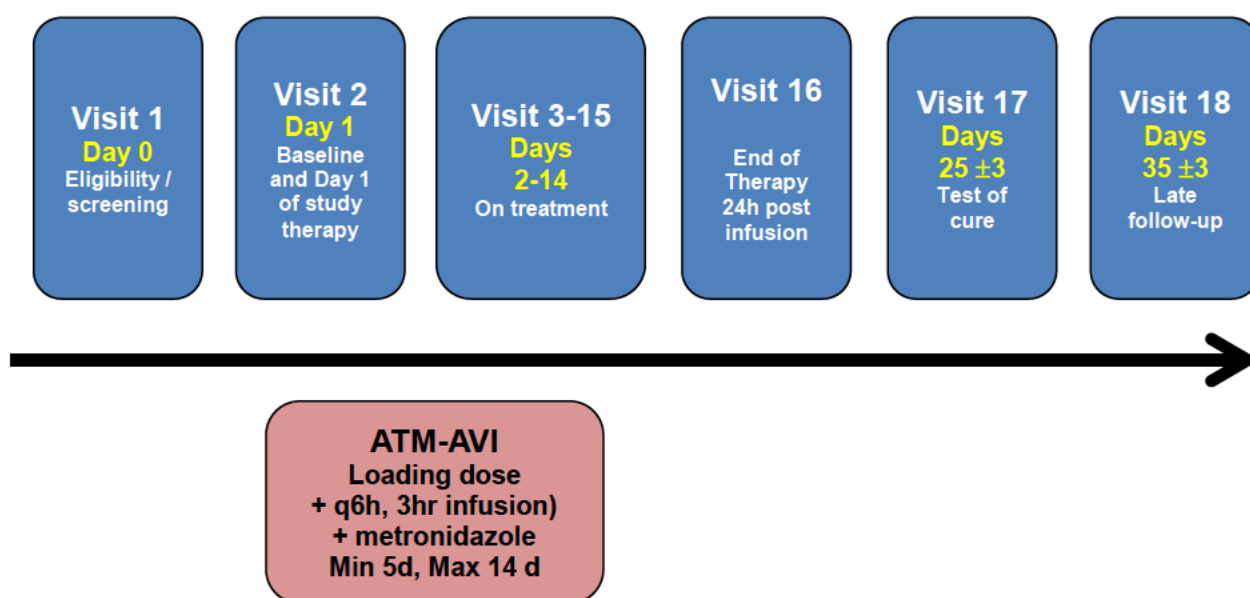
1.3   Number of subjects

Up to 40 patients will be enrolled into the study in total. Although the study is not powered to perform statistical tests, assessment of safety, and complete PK assessments from at least 30 patients with cIAI, is considered sufficient to adequately confirm the PK and safety profile of ATM-AVI in a population with a representative burden of disease. It is expected that 10 patients having completed all safety assessments are sufficient for an initial review of key safety criteria of ATM-AVI in patients in order to continue treating patients in the Phase IIa and Phase III studies.

[CSP section 8.2, p.81]

Table 1   **Study Plan detailing the procedures**

Procedures and Assessments	Eligibility/ Screening Visit 1 Day 0	Treatment Period[a] Visit 2 Day 1 (Baseline)	Treatment Period[a] Visit 3 - 15 Days 2 to 14	EOT Visit 16 Within 24 hours after last infusion	TOC Visit 17 Day 25 ± 3	LFU Visit 18 Day 35 ± 3
Informed consent	X					
Inclusion and exclusion criteria	X	X				
Demographics	X					
Smoking and alcohol history	X					
Medical and surgical history	X					
Review prior and concomitant medications (including prior antibiotic therapy)	X	X	Daily	X	X	X
Complete physical examination	X			X	X	X
Assess infection-related signs and symptoms and perform focused physical / wound examination	X	X[b]	Daily	X	X	X
Vital sign measurements	X	X	Daily	X	X	X
12-Lead digital ECG		X	Day 3	X		
Adverse Events	X	X	Daily	X	X	X
Obtain clinically relevant culture and send to central laboratory	As clinically indicated					
Blood cultures	X	As clinically indicated. If blood cultures are positive: repeat at least every 3 days until negative				
Culture from intra-abdominal site of infection	At surgical intervention					
Safety Labs (Chemistry, Hematology, Urine, CrCl[c])	X	X[b]	Days 4, 7, 10, and 13[e]	X	X	X
Serum β-hCG for women of childbearing potential	X					X
Pharmacokinetic sample: intensive regimen		Day 1 4 samples	Day 4[f] 11 samples			
Pharmacokinetic sample: sparse regimen		Day 1 4 samples	Day 4[f] 3 samples			
Description of operative procedures	As available	As available	As available	As available	As available	As available
Administer study therapy		X	X			
Clinical response assessment				X	X	X
Record radiologic examination	X[d]					
Mortality assessment				X	X	X

a: Treatment period is defined as a minimum of 5 full days to a maximum of 14 full days, where a full day is defined as a 24-hour period. Treatment period starts with administration of the first dose of IV study therapy which marks the beginning of Study Day 1. Visit 2 includes the baseline assessments and Day 1.

b: Repeat assessments are only required if Visit 1 and Visit 2 are separated by surgery OR are >12 hours apart

c : Following each determination of serum creatinine until LFU (inclusive), also the estimate creatinine clearance will be calculated.

d: Radiological examinations are not required for the study but the results should be recorded if done as part of the cIAI diagnosis. Radiological examinations include but are not limited to white blood cell scans, PET scans, plain abdominal radiographs, computed tomography scans, ultrasound, and/or magnetic resonance image scans with or without contrast.

e : On days 4, 7, 10 and 13, samples can be collected ± 1 day.

f : To provide greater flexibility, these samples can be collected on Day ±1 day.

[CSP section 1.4, p.25-27; Table 1 p. 42]

2 Analysis sets

2.1 Definitions

The modified intent-to-treat (MITT) population will include all enrolled patients who receive any amount of study drug.

The microbiologically modified intent-to-treat (mMITT) population is a subset of the MITT population and includes all enrolled patients who have a diagnosis of cIAI (i.e. meet inclusion criterion 4; CSP, p. 31-32) and have an intraabdominal pathogen at baseline (regardless of susceptibility to the study drug). For example, a reason for exclusion from the mMITT population would be (amongst others) lack of a pathogen collected from a surgical procedure at baseline, whether that be because the surgery was not performed, or was performed outside protocol time frame (see inclusion criteria, with an extra tolerance of ±1 hour around the specified time intervals).

The pharmacokinetic (PK) population includes all patients who have at least 1 plasma concentration data assessment available for ATM-AVI, no fundamental violations of the inclusion and exclusion criteria and no important protocol deviations affecting assessment of PK as defined in section 2.3. The reason for exclusion will be listed for all subjects in question. [CSP section 8.3, p.81]

2.2 Application

There are two efficacy analysis sets: the MITT and mMITT population. The safety analysis set is the MITT population. The PK analysis set is the PK population.

2.3 Major protocol deviations for definition of PK population

Patients who fail to qualify according to the following inclusion/exclusion criteria will be excluded from the PK analysis set [CSP sections 3.1 and 3.2, p.29-36]:

- Inclusion criterion 4: diagnosis of cIAI
- Exclusion criterion 3: prior (within 3 months of starting study medication) or concomitant administration of another investigational medication
- Exclusion criterion 25: current probenecid treatment

These criteria have been selected as essential for restricting the PK population because they relate either to the fundamental target population (cIAI) of the study or to the pharmacokinetic system being studied.

Patients with the following protocol deviations will be excluded from the PK analysis set:

- taking prohibited medication, i.e. probenecid

3   Trial centres

The study takes place in 22 trial centres in Germany, France and Spain. Each side is expected to recruit two patients.

Site code	Country	Organisation
PPD	France	PPD
	France	
	France	
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4 Analysis variables

4.1 Demography and baseline characteristics

The demographic and baseline variables are measured at visit 1 (day -1 to 0) or 2 (day 1).

The following demographic variables will be analysed:
- Age [years]
- Sex [male, female]
- Ethnicity [Hispanic/non-Hispanic]
- Race [caucasian/white, black, Asian]
- Smoking status [never smoker, ex-smoker, smoker]
- Cumulative pack years
- Ex-smoker since [years]
- Alcohol history (number of glasses of beer, wine, spirits per week)

The following baseline variables will be analysed:
- Height [cm]
- Weight [kg]
- Body mass index [kg/m^2] (computed as Weight [kg]/(Height [cm]/100)^2)
- Physical examination
- Vital signs
- Infection-related signs and symptoms
- Diagnosis
- Location of infection
- Infection due to complications of previous abdominal surgery
- Infection due to complication of previous treatment failure
- Pathogens identified at baseline: genus and (if identified) species
- Monomicrobial (manifestation of only one bacterial species from any source of infection [cIAI site or blood]) or polymicrobial (manifestation of more than one bacterial species from any source of infection [cIAI site or blood]) bacterial species
- Minimum inhibitory concentrations (MIC) of each tested antimicrobial agent for each tested pathogen: possible values are ≤0.008, 0.015, 0.03, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, >256 µg/ml.
- Disk zone of ATM-AVI for each tested pathogen

4.2 Primary variable

The PK analyses of the concentration (µg/mL) data for ATM and AVI respectively, will be performed by Standard Operating Procedures; Work Instructions will be used as the default methodology if not otherwise specified. The actual sampling times will be used in the final PK parameter calculations. Nominal sampling times (see CSP Table 4 below) will be used for all interim PK analyses. All PK computations will be performed using Phoenix®WinNonlin 6.2, or higher (Certara L.P., St. Louis, Missouri); or SAS® Version 9.2, or higher (SAS Institute,Inc., Cary, North Carolina). Pharmacokinetic parameters will be derived using non-compartmental methods.

The primary outcome measures will be:

- Derived PK parameters for ATM and AVI, respectively:
  - C_{max}: Maximum plasma concentration
  - t_{max}: Time of observed maximum concentration
  - $AUC_{(0-6)}$: Area under the plasma concentration vs. time curve from time point zero up to 6 hours
  - $AUC_{(0-last)}$: Area under the plasma concentration vs. time curve from time point zero up to the last measured concentration above Limit of quantification (LOQ)
  - t_{last}: Time of last measured concentration above LOQ
  - $t_{1/2}$: Plasma elimination half-life
  - V_{ss}: Apparent volume of distribution at steady state after IV administration
  - V_z: Volume of distribution during terminal phase after IV administration and
  - CL: clearance

for the first 25 patients undergoing intensive sampling on day 4. They will be analysed in the PK analysis set.

The pharmacokinetic sampling schedule is displayed below:

Day	Sample time
Day 1	All patients: sparse sampling (4 samples/patient)
	Sample 1: 0h (within 1h before start of loading dose)
	Sample 2: within 5 min before the end of the loading dose infusion
	Sample 3: anytime within the 15 minutes prior to the end of the second IV infusion (3.25h – 3.5h after start of loading dose infusion)
	Sample 4: within 5-6 h after start of loading dose infusion
Day 4	First 25 patients: intensive sampling (11 samples/patient)
	Trough (within 10 min prior to IV infusion start), 0.5h 1, 2, 3 (within 15 min before IV infusion stop), 3.25, 3.5. 3.75, 4, 5, and 6 h after start of IV infusion (just before start of next infusion)
	Remaining 15 patients: sparse sampling (3 samples/patient)
	Sample 1: within 1 h of start of IV infusion
	Sample 2: within the 15min prior to the end of IV infusion (within 2.75 to 3 h after start of IV infusion)
	Sample 3: within 5-6 h after start of IV infusion (within one hour prior to start the next infusion)

[CSP Table 4, p.60]

Safety and tolerability as assessed by

- Number and severity of adverse events and serious adverse events

- Physical examination (at EOT and TOC)
  - Weight [kg] (only if clinically relevant change from baseline)
  - General appearance
  - Skin, head, eyes, ears, nose, throat
  - Lymph nodes

- o Respiratory
- o Cardiovascular Abdomen
- o Musculoskeletal
- o Neurological Other

All variables (except weight) are classified as normal or abnormal (clinical significance [yes, no] if abnormal).

- Vital signs (daily assessment, day 2-14 = visit 3-15; EOT, TOC, LFU)
  - o Systolic blood pressure [mmHg]
  - o Diastolic blood pressure [mmHg]
  - o Heart rate [beats/min]
  - o Respiratory rate [breaths/min]
  - o Temperature [°C]

- ECGs

- Laboratory assessments (baseline, day 4, 7, 10, 13, EOT, TOC, LFU and possibly further samples as clinically indicated to be analysed at the local laboratory)
  - **Clinical chemistry**
  - o Alanine aminotransferase
  - o Albumin
  - o Alkaline phosphatase
  - o Aspartate aminotransferase
  - o β-hCG (β-human chorionic gonadotropin; will be determined for woman of childbearing potential at screening (visit 1) and LFU only)
  - o Bicarbonate
  - o Blood urea nitrogen
  - o Calcium, total
  - o Chloride
  - o Creatinine
  - o Estimated creatinine clearance (to be calculated by study center personnel following each determination of serum creatinine until EOT (inclusive) according to CSP Appendix E. TOC and LFU: Calculation of CrCl only if clinically indicated.
  - o Glucose (nonfasting)
  - o Inorganic phosphorus
  - o Potassium
  - o Sodium
  - o Bilirubin (total, direct and indirect)
  - o Total protein
  - **Hematology**
  - o Hematocrit
  - o Hemoglobin
  - o Platelet count
  - o Red blood cell count
  - o White blood cell count (total and differential)

- ▪ **Urinalysis**
  - ○ Appearance (color, clarity) Bilirubin
  - ○ Glucose
  - ○ Ketones
  - ○ Leukocyte esterase
  - ○ Nitrite
  - ○ pH
  - ○ Protein
  - ○ Specific gravity
  - ○ Urobilirubin
  - ○ Microscopic examination:
    - Red blood cells
    - White blood cells
    - Casts
    - Crystals
    - Bacteria, yeast cells, or parasites
- ▪ **Other**
  - ○ Blood culture (baseline and as clinically indicated thereafter)

They will be analysed in the safety analysis set.

4.2.1 Definition of AEs and SAEs

An adverse event (AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). For the purposes of the present study, an AE can include an undesirable medical condition occurring at any time following informed consent, even if no IV study treatment has been administered.

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

A serious adverse event (SAE) is an AE occurring during any study phase after the patient has signed the ICF (i.e., treatment, 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 excluding hospitalization due to worsening or failure of treatment for primary infection under study

- 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, including suspected transmission of an infectious agent via the IV study therapy

Liver dysfunction criteria:

- A Potential Hy's Law case is defined as any situation where a patient has an increase in both AST or ALT ≥3xULN and total bilirubin ≥ 2xULN, irrespective of the patient's alkaline phosphatase value, at any point during the study following the start of study medication.

- A Hy's Law case is defined as a patient with an increase in serum AST or ALT ≥3xULN together with total bilirubin ≥2x ULN, where no other reason than the IMP can be found to explain the combination of increases, e.g., elevated serum ALP indicating cholestasis, viral hepatitis, another drug.

For potential and Hy's Law cases the elevation in transaminases must precede or be coincident with (i.e., on the same day) the elevation in total bilirubin, but there is no specified timeframe within which the elevations in transaminases and total bilirubin must occur.

[CSP Appendix D]

The following cases of liver dysfunction are defined and reported as AE or SAE:

(1) Hy's law case: SAE

(2) Potential Hy's law case with an alternative explanation which is clinically significant: AE, or if seriousness criteria are met at any time, SAE

(3) Potential Hy's law cases where more than 3 weeks elapse without further confirmatory information: SAE (report term 'Hy's law').

Planned procedures or hospitalizations should not be recorded as SAEs, but complications arising from planned procedures or hospitalization / prolongation of hospitalizations meeting a seriousness criterion should be recorded and reported as SAEs.

Adverse Events and Serious Adverse Events must be collected for each patient from informed consent until the late follow-up visit (irrespective of whether the last follow-up visit attended by the patient is TOC or LFU; i.e. at the latest until Day 38).

[CSP section 6, p.65-71]

4.3 Secondary variables

The secondary outcome measures will be:

- Proportion of patients with clinical cure at the TOC visit. This will be analysed in the MITT and mMITT populations.
- Correlation of derived PK parameters for ATM-AVI and clinical cure at TOC. This will be analysed in the mMITT population.

4.3.1 Clinical cure

Clinical cure is defined as follows:

Clinical Response	Definition
Cure	Complete resolution or significant improvement of signs and symptoms of the index infection (cIAI) such that no further antimicrobial therapy, drainage, or surgical intervention is necessary and does not meet any of the failure criteria listed below.
Failure	Patients who meet any 1 of the following criteria will be considered a treatment failure: • Death related to intra-abdominal infection • Patient who received treatment with additional antibiotics for ongoing symptoms of cIAI (including patients prematurely discontinued from study therapy due to an adverse event who require additional antibiotics for cIAI) • Patient previously met criteria for failure (i.e. prior to respective visit) • Persisting or recurrent infection within the abdomen documented by the findings at re-intervention either percutaneously or operatively (exception: the re-intervention was already planned at initial surgery to check proper infection/focus control, occurs within 96 hours after enrolment, does not show deterioration and includes fascial closure) • Post-surgical wound infections defined as an open wound with signs of local infection such as purulent exudates, erythema, or warmth that requires additional antibiotics and/or non-routine wound care
Indeterminate	Study data are not available for evaluation of efficacy for any reason, including: Patient lost to follow-up or assessment not undertaken such that a determination of clinical response cannot be made

Abbreviations: cIAI, complicated intra-abdominal infection; EOT: End of Treatment (with study therapy); LFU: Late follow-up; TOC: Test of Cure.

[CSP table 2, p.52]

In order to confirm the clinical response classification (cure, failure or indeterminate) of a patient, all criteria for failure (see table above) will be checked. This will involve data from clinical response assessment (at EOT and TOC), mortality assessment (at EOT and TOC), concomitant antibiotic medication, study completion/discontinuation, surgical procedure and post-operative wound examination. Doubtful cases will be listed for scrutiny by the study physician.

The proportion of patients with clinical cure is defined as the number of patients in the efficacy analysis set (MITT or mMITT population respectively) with clinical cure at TOC visit divided by the total number of patients in the efficacy analysis set. Indeterminate or missing assessment will be included in the denominator for calculation of Patients in the efficacy analysis set.

Any patients with a missing assessment at TOC visit (along with any other reasons leading to an assignment of indeterminate response) will be regarded as failures in the calculation of the proportion cured.

5 Handling of missing values and outliers

Because of the small sample size and the explorative nature of the study missing values will not be imputed. Because the data will only be reported descriptive outliers will not be removed from the dataset.

6 Statistical analyses / methods

Because of the explorative nature of the study no complicated statistical methods will be used for the analysis of the data. The tables, figures and listing used for the analysis are displayed in the TFL document in the appendix 12.3.

6.1 Patients

The study population will be described as follows:

- number (and percentage) of patients enrolled (overall and by country and site), received treatment, completed treatment and completed the study with reasons for not receiving treatment, discontinuing treatment and withdrawal from the study
- number and percentage of patients with at least one important protocol deviation and reason for protocol deviation
- number and percentage of patients in the safety, efficacy and PK analysis set and reasons of exclusion

6.2 Demography and baseline characteristics

If not stated otherwise the last measurement before the first dose is the baseline value. Demographic and baseline variables will be summarised as follows:

- for quantitative variables: number of observations, arithmetic mean, standard deviation (SD), median, 1st quartile, 3rd quartile, minimum and maximum
- for qualitative variables: absolute and relative frequencies

6.3 Prior or concomitant medication, surgery and diseases

Both ATM and AVI are predominantly eliminated by the kidney, partly by active tubular excretion. Probenecid and furosemide interfere with the active tubular excretion, resulting in increased plasma concentrations of the study drugs. While these increases are considered to be clinically insignificant, concomitant administration of probenecid is prohibited and furosemide should be avoided if at all possible during IV study therapy. Patients being treated with probenecid are not eligible for this study. Based on current knowledge, further relevant drug-drug interactions with regard to ATM-AVI administration in this study are not to be expected.

All prescription and over the counter medications being taken by the patient for the 2 weeks prior to study entry (considered prior treatment) and from enrolment through the LFU visit (considered concomitant treatments) must be documented on the appropriate pages of the eCRF. Systemic antibiotics should be documented for the entire duration of the study (from 2 weeks prior to enrolment through the LFU visit). Also application of topical antibacterial and antifungal agents and antibiotic peritoneal lavage need to be recorded in the eCRF.

If *Enterococcus* species or MRSA is one of the pathogens suspected or isolated and, in the opinion of the investigator, specific therapy is indicated, then open-label vancomycin,

linezolid, or daptomycin may be added to the study regimens according to the usual practice of the investigator. If vancomycin, linezolid, or daptomycin are started empirically to cover MRSA or *Enterococcus* species, and if final culture results did not isolate MRSA or *Enterococcus* species, then the investigator should discontinue the additional Gram-positive coverage that was empirically added.

The use of other systemic antimicrobials not specified by this protocol is not permitted during the study. However, if a new infection develops at a remote site (i.e., outside of the abdomen) between the date and time of enrolment and the LFU visit, and the investigator considers addition of non-study antibiotics essential to the safety and wellbeing of the patient, additional antibiotics may be added.

Also it is anticipated that in instances of suspected clinical failure, alternative or additional antibiotic therapy to treat the cIAI may be required. Prior to administering additional or alternative antibiotics the investigator should contact the national coordinating investigator to confirm the clinical evaluation and microbiological identification of an isolate not covered by ATM-AVI and metronidazole. It is anticipated that in instances of suspected clinical failure, alternative or additional antibiotic therapy to treat the cIAI may be required, and where rescue therapy is provided the patient should be assessed as a clinical failure. An appropriate antibiotic should be selected, taking into account results of sensitivity testing.

Also antifungal therapy to treat the cIAI should be avoided unless clinically indicated.

All actions related to the administration of concomitant antibiotics should be documented in the eCRF.

Further medication (other than that described above), which is considered necessary for the patient's safety and well-being, may be given at the discretion of the investigator without delay but should also be documented in the eCRF.

If analgesic medication is needed for pain, the use of analgesics without antipyretic properties is preferred. Should a patient require immunosuppressive agents or chemotherapy after enrolment, the investigator should contact the AstraZeneca physician or delegate before initiating therapy. Continued patient study participation will be determined based upon assessment of the safety risk to the patient if he or she were to continue in the study. Patients who have already completed the IV study therapy should remain in the study until LFU assessment as they are not actively on study therapy but being followed up for outcomes.

Restricted Medication/Class of drug:	Usage:
Systemic anti-infective drugs* (antibiotics and antifungal drugs) *other than vancomycin, linezolid, or daptomycin which are permitted for treatment of suspected or isolated MRSA or Enterococcus species	Prior to administration: • contact national coordinating investigator • obtain a microbiological specimen from the infection site • confirm infection due to a pathogen not covered by ATM-AVI and metronidazole • review result of sensitivity testing • select appropriate antibiotic drug, taking the above into account as well as the corresponding Summary of Product Characteristics (SmPC) for further prescribing information
Analgesic medication	Drugs without antipyretic properties are preferred

Prohibited Medication/Class of drug:	
Probenecid	Prohibited from enrolment to end of IV study therapy

[CSP section 7.7, p.79-80]

All medical/surgical events prior to start of the study will be listed for each patient by date. Additionally, disease related events prior to study start will be tabulated by system organ class.

The prior (within the last two weeks before the study) and concomitant medication and antibiotic medication will be listed for each patient by date. Number and percentage of patients with prior and concomitant antibiotic medication will be given.

All prior systemic antibiotic and concomitant medications will be coded using the AZ Drug dictionary medication code and summarized by ATC classification and preferred term.

6.4   Microbiology

The local laboratory must identify all *aerobic* bacterial pathogens to the genus and species level using confirmatory (not presumptive) identification methods from blood, intraabdominal or other specimens. Antimicrobial susceptibility should be determined for each aerobic pathogen isolated according to local practices. In addition, a disk diffusion test for ATM-AVI must be performed on each aerobic isolate using CLSI methods. The local laboratory can perform any additional testing on further agents as they normally do to provide susceptibility results of isolated aerobic microorganisms.

All *anaerobic* bacterial pathogens must be identified to at least the genus level. If the local laboratory cultures and performs susceptibility testing on anaerobic organisms, it should follow CLSI methodologies by either broth microdilution (Bacteroides fragilis group) or agar dilution with minimum inhibitory concentration (MIC) testing only (not disk diffusion) on metronidazole.

However, all anaerobic isolates must be sent to the central reference laboratory for confirmation of identification and susceptibility testing.

The investigator should record information on all specimens according to the microbiological investigator's manual supplied by the central reference laboratory. All specimens collected at baseline or post baseline should be entered in the eCRF with regards to a) day, time and site of sampling b) local identification for each pathogen and c) disc susceptibility / MIC to the study therapy for each pathogen.

The central reference laboratory will confirm pathogen identifications and susceptibility test results on all clinical isolates reported and shipped by the local laboratory. Thus, the central laboratory is responsible for the definitive identification for each microbiological organism and determination of MICs for ATM-AVI and comparator antibiotics. If a) discrepancies occur between the results obtained at the central lab and those obtained at the local lab or b) microorganisms that are isolated at the local lab do not survive shipping to the central lab, an AstraZeneca representative or delegate may request to ship a second sample of the isolate in question. In the instance of differences in pathogen identification or susceptibilities, the central reference laboratory results will take precedence over the local laboratory results.

The data obtained at the central reference lab will be used to determine descriptively a) the pathogen(s) causing the patient's infection and b) the efficacy of the study therapy at the

pathogen level. Local laboratory results may be used if a microorganism does not survive shipping or is not recoverable from the local laboratory. The central reference lab will transfer all microbiological data directly to CTCC where they will be merged into the study database (without being entered in the eCRF). The isolate ID number will be used to match the data obtained at the central lab with the data obtained at the local lab. Details will be described in a data transfer agreement.

Adequate samples are defined as samples from pus, tissue, peritoneal fluid or swab collected at surgical intervention, or blood; data analysis will be restricted to adequate samples.

Number and percentage of patients with pathogenic organisms identified at baseline will be summarized separately according to specimen type (intra-abdominal, blood) and shown by categorization into gram positive / gram negative and aerobes / anaerobes. Patients with pathogens categorized as *Enterobacteriaceae* will also be summarised.

Number and percentages of patients having monomicrobial or polymicrobial infections at baseline will be summarised.

Disk zone diameters from local lab will be categorised into susceptible, intermediate and resistant and will be tabulated. MIC from central lab will be tabulated as well (in case of multiple isolates with the same pathogen, that with the highest MIC will be reported). The isolates will be classified and tabulated as well.

Any pathogenic organisms identified post-baseline will be listed.

6.5 Exposition to treatment/Compliance

The exposure (in days; calculated as "last dose date – first dose date + 1") to study therapy will be listed by patient. If the treatment duration is less than 5 days or beyond 14 days, the patient will be flagged. Additionally, the number of IV infusions and the number of each individual component of study therapy (ATM, AVI, metronidazole) will be summarized by baseline renal function (normal renal function/mild renal impairment (CrCl >50 mL/min) and moderate renal impairment (CrCl of 31 to 50 mL/min).

Compliance for each IV therapy will be calculated as follows: (sum of actual infusions / expected infusion) × 100. The overall compliance will be an average of the ATM, AVI and metronidazole compliance.

The number of expected infusions for each IV therapy will be calculated as A/B rounded to the nearest integer with

- A: "stop date and time of the last dose of maintenance IV therapy – start date and time of the first dose of maintenance IV therapy" in hours

- B: interval of infusion (6 for ATM and AVI, 8 for metronidazole)

For ATM and AVI it is necessary to add 1 for the loading dose.

These analyses will be performed in the safety analysis set.

6.6 Primary analysis

6.6.1 ATM-AVI concentrations and PK parameters

There will be separate listings and summaries for ATM and AVI.

Plasma concentrations and PK parameters will be summarized using descriptive statistics by by baseline dose group/renal function (the number of patients (N), geometric mean, geometric coefficient variation (CV%), arithmetic mean, standard deviation (SD), median, minimum and maximum). For t_{max} and t_{last} only n, median, minimum and maximum will be used. Samples which deviate markedly from the planned nominal time may be omitted from summary statistics. Further, values deemed to be outliers (decisions to be reviewed by pharmacologists) may also be excluded, but will be reported separately in the CSR.

The geometric mean is calculated as the exponential of the arithmetic mean calculated from data on a log scale. The CV% is calculated as $100 \cdot \sqrt{\left(\exp\left(s^2\right)-1\right)}$ where s is the standard deviation of the data on a log scale.

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided. Plasma concentrations will be summarised by visit day, sampling (intensive vs reduced) and dose group/renal function and concentrations per nominal time point will be listed by patient. ATM and AVI concentrations will be plotted for each patient for each visit, as well as all visits (Day 1 and 4) in the same plot. For patients (n=25) with intensive blood sampling on Day 4 combined (all subjects) individual plasma concentration versus actual times will be plotted in linear and semi logarithmic scale. Separate plots will be grouped by each dose group/renal function.

Geometric mean plasma concentration (± SD) versus nominal sampling time will be plotted in linear and semi logarithmic (no SD presented) scale with all dose groups/renal functions overlaid on the same figure.

PK parameters will be summarised and listed by patient. PK parameters of the intensive sampling schedule will be summarised by visit day, and baseline renal function (normal renal function/mild renal impairment (CrCl >50 mL/min) and moderate renal impairment (CrCl of 31 to 50 mL/min). Box plots of PK parameters (C_{max}, $AUC_{(0-6)}$, $AUC_{(0-last)}$ and CL) stratified by dose group/renal function will be produced. Additional exploratory box plots (e.g. stratified by sex, age etc) may be produced if data allow.

Plasma concentrations that are not quantifiable (NQ) or if there are missing values (e.g., no result [NR]) will be handled as follows:

•Where there is NR, these will be set to missing.

•At a time point where less than or equal to 50% of the values are NQ, all NQ values will be set to the LLOQ, and all descriptive statistics will be calculated.

•At a time point where more than half of the values are NQ, the mean, SD, geometric mean and CV% will be set to Not Calculated (NC). The maximum value will be reported from the individual data, and the minimum and median will be set to NQ.

•If all values are NQ at a time point, no descriptive statistics will be calculated for that time point. Not calculated (NC) will be written in the field for standard deviation and CV% and NQ will be written in fields for mean, geometric mean, minimum, median and maximum.

•The number of NQ values (n below LLOQ) will be reported for each time point.

Data from subjects excluded from the PK analysis set will be included in the data listings, but not in the descriptive statistics.

6.6.2 Adverse Events and Serious Adverse Events

The numbers and percent of patients with AEs occurring from the time when informed consent is obtained at screening up to the late follow-up visit will be summarised with absolute and relative frequencies. The following summaries will be presented:

- Overall summary of AEs

- Incidence of AEs by system organ class (SOC) and preferred term

- Incidence of AEs by SOC, preferred term, and relationship to study therapy

- Incidence of AEs by SOC, preferred term, and severity

AEs which occur before the first dose of study medication will be identified as such in frequency tables (by inserting the corresponding frequency of such AEs in parentheses) and in listings.

In addition, AE will be summarized by patients who received other antibiotics (i.e. excluding ATM-AVI, metronidazole) at any time during the treatment period versus those who did not receive antibiotics during the treatment period. In this summary all adverse events will be included regardless of whether they occurred prior to starting the antibiotic or not.

A summary for deaths up to LFU visit will be presented; all deaths up to LFU visit with key patient information will be presented in a listing. Adverse events with outcome of death up to LFU visit will be summarized by system organ class and by preferred term; all adverse events with outcome of death up to LFU visit for key patient information will be presented in a listing.

Incidence of SAEs up to LFU visit will also be presented by system organ class (SOC) and preferred term; all SAEs up to LFU visit for key patient information will be presented in listings.

Incidence of adverse events of special interest will also be presented for each category by preferred term. Categories include: AEs potentially related to drug-induced liver injury, clostridium difficile colitis and hypersensitivity/anaphylaxis

Incidence of AEs leading to discontinuation of investigational product by SOC and preferred term will be presented in a table and the patients discontinued the investigational product due to AEs will also be presented in a data listing.

6.6.3 Laboratory safety assessment

Central laboratory data for chemistry, hematology, and urinalysis will be summarized and plotted (box plots) by scheduled visit for the observed values and for the corresponding change from baseline (CFB) values. Continuous laboratory parameters will be summarized as number of observations, arithmetic mean, standard deviation (SD), median, 1st quartile, 3rd quartile, minimum and maximum. If a patient has multiple results for a particular test at a

particular visit, the first non-missing value will be used for the summary. Summaries will be based on data of the central lab unless the data are missing, in which case local lab data will be used if available.

In addition, the following summaries at each applicable visit will also be provided:

- Shift tables (from baseline) showing the number and percentages of patients on treatment at the applicable visit with low, normal, or high values for hematology, chemistry and coagulation tests, defined according to the LLN and ULN provided by the respective laboratory; for urinalysis negative (or normal), trace and positive will be tabulated.

- The numbers and percentage of patients with potentially clinically significant abnormal values based on percent CFB using the criteria for potentially clinical significance detailed in appendix 12.4, by scheduled assessments and separately by scheduled as well as unscheduled.

In addition, a summary table will be presented which will indicate the number of patients who separately meet the criteria for potential Hy's Law or Hy's Law (section 4.2.1 above) after the start of study treatment at any time up to the LFU visit:

The AST, ALT, total bilirubin and ALP elevations can occur at any time in the specific review period and do not need to occur simultaneously.

A listing of patients with a value of ≥3xULN for ALT or AST or a value of ≥2xULN for total bilirubin in any one of the AST, ALT, total bilirubin parameters will be also presented. This listing will contain all the ALT, AST, total bilirubin and ALP study data for such patients.

6.6.4   Physical examinations

The numbers and percentage of patients with normal or abnormal assessment for each body system will be displayed by scheduled assessment. The abnormal results at the applicable post-baseline visits will be further classified into two categories: 1) same as baseline and 2) new or aggravated.

All the physical examination data will be listed.

6.6.5   Resting ECG

The number and percentage of patients within overall ECG interpretation category (normal, abnormal) for baseline and post-baseline (day 3 and EOT) by visit will be summarized.

The number of patients with normal, abnormal ECG interpretation shift results from baseline to the EOT visit will be provided.

ECG variables (PR, QRS, QT, QTcB mean, QTcF mean) all visits will be listed for all patients with abnormal results or shifts.

6.6.6   Vital signs

Vital signs will be summarized (number of observations, arithmetic mean, standard deviation (SD), median, 1st quartile, 3rd quartile, minimum and maximum) and plotted (box plots) for the observed values and for the corresponding change from baseline (CFB) values at each applicable visit.

Vital signs for all patients will be listed.

6.7 Secondary analyses

The number and percentage of patients with clinical cure (shown with 80% and 95% confidence intervals), clinical failure and indeterminate response at the TOC visit will be tabulated for the efficacy analysis set in both the MITT and mMITT populations).

The association of derived PK parameters for ATM-AVI and clinical cure at TOC will be analysed in the mMITT population. The PK descriptive statistics and plots will be produced separately for each clinical response group and informally compared.

Patients with extended-spectrum β-lactamase [ESBL]-phenotype pathogens will be summarised in the same way, and illustrated graphically as a forest plot. As an exploratory analysis, the proportion of clinical cure at the TOC visit will be determined excluding any patients with concomitant antibiotics (mMITT).

No inferential statistical tests will be implemented, given the small sample sizes with respect to any efficacy objective.

6.8 Planned subgroup analyses

A subgroup analysis will explore differences according to sex. The following outcomes will be analysed comparatively in males and females:

- ATM and AVI plasma concentrations (time series plots)

- Derived PK parameters (summary statistics)

- Severe AEs, SAEs, clinically significant lab values, vital signs, physical examination and ECG results

- Clinical cure rates

In accordance to Lucasti et al (2013, 2014) a male-female ratio of 2:1 is expected. From a clinical point of view no pharmacological or physiological differences between sexes are expected because metabolism of ATM-AVI mainly depends on renal function that is influenced by severity of disease and age.

Similarly, subgroup analyses will be performed for renal function (normal, mild, moderate according to amendment of inclusion criteria) and for age.

6.9 Early patient review

The safety and tolerability of the ATM-AVI dosing regimen (500 mg ATM plus 137 mg AVI loading dose by IV infusion over a 30 minute period, immediately followed by a maintenance dose of 1500 mg ATM plus 410 mg AVI by IV infusion over a 3 hour period every 6 hours) will be assessed based on the impact on patient liver transaminases. The assessment will be both a per patient assessment throughout the study period and per a cohort review of the first 10 patients having completed all PK and safety assessments.

The initial safety review (together with the parallel evaluation of the pharmacokinetics of ATM-AVI) will be assessed by a scientific advisory committee and AstraZeneca and input to a decision on whether

a) the remainder of the study will continue with the same dose regimen for patients with normal renal function or mild renal impairment (CrCl >50 mL/min)

b) patients with moderate renal impairment (CrCl 31 to 50 mL/min) should be included and administered the dosing regimen to be confirmed by amendment to the protocol

c) the dose regimen is confirmed as the regimen for the ATM-AVI Phase III program.

[CSP section 5.2.5, p.57-59]

6.9.1 Criteria for immediate progression

The criteria for immediate progression beyond the initial 10 patients are as follows:

- All transaminase rises are asymptomatic and rapidly reversible upon end of (or discontinuation of) therapy.
  Individual patient transaminase elevations:
  - o Patients with normal baseline transaminases (elevations ≥ 3xULN but < 5xULN)
  - o Patients with baseline transaminase > 3xULN and < 5xULN documented as being directly related to infection being treated (inclusion criteria) - elevations ≥ 5xULN but < 8xULN
- No more than 2 individuals have transaminase elevation as described above

If the criteria for immediate progression are met, investigators will be informed of the decision to continue recruiting as soon as feasible (within a timeframe to be confirmed). The pharmacokinetic data will be evaluated when available but will not be required to confirm the decision for immediate progression.

[CSP section 5.2.5.1]

6.9.2 Criteria for stop and review

The criteria for stop and review before progression beyond the initial 10 patients are as follows:

- Any one transaminase elevation is not rapidly reversible (in the absence of an alternative explanation for transaminase elevation)

- Between 3 to 5 individuals have a transaminase elevation as described in section 6.9.1

- Any one patient meets the following individual transaminase discontinuation criteria in the absence of an alternative explanation:
  Patients with initial normal baseline transaminases progressing to:
  - o ALT or AST ≥ 3×ULN and evidence of coagulopathy
  - o ALT or AST ≥ 3×ULN with symptoms suggestive of new or progressive liver disease

Patients with initial baseline transaminases > 3xULN and < 5xULN, documented as being directly related to infection being treated, progressing to:
  - o ALT or AST ≥ 5×ULN and evidence of coagulopathy
  - o ALT or AST ≥ 5×ULN with symptoms suggestive of new or progressive liver disease

In the event of a stop and review before progression, available pharmacokinetic data will be evaluated to determine the relationship between exposure and transaminase elevation. More extensive modelling may be required to determine if a change in dose is required and to identify an appropriate dose for the remaining patients.

[CSP section 5.2.5.2]

6.9.3 Criteria for non-progression

The criteria for non-progression beyond the initial 10 patients are as follows:

- 1 patient meets Hy's Law criteria

- More than one transaminase rise is not rapidly reversible (in the absence of an alternative explanation for transaminase elevation)

- More than 5 individuals have a transaminase rise as described in section 6.9.1

- Three or more patients meet the following individual transaminase discontinuation criteria in the absence of an alternative explanation:
  Patients with normal baseline transaminases

  - ALT or AST ≥ 3×ULN and evidence of coagulopathy

  - ALT or AST ≥ 3×ULN with symptoms suggestive of new or progressive liver disease

  Patients with baseline transaminases > 3xULN and < 5xULN documented as being directly related to infection being treated

  - ALT or AST ≥ 5×ULN and evidence of coagulopathy

  - ALT or AST ≥ 5×ULN with symptoms suggestive of new or progressive liver disease

If any one of the above criteria for non-progression occurs before 10 patients have been recruited for the early safety review, then the study will be stopped.

These criteria are to be interpreted by the scientific advisory committee in the light of all relevant clinical data on the patients concerned. The statisticians will translate the criteria to a programmable algorithm to indicate the numbers of patients fulfilling each of the component criteria, in order to assist the committee.

[CSP section 5.2.5.3]

For the early patient review the following will be presented:

- Diagnosis [listing]

- Safety local lab values (primarily AST, ALT, ALP, TBili, CrCl) [listing with flag for out of range values, graphs]

- Safety central lab values (primarily AST, ALT, ALP, TBili, CrCl) [listing with flag for out of range values, graphs*]

- PK parameters [listing, summary table]

- ATM and AVI concentrations [listing, graph]

As required for critical cases (such as potential Hy's law, discontinuations, withdrawals, deaths, SAE):

- AEs and SAEs [listing]

- Concomitant medication [listing]

- Patient profiles for potential Hy's Law cases

- Patient profiles for study drug discontinuations and withdrawals

- Patient profiles for any overdose or pregnancy report eCRFs

- Vital signs [listing with flag for out of range values, summary table]

- ECG [listing, graph]*

  * direct from respective online portal as far as possible

7 Deviations from the protocol

Not applicable

8 Interpretation of results

No complex statistical methods are used in the study, therefore (from a statistical point of view) there are no special issues concerning the interpretation of the results. Because of the small sample size a careful interpretation of the results is essential.

9 Data problems

Not expected. Will be recorded in a separate document.

10 Software

SAS 9.4 (SAS Institute Inc., Cary, NC, USA) will be used for the analysis of the data. Mainly the SAS procedure PROC TABULATE, PROC REPORT and PROC SGPLOT will be used to generate the TFL. Own macros will be validated according to IMSIE SOPs before using them for the analysis.

11 References

- Clinical Study Protocol of REJUVENATE, Version 2.0, 02.03.2016

- Lucasti C, Popescu I, Ramesh MK, Lipka J, Sable C. Comparative study of the efficacy and safety of ceftazidime/avibactam plus metronidazole versus meropenem in the treatment of complicated intra-abdominal infections in hospitalized adults: results of a randomized, doubleblind, Phase II trial. J Antimicrob Chemother. 2013;68(5):1183-92.

- Lucasti C, Hershberger E, Miller B, Yankelev S, Steenbergen J, Friedland I, Solomkinc J. Multicenter, double-blind, randomized, Phase II trial to assess the safety and efficacy of ceftolozane-tazobactam plus metronidazole compared with meropenem in adult patients with complicated Intra-abdominal infections. Antimicrobial Agents and Chemotherapy. 2014;58(9):5350-7.

12 Appendices

12.1 Reference ranges of laboratory parameters

For all local laboratory parameters, upper and lower normal limits are entered in the eCRF together with the measured values.

For central laboratory parameters, limits are specified in the Covance document *D4910C00009_RefRanges_20160308.xlsx*.

Physical examination results are assessed as normal/abnormal and (if abnormal) clinically significant yes/no in the eCRF.

Vitals signs will be assessed by the investigator with regard to clinical abnormality and the result entered in the eCRF.

12.2 Expected pathogens and their possible groupings

The following common pathogens are expected in this study. This list will be used for frequency tables, according to which pathogens actually occur:

Gram-negative bacteria

- Escherichia coli
- Citrobacter koseri
- Enterobacter cloacae
- Klebsiella pneumoniae
- Klebsiella oxytoca
- Proteus mirabilis
- Proteus vulgaris
- Providencia stuartii
- Serratia marcescens
- Pseudomonas aeruginosa

Anaerobes

- Bacterioides fragilis group
- Bacterioides spp.
- Finegoldia magna
- Peptostreptococcus spp.
- Prevotella spp.
- Fusobacterium spp.

Gram-positive bacteria

- Staphylococcus aureus
- Viridans streptococci (anginosus group)
- Enterococcus faecalis/faecium
- Coagulase-negative Staphylococci

The following groupings of isolates may be employed for frequency tables and listings according to the information available and observed frequencies:

- Carbapenem non-susceptible isolates
- Colistin non-susceptible isolates
- Multidrug-resistant isolates
- MBL-producing isolates
- ESBL-producing isolates

12.3 Planned tables, listing, graphics

See separate TFL document

12.4 Criteria for clinical significance of changes from baseline

See separate document

Approved by

Prof. PPD ,
Principal Investigator

PPD

PPD

Place and date   Signature

Dr. PPD
Statistician, CTCC

_____   _____
Place and date   Signature

PPD MSc
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