25 January 2024
EMA/63929/2024
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Exblifep

International non-proprietary name: cefepime / enmetazobactam

Procedure No. EMEA/H/C/005431/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

6-APA	6-aminopenicillanic acid
ADME	Absorption, distribution, metabolism, and excretion
ADR	Adverse drug reaction
AE	Adverse event
AESI	Adverse event of special interest
AET	Analytical evaluation threshold
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AP	Acute pyelonephritis
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area under the concentration-time curve
AUC_{0-8}	Area under the mean concentration-time curve from time 0 to 8 hours
AUC_{0-24h}	Area under the mean concentration-time curve from time 0 to 24 hours
AUC_{inf}	Area under the mean concentration-time curve from time 0 to infinity
AUC_{last}	Area under the mean concentration-time curve from dosing to the time of the last measured concentration
AUC_{tau}	Area under the mean concentration-time curve for the dosing interval
BID	bis in die (twice daily)
BL	Beta-lactam
BLI	Beta-lactam inhibitor
BLQ	Below the limit of quantification
BSE	Bovine Spongiform Encephalopathy
BUN	Blood urea nitrogen
CCS	Container closure system
CE	Clinically evaluable
CEP	Certificate of Suitability of the European Pharmacopoeia
CFU	Colony forming units
CHMP	Committee for Medicinal Products for Human use
CHO	Chinese hamster ovary
CI	Confidence interval
CL	Total plasma clearance
CLcr	Creatinine clearance
CL_{CRRT}	CRRT clearance
CLSI	Clinical and Laboratory Standards Institute
C_{max}	Maximum (peak) plasma drug concentration
CRO	Contract research organisation
CRRT	Continuous renal replacement therapy
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	Trough concentration
cUTI	Complicated urinary tract infections
CYP	Cytochrome P450 enzymes
DDI	Drug-drug interaction
DMET	Drug metabolising enzymes and transporter
DMF	N,N-dimethylformamide
DNA	Deoxyribonucleic acid

DSC	Differential Scanning Calorimetry
EC	European Commission
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
EDQM	European Directorate for the Quality of Medicines
EFD	Embryofoetal development
eGFR	Estimated glomerular filtration rate
ELF	Epithelial lining Ffuid
EMA	European Medicines Agency
EOT	End of treatment
ERA	Environmental risk assessment
ESBL	Extended spectrum beta-lactamases
ESRD	End-stage renal disease
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EURD	European reference date
fAUC	Area under the plasma concentration-time curve corrected for plasma protein binding
$fAUC_{24/MIC}$	Ratio of free delafloxacin area under the mean concentration-time curve from time 0-24 hours to pathogen minimum inhibitory concentration
FDA	US Food and Drug Administration
GC	Gas Chromatography
GCP	Good Clinical Practice
GPCR	G-Protein Coupled Receptors
GGT	Gamma-glutamyltransferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
h	hour/hours
HA	Influenza hemagglutinin
HAP	Hospital-acquired pneumonia
HDL	High-density lipoprotein
hERG	Human Ether-à-go-go-Related Gene
HPLC	High performance liquid chromatography
HPLC-MS/MS	High-performance liquid chromatography-tandem mass spectrometry
IBD	International birth date
IC	Ion chromatography
IC_{50}	Concentration giving 50% of the drug-induced inhibitory effect
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IR	Infrared
IRT	Interactive response technology
ITT	Intent-to-treat
IU	International Units
IV	Intravenous(ly)
KF	Karl Fischer titration
KPC	*K. pneumoniae* carbapenemases
LC-MS	Liquid chromatography-mass spectrometry
LFT	Liver function test
LFU	Late follow-up

LOQ	Limit of quantification
LSC	Lquid scintillation counting
MAA	Marketing authorisation application
MAD	Multiple ascending dose
MAH	Marketing authorisation holder
MDRD	Modification of Diet in Renal Disease
ME	Microbiologically evaluable
ME+R	Microbiologically evaluable with resistant isolates
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum inhibitory concentration
MIC_{90}	Concentration of drug required to inhibit growth of 90% of pathogens
m-MITT	Microbiological Modified Intent-to-Treat
m-MITT+R	Microbiological Modified Intent-to-Treat with resistant isolates
MITT	Modified Intent-to-treat
MHRD	Maximum recommended human dose
mRNA	messenger RNA
MS	Mass Spectrometry
MTD	Maximum tolerated dose
NAS	New active substance
NDBA	N-nitroso-di-n-butylamine
NDEA	N-nitroso-diethylamine
NDMA	N-nitroso-dimethylamine
NI	Non-inferiority
NLT	Not less than
NMBA	N-nitroso-N-methyl-4-aminobutyric acid
NOAEL	No observed adverse effect level
NOEL	No-observed-effect level
NMR	Nuclear Magnetic Resonance
NMT	Not more than
OOS	Out of specification
PAS	Periodic Acid-Schiff
PBPK	Physiologically-based pharmacokinetic
PBP	Penicillin-binding proteins
PD	Pharmacodynamic(s)
PDE	Permitted daily exposure
PDT	Pharmacodynamic targets
PE	Polyethylene
PEC	Predicted environmental concentration
Ph. Eur.	European Pharmacopoeia
PI	Product information
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic(s)
PL	Package Leaflet
PopPK	Population PK
PRAC	Pharmacovigilance Risk Assessment Committee
PTA	Probability of target attainment
PVC	Polyvinyl chloride
QTcB	Corrected QT interval using Bazett’s formula
QTcF	Corrected QT interval using Fridericia’s formula
REC	CHMP recommendation, EMA Post-Authorisation Measure (PAM)

RH	Relative Humidity
RI	Renal impairment
RMP	Risk management plan
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SAWP	Scientific Advice Working Party
SC	Subcutaneous(ly)
SD	Standard deviation
SHV	Sulfhydryl-variable
SmPC	Summary of Product Characteristics
SMQ	Standardised MedDRA Query
SOC	System organ class
SOE	Strength of evidence
TEAE	Treatment-emergent adverse event
TEM	Temoneira
TID	Tr in die (three times a day)
TK	Toxicokinetic(s)
TOC	Test of cure
TSE	Transmissible Spongiform Encephalopathy
ULN	Upper limit of normal
USP	United States Pharmacopoeia
USPI	United States Product Information
UTI	Urinary tract infections
UV	Ultraviolet
VAP	Ventilator-acquired pneumonia
VPC	Visual Predictive Check
V_{ss}	Volume of distribution at steady state
WFI	Water for injection
XRD	X-Ray Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Advanz Pharma Limited submitted on 11 November 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Exblifep, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 25 July 2019.

The applicant applied for the following indications:

Exblifep is indicated for the treatment of the following infections in adults (see section 5.1):

- Complicated urinary tract infections (including pyelonephritis)
- Hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)
- Treatment of patients with bacteraemia that occurs in association with or is suspected to be associated with any of the infections listed above.

Exblifep is also indicated for the treatment of infections due to aerobic Gram-negative organisms in adults with limited treatment options.

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

1.2. Legal basis and dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0047/2023 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0047/2023 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's requests for consideration

1.5.1. New active substance status

The applicant requested the active substance cefepime / enmetazobactam contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Scientific advice

The applicant received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
12 March 2018	EMA/CHMP/SAWP/415567/2018	*Serena Marchetti and Filip Josephson*
17 April 2019	EMA/CHMP/SAWP/338802/2019	*Filip Josephson and Mair Powell*

The Scientific advice EMA/CHMP/SAWP/415567/2018 pertained to the following non-clinical, and clinical aspects:

- Suitability of the animal PK/PD information and correlations to the dose-response relationship to support MAA; acceptability of the results of the completed PK/PD Phase 2 study in cUTI including the report of the PopPK analyses and quantitative models of drug-exposure-response (efficacy and tolerability) to support MAA.
- Acceptability of the plans for the dose selection large scale pivotal Phase 3 trial (including dosing adjustment in patients with mild and moderate impaired renal function), acceptability of the design of the Phase 3 study (*e.g.;* randomisation scheme, comparator drug, N.I. margin, in/exclusion criteria and efficacy/safety variables) and agreement that the planned single N.I. cUTI study will be sufficient and adequate for a MAA for cUTI indication.

The Scientific advice EMA/CHMP/SAWP/338802/2019 pertained to the following clinical aspects:

- Acceptability of the PopPK model to estimate the ELF/plasma AUC ratios.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Filip Josephson Co-Rapporteur: Maria Concepcion Prieto Yerro

It was noted that the appointed CHMP Rapporteur was SAWP coordinator for EMA/SA/SAWP/415567/2018 and EMA/CHMP/SAWP/338802/2019 for Exblifep. This was exceptionally considered acceptable, considering the strong expertise in antibiotics required for the assessment, and the fact that the appointed CHMP Co-Rapporteur was not involved in scientific advice activities for the product.

The application was received by the EMA on	11 November 2022
The procedure started on	26 January 2023

The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	17 April 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	26 April 2023
The CHMP Co-Rapporteur's critique was circulated to all CHMP and PRAC members on	03 May 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 May 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	08 September 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	16 October 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	26 October 2023
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	31 October 2023
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	9 November 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	19 December 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	19 January 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Exblifep on	25 January 2024
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	25 January 2024

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease epidemiology

Complicated urinary tract infections (cUTI), including pyelonephritis, and hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP), are common infection types that sometimes are associated with bacteraemia. Acute pyelonephritis (AP) may result from an ascending uncontrolled

bladder infection or may be haematogenous, while cUTI is usually associated with anatomical abnormalities or foreign bodies placed in the tract, such as catheters and renal stents. HAP/VAP is a major resource-consuming problem especially associated with patients who have had a complication of an underlying illness or medical intervention. Mortality rates are commonly at least 20%. In each case the severity of the underlying disease and inappropriate antimicrobial therapy, due in part to increased antimicrobial resistance, significantly contribute to the mortality rates.

The applicant has in addition proposed an indication of treatment of infections due to aerobic Gram-negative organism in adults with limited treatment options. According to CHMP guidance (CPMP/EWP/558/95 Rev 3), pathogen-specific indications in patients with limited treatment options may be considered appropriate for antibacterial products expected to be clinically active against target organisms that are resistant to most or all of the relevant licensed antibacterial agents. The current position of the CHMP is that beta-lactams (BL) or BLs combined with beta-lactamase inhibitors (BLI) addressing carbapenem-resistant pathogens may be eligible for such an indication in that the remaining treatment options are very few and, in some cases, limited to antibacterials with less favourable safety profiles. The therapeutic utility of BLs against gram-negative pathogens is also threatened by increased dissemination of extended spectrum beta-lactamases (ESBL), which confer resistance to most BLs, including 3rd generation (ceftriaxone, ceftazidime, cefotaxime) and 4th-generation cephalosporins (cefepime), but not against carbapenems. New antibacterial agents addressing ESBL-producing pathogens are valuable in the armamentarium although they are not considered eligible for a pathogen-specific indication in patients with limited treatment options.

2.1.2. Aetiology and pathogenesis

Complicated UTIs are UTIs complicated by involvement of the upper urinary tract (pyelonephritis) or by underlying functional or anatomic abnormalities of the urinary tract. Common uropathogens causing cUTI are *E. coli*, other Enterobacterales and *P. aeruginosa*.

HAP and VAP are infections in hospitalised (or recently hospitalised) patients. Colonisation of the respiratory tract with a variety of Gram-positive and Gram-negative bacteria may lead to infection. Among the most encountered pathogens in HAP/VAP are *Staphylococcus aureus*, Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

2.1.3. Clinical presentation, diagnosis

Infection types included in the proposed indication are diagnosed based on clinical presentations and radiologic imaging in addition to microbiologic investigations to characterise the pathogens causing the infections.

2.1.4. Management

Treatment of cUTI/acute pyelonephritis and HAP/VAP is in general initially empirically chosen based on the knowledge of the main causative pathogens and their likelihood of carrying resistance mechanisms. The selection of antibacterial agent(s) is further guided by results of pathogen identification and susceptibility testing.

Beta-lactam antibacterial agents including 3rd- and 4th-generation cephalosporins are commonly used as empirical treatment. However, treatment has become increasingly more challenging because of the rising rates of antimicrobial resistance among pathogens causing these infections including pathogens harbouring ESBLs. Treatment options for infections caused by ESBL-producing pathogens generally

require the use of carbapenems. Treatment options for infections caused by carbapenemase-producing pathogens are even more limited although there have been some additions to the treatment armamentarium during the recent years including new BL/BLI combinations such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam and cefiderocol. Despite this, there is still a need for additional antibacterial agents addressing infections caused by resistant pathogens including resistance to cephalosporins and carbapenems.

2.2. About the product

Cefepime is a 4th-generation cephalosporin beta-lactam antibacterial agent that inhibits bacterial cell-wall synthesis by targeting penicillin-binding proteins (PBPs).

Enmetazobactam is a novel penicillanic acid sulfone beta-lactamase inhibitor (BLI), structurally related to tazobactam, with inhibitory activity against certain beta-lactamases which prevents the hydrolysis of cefepime.

The applicant has applied for the following indications:

Exblifep is indicated for the treatment of the following infections in adults (see section 5.1):

- *Complicated urinary tract infections (including pyelonephritis)*
- *Hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)*
- *Treatment of patients with bacteraemia that occurs in association with, or is suspected to be*
- *associated with, any of the infections listed above*

Exblifep is also indicated for the treatment of infections due to aerobic Gram-negative organisms in adults with limited treatment options

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

The proposed dose is 2 g/0.5 g cefepime/enmetazobactam every 8 hours for a treatment duration of 7 to 10 days (up to 14 days in patients with bacteraemia). Dose adjustments are proposed in patients with various degree of renal impairment.

2.3. Quality aspects

2.3.1. Introduction

The finished product is presented as powder for concentrate for solution for infusion containing 2 g of cefepime (as dihydrochloride monohydrate) and 0.5 g of enmetazobactam.

The other ingredient is: L-arginine.

The product is available in a 20 mL vial (Type I clear glass) with stopper (bromobutyl rubber) and flip-off seal.

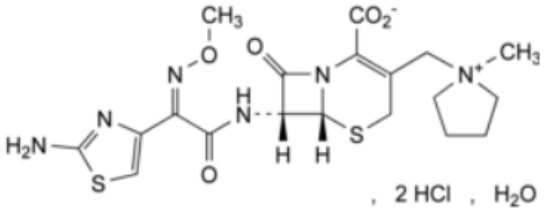
2.3.2. Active substance (cefepime dihydrochloride monohydrate, sterile)

2.3.2.1. General information

The chemical name of cefepime dihydrochloride monohydrate is (6*R*,7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-

azabicyclo[4.2.0]oct-2-ene-2-carboxylate dihydrochloride monohydrate corresponding to the molecular formula $C_{19}H_{26}Cl_2N_6O_5S_2$. It has a relative molecular mass of 571.5 g/mol and the following structure:

Figure 1: Cefepime dihydrochloride monohydrate structure



Cefepime dihydrochloride monohydrate is a hygroscopic white or almost white crystalline powder, with a pK_a-value of 1.5-1.6 and 3.1-3.2. Polymorphism and particle size have not been investigated since they are not considered important characteristics for the finished product, as the active substance is administered following complete dissolution (cefepime dihydrochloride monohydrate is freely soluble in water) by intravenous infusion. Enantiomeric purity is controlled routinely at active substance level by specific optical rotation.

As there is a monograph of cefepime dihydrochloride monohydrate in the *European Pharmacopoeia* (*Ph. Eur.*), the manufacturer of the active substance has been granted a Certificate of Suitability of the *European Pharmacopoeia* (CEP) for cefepime dihydrochloride monohydrate active substance which has been provided within the current Marketing Authorisation Application (MAA).

2.3.2.2. Manufacture, characterisation and process controls

The relevant information regarding characterisation of the active substance and impurities, manufacture, process controls, control of materials, control of critical steps and intermediates, process validation and manufacturing process development has been assessed by the European Directorate for the Quality of Medicines (EDQM) before issuing the CEP. The active substance is sterilised by sterile filtration. The sterilisation process has been assessed and approved by EDQM. In addition, data from the validation of sterilising filters and media fill simulations for the aseptic part of the manufacturing process are presented. The provided information is considered acceptable.

The container closure system (CCS) used for the cefepime dihydrochloride monohydrate active substance is either a sterile anodised aluminium tin with a chlorobutyl stopper and an aluminium tear-off cap or a sterile anodised aluminium tin with a bromobutyl rim, an aluminium cover, and an aluminium tear-off cap. The information presented regarding the packaging material is in accordance with the CEP and is considered acceptable.

2.3.2.3. Specification

The active substance is controlled in accordance with the *Ph. Eur.* monograph for cefepime dihydrochloride monohydrate and additional tests for residual solvents (GC - gas chromatography) and sterility (*Ph. Eur.*) are included.

The active substance is controlled using the methods described in the relevant Ph. Eur. monograph. The in-house method for the additional test residual solvents by GC is provided in an annex of the CEP. The test for sterility is performed according to the current Ph. Eur. 2.6.1. monograph. The information

has been assessed by the EDQM and found to be suitable. The reference standards used by the finished product manufacturer are also considered acceptable.

Batch analysis data are presented for three commercial scale batches of cefepime dihydrochloride monohydrate. The batch analysis results comply with the active substance specification and are consistent from batch to batch.

In conclusion, the specification applied by the applicant for the cefepime dihydrochloride monohydrate active substance is considered adequate.

2.3.2.4. Stability

Stability data from twelve commercial scale batches of cefepime dihydrochloride monohydrate active substance, stored in both intended commercial packages, for up to 36 months under long term conditions (25 °C / 60% RH) and up to 6 months under accelerated conditions (40 °C / 75% RH) according to the ICH guidelines were provided.

The parameters addressed in the stability studies were appearance, appearance of solution (clarity and colour), Impurity G, assay, related substances (Impurity A, Impurity B, Impurity E, Impurity F, max unspecified impurity and total impurities), water content, bacterial endotoxins and sterility.

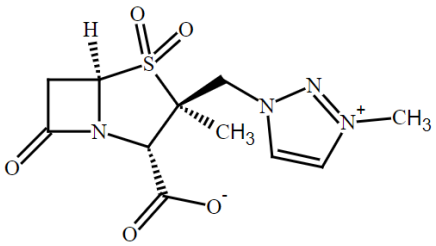
The stability results indicate that the cefepime dihydrochloride monohydrate active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed re-test period of 36 months when stored in the commercial packaging, without any special storage conditions.

2.3.3. Active Substance (enmetazobactam, sterile)

2.3.3.1. General Information

The chemical name of enmetazobactam is (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1H-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide corresponding to the molecular formula $C_{11}H_{14}N_4O_5S$. It has a relative molecular mass of 314.318 g/mol and the following structure:

Figure 2: Enmetazobactam structure



The chemical structure of enmetazobactam was elucidated by a combination of the following techniques: elemental analysis, ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).

The solid-state properties were measured by X-ray diffraction (XRD) and thermal analysis (DSC and TGA). Enmetazobactam is a deliquescent white to off-white crystalline powder, with a pK_a-value of

2.37. The XRD spectrums of three enmetazobactam batches show that the active substance exhibits polymorphism and the manufacturing process ensures that the desired polymorphic form is produced consistently. However, polymorphism and particle size are not considered important characteristics for the finished product, as the active substance is administered following complete dissolution (enmetazobactam is freely soluble in water) by intravenous infusion.

Enmetazobactam has a 2*S*, 3*S*, 5*R* configuration that is kept unchanged starting with the tazobactam intermediate step. Enantiometric purity is controlled routinely by specific optical rotation at the level of the active substance. No other stereoisomers were found in enmetazobactam batches during testing.

A forced degradation study has been performed and the results provided show that the active substance is sensitive to acid, alkali, oxidation, high temperature (60 °C for 5 days) and high temperature hydrolysis. Sulfinic acid and methyl triazole are the main impurities identified.

2.3.3.2. Manufacture, characterisation and process controls

Detailed information on the manufacturing of the enmetazobactam active substance has been provided in the dossier and is considered satisfactory. The proposed synthesis of enmetazobactam includes the synthetic process for an intermediate compound (tazobactam acid), at a different manufacturer, starting from the commercially available compound 6-aminopenicillanic acid (6-APA). The synthesis is continued at the active substance manufacturer from the intermediate, using a 3 steps process involving methylation of tazobactam acid and aseptic isolation of sterile enmetazobactam.

The proposed starting material and its specification are acceptable. The manufacturing process from 6-APA via tazobactam acid to enmetazobactam is sufficiently described. The control of reagents, solvents and auxiliary materials used in the synthesis of the enmetazobactam is considered acceptable. Critical process parameters and critical in-process controls in the manufacture of enmetazobactam are discussed and justified. The definition and control of critical steps and intermediates is endorsed. The description of the manufacturing process development is considered comprehensive and adequate. No rework is performed in the manufacturing of tazobactam acid intermediate and enmetazobactam active substance.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Details of the changes in manufacturing process used for the clinical batches versus the proposed manufacturing process are provided in the dossier. It has been demonstrated that the changes did not have a significant impact on the quality of the product.

The process validation for the production of sterile enmetazobactam, including filter validation, sterile filtration and media fills is considered adequate. The justification for the choice of sterilisation method is considered acceptable.

The characterisation of enmetazobactam active substance and its impurities is in accordance with the EU guideline on chemistry of new active substances. The formation, control and qualification of related substances are well discussed in the impurities section of the dossier. Impurities with potential genotoxicity has been investigated with two complementary (Q)SAR prediction methodologies, one expert rule-based (DEREK) and one statistically based (Leadscope Model Applier), and no risk has been identified. This is considered sufficient.

The information presented regarding residual solvents (controlled in accordance with ICH Q3C), reagents and catalysts potentially present in enmetazobactam active substance is considered sufficient.

The information presented regarding elemental impurities is considered acceptable and all relevant elements are below their respective Option 1 limits, in line with ICH Q3D. A test for elemental impurities in the enmetazobactam active substance is therefore not considered necessary.

A nitrosamine risk assessment report is included in the dossier. Relevant route causes including environment, equipment, starting material (6-APA), packing material, reagents, solvents and auxiliary materials, recovered solvent in the production of tazobactam, water, disinfectant and cleaning agents, human impact, reaction conditions are considered in the risk assessment. A risk of *N*-nitroso-dimethylamine (NDMA) and *N*-nitroso-di-n-butylamine (NDBA) formation has been identified by the applicant since nitrite, azide, tetrabutylammonium bromide and *N*,*N*-dimethylformamide (DMF) are used in the synthesis of tazobactam acid and DMF in the synthesis of enmetazobactam. Confirmatory testing of potential *N*- nitrosamine impurities has been performed on twenty-five tazobactam acid intermediate batches and three enmetazobactam commercial batches, showing NDMA and NDBA levels below 10 % of the Acceptable Intake (AI) limit. Based on the outcome of the risk assessment, it is concluded that nitrosamine testing at the level of enmetazobactam active substance is not deemed necessary.

The enmetazobactam active substance is packaged inside sterile anodised aluminium tins with a bromobutyl rubber rim, an aluminium cover and an aluminium tear-off cap. The CCS materials comply with Commission Regulation (EU) 10/2011, as amended.

2.3.3.3. Specification

The enmetazobactam active substance specification contains tests for appearance, identification (IR, HPLC - High performance liquid chromatography), specific rotation (Ph. Eur.), visible and sub-visible particles (Ph. Eur.), clarity of solution (Ph. Eur.), pH (Ph. Eur.), water content (Ph. Eur.), sulfated ash (Ph. Eur.), assay and related substances (HPLC), residual solvents (GC), triflate ion (IC – ion chromatography), DMF (GC), 2-ethylhexanoic acid (GC), bacterial endotoxins (Ph. Eur.) and sterility (Ph. Eur).

The proposed enmetazobactam active substance specification includes relevant parameters. The specification was established taking into account applicable ICH and EU guidelines and compendial considerations, as well as manufacturing capability, batch analysis data and stability results. Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set. The control strategy for residual solvents and for the elemental impurities has been detailed in the characterisation of the active substance section and the applied limits are in line with ICH Q3C and ICH Q3D, respectively.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data for three commercial scale enmetazobactam batches are provided. The results are within the specifications and demonstrate batch to batch consistency.

In conclusion, the enmetazobactam active substance specification is considered adequate.

2.3.3.4. Stability

Stability study data are presented for three commercial scale enmetazobactam batches, stored in the proposed commercial package, for up to 36 months under long-term storage conditions (-20 °C ± 5 °C and 5 °C ± 3 °C) and for up to 6 months under accelerated conditions (25 °C ± 2 °C/60 ± 5% RH).

Photostability stability studies in accordance with ICH Q1B have been performed and data from forced degradation studies under various stress conditions (thermal, acidic, alkaline and oxidative) are also presented.

The following parameters were tested: appearance, water content, specific rotation, pH, assay, related substances, sterility and bacterial endotoxins, with the same specification limits and analysis methods used as for release testing.

The stability results under stress conditions show that the enmetazobactam is relatively stable when exposed to light irradiation and high temperature (40 °C for 10 days), but is unstable when exposed to acid, alkali, oxidation, high temperature (60 °C for 5 days) and high temperature hydrolysis.

The results under long-term and accelerate conditions were within specifications, indicating that enmetazobactam is stable under frozen (-20 °C ± 5 °C) and refrigerated (5 °C ± 3 °C) conditions for up to 36 months. The proposed re-test period of 36 months when stored under refrigerated conditions in the commercial packaging is therefore considered acceptable.

A post-approval commitment to continue the long-term stability study up to 48 months at frozen and refrigerated conditions and to inform the relevant health authorities in the event of an out of specification result has been included in the dossier and is considered acceptable.

2.3.4. Finished medicinal product

2.3.4.1. Description of the product and Pharmaceutical Development

The finished product is a powder for concentrate for solution for infusion consisting of sterile cefepime dihydrochloride monohydrate blended with sterile L-arginine, in combination with sterile enmetazobactam, stored in 20 mL Type I glass vials. Each vial contains 2000 mg of cefepime in combination with 500 mg of enmetazobactam, which is intended to be reconstituted and further diluted with either 0.9% sodium chloride or dextrose 5%, or a sodium chloride/dextrose combination containing 4.5% sodium chloride and 2.5% dextrose.

The aim of the development of the finished product was to obtain a fixed combination of the 4th-generation cephalosporin cefepime (as dihydrochloride monohydrate) and the new β-Lactamase inhibitor enmetazobactam, in a single unit dose vial.

The only excipient used for the preparation of the finished product is sterile L-arginine which complies with its Ph. Eur. monograph. There are no novel or animal/human-derived excipients used in the finished product formulation. Manufacturing is carried out under a protective nitrogen atmosphere.

The two sterile components (cefepime dihydrochloride monohydrate/arginine blend and enmetazobactam) are sequentially and aseptically filled into a single vial to improve finished product storage and handling, to avoid preparation of two separate reconstitution solutions and to avoid dosing errors. Flowability, hygroscopicity and stability are amongst the main cefepime dihydrochloride monohydrate and enmetazobactam attributes that were considered during the process development. The compatibility of the two active substances with each other and L-arginine in the final powder has been demonstrated in the ICH stability study.

During the clinical studies, separate vials of sterile cefepime for injection and sterile enmetazobactam powders were used. The two compounds were reconstituted and mixed into a single infusion bag prior to intravenous infusion. This was necessary since the optimal ratio of the 2 active substances was still under investigation. The quantitative compositions after reconstitution and dilution of the Phase 3 product and the proposed commercial product are identical. The mode of administration in the clinical

studies was to precisely mimic the administration of the two actives substances mixed in a single vial. This approach is endorsed and does not raise any concerns.

An overfill is used during filling of vials to account for handling losses during reconstitution and dilution of the finished product. The overfill amount was adequately justified.

Both cefepime dihydrochloride monohydrate and enmetazobactam are supplied as sterile active substances. Concerning the excipient L-arginine, this is sourced as non-sterile L-arginine. A Major Objection was raised as sterilisation of L-arginine by filtration had not been adequately justified. The applicant provided further justification and the Major Objection was considered resolved.

The finished product CCS is a 20 ml Type I glass vial closed with a butyl rubber stopper coated with PET film and an aluminium-plastic cap. All the CCS components are compliant with Ph. Eur./European Commission (EC) requirements and are suitable for use in food and medicines packaging. The CCS used for the finished product is sufficiently described and the information presented is acceptable.

The compatibility of the CCS with the finished product has been investigated in the long-term and accelerated ICH stability studies and in a separate extractables study for the rubber stopper, demonstrating that the CCS is adequate for the intended use of the product. Nine organic impurities were detected in the extractables study of the stopper, conducted in buffers pH 2.5 and pH 9.5, isopropanol/water and dichloromethane, at levels well below the analytical evaluation threshold (AET) and do not give raise to any concerns.

2.3.4.2. Manufacture of the product and process controls

The finished product is manufactured, filled, packaged, inspected and tested in accordance with Good Manufacturing Practice (GMP). The process is considered to be a non-standard manufacturing process and consists of three steps:

1. Production of sterile L-arginine from non-sterile L-arginine by dissolving non-sterile L-arginine in water for injection (WFI), sterile filtration, crystallisation, washing with water, drying, homogenising and filling into the sterilised aluminium tins.
2. Production of a sterile blend of cefepime and L-arginine (intermediate) by blending of sterile cefepime and sterile L-arginine, followed by filling into sterile aluminium tins.
3. Production of cefepime-enmetazobactam powder for concentrate for solution for infusion by sequential filling of cefepime-arginine blend and enmetazobactam under nitrogen atmosphere into washed and sterilised vials closed with washed/depyrogenised and sterilised rubber stoppers.

The description of the finished product manufacturing process is considered adequate and covers flowcharts, amounts charged, manufacturing parameters, sterilisation by filtration, holding times, yield, sterilisation of nitrogen and information regarding the conditions and validation data for the sterilisation of the packaging materials. The maximum holding times during and after manufacture of sterile L-arginine, sterile cefepime with L-arginine blend and finished product are stated in the dossier. Stability data supporting the claimed holding times are presented. The start of shelf-life is defined as the manufacturing date for preparation of sterile cefepime with L-arginine bulk powder.

The in-process controls are adequate for this type of manufacturing process and include testing of vials and stoppers for visible particles, 100% testing of fill weight during filling, uniformity of filling mass after capping, residual oxygen, capping quality during capping, leak test during capping, clarity of solution and visible particles, capping quality of semi-finished product. The filled vials are capped, cleaned and dried and subjected to a 100% visual inspection for defects. Vials are sampled for leak test at different stages of production.

Non-sterile arginine is tested for microbial content before sterilisation in accordance with Ph. Eur. and L-arginine is tested for sterility and endotoxins by sufficiently described and validated methods. The endotoxin limit proposed for L-arginine is sufficiently justified.

The specifications for the sterile cefepime with arginine intermediate comply with the USP monograph for cefepime injection and the test of residual solvents complies with ICH Q3C. The specifications for the intermediate are considered acceptable. The analytical methods used in the intermediate specification are considered sufficiently described and validated. Satisfactory batch analysis data for three batches of intermediate complying with the proposed specifications are presented. The stability results for seven intermediate batches stored at 25 °C/60% RH for up 36 months and six intermediate batches stored at 40 °C / 75% RH for up to 6 months support the proposed holding time of 36 months when stored in a well closed container, at a temperature below 25 °C.

Two batch size formulas are described in the dossier.

The manufacturing process has been validated by a number of studies and process validation results are presented for the manufacture of sterile L-arginine and sterile cefepime/L-arginine blend, and for the manufacture of sterile cefepime-enmetazobactam finished product, respectively.

The process validation of the manufacture of sterile L-arginine and sterile cefepime/L-arginine blend comprises of validation of sterile filtration, blending validation for three batches of intermediate and process simulation. The process validation of the manufacture of sterile cefepime/L-arginine-enmetazobactam finished product comprises validation of the aseptic filling of three finished product batches into vials, the capping process, the washing of vial external surface, the vial inspection for defects, the packaging process, process simulation and container closure integrity testing.

Initially, the applicant only provided full-scale process validation data for the smaller scale batch size. Due to the complex nature of the manufacturing process, extrapolation of process validation data from the smaller scale batch size to account for the larger scale batch size could not be accepted. Therefore, a Major Objection was raised during the assessment to ask for full-scale process validation for the larger scale batch size. In response, the applicant provided the requested information, adequately addressing the Major Objection.

In conclusion, the manufacturing process of the sterile cefepime-enmetazobactam finished product is considered adequately validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

2.3.4.3. Product specification

The finished product release and shelf-life specifications include appropriate tests for this type of product: identification of the active substances (HPLC), appearance (visual), colour and clarity of reconstituted solution (Ph. Eur.), completeness and clarity of reconstituted solution (Ph. Eur.), reconstitution time (in-house method), visible particulate matter (Ph. Eur.), pH (Ph. Eur.), water content (Karl Fischer, KF/Ph. Eur.), sub-visible particles (Ph. Eur.), uniformity of dosage units (Ph. Eur.), assay of the active substances (HPLC), related substances and total impurities (HPLC), N-metylpyrrolidine content (IC), bacterial endotoxins (Ph. Eur.) and sterility (Ph. Eur.). Particle size distribution is not included in the specifications, since this parameter is not considered important for a product intended to be further dissolved and diluted prior to intravenous infusion.

The proposed specification tests are in line with ICH Q6A and Ph. Eur. requirements. The parameters included in the finished product specification are found adequate to control the quality of the finished product at release and during shelf-life. The proposed limits (including those applicable to specified, unspecified and total impurities) have been adequately justified and are considered appropriate. All

known impurities are considered process impurities originating from the active substances and no additional degradation products have been identified in the finished product.

Water is the only solvent used in the manufacturing process of the finished product. Both active substances are tested for residual solvents and the results comply with the requirements of ICH Q3C Option 1. Therefore, no test for residual solvents is necessary to be added to the finished product specification.

The risk assessments presented regarding elemental impurities are considered acceptable and the levels observed in finished product batches are consistently below 30% of the applicable Permitted Daily Exposure (PDE). Therefore, in accordance with ICH Q3D, routine control of elemental impurities in the finished product specification is not necessary.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report - Procedure under Article 5(3) of Regulation EC (No) 726/2004 - Nitrosamine impurities in human medicinal products" (EMA/369136/2020). The analysis results presented by the applicant for the potential impurities NDMA, *N*-nitroso-diethylamine (NDEA), NDBA and *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA) showed that none of them could be detected, which indicate that these *N*-nitrosamines are present at a level below 10% of their respective AI limit. It is also noted that the Limit of Quantitation presented for each of these *N*-nitrosamines is below 10% of their respective AI limit. Based on the information provided, the results show that the theoretical maximum levels of these potential *N*-nitrosamine impurities would constitute a negligible toxicological risk and that no routine tests for nitrosamine impurities is necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are presented for three batches of finished product manufactured at each of the proposed scales. The batches were tested in accordance with the proposed finished product specification. The results confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

2.3.4.4. Stability of the product

Stability data from three smaller scale batches of finished product stored for up to 24 months under long-term conditions (5°C ± 3°C and25°C/60% RH) and for up to 6 months under accelerated conditions (40°C/75% RH), in both upright and inverted position, and for three larger scale batches of finished product stored for up to 12 months under long-term conditions and for up to 6 months under accelerated conditions, in inverted position, according to the ICH guidelines, were provided. The stability samples were tested for all tests included in the finished product release specification, except for identification and uniformity of dosage units. The batches are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

All parameters evaluated complied with the specifications during storage under long-term conditions after 24 months for smaller scale batches and 12 months for larger scale batches. However, until more extensive data will be generated on the larger scale batches, the applicant will store the finished product at 5 °C ± 3°C, as a precautionary measure. All parameters evaluated complied with the specifications after 6 months of storage under accelerated conditions for both batch sizes of finished

product, with the exception of methyl triazole impurity for which out-of-specification (OOS) results were observed. In addition, OOS results were observed for any individual unspecified impurity for the smaller scale batches, however an increase up to the specification limit was noted also for the larger scale batches. There were no significant differences in results between vials stored in upright or inverted positions.

A photostability study in accordance with ICH 1B has also been conducted on one batch manufactured at the smaller scale stored in the proposed primary and secondary commercial packaging. The results for non-stressed samples were identical to the results for light-stressed samples packaged in the proposed primary and secondary packaging. However, the light-stressed samples packaged only in the proposed primary package showed an increase of cefepime related compound A and total impurities and a concomitant decrease of cefepime assay. The results indicate that the finished product in the primary package needs to be protected from light by being kept in the outer carton.

The stability of the finished product after reconstitution and dilution using three diluents (NaCl 0.9% solution for injection, dextrose 5% solution for injection, and NaCl 0.45% and dextrose 2.5% solution for injection) was also investigated during storage for up to 6 hours at 5 °C ± 3 °C followed by 2 hours at controlled room temperature. Throughout the in-use stability study, all parameters evaluated complied with the specifications.

An in-use compatibility study using standard polypropylene (PE) bags and polyvinyl chloride (PVC) tubing was conducted for the diluted finished product. During this study, no change in any investigated parameter was observed. The applicant's conclusion that the composition of the bags and tubes is not critical for the quality of the reconstituted finished product is therefore endorsed.

A comparison of the in-use stability of the finished product and the cefepime/enmetazobactam combination originating from two separate vials (as used in the clinical program) was conducted, both products being diluted in NaCl 0.9% and held for 2 hours at controlled room temperature. The results were within the proposed release specification limits and were comparable for the diluted commercial product and the diluted clinical trial product.

A freeze-thaw study of one finished product batch was undertaken by storing the samples at -20 °C ± 5 °C for 2 days and then in a 50 °C oven for an additional 2 days. The 4 day exposure represented one study cycle. The study continued for 3 consecutive cycles. The results indicate that the finished product can withstand freeze-thaw cycling which may occur e.g. during transport.

In conclusion, the stability data presented for the finished product support the proposed shelf-life of 24 months for the finished product when stored in the commercial packaging material at 5 °C ± 3 °C and when applying the special storage condition "Keep the vial in the outer carton in order to protect from light".

Chemical and physical in-use stability has been demonstrated for 6 hours at 5 °C ± 3 °C followed by 2 hours at 25 °C. From a microbiological point of view, unless the method of opening/reconstitution/dilution precludes the risk of microbial contamination, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user.

2.3.4.5. Adventitious agents

None of the ingredients are of human or animal origin. According to the CEP, no material of human or animal origin is used in the manufacture of cefepime dihydrochloride monohydrate. Bovine Spongiform Encephalopathy/Transmissible Spongiform Encephalopathy (BSE/TSE) statements are presented for tazobactam, L-arginine and enmetazobactam.

In conclusion, the information presented regarding the TSE/BSE risk associated with the manufacture of the finished product is considered acceptable.

2.3.5. Discussion and conclusions on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During the procedure, two Major Objections with regards to quality aspects were raised, concerning (1) inadequate justification for the selection of sterile filtration technique for L-arginine sterilisation and (2) lack of process validation data for the maximum finished product batch size. The Major Objections, as well as all the other concerns, have been satisfactorily resolved.

2.3.6. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.3.7. Recommendations for future quality development

Not applicable.

2.4. Non-clinical aspects

2.4.1. Introduction

The non-clinical program consisted of primary and secondary, and a core battery of standard pharmacology studies, pharmacokinetics and ADME studies, and toxicology studies with enmetazobactam or cefepime alone or with cefepime and enmetazobactam in combination. The primary pharmacology and the pharmacodynamic drug interaction studies are assessed in the clinical section. No safety pharmacology or toxicology studies were done with cefepime alone as the applicant relies on EMA's and FDA´s previous determination of the safety of cefepime at the same dose and regimen proposed for approval in combination with enmetazobactam (Maxipime USPI; Renapime SmPC; NDA 4601712 1996). All pivotal studies were done in accordance with relevant guidelines (i.e., ICH M3).

2.4.2. Pharmacology

2.4.2.1. Primary pharmacodynamic studies

The non-clinical primary pharmacodynamic studies are assessed in the clinical section.

2.4.2.2. Secondary pharmacodynamic studies

The secondary pharmacodynamics of enmetazobactam alone was tested in one study by applying Fluorescence Imaging Plate Reader assays with enmetazobactam at 10 or 12.5 µM on relevant G-Protein Coupled Receptors (GPCRs), cardiac ion channels, and kinases and phosphatases. No activity was recorded when tested on 76 GPCRs, 8 cardiac ion channels, and 36 kinases and phosphatases. The tested concentrations of enmetazobactam does not cover the clinical C_{max} exposure at ~63 µM (free fraction). However, this study was conducted before the clinical exposure was established.

2.4.2.3. Safety pharmacology programme

The impact of enmetazobactam on vital functions has been evaluated in the core battery of safety pharmacology investigations in adherence to prevailing ICH S7A and B guidelines (CPMP/ICH/539/00; CPMP/ICH/423/02).

The hERG study revealed an IC_{50} -value of 1,254.22 µg/mL, corresponding to 3,990.30 µM, for enmetazobactam which translates into a 63-fold safety margin compared to the C_{max} level of 19.8 µg/ml at the MRHD. Thereby, the potential risk for QT prolongation in humans is low.

No effects on the cardiovascular in dogs dosed up to 240 mg/kg/day or on respiratory and nervous system in rats dosed up to 500 mg/kg/day were recorded. The only notable findings where a non-dose dependent decrease of systolic pressure was recorded in dogs. This alteration in systolic pressure was within normal ranges for beagle dogs at the test facility and regarded as incidental which is agreed. Further, a statistical but minimal decreased respiratory rate was seen in the mid and high groups at 0.5 and 1 h post-dose. The decrease was seen also at pre-dose and were generalised across all groups although slightly lower but statistically significant in mid and high dose group. Anyway, the decrease was minimal and is regarded as non-adverse effects.

The NOAELs was set to 240 mg/kg/day in dogs and 500 mg/kg/day in rats. At those doses toxicokinetic analysis revealed a corresponding gender mean C_{max} of about 865 µg/ml in dogs and 1345 µg/ml in rat that is approximately 44-fold and 68-fold, respectively, above the C_{max} at the human peak plasma levels at the MRHD (0.5 g enmetazobactam and 2 g cefepime TID).

2.4.2.4. Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies are assessed in the clinical section.

2.4.3. Pharmacokinetics

Studies have been performed to characterise the absorption, distribution, metabolism, and excretion (ADME) of enmetazobactam, using the intended clinical route of administration (IV infusion), and the species selected for non-clinical safety testing, e.g., Wistar Han rats and Beagle dogs as the main non-clinical species but also New Zealand White rabbits and Spraque Dawley rats. In addition, the PK/TK profiles of enmetazobactam when administered in combination with cefepime and of cefepime when administered alone and in combination with enmetazobactam were studied in rats and dogs.

Methods of analysis

Enmetazobactam was quantified by LC-MS/MS in blood or plasma from rats, dogs and rabbits and cefepime was quantified by LC-MS/MS in plasma of rats and dogs. The methods used in pivotal toxicity studies in rats, dogs and rabbits were validated in accordance with the guideline on bioanalytical method validation and the principles of GLP. All TK analyses of enmetazobactam in the systemic

circulation were performed on plasma after stabilisation with the esterase inhibitor pefabloc except for the TK analysis in the pivotal repeated dose toxicity of enmetazobactam in rats which was performed using blood. Radioactivity in blood, plasma, urine, faeces, and tissues from ADME-studies of [^{14}C]-enmetazobactam in rats was assessed by liquid scintillation counting (LSC), quantitative whole-body autoradioluminography and/or profiling by LC-^{14}C-HRMS/MS. Enmetazobactam in faeces and urine from rats and dogs was quantified by a HPLC-MS/MS method.

Plasma kinetics

Enmetazobactam

Single dose pharmacokinetics

Single dose pharmacokinetics following IV administration of enmetazobactam alone were determined from blood in rats and from plasma in dogs in pivotal general toxicity studies. Calculated PK variables following administration of 125 to 500 mg/kg to rats and 60 to 240 mg/kg to dogs indicated a distribution comparable to body water (mean V_{ss}: 0.64 to 1.48 L/kg in rats, 0.32 to 0.41 L/kg in dogs) and a rapid clearance from blood/plasma equal to or higher than the glomerular filtration rate (mean CL: 17.9 to 32.4 ml/min/kg in rats, 4.24 to 5.02 ml/min/kg in dogs) resulting in a short elimination half-life (mean t½: 0.89 to 2.16 h in rats, 0.93 to 4.38 h in dogs). Concentrations of enmetazobactam were quantifiable during ≤ 8 hours in blood of rats and in plasma of dogs. The exposure in terms of AUC_{last} (i.e $AUC_{0\text{-}8\ h}$ or less) increased with increasing dose at the studied dose range, generally in an approximately dose proportional manner in both rats and dogs, although somewhat less than dose proportional between 125 and 250 mg/kg in male rats. No important (>2-fold) gender difference was observed for any of the species.

Single dose pharmacokinetics in plasma following IV administration of enmetazobactam together with cefepime were determined from pivotal combination toxicity studies in rats and dogs. Calculated PK variables following administration of enmetazobactam/cefepime at doses of 125/250 and 250/500 mg/kg to rats and 50/100 and 200/400 mg/kg to dogs, showed that coadministration did not affect the single dose TK parameters for enmetazobactam in dogs. In rats, the estimated values for CL and t½ were however lower and longer, respectively, and the AUC_{last} was higher when enmetazobactam was given together with cefepime (for AUC_{last} see Toxicology section).

Repeated dose pharmacokinetics

Steady state toxicokinetics of enmetazobactam following IV administration of enmetazobactam alone were determined from blood in rats and from plasma in dogs in pivotal general toxicity studies. Calculated PK variables on Day 28 following once daily administration of 125 to 500 mg/kg to rats and 60 to 240 mg/kg to dogs (V_{ss}: 1.41 to 4.44 L/kg in rats, 0.37 to 0.49 L/kg in dogs, CL: 29.2 to 70.0 ml/min/kg in rats, 4.35 to 4.88 ml/min/kg in dogs, t½: 0.54 to 2.36 h in rats, 0.90 to 2.36 h in dogs) were comparable to those obtained following single dose administration for dogs indicating time independent kinetics. In rats the CL was however higher and the AUC_{last} was lower at steady state, indicating time-dependent PK in blood (for AUC_{last}, see Toxicology section). Concentrations of enmetazobactam were quantifiable during ≤ 8 hours in blood of rats and in plasma of dogs. The exposure at steady state in terms of AUC_{last} (i.e $AUC_{0\text{-}8\ h}$ or less) increased with increasing dose at the studied dose range, generally in an approximately dose proportional manner in both rats and dogs, although somewhat less than dose proportional between 125 and 250 mg/kg in male rats. No important (>2-fold) gender difference and no accumulation were observed for any of the species.

Steady state toxicokinetics in plasma following IV administration of enmetazobactam together with cefepime were determined from pivotal combination toxicity studies in rats and dogs. Calculated PK variables on Day 28 and 91 following once daily administration of enmetazobactam/cefepime at doses of 125/250 and 250/500 mg/kg to rats and 50/100 and 200/400 mg/kg to dogs showed that whereas

the PK in dogs were in general not affected the estimated values for CL and t½ in rats were lower and longer, respectively, and the AUC_{last} was higher when enmetazobactam was given together with cefepime. This may be due to the use of different matrices for the TK analyses. Based on the low blood to plasma ratio for enmetazobactam (between 0.591 and 0.608 in rat) the observed differences with lower AUC_{last} in blood compared to plasma when given alone and together with cefepime, respectively, seems reasonable and not an indication of that concomitant administration of cefepime affects the PK of enmetazobactam.

Cefepime

Single dose pharmacokinetics

Single dose pharmacokinetics in plasma following IV administration of cefepime alone and together with enmetazobactam were determined from pivotal combination toxicity studies in rats and dogs. Calculated PK variables following administration of 500 mg/kg cefepime to rats and 400 mg/kg to dogs indicated a distribution comparable to body water (V_d: 1.12 to 1.20 L/kg in rats, 0.24 to 0.98 L/kg in dogs) and a rapid clearance from plasma equal to or higher than the glomerular filtration rate (CL: 14.1 to 17.5 ml/min/kg in rats, 3.22 to 3.73 ml/min/kg in dogs) resulting in a short elimination half-life (t½: 0.60 h in rats, 0.87 to 3.06 h in dogs).

Calculated single dose PK variables following administration of enmetazobactam/cefepime at doses of 125/250 and 250/500 mg/kg to rats and 50/100 and 200/400 mg/kg to dogs showed that coadministration with enmetazobactam did not affect the single dose PK parameters for cefepime.

Repeated dose pharmacokinetics

Steady state toxicokinetics in plasma following IV administration of cefepime alone were determined from rats and dogs in pivotal general toxicity studies. Calculated PK variables on Day 28 and Day 91, respectively, following once daily administration of 500 mg/kg to rats and 400 mg/kg to dogs (Vd: 1.20 to 1.72 L/kg in rats, 0.38 to 0.71 L/kg in dogs, CL: 16.3 to 22.8 ml/min/kg in rats, 3.73 to 4.65 ml/min/kg in dogs, t½: 0.50 to 0.60 h in rats, 0.75 to 2.00 h in dogs) were in general comparable to those obtained following single dose administration indicating time independent kinetics. Concentrations of cefepime were quantifiable in plasma up to 8 hours in rats and dogs.

Calculated TK variables on Day 28 and 91 following once daily administration of enmetazobactam/cefepime at doses of 125/250 and 250/500 mg/kg to rats and at 50/100 and 200/400 mg/kg to dogs showed that coadministration did generally not affect the steady state PK parameters for cefepime.

Distribution

Enmetazobactam

Tissue distribution of enmetazobactam in pigmented (Lister Hooded) and non-pigmented (Spraque Dawley) rats following a single IV bolus dose of [^{14}C]-labelled enmetazobactam was evaluated by quantitative whole-body autoradioluminography. In non-pigmented rats, distribution of [^{14}C]-enmetazobactam-derived radioactivity was in general similar in males and females. The radioactivity was widely and rapidly distributed with a maximum concentration noted at 0.25 h, the first time point analysed, for most of the tissues. The radioactivity was preferentially distributed into organs of elimination with the highest levels detected in urinary bladder (urine content included), mostly at the early time points, which suggests a rapid renal excretion of drug-derived radioactivity. Besides the urinary bladder, the highest radioactivity concentration, markedly exceeding those in blood during the first 6 hours, were found in the kidney. At 24 h, the last time point analysed, the levels in the kidney and the liver were comparable. The highest exposure in terms of AUC_{last} was found in the kidney (2007 µgeq·h/g) and in the liver (651 µgeq·h/g), followed by adrenal glands (314 µgeq·h/g). For the other

tissues the extent of exposure was in general comparable to or lower than that in blood, indicating distribution of radioactivity without a preferential uptake. Whereas the AUC_{last} for the ovary was lower than in blood (ratio of 0.68) the radioactivity concentration at 24 h exceeded the concentration in blood and was comparable to that in the kidney. Furthermore, the radioactivity concentration in the spinal cord of females was higher than in blood from 6 to 24 h post-dose. The AUC_{last} for the spinal cord and brain was however markedly lower than in blood (ratio ≤ 0.02). At 24 hours post-dosing, radioactivity was cleared from all tissues except kidney, liver, adrenal glands, spleen (males), lung (females), spinal cord (females) and ovary.

The distribution pattern in male pigmented rats was similar to that in male non-pigmented rats. There was no preferential distribution to or retention in the skin, the eye or the uveal tract, i.e., no specific binding of [^{14}C]-enmetazobactam and drug related radioactivity to melanin was indicated.

Plasma protein binding of enmetazobactam was concentration independent and very low, it was reported as 0% in all species tested including rats, dogs, rabbits and humans, indicating that measured concentrations in plasma represent free enmetazobactam.

Blood cell partitioning of enmetazobactam in rats, dogs and humans was concentration independent and showed minimal binding to red blood cells. Blood to plasma ratios ranged between 0.591 and 0.608 in rats, between 0.523 and 0.549 in dogs and between 0.491 and 0.660 in humans.

Concentration of enmetazobactam was determined in milk collected 0.5 h after dosing on Day 14 postpartum from female rats dosed IV once daily with 500 mg/kg/day enmetazobactam from Day 6 of presumed pregnancy to Day 20 postpartum. Milk concentrations ranging from 34.3 to 317 ug/mL were measured, indicating that enmetazobactam is excreted into milk and that nursed pups are exposed to enmetazobactam.

Metabolism

Enmetazobactam

The metabolism of enmetazobactam was investigated *in vitro* in liver microsomes and/or hepatocytes of rats, dogs, rabbits and humans and *in vivo* in rats, dogs and humans.

In humans, enmetazobactam was the major component of drug related material in plasma and there were no major (>10% of total drug related materials in plasma) or unique metabolites identified after treatment with enmetazobactam. Based on $AUC_{0\text{-}24h}$ unchanged compound represented 94.7% of the [^{14}C]-enmetazobactam-derived components whereas M3 (formed by cleavage of the enmetazobactam thiazolidine ring either through metabolism or extraction process degradation) represented ≤6% of the total radioactivity. No qualification or characterisation of metabolites in non-clinical toxicity studies is therefore needed.

In vitro

Unchanged enmetazobactam accounted for 82 to 107%, 100 to 117% and 108 to 114% of added compound in liver microsomes of rats, dogs and humans, respectively, incubated with 1 and 10 µM enmetazobactam, indicating that enmetazobactam is stable in liver microsomes.

Following incubation of rat, dog, rabbit and human hepatocytes with 10 µM [^{14}C]-enmetazobactam unchanged enmetazobactam represented 94 to 97% of total radioactivity, indicating limited metabolism of enmetazobactam by hepatocytes of all studied species. The only relevant metabolite detected was M2, formed by cleavage of the enmetazobactam thiazolidine ring followed by dehydrogenation, which accounted for 3, 3, 6 and 5% of total radioactivity in rat, dog, rabbit and human hepatocytes, respectively. Trace amounts of M1 (0.5% of total radioactivity) were detected following incubation of rat hepatocytes. Metabolite M2 was found at lower amounts in control

incubation samples (2% after 1.5 hours incubation) and test item standard solution as well. No metabolite unique to human was observed and no species-specific metabolites were detected.

In vivo

In rats and dogs unchanged enmetazobactam represented the predominant part of total radioactivity in pooled plasma samples from both males and females, accounting for 82 or above in rats up to 1 hour after a single IV administration of 125 mg/kg (50 µCi/kg), and for 66% or above in dogs up to 2 hours after a single IV administration of 50 mg/kg (20 µCi/kg). Metabolite M3 represented the main biotransformation product in plasma of both genders in rats (up to 18%) and dogs (up to 29%). A minor unknown metabolite M7 was found in male rat plasma (0.5%) and in dog plasma (less than 1.1%), where also M2 constituted a minor metabolite (less than 3.8%).

In urine, unchanged compound accounted for a large or major part of enmetazobactam-derived radioactivity obtained during the 8-24 h collection period in rats of both genders (78%) and during the 0-48 h collection period in dogs of both genders (46 to 81%). Metabolite M3 was the main metabolite and accounted for 21% in both genders of rats and from 44 to 52% in female and male dogs. In rats, traces of metabolite M7 (0.2%) were found in male urine only and in dogs M2 was found in lower amounts in males (up to 4.3% of radioactivity) and females (1.9%).

In faeces from male and female rats unchanged compound represented a large or major part of total radioactivity (29-72%). Metabolite M3 was found in the range of 2-57% and M2, formed by dehydrogenation of M3, was the main metabolite (9-22%) except in one female for which unchanged compound accounted for 29% and M3 accounted for 57%. Minor unidentified metabolites (M5, M6 and M7) were also found.

Excretion

Enmetazobactam

Excretion of [^{14}C]-enmetazobactam in urine and faeces following a single IV administration to rats and dogs was well characterised, total recovered radioactivity at 168 hours post-dose was >95% and >90%, respectively. The excretion was fast with more than 80% excreted within the first 24 hours for both species. There were no gender differences in the excretion pattern in rats and dogs. As for humans, urine was the major route of excretion (90 and 89%, respectively, of the administered dose when cage washing was included) with minimal excretion via faeces (5.3 and 1.5%, respectively, of the administered dose). Unchanged drug represented the predominant part of the total radioactivity in urine of rats, dogs and humans. Enmetazobactam is thus eliminated primarily in urine as unchanged drug in all species.

Conclusion

The non-clinical pharmacokinetic studies, including toxicokinetics for single dose and repeat dose pharmacokinetics, of enmetazobactam and cefepime are in general considered sufficient and cover the main animal species used in the pharmacological and toxicological studies, i.e. rats, dogs and rabbits. The enmetazobactam metabolic pathway in humans is similar to that observed in the preclinical species, i.e. with the pharmacologically inactive metabolite M3 (sulfinic acid metabolite formed by cleavage of the enmetazobactam thiazolidine ring) representing the main biotransformation product. Metabolite M3 is also a degradation product. The pharmacokinetic/toxicokinetic profile of enmetazobactam given alone and in combination with cefepime as well as the pharmacokinetic/toxicokinetic profile of cefepime given alone and in combination with enmetazobactam are overall considered well characterised. It is however noted that blood or plasma exposures for enmetazobactam and cefepime in pivotal GLP-compliant repeated dose toxicity studies are low-compared-to the clinical plasma exposure.

2.4.4. Toxicology

Enmetazobactam is a novel penicillanic acid sulfone beta-lactamase inhibitor (BLI), active against a wide range of beta-lactamases, which restores the antibacterial activity of beta-lactam antibiotics against pathogens producing such beta-lactamases including cefepime. It will be marketed as a fixed dose combination with cefepime for intravenous (IV) infusion and treatment of various serious infections in adults. The toxicological profile of enmetazobactam has been characterised in a program compliant with ICH M3. Pivotal toxicology studies are a in accordance with GLP regulations.

The metabolic profiles of enmetazobactam in rats, dogs, rabbits and humans were similar which supports the selection of these animal species as the primary toxicology species. Enmetazobactam was the major component of drug related material and there were no human major or unique metabolites identified after treatment with enmetazobactam. No qualification or characterisation of metabolites in non-clinical toxicity studies is therefore needed.

2.4.4.1. Single dose toxicity

Two GLP single-dose acute toxicity studies of enmetazobactam have been performed, one in mouse and one in rat.

No mortality and no clinical signs of toxicity were observed for any of the 3 female Swiss albino mice given a single IV dose of 5000 mg enmetazobactam/kg. Normal body weight gain was observed for all mice on Day 7 whereas a slight decrease in body weight was evident in 2 mice on Day 14. Gross pathological examination on Day 14 did not reveal any treatment related abnormality.

No test-article related mortality or clinical signs of toxicity were observed for any of the 5 female Wistar rats given a single IV dose of 2000 mg enmetazobactam/kg. There were no significant changes in mean body weight of the treated group (9.9% weight gain) compared to the control group (5 animals; 11.1% weight gain) and gross pathological examination on Day 14 did not reveal any test article related abnormality on any of the animals.

2.4.4.2. Repeat dose toxicity

Pivotal studies of enmetazobactam given alone in 28-days studies in Wistar rats and Beagle dogs and of the combination cefepime/enmetazobactam and enmetazobactam or both substances alone in Wistar rats and Beale dogs for 28 days and 13 weeks, respectively, were provided. All studies used once daily IV administration and included a recovery phase.

Enmetazobactam given alone to rats at doses of 125, 250 and 500 mg/kg/day and at doses of 60, 120 and 240 mg/kg/day to dogs was in general well tolerated. In the combination studies, treatments with enmetazobactam/cefepime in a ratio of 1:2 at low and high dose levels in rats (125/250 and 250/500 mg/kg/day) and in dogs (50/100 and 200/400 mg/kg/day), enmetazobactam at 200 mg/kg/day in dogs and with cefepime at 500 and 400 mg/kg/day in rats and dogs, respectively, were also generally well tolerated. The recommended clinical dosing regimen is 500 mg enmetazobactam/2 g cefepime (1:4) TID.

No test-article related mortality occurred in any of the studies. Treatment-related findings were seen in the liver and kidney of both species following treatment with enmetazobactam alone and in combination with cefepime and in spleen and thymus of rats following treatment with cefepime alone and in combination with enmetazobactam.

Liver effects

Based on the 28-day Repeated Dose Toxicity (RDT) studies of enmetazobactam and the 28-day and 13-week combination studies in rats and dogs, the liver appears to be a primary target of toxicity for enmetazobactam. Findings common for both species and sexes included a dose dependent increase in hepatocyte vacuolisation/granular hepatocellular inclusions, accompanied in rats by up to ~20% increase in liver weights. Furthermore, in the two studies using Periodic Acid-Schiff, PAS, staining (the 28-day study of enmetazobactam in rats and the 13-week combination study in dogs), the vacuolisations/granular hepatocellular inclusions were found to correlate with an increase in hepatocellular glycogen accumulation. This glycogen accumulation was detectable in both species at all dose levels of enmetazobactam. In dogs, other notable findings attributed to enmetazobactam included hepatocytes with ground glass cytoplasm, single cell cystic degeneration/necrosis accompanied by elevations of liver enzymes and decreases in total and HDL cholesterol. Liver findings attributed to cefepime based on data from the combination studies included a minimal to slight increase in Kupffer cell cellularity in rats of both sexes. No aggravation of the findings related to enmetazobactam or cefepime was observed when these substances were used in combination.

In the rat, the finding of hepatocellular glycogen accumulation was graded minimal-mild at 125 mg/kg enmetazobactam and increased to moderate-severe from 250 mg/kg in the 28-day RDT study of enmetazobactam. As stated above the finding was accompanied by an increase in liver weights. In addition, a small but significant increase in alkaline phosphatase was detectable at the 500 mg dose in the enmetazobactam only study and from 125/250 mg enmetazobactam/cefepime (up to 53% across dose levels). Glycogen accumulation (and hepatocyte vacuolisation) was still detectable in enmetazobactam exposed rats in recovery animals, but at a reduced severity in particular after the longer recovery period used in the combination study (8 weeks) compared to the 28-day enmetazobactam study (2 weeks). Notably, in the 28-day study of enmetazobactam only, glycogen accumulation was still graded severe in the highest dose (500 mg/kg) although slightly reduced in comparison to animals sacrificed at end of treatment phase.

As the hepatocellular glycogen accumulation in rats was not associated with any changes in tested plasma liver enzymes, except for a slight increase in ALP, and showed some reversibility, the applicant classified this finding as non-adverse. This is questioned based on the moderate-severe grading of glycogen accumulation and limited reversibility for this finding. Nonetheless, the applicant defined a NOAEL for the 28-day enmetazobactam study to 125 mg/kg taking into account the increased liver weight and decreased body weight observed at the 250 mg/kg/day dose. This NOAEL can be agreed. However, for the combination study the applicant has set the NOAEL to the highest dose level 250/500 mg/kg enmetazobactam/cefepime. Based on the lower than dose proportional increase in exposure to enmetazobactam between the low and high dose combination the increased levels of alkaline phosphatase at the 250/500 mg/kg dose level should also be taken into account. As the glycogen accumulation at the 250/500 mg/kg dose level is moderate with limited reversibility and related to increased liver weights (~20% or more) the overall results suggest that the liver effects at this level should be considered adverse and that the proposed NOAEL should be reduced to 125/250 mg/kg enmetazobactam/cefepime. Anyway, as there is no margin to the clinical exposure for any of the dose levels in rats or for the NOAELs for corresponding effects in dogs, and risk for increased liver enzymes and liver effects has been observed in the clinic, information on the non-clinical liver effects have been included in the SmPC.

In dogs, increased hepatocellular glycogen was detectable by PAS staining from 50/100 mg/kg enmetazobactam/cefepime based on the combination study report. At 200/400 mg/kg enmetazobactam/cefepime and 200 mg/kg enmetazobactam only, increased condensation of glycogen was also observed. This finding seems to be correlating with the observation of a ground glass cytoplasm, a histological feature that can appear in association with abnormally structured glycogen

(Hsiang-Chih et al. Annals of Diagnostic Pathology 52 (2021) 151740) noted in the same treatment groups in the combination study and from 120 mg/kg enmetazobactam in the 28-day dog study. Of note, the mentioned signs of altered glycogen content in dogs were not adequately described or discussed by the applicant in the non-clinical overview. Instead, the applicant notes a seemingly contrasting finding: a minimal to mild glycogen depletion. However, it seems that the term glycogen depletion (or decreased glycogen), which is not based on PAS staining, relates to the content of normal glycogen in the cell and is probably part of the same ethology as the observations of glycogen accumulation/condensation. Further, electron microscope examination of liver samples from two high dose animals in the combination study indicated that the large vacuoles observed in dog hepatocytes contained clumpy material considered by the pathologist to consist of glycogen alongside of intact and degenerated organelles (likely ER and mitochondria), indicating that the glycogen alteration was toxic to the cell.

With regards to a potentially adverse outcomes of these alterations at a cellular level, minimal-mild single cell cystic degeneration/necrosis of hepatocytes was indeed observed in the 28-day study of enmetazobactam from 120 mg/kg accompanied by elevations in liver enzymes (ALT up to 6.4x, AST up to 2.4, ALP 1.9x and GGT up to 2.1 at 240 mg/kg) and decreases in total and HDL cholesterol (down 37 and 36 % respectively). No change in liver weight was observed which is contrasting to the findings in rat. Notably, no significant reversibility of these findings was noted after the 2-week recovery period. The NOEL for the liver findings and this study was concluded by the applicant to be 60 mg/kg at an AUC_{last} of 216 µg*h/mL for males and 208 µg*h/mL for females. This is agreed also noting the lack of margin to clinical exposure.

In the dog combination study, the only indication of an adverse outcome was the observation of marked granular inclusions indicative of degenerating hepatocytes observable in all animals treated in the enmetazobactam/cefepime high dose and enmetazobactam alone at 200 mg/kg in the combination study. Liver transaminases (AST and ALT) were slightly increased (1.4–2.6 fold) in animals in the same dose groups. After the 8-week recovery period, these findings appeared to be at least partly resolved in recovery animals.

To conclude, the liver effects induced by enmetazobactam in rats and dogs and by cefepime in rats did not change or exacerbate when the substances were given in combination. Adverse toxic effects in terms of hepatocyte degeneration/necrosis, most likely attributed to an enmetazobactam-related disturbance of glycogen storage mechanisms, occurred in dogs at 120 mg/kg/day and in terms of moderate to severe hepatocellular accumulation of glycogen in rats from 250 mg/kg/day when enmetazobactam was given alone. When enmetazobactam was given in combination with cefepime corresponding adverse liver effects were observed at 200/400 mg/kg/day in dogs (639 µgxh/mL) and at 250/500 mg/kg/day in rats (195 µgxh/mL). These doses correspond to an AUC exposure margin of 0.86 in rats and 2.8 in dogs when compared with the AUC exposure at MRHD (226 µgxh/mL). Regarding the enmetazobactam-mediated liver findings, there seems to be a common ethology behind the findings in rats and dogs, namely a potential of enmetazobactam to alter glycogen storage and/or structure in hepatocytes. Moreover, the dog appeared to be more sensitive to these alterations based on the presence of single cell cystic degeneration/necrosis accompanied by significant elevations of liver enzymes and decreases in total and HDL cholesterol. At the doses evaluated in rats and dogs there were no major functional consequences detected. Moreover, while most findings were still detectable in recovery animals there was indication of reversibility, albeit at a slow pace. This is reassuring. However, given that the highest dose levels tested in the non-clinical safety evaluations did not allow for an appropriate definition of MTD in animals at a reassuring margin to clinical exposure, and further, that there is an identified risk for elevated liver enzymes in patients, there is an uncertainty whether there could be risk for more severe hepatotoxicity if plasma exposures to enmetazobactam would reach a supratherapeutic level in a patient. As adverse liver effects in terms of

hepatocyte degeneration/necrosis and related hepatocellular accumulation of glycogen in dogs and hepatocellular accumulation of glycogen in rats, which seem to be clinically relevant, are induced by enmetazobactam at doses with low or no margin to the exposure at the MRHD this information have been included in SmPC 5.3. For the NOAELs in the combination studies of enmetazobactam given together with cefepime, the margin to the exposure at the MRHD was 0.71 in dogs and 0.86 or lower in rats. Furthermore, as also noted by the applicant, glycogen accumulation has been reported in non-clinical studies with the structurally related tazobactam and sulbactam possibly indicating that a disturbance of glycogen storage may be a class effect.

A modest, but widespread suppression of drug metabolising enzymes and transporter (DMET) mRNAs, including BCEP, MDR3, and ABCG5/8 was also noted. Loss of function of these transporters may induce liver injury due to the toxic effects of bile acids on both hepatocytes and biliary epithelial cells.

Increased liver weights were observed in adult rats given IV doses of enmetazobactam alone and in combination with cefepime and, together with microscopic hepatocyte hypertrophy. In juvenile rats, increased liver weights were observed following IV and SC doses of enmetazobactam, also associated with hepatocyte hypertrophy which continued until end of recovery after SC administration at 500 mg/kg/day and a trend towards recovery after IV administration. On the contrary, no liver weight changes in dogs were reported, indicating that the cause of the increased liver weight could not be attributed to an increase in elimination pathways associated with this organ.

Kidney effects

When enmetazobactam was given alone to rats, kidney findings of tubular dilatation with diffuse intraluminal casts were observed at ≥250 mg/kg in both sexes, accompanied by increased kidney weight in males. This effect is proposed to be related to increased excretory workload. Enmetazobactam/cefepime-related microscopic findings in the kidney consisted of minimal hyaline droplet accumulation and tubular dilatation which were accompanied by increased kidney weights in both genders from 125/250 mg/kg/day. Cefepime-related microscopic findings in the kidney consisted of minimal hyaline droplet accumulation in both sexes. The hyaline droplet accumulation and tubular dilatation observed for the combination are therefore considered related to cefepime and enmetazobactam, respectively. As the effects in kidneys were unaccompanied by evidence of parenchymal degeneration and necrosis, they were not considered adverse at the studied dose levels up to 250/500 mg/kg/day.

In dogs, findings seen in the kidney occurred only in males at ≥ 120 mg/kg/day and consisted of minimal to mild basophilic and dilated tubules, and cell debris. These changes were not associated with alterations in BUN and cholesterol whereas the increased BUN and creatinine levels at 240 mg/kg/day in females where not associated with any kidney lesions. No findings in the kidney were seen in the 13 weeks dog combination study.

Spleen effects

Effects in spleen were only observed in the combination study in rats. Enmetazobactam/cefepime-related microscopic findings in the spleen included increased extramedullary haematopoiesis and cellularity of the follicles and germinal centres in both sexes, not correlated with changes in organ weight. Cefepime-related microscopic findings in spleen consisted of decreased and increased extramedullary haematopoiesis in males and females, respectively, which correlated with cefepime-related organ weight changes (decreases in males and increases females). No microscopic changes were reported for the spleen after treatment with enmetazobactam alone, suggesting that the findings for the combination observed in the spleen are induced or exacerbated by cefepime. Increased extramedullary haematopoiesis and cellularity of the follicles and germinal centres are noted also in control animals. These findings may thus be attributed to procedure-related factors such a local

tissue/vascular trauma and potential bacterial contamination and therefore not adverse effects clearly related to the test-article related.

Thymus effects

Enmetazobactam/cefepime-related microscopic findings in the thymus consisted of increased cortical lymphocyte apoptosis in males which was correlated with decreased organ weight. Cefepime-related microscopic findings in thymus also consisted of increased cortical lymphocyte apoptosis in males. No microscopic changes were reported for the thymus after treatment with enmetazobactam alone, suggesting that the findings of increased cortical lymphocyte apoptosis in the thymus are induced or exacerbated by cefepime. They are however noted also in control animals and may thus be attributed to procedure-related factors such a local tissue/vascular trauma and potential bacterial contamination and therefore not considered adverse effects clearly related to the test article.

Toxicokinetics

When given alone or in combination the plasma exposure of enmetazobactam and cefepime at steady state (AUC_{last}; $AUC_{0\text{-}8\ h}$ or less) generally increased with increasing dose in an approximately dose proportional manner, although less than dose proportional for enmetazobactam in male rats between 125 and 250 mg/kg when given alone and between 125/250 and 250/500 mg/kg when given in combination. There were no important differences in exposure to enmetazobactam or cefepime between genders (less than 2-fold) and no signs of accumulation. In dogs, the exposure (AUC and C_{max}/C_0) of enmetazobactam or cefepime revealed a general dose proportionality and no obvious signs of accumulation and no or minimal difference between genders.

The NOAELs in the repeated dose toxicity studies were set to 60 mg/kg/day in dog (AUC_{last}: 212 µgxh/mL) and 125 mg/kg/day in rat (AUC_{last}: 56.7 µgxh/mL) for enmetazobactam administered alone and to 50/100 in dog (AUC_{last} for enmetazobactam: 160 µgxh/mL/AUC_{last} for cefepime: 387 µgxh/mL) and 250/500 mg/kg/day or lower in rat (AUC_{last} for enmetazobactam: 129 µgxh/mL/AUC_{last} for cefepime: 438 µgxh/mL) for enmetazobactam/cefepime. At these NOAELs there were no or limited exposure margins (approximately 0.94 and 0.71/0.34X in dogs, and 0.25 and 0.86 or lower/0.48 or lower X) to the clinical AUC exposure to enmetazobactam/cefepime at MRHD of 0.5 g enmetazobactam and 2.0 g cefepime TID (226/1139 µgxh/mL) predicted in Study AT-301.

When enmetazobactam was given alone to rats the TK of enmetazobactam was measured in blood which is in contrast to the combination study in rats where the TK of enmetazobactam was measured in plasma. Due to low blood to plasma ratio for enmetazobactam (between 0.591 and 0.608 in rat) the margins between the systemic exposure in the pivotal rat toxicity studies and the clinical plasma exposure at the maximum recommended human dosing regimen are therefore lower than reported.

2.4.4.3. Genotoxicity

Cefepime

In chromosomal aberration studies using primary human lymphocytes, cefepime was positive for clastogenicity. However, cefepime was negative for clastogenicity *in vivo* in mice (2 chromosomal aberration and 2 micronucleus studies) and in Chinese hamster ovary cells. In addition, other *in vitro* assays (bacterial and mammalian cell mutation, DNA repair in primary rat hepatocytes, sister chromatid exchange in human lymphocytes), showed that cefepime was negative for genotoxicity.

Enmetazobactam

Enmetazobactam was negative for mutagenicity in two *in vitro* reverse mutation assays (Ame's) and negative for clastogenicity in Chinese hamster ovary (CHO) cells as well as *in vivo* bone marrow

erythrocyte micronucleus in rat. It was concluded that enmetazobactam does not possess any genotoxic potential.

2.4.4.4. Carcinogenicity

Cefepime

No long-term studies have been performed on carcinogenicity of cefepime but cefepime is not regarded to possess a genotoxic potential.

Enmetazobactam

Carcinogenicity studies of enmetazobactam have not been conducted and are not planned based on the intended duration of therapy (≤ 14 days). Enmetazobactam demonstrated no potential to cause genotoxicity.

2.4.4.5. Reproductive and developmental toxicity

Cefepime

In vivo studies on cefepime have not exhibited any effects on reproduction, embryonal development, gestational length, or peri- post-natal development up to 10 times the recommended dose in human. Cefepime is excreted in breast milk.

Enmetazobactam

The data package is considered sufficient to cover all parts of reproductive toxicity, and the pivotal studies were conducted in accordance with GLP regulations and in accordance with appropriate guidelines. Enmetazobactam exposure were not affected by pregnancy as shown in the EFD study on rats.

The safety margins from animal to human exposure ratio for enmetazobactam in the reproduction and development studies were very low or undetermined.

Enmetazobactam

Fertility

Male and female fertility and early embryonic development was analysed at doses of 125, 250, and 500 mg/kg once daily. In females, a reduction in body weight gain and food consumption was observed at the high dose during the period of enmetazobactam treatment. No other treatment-related findings were observed in females regarding reproductive organs, oestrous cycle, mating behaviour, reproductive ability and early embryonic development at any dose level. Based on the maternal reduction in body weight gain in the high dose at the time of pregnancy, NOEL was estimated to 250 mg/kg/day for maternal toxicity and 500 mg/kg/day for fertility. For males, a dose dependent reduction in body weight gain was observed, reaching statistical significance in all dose groups before end of dosing. This correlated to intermittent decreases in food intake. Pregnancy induction was successful the first week of cohabitation and no treatment related findings were observed in males at necropsy regarding reproductive organs or fresh sperm parameters or in pregnancy parameters in untreated females. NOEL for male fertility was 500 mg/kg/day. Margins to estimated human exposure was x 2.54 for female fertility and x 2.42 for male fertility.

Embryofoetal development

Rat

The maternal body weight gain from GD8 up to GD20 was significantly lower compared to the control group after uterus excision. A marginally lower but significant mean foetal weight was observed at 500 mg/kg dose level (3.71 and 3.84 g for treated vs. control pups). No external, visceral, or skeletal malformations were reported at any dose level. Variations in ossification at the skeletal observation appeared to be evenly distributed when vehicle-treated pups were compared to 500 mg/kg treatment, except for incomplete ossification of the skull bones. In comparison to historical controls, delayed ossification of the interparietal and occipital lobes was exceeded at the 500 mg/kg dose level.

These observations were considered to be at a clinically relevant exposure and information has been added to section 4.6 and 5.3 of the SmPC.

Rabbit

Embryofoetal development was assessed in pregnant rabbits with enmetazobactam treatment during organogenesis in the dose levels of 50, 150 and 300 mg/kg/day. A higher mean % of post-implantation loss at all dose levels was observed in the EFD study in rabbit (x2.3-3.3 times of the findings in control animals). One female underwent total abortion on GD22 at 150 mg/kg/day, which was attributed to maternal toxicity. All aborted foetuses showed normal development in accordance with the gestational age and exhibited no external malformations. The mean foetal weight was -17.9% at 150 mg/kg/day compared to control values. At 300 mg/kg/day, the mean foetal weight was -26.7% and the placental weight were -24.5% compared to controls.

For the external examination at 300 mg/kg/day, all foetuses in a full litter showed malformations with cleft palate (7/8 foetuses) and open eyes (8/8 foetuses). The external examination was followed by a detailed head visceral examination of the half litter, showing either absence or discoloration of the lens. At skeletal examination, shortening of sternum with fused sternebrae from 2nd to 5th were observed. The malformations seen in all foetuses from this litter was considered incidental as the external malformations were exclusively found in this litter. All foetuses in this litter exhibited sternebrae fusion to some extent. In addition, variations of the sternum, with fused sternebrae and all at the same location, was observed in both 150 and 300 mg/kg/day (3 foetuses from 2 litters at 150 mg/kg/day and 10 foetuses from 4 litters at 300 mg/kg/day) which according to the CRO was difficult to interpret. Variations such as ossification of the skull bones were also observed at 300 mg/kg day in 7 foetuses from 4 different litters. NOEL for maternal toxicity and for the embryofoetal development was 50 mg/kg/day and the margins to estimated clinical exposure was x 1.10.

Prenatal and post-natal development, including maternal function

The toxicity of enmetazobactam was investigated in pregnant/lactating F0 female rats and on pre- and post-natal development to sexual maturity of the F1 generation following IV administration from implantation on Day 6 post-coitum to Day 20 post-partum. Milk was collected F0 satellite females (500 mg/kg, n=5) and analyses was performed to demonstrate maternal exposure to the F1 generation. The F1 generation was analysed from birth to weaning and one male and female respectively from each litter was selected to produce the F2 generation. The F2 generation were followed from post-partum through pre-weaning.

F0: A slight maternal toxicity was confirmed by reduced mean body weight and food consumption post-partum. Increased relative kidney and liver weight was recorded in F0-females for all dose levels. Three F0 females, one low dose and two high doses, were sacrificed due to complications associated to giving birth. No effects on cumulative pup loss and no intergroup differences in lactation index were seen in treated compared to control females. Milk exposure investigated in satellite animals showed a

large variation in concentration. A conclusion regarding effects from milk exposure to pups cannot be drawn.

F1: Lower mean pup weight was observed from day 4 post-partum for the highest dose level and from day 7 for the low and mid-dose level. A slight delay in the pre-weaning development were observed at the mid- and high dose group, (pupil reflex and pinna detachment). At weaning on day 21, a lower mean body weight was noted for all dose levels in females and from the mid-dose level in males. In the water filled Y maze, maintained improvement in swimming time was considered an indication of learning. Mean group data showed an improvement in swimming time. Mean scores for motor activity on week 6/7 for males in the high dose was 79% of that in control. The low score was attributed to a few males and did not reach a level of significance when compared to controls. Considering the large individual variation in motor activity scores and the large variation in breast milk concentrations, measured in in five satellite F0 females at 500 mg/kg/day, a correlation between the over-all exposure and motor activity performance cannot be excluded. A NOAEL of 125 mg/kg/day was set for the F1 parental generation and 250 mg/kg/day was set for the F0 generation, as well as F2 pups based on reduced pup weight, corresponding to a margin of 0.68 and 1.14 respectively, to the clinical exposure at maximum recommended dose.

Juvenile toxicity

In the pivotal study, toxicity and toxicokinetics was investigated in juvenile animals after SC administration from PND7 and IV administration from PND23 for a period of 14 consecutive days. Reversibility was assessed after a 28-day recovery period.

No relevant toxicological effect was observed on bodyweights or food consumption for either sex or administration route during the treatment period. Remarkably, at the end of recovery mean body weight and absolute body weight gain was higher in the subcutaneously enmetazobactam treated group for both sexes in comparison to control animals. A higher food intake was recorded in females from day 31-42. A few haematology parameters, and clinical chemistry parameters that may be associated to liver and/or kidney effects showed a slight but significant deviation from control group at the end of dosing, most of which disappeared following the recovery period. Increased liver to body weight ratios were observed at end of administration in both sexes and for both administration routes and was persistent after the 28 days recovery period except for IV-treated females. Increased liver weight was associated to hepatocyte hypertrophy from 250 mg/kg (SC administration) and 500 mg/kg (IV administration) which continued until end of recovery at 500 mg/kg/day, however, with a tendency to recovery for the IV-treated group. In addition, after 28 days of recovery, a higher spleen and thymus weight (34 and 22%, respectively) was noted in males in the subcutaneously treated group, however, no histopathological changes were observed. Since the product is intended for IV use in the adult population, this issue is not pursued.

No relevant sex-dependent difference was observed for either SC or IV administration. Following IV administration from PND23, C_{max} and AUC_{last} increased proportionally with dose level. Following SC administration from PND7, a supra-proportional increase in exposure ($AUC_{last)}$ was observed Day 1, while C_{max} increased proportionally with dose. The systemic exposure Day 1 was up to 8 times higher compared to Day 14. In human, PND7 equals to Pre-Term/Term Neonate and PND21 equals to a child of approximately 2 years.

In conclusion, findings persistent after recovery include increased liver to body weight ratios for both sexes and administration routes except for IV-treated females after recovery. Low albumin/globulin ratio resolved after recovery except for SC-treated males. The SC administration group exhibited relatively more test item-related observations compared to the IV administration groups. In addition, altered clinical chemistry parameters, possibly liver-associated, was observed. A NOAEL value of 250 mg/kg/day for IV administration, is appropriate. The reduced tolerability seen after SC administration

may be related to the comparable higher systemic exposure the first day(s) of administration as well as greater vulnerability in such young animals. Since the product is aimed for treatment of the adult population via intravenous route, issue is not pursued.

2.4.4.6. Interspecies comparisons

Table 1: Enmetazobactam

	NOAEL	Exposure variables (Gender mean)		Animal to Human Exposure Ratios	
Study	**Daily Dose (mg/kg)**	**C_{max}/C_o (µg/mL)**	**AUC_{0-24} (µg*h/mL)**	**C_{max}/C_o**	**AUC_{0-24}**
Repeated tox, rat (day 28)	125	0	56.7	**0**	**0.25**
Repeated combination tox, rat (day 28)	250[b]/500[c]	134	195	**6.8**	**0.86**
Repeated tox, dog (day 28)	60	253	212	**12.8**	**0.94**
Repeated combination tox, dog (day 91)	50[b]/100[c]	62.5	160	**3.2**	**0.71**
Female rat, Fertility	500	1510	574	**76 .3**	**2 .54**
Male rat, Fertility	500	1630	547	**82 .3**	**2 .42**
Rat, EFD, AAI101-TO-18-09	125*	376	153	**19 .0**	**0 .68**
Rat, EFD, AAI101-TO-18-09	250*	609	257	**30 .8**	**1 .14**
Rabbit, EFD, AAI101-TO-19-01	50	206	206	**10 .4**	**1.10**
Female juvenile rat, IV PND23, AAI101-TO-19-04	250	715	281	**36 .1**	**1 .24**
Male juvenile rat, IV PND23, AAI101-TO-19-04	250	706	300	**35 .7**	**1 .33**
Estimated human exposure	**500 mg**	**19 .8**	**226[a]**	**N/A**	**N/A**

*No estimated NOAEL levels; a) Based on $AUC_{0-8\ h}$ x 3. b) enmetazobactam c) cefepime

Table 2: Cefepime

	NOAEL	Exposure variables (Gender mean)		Animal to Human Exposure Ratios	
Study	**Daily Dose (mg/kg)**	**C_{max}/C_o (µg/mL)**	**AUC_{0-24} (µg*h/mL)**	**C_{max}/C_o**	**AUC_{0-24}**
Repeated combination tox, rat (day 28)	250[b]/500[c]	360	551	**3.6**	**0.48**
Repeated combination tox, dog (day 91)	50[b]/100[c]	150	387	**1.5**	**0.34**
Estimated human exposure	**2 g**	**99.8**	1139[a]	**N/A**	**N/A**

a) Based on $AUC_{0-8\ h}$ x 3. b) enmetazobactam, c) cefepime

2.4.4.7. Impurities

Sulfinic acid

The proposed NMT 1.0 % limit for the sulfinic acid impurity is considered qualified since sulfinic acid is an inactive, non-major metabolite (M3, or M2 as dehydrated) of enmetazobactam. It is not known how this metabolite/degradation product is formed, but it has been identified in plasma and urine (see also non-clinical and clinical pharmacokinetics).

*Methyl este*r

The methyl ester impurity has a limit of 0.07% (IC threshold).

1-methyltriazole

Since no toxicological data was available for the 1-methyltriazole impurity, mutagenicity was analysed in QSAR and Ames test based on structurally similar analogues and was considered non-mutagenic. The limit of 1-methyltriazole impurity has been adjusted to 0.07%.

Tazobactam impurity

Tazobactam is a well-known drug substance marketed in combination with piperacillin at a dose of 1.5 to 2.0 g daily. The proposed NMT 0.20 % limit set for the tazobactam impurity is acceptable since this limit is the qualification threshold for a MDD of 1.5 g/day.

2.4.4.8. Other studies

Phototoxicity

The molar extinction coefficient for enmetazobactam at a wavelength scan from 290nm to 700nm was lower than 1000 L mol-1 cm-1. Enmetazobactam is therefore not considered to be sufficiently photoreactive to result in direct phototoxicity.

2.4.5. Ecotoxicity/environmental risk assessment

Cefepime

The applicant has submitted a declaration over the use of cefepime in all members states concerned, including historical consumption data. Consumption has decreased over the last four years, despite a slight increase in the population. This supports the conclusion that total environmental exposure is unlikely to increase and that a full ERA procedure is not necessary.

Enmetazobactam

A Log Kow determination was performed according to OECD 107 and in compliance with GLP. The experimental Log Kow value is -3.44 at 20°C±0.5 and at pH 5.2 which is appropriate for such a weak acid.

The Phase I surface water PEC (PECsw) value is > 0.01µg/L, and a Phase IIA evaluation is necessary (a tailored approach in line with Draft CHMP ERA guideline recommendations from 2018). Phase IIA studies for aquatic toxicity (tailored with focus on OECD TG201) and activated sludge (OECD TG209) have been submitted. However, the concentration ranges chosen in all three submitted OECD 201 test reports cover effects of 5-75%, the lowest concentration in the definitive test with *A. flos-aquae* indicated a growth inhibition of >50%. The EC10 was highly extrapolated, and the NOEC couldn't even be estimated. Effect values are therefore not reliable and new studies according to OECD TG 201 are considered necessary with NOEC/LOEC values using *A. flos-aquae*, which so far is indicated to be the most sensitive species. In addition, the necessary environmental fate studies (required by the presently active ERA guideline and to modified extent by the Draft ERA guideline) are currently missing and should be considered in the updated ERA.

An additional issue is that the tailored approach, while likely relevant for this drug candidate, is based on the Draft ERA guideline from 2018 which is still superseded by the presently active ERA guideline from 2006. As such, formally, an OECD TG210 study must be included in the updated ERA.

As the ERA has no full study package at time of CHMP Opinion, a full regulatory assessment – and any formal decision on environmental risk – needs to be delayed to when the full ERA study package has been submitted, which the applicant has committed to submit within maximum 2 years post-approval, covering all the above aspects, as a follow-up post-authorisation measure (see letter of Recommendations).

2.4.6. Discussion on non-clinical aspects

Pharmacology

In vitro off-target screening displayed no effects when enmetazobactam was screened against 76 GPCRs, 8 cardiac ion channels, and 36 kinases and phosphatases. However, the analysed maximum concentration of 12.5 µM enmetazobactam in the secondary pharmacodynamics studies (AAI101-PH-12-02) is considered too low and does not even cover the therapeutic C_{max} exposure of ~63 µM (free fraction) but the studies was conducted before the clinical exposure was established. It remains whether enmetazobactam at a therapeutic exposure would produce any additional off-target effects. However, based on the clinical experience it is considered a low risk.

The potential risk for QT prolongation in humans given enmetazobactam is considered to be minimal as the IC_{50}-value is 1254.22 µg/mL, corresponding to 3990.30 µM which translates into a 63-fold safety margin compared to the C_{max} level of about 19.8 µg/ml at the MRHD.

In vivo safety pharmacology studies with enmetazobactam showed no major effects on the cardiovascular, respiratory and nervous system at doses up to 240 mg/kg/day in dogs and 500 mg/kg/day in rats. These doses correspond to an exposure C_{max} of approximately 44-fold and 68-fold in dogs and rats, respectively, compared to the recommended clinical dose at 0.5 g enmetazobactam and 2 g cefepime TID.

Overall, the conducted secondary and safety pharmacology studies with enmetazobactam do not indicate a risk for human safety.

Concerning cefepime, the safety data (Renapime; SmPC) revealed no major effects on the cardiovascular, respiratory or nervous system, and considering the clinical experience there is no concern for human use.

Pharmacokinetics

It is noted that the systemic exposure reached in pivotal toxicity studies are in general low which results in low or no margins to the clinical exposure at the maximum recommended human dosing regimen. In order to reach higher systemic exposure during 24 hours at steady state, dosing twice daily in the pivotal toxicity study may have been more appropriate.

Whereas coadministration did not affect the single dose or steady state TK parameters for enmetazobactam in dogs or for cefepime in rats and dogs, the AUC_{last} for enmetazobactam was higher and the estimated values for CL and t½ were lower and longer, respectively, when given together with cefepime in rats. This may be due to the use of different matrices for the TK analyses. Based on the low blood to plasma ratio for enmetazobactam (between 0.591 and 0.608 in rat) the observed differences with lower AUC_{last} in blood compared to plasma when given alone and together with cefepime, respectively, seems reasonable and not an indication of that concomitant administration of cefepime affects the PK of enmetazobactam. Of the same reason the margins between the systemic exposure in the pivotal rat toxicity studies and the clinical exposure at the maximum recommended human dosing regimen are lower than reported.

Repeated dose toxicity

In rats and dogs given IV-administrations of enmetazobactam or cefepime the main toxic effects occurred in the liver. The liver effects induced by enmetazobactam in rats and dogs and by cefepime in rats did not change or exacerbate when the substances were given in combination (enmetazobactam/cefepime 1:2). The recommended clinical dosing regimen is 500 mg enmetazobactam/2 g cefepime (1:4) TID. The Applicant acknowledges that the combination ratio was dissimilar between non-clinical and clinical studies, as the exact ratio was not defined during the preclinical development.

As the hepatocellular glycogen accumulation observed in rats was not associated with any changes in tested plasma liver enzymes, except for a slight increase in ALP, and showed some reversibility, the applicant classified this finding as non-adverse. This is questioned based on the moderate-severe grading of glycogen accumulation and limited reversibility for this finding. Nonetheless, the applicant defined a NOAEL for the 28-day enmetazobactam study to 125 mg/kg taking into account the increased liver weight and decreased body weight observed at the 250 mg/kg/day dose. This NOAEL can be agreed. However, for the combination study the applicant has set the NOAEL to the highest dose level 250/500 mg/kg enmetazobactam/cefepime. Based on the lower than dose proportional increase in exposure to enmetazobactam between the low and high dose combination the increased levels of alkaline phosphatase at the 250/500 mg/kg dose level should also be taken into account. It is therefore appropriate to consider the moderate hepatocyte vacuolation at the 250/500 mg/kg dose level as adverse and to decrease the NOAEL of the rat combination toxicity study from 250/500 mg/kg/day, as proposed by the Applicant, to 125/250 mg/kg/day.

The liver effects in terms of hepatocyte degeneration/necrosis were most likely attributed to an enmetazobactam-related disturbance of glycogen storage mechanisms, which occurred in dogs at 120 mg/kg/day and in terms of moderate to severe hepatocellular accumulation of glycogen in rats from 250 mg/kg/day when enmetazobactam was given alone. When enmetazobactam was given in combination with cefepime corresponding adverse liver effects were observed at 200/400 mg/kg/day in dogs (639 µgxh/mL) and at 250/500 mg/kg/day in rats (195 µgxh/mL). These doses correspond to an AUC exposure marginal of 0.86 in rats and 2.8 in dogs when compared with the AUC exposure at MRHD (226 µgxh/mL). Regarding the enmetazobactam-mediated liver findings, there seems to be a common ethology behind the findings in rats and dogs, namely a potential of enmetazobactam to alter glycogen storage and/or structure in hepatocytes. Moreover, the dog appeared to be more sensitive to these alterations based on the presence of single cell cystic degeneration/necrosis accompanied by significant elevations of liver enzymes and decreases in total and HDL cholesterol. At the doses evaluated in rats and dogs there were no major functional consequences detected. Moreover, while most findings were still detectable in recovery animals there was indication of reversibility, albeit at a slow pace. This is reassuring. However, given that the highest dose levels tested in the non-clinical safety evaluations did not allow for an appropriate definition of MTD in animals at a reassuring margin to clinical exposure, and further, that there is an identified risk for elevated liver enzymes in patients, there is an uncertainty whether there could be risk for more severe hepatotoxicity if plasma exposures to enmetazobactam would reach a supratherapeutic level in a patient. As adverse liver effects in terms of hepatocyte degeneration/necrosis and related hepatocellular accumulation of glycogen in dogs and hepatocellular accumulation of glycogen in rats, which seem to be clinically relevant, are induced by enmetazobactam at doses with low or no margin to the exposure at the MRHD this information have been included in SmPC 5.3. For the NOAELs in the combination studies of enmetazobactam given together with cefepime, the margin to the exposure at the MRHD was 0.71 in dogs and 0.86 or lower in rats. Furthermore, as also noted by the applicant, glycogen accumulation has been reported in non-clinical studies with the structurally related tazobactam and sulbactam possibly indicating that a disturbance of glycogen storage may be a class effect.

A modest, but widespread suppression of drug metabolising enzymes and transporter (DMET) mRNAs, including BCEP, MDR3, and ABCG5/8 was also noted. Loss of function of these transporters may induce liver injury due to the toxic effects of bile acids on both hepatocytes and biliary epithelial cells.

Increased liver weights were observed in adult rats given IV doses of enmetazobactam alone and in combination with cefepime and, together with microscopic hepatocyte hypertrophy, in juvenile rats given SC doses of enmetazobactam. On the contrary, no liver weight changes in dogs were reported. The Applicant suggested that the cause of the increased liver weight could be attributed to an increase in elimination pathways associated with this organ. On the contrary, no liver weight changes in dogs

were reported, indicating that the cause of the increased liver weight could not be attributed to an increase in elimination pathways associated with this organ.

The NOAELs in the repeated dose toxicity studies were set to 60 mg/kg/day in dog (AUC_{last}: 212 µgxh/mL) and 125 mg/kg/day in rat (AUC_{last}: 56.7 µgxh/mL) for enmetazobactam administered alone and to 50/100 in dog (AUC_{last} for enmetazobactam: 160 µgxh/mL/AUC_{last} for cefepime: 387 µgxh/mL) and 250/500 mg/kg/day or lower in rat (AUC_{last} for enmetazobactam: 195 µgxh/mL/AUC_{last} for cefepime: 551 µgxh/mL) for enmetazobactam/cefepime. At these NOAELs there were no or limited exposure margins (approximately 0.94 and 0.71/0.34X in dogs, and 0.25 and 0.86 or lower/0.48 or lower X) to the clinical AUC exposure to enmetazobactam/cefepime at MRHD of 0.5 g enmetazobactam and 2.0 g cefepime TID (226/1139 µgxh/mL) predicted in Study AT-301.

In the pivotal rat toxicity studies the TK of enmetazobactam was measured in blood. Due to low blood to plasma ratio for enmetazobactam (between 0.591 and 0.608 in rat) the margins between the systemic exposure in the pivotal rat toxicity studies and the clinical plasma exposure at the maximum recommended human dosing regimen are therefore lower than reported.

Reproductive toxicity

The were no safety margins from animal to human exposure ratio for enmetazobactam in the reproduction and development studies. The results from the reproductive toxicity studies of enmetazobactam resembles those of the comparator, and a class effect to tazobactam cannot be excluded, which is mirrored in the SmPC section 4.6 and 5.3.

EFD: In the rat study, a lower foetal bodyweight correlating with a higher number of incomplete ossifications of the skull bones in comparison to the submitted historical control data was recorded for the group treated with 500 mg/kg/day. Since no other variations/malformations were detected at other dose levels, a NOAEL at the mid-dose group (250 mg/kg/day) can be agreed. In the EFD study in rabbit malformations of sternum were observed, which according to the CRO, was difficult to interpret and consisted of variations at the same site with fused sternebrae, but with preserved length of the sternum and was recorded in individual foetuses at 150 and 300 mg/kg/day (3 foetuses from 2 litters at 150 mg/kg/day and 10 foetuses from 4 litters at 300 mg/kg/day). Margins to human exposure in rabbit at 50 mg/kg (NOAEL) was x 1.10 and at 150 mg/kg/day, x 3.7 times the human exposure at the intended clinical dose level. Moreover, a higher mean % of post-implantation loss at all dose levels was observed in the EFD study in rabbit (x2.3-3.3 times of the findings in control animals). Collectively, data from the EFD studies suggest that there is a risk for foetal toxicity, and it cannot be assumed that the developmental effects reported in the EFD studies are secondary to maternal toxicity and growth retardation. These observations have been described in SmPC section 4.6 and 5.3.

PPND: In the PPND study, the body weight in males appears to be lower with increased dose until end of maturation phase, however, only reaching a level of significance on post-natal day 42 and 49. A slight delay in the pre-weaning development were observed at the mid- and high dose group, (pupil reflex and pinna detachment). Milk exposure investigated in F0 satellite animals at one time point showed a large variation in concentration. A conclusion regarding effects from milk exposure cannot be drawn. Mean scores for motor activity on week 6/7 for males in the high dose was 79% of that in control. Findings in the PPND study were observed at clinically relevant exposure levels and appropriate information have been included in the SmPC section 4.6 and 5.3.

Juvenile toxicity: At the end of dosing in the juvenile toxicity study, treatment did not affect body weights in either sex in comparison to vehicle treated animals following either administration route. Remarkably, at the end of recovery mean body weight and absolute body weight gain was higher in the subcutaneously enmetazobactam treated group for both sexes in comparison to control animals. A higher food intake was recorded in females from day 31-42. Findings persistent at the high dose level

after recovery was observed and include increased liver to body weight ratios for both sexes and administration routes except for IV-treated females after recovery. The tolerability was lower to SC administration. Since the product is aimed for treatment of the adult population via intravenous route, issue is not pursued.

Environmental risk assessment

Cefepime

The applicant has submitted a declaration over the use of cefepime in all members states concerned, including historical consumption data, supporting the conclusion that total environmental exposure is unlikely to increase and that a full ERA procedure is not necessary.

Enmetazobactam

As the ERA has no full study package at time of CHMP Opinion, a full regulatory assessment – and any formal decision on environmental risk – needs to be delayed to when the full ERA study package has been submitted, which the applicant has committed to submit within maximum 2 years post-approval, covering all the above aspects, as a follow-up post-authorisation measure (see letter of Recommendations).

2.4.7. Conclusion on the non-clinical aspects

The CHMP considers the following measures necessary to address the non-clinical issues:

The MAH is recommended to submit an updated ERA within two years of approval.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Overview of clinical studies

The clinical development program for cefepime-enmetazobactam included 6 clinical studies:

- Study AT-101, a Phase 1 study with both single-ascending and multiple-ascending doses of enmetazobactam alone and in combination with cefepime in healthy adult male subjects
- Study AT-102, a Phase 1 study of cefepime-enmetazobactam in adults with varying degrees of renal impairment or normal renal function. The effect of dialysis in subjects with end-stage renal disease (ESRD) was also assessed in this study
- Study AT-103, a Phase 1 study evaluating the concentrations of cefepime-enmetazobactam in plasma and epithelial lining fluid (ELF)
- Study AT-104, a mass balance and metabolite identification study in healthy adult male subjects
- Study AT-201, a Phase 2 study to determine the optimal cefepime-enmetazobactam combination dose and the probability of target attainment (PTA) in subjects with cUTI, including AP

- Study AT-301, a Phase 3 study to assess the efficacy of cefepime-enmetazobactam compared with piperacillin-tazobactam in the treatment of cUTI, including AP

The clinical studies mainly relevant to the applied indications is study AT-301 to provide comparative safety and efficacy data and PK-data from patients to update the PopPK model for PTA analyses and study AT-103 to provide enmetazobactam PK-data from ELF to support treatment of lung infections.

It should be noted that phase 3 studies evaluating a beta-lactam (BL) at its approved dose combined with a new beta-lactamase inhibitor (BLI) may not provide stand-alone efficacy data to support the dose regimen for the BLI. This is because it is not a strict requirement to confine such studies to infections caused by pathogens resistant to the BL but susceptible to the BL-BLI combination. Therefore, the PK/PD analyses incorporating the non-clinical PK/PD data and patient PK data are generally considered pivotal to support the dose regimen of the BLI for the proposed indication(s).

The applicant has received CHMP scientific advice (2018 and 2019) regarding the completeness and robustness of the non-clinical animal PK/PD package, the proposed Phase 3 trial design and the adequacy of the Phase 1 ELF penetration study results in support of the use of cefepime-enmetazobactam in HAP and VAP indications. In general, the CHMP considered the proposed non-clinical package and clinical programme appropriate to support the proposed indications. However, the applicant was informed that cefepime-enmetazobactam would not be eligible for a pathogen-specific indication in patients with limited treatment options as currently proposed.

2.5.2. Clinical pharmacology

2.5.2.1. Pharmacokinetics

Cefepime/enmetazobactam was developed as a fixed dose combination of cefepime 2 g and enmetazobactam 0.5 g for administration by intravenous (IV) infusion. Cefepime is a well-known cephalosporin and enmetazobactam is a new beta-lactamase inhibitor developed to enhance the activity of cefepime by inhibiting beta lactamases in resistant bacteria.

Enmetazobactam is a new chemical entity, and the pharmacokinetic studies should thus aim at describing the disposition and also to identify subgroups where an altered exposure can be expected based on the pharmacokinetic properties. Potential interactions should also be evaluated.

Methods

Bioanalytical methods

Enmetazobactam and cefepime have been measured in plasma, urine and bronchoalveolar lavage fluid using validated HPLC/MS methods. Piperacillin was investigated in the early phase of the program, but it was decided to select cefepime for the dose combination which led to the discontinuation of the piperacillin-enmetazobactam program.

Non-compartment data analysis

Standard non-compartmental analysis was performed in all studies where rich sampling was applied.

Population PK (PopPK)

Pharmacokinetic data was analysed using non-compartmental methods as well as population pharmacokinetic analyses.

2.5.2.2. Evaluation and Qualification of Models

Several PopPK analyses have been submitted. The combined cefepime-enmetazobactam PopPK model from Report AAI101-PK-21-01 was used for the PTA simulations and the model is described below. In addition, a PopPK analysis of cefepime and enmetazobactam in ELF was conducted which is described further down in this section.

PopPK analysis of cefepime and enmetazobactam

Models for cefepime and enmetazobactam were originally constructed using data from the Phase 1 and Phase 2 studies and were updated with data from the Phase 3 study, AT-301 (Report AAI101-PK-21-01). This model was used for PTA simulations.

The objectives of the PopPK analysis included the following:

- To characterise PopPK for enmetazobactam and cefepime using combined data from the Phase 1, Phase 2, and Phase 3 studies.
- To confirm subject factors that impact exposure to enmetazobactam and/or cefepime that were identified in previously conducted combined analysis of the Phase 1 and Phase 2 studies
- To determine if there are any other covariate effects.

Dataset

PopPK modelling included data from studies AT-101, AT-102, AT1-03, AT-201 and AT-301 (Table 3). All studies were in adult subjects. Most of the subjects in the dataset were from the phase III study (488 out of 663 subjects).

Table 3: Studies included in PopPK analysis.

Study number	Phase	Population	Part or group	FEP/ENM doses	Number of individuals in PK analysis dataset
AT-101	Phase I	Healthy volunteers	SAD	C1: 0/13.44 IV 30 min	2
				C2: 0/600 IV 30 min	3
				C3: 0/1000 IV 30 min	6
				C4: 0/2000 IV 30 min	6
				C5: 0/4000 IV 30 min	6
				C6: 0/1000 IV 30 min q6h x4	6
				C7: 0/2000 IV 30 min q6h x4	6
			DDI[†]	C8: 0/2000 IV 30 min and 2000/0 IV 30 min and 2000/2000 IV 30 min	8
			MAD Solo	C9: 0/1000 IV 30 min q1d x14	8
				C10: 0/2000 IV 30 min q1d x14	8
			MAD Combo	C11: 1000/500 IV 30 min q1d x14	8
				C12:500/0 IV 30 min q1d x14	8
			MAD CInf	C13: 0/4'000/day IV continuous infusion over 14 days	8
AT-102	Phase I	Subjects with varying degrees of renal function	Normal	1000/500 IV 2 hr	6
			Mild renal impairment	1000/500 IV 2 hr	6
			Moderate renal impairment	1000/500 IV 2 hr	6
			Severe renal impairment	500/250 IV 2 hr	6
			ESRD	500/250 IV 2 hr	6
AT-103	Phase I	Healthy volunteers		2000/1000 IV 2 hr	19
AT-201	Phase II	Adults with cUTI, including AP	Cohort 1	1000/500 IV 2hr q8h	15
				1000/0 IV 2hr q8h	6
			Cohort 2	2000/750 IV 2hr q8h	14
				2000/0 IV 2hr q8h	8
AT-301	Phase III	Adults with cUTI, including AP	eGFR ≥60 mL/min/1.73m^2	2000/500 IV 2 hr q8h	390
			eGFR <60 mL/min/1.73m^2 and ≥30 mL/min/1.73m^2	1000/250 IV 2 hr q8h	98

[†]Randomized, two-sequence, 5-way crossover study to evaluate the potential interaction between a single IV dose of enmetazobactam and piperacillin on one hand, and between a single IV dose of enmetazobactam and cefepime on the other hand, in one cohort of 8 subjects. Abbreviations: SAD – single ascending dose, MAD - multiple ascending dose, DDI – drug-drug interaction, CInf – Continuous infusion.

Table 4: Covariate summary

Table 6: Covariate summary statistics from the combined FEP/ENM PK analysis data set.

Covariate (units)	Mean (min – max) or N (%)					
	Overall	AT-101	AT-102	AT-103	AT-201	AT-301
Number of subjects	663	83	30	19	43	488
Age (years)	51.62 (17 - 94)	33.57 (21 - 45)	57 (35 - 70)	33.32 (19 - 64)	52.07 (18 - 82)	55.04 (17 - 94)
Body weight (kg)	76.52 (45 - 135)	74.3 (49 - 97)	81.04 (55.5 - 108)	78.05 (59 - 99)	81.62 (50 - 122)	76.11 (45 - 135)
BMI (kg/m^2)	26.54 (15.9 - 45.9)	23.87 (18.2 - 30)	28.13 (17.9 - 34.8)	25.73 (21.1 - 32)	28.64 (18.4 - 38.5)	26.75 (15.9 - 45.9)
BSA (m^2)	1.89 (1.37 - 2.63)	1.9 (1.49 - 2.24)	1.95 (1.64 - 2.31)	1.94 (1.62 - 2.2)	1.94 (1.49 - 2.46)	1.88 (1.37 - 2.63)
Albumin (g/dL)	40.38 (21 - 51)	43.2 (43.2 - 43.2)	41.24 (33.6 - 47.7)	46.11 (43 - 51)	38.21 (22 - 47)	39.81 (21 - 50)
Bilirubin (µmol/L)	11.91 (3 - 51.5)	15.91 (10 - 51.1)	10.04 (4 - 30.5)	7.79 (3 - 12)	9.96 (3.6 - 27.5)	11.67 (3.6 - 51.5)
Creatinine clearance (mL/min)	96.77 (8.9 - 246.9)	120.51 (84.4 - 164.8)	55.82 (8.9 - 171)	138.05 (96.1 - 193)	111.39 (51.6 - 246.9)	92.35 (26.1 - 244.1)
eGFR (mL/min/1.73m^2)	81.03 (4.4 - 166)	96.97 (75.1 - 142.9)	41.54 (4.4 - 104.9)	102.79 (75.9 - 140)	89.5 (31.2 - 166)	79.15 (17.7 - 164.8)
De-indexed eGFR (mL/min)	87.98 (5.21 - 195.84)	106.35 (79.03 - 140.03)	47.22 (5.21 - 140.12)	114.98 (83.23 - 160.37)	98.58 (42.8 - 195.84)	85.37 (21.36 - 193.45)
Sex						
Female	310 (47 %)	0 (0 %)	11 (37 %)	10 (53 %)	27 (63 %)	262 (54 %)
Male	353 (53 %)	83 (100 %)	19 (63 %)	9 (47 %)	16 (37 %)	226 (46 %)
Race						
American Indian or Alaska Native	3 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	3 (1 %)
Asian	4 (1 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	4 (1 %)
Black or African American	1 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	1 (0 %)
Other	14 (2 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	14 (3 %)
White/Not Hispanic or Latino	641 (97 %)	83 (100 %)	30 (100 %)	19 (100 %)	43 (100 %)	466 (95 %)
Infection						
AP	252 (38 %)	0 (0 %)	0 (0 %)	0 (0 %)	26 (60 %)	226 (46 %)
cUTI	17 (3 %)	0 (0 %)	0 (0 %)	0 (0 %)	17 (40 %)	0 (0 %)
cUTI with removable source of infection	111 (17 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	111 (23 %)
cUTI without removable source of infection but with other risk factors	151 (23 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	151 (31 %)
Healthy	132 (20 %)	83 (100 %)	30 (100 %)	19 (100 %)	0 (0 %)	0 (0 %)

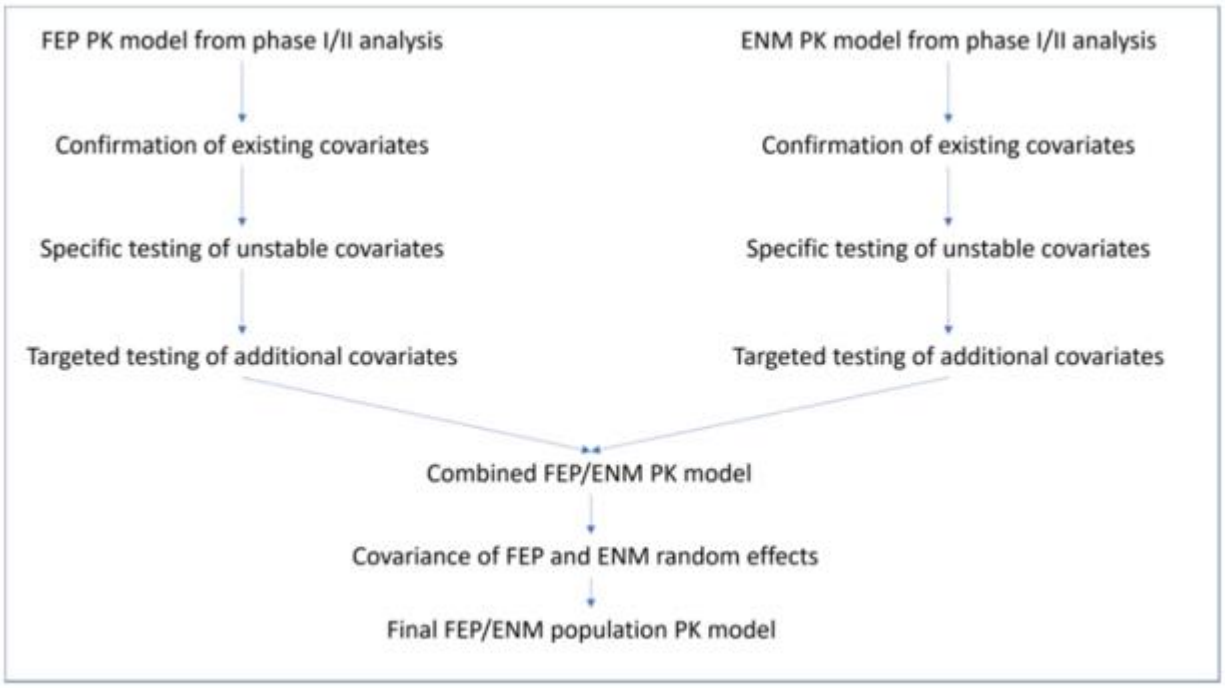
Methods

The starting model for cefepime was the final PK model from the combined phase I+II analysis (Allecra Document Number AAI101-PK-19-07). Data BLQ were included in the PopPK analysis using the censored methodology available with Monolix.

The steps of the modelling are summarised in Figure 3.

Population parameters were estimated using the stochastic approximation of expectation-maximisation (SAEM) algorithm implemented in Monolix. The maximum (minimum) number of iteration steps were set to at least 2500 (1500) and 1000 (200) in all modelling runs. For the covariate model building, a stepwise forward inclusion and stepwise backward elimination approach (stepwise covariate modelling method) was used.

Figure 3: PopPK modelling approach



Source: Study AAI101-PK-21-01, Figure 1

Final cefepime-enmetazobactam joint model evaluation

A final joint model was developed. Both cefepime and enmetazobactam plasma PK were best described with two-compartmental PK models with linear clearances from the central compartment after IV infusion. The sparse sampling in AT-301 did not allow to characterise the distribution into the peripheral compartment. The estimate of Q was therefore fixed to the final estimate from the phase I+II analysis. The cefepime part of the model and the enmetazobactam part of the model are presented separately further down in this section for the report.

Cefepime part of the combined model

Cefepime plasma PK were best described with two-compartmental PK models with linear clearances from the central compartment after IV infusion. PopPK results for cefepime in the combined PopPK model are provided in Table 5 for a typical subject with a body weight of 70 kg, de-indexed eGFR of 100 mL/min, and an age of 50 years (Note: sex was not identified as a significant covariate on cefepime PK and thus, was not used in defining a reference individual). From these parameters, a terminal plasma half-life of 2.2 hours was calculated. The cefepime VPCs stratified by study showed good agreement of the model predicted percentiles with the observed percentiles.

Table 5: PopPK results for Cefepime

	Final Combined Cefepime-Enmetazobactam PK Model
Fixed effects	
Clearance (L/h)	5.95 (16, 30)[a]
Central volume of distribution, V1 (L)	11.2 (-, 44)
Intercompartmental clearance (L/h)	7.22 (-)
Peripheral volume of distribution, V2 (L)	5.7 (20)
Correlations	
V1, Cl in cUTI	0.261
Covariate coefficients	
Age on Cl	-
De-indexed eGFR on clearance	0.834
Age on V1	0.176
Body weight on V1	0.802
Albumin level on V2	-
De-indexed eGFR on V2	-
Observational error	
Constant error	-
Proportional error	0.253

Note: for a typical subject with a body weight of 70 kg, de-indexed eGFR of 100 mL/min, and an age of 50 years.
[a]For values in parentheses, the first value is the %CV for healthy subjects and the second value is the %CV for infected subjects.
cUTI=complicated urinary tract infection; eGFR=estimated glomerular filtration rate; V1=central volume of distribution; V2=peripheral volume of distribution
Source: Study AA101-PK-21-01, Table 19.

Enmetazobactam part of combined model

Enmetazobactam plasma PK were best described with two-compartmental PK models with linear clearances from the central compartment after IV infusion PopPK results for enmetazobactam in the joint PK model are provided in Table 6 for a typical, non-infected, female subject with a body weight of 70 kg, de-indexed eGFR of 100 mL/min, and an age of 50 years. For infected subjects, the estimated central volume of distribution was slightly larger with 15.3 L.

From these parameters, a terminal plasma half-life of 2.0 hours in infected subjects was calculated.

The enmetazobactam VPCs stratified by study showed good agreement of the model predicted percentiles with the observed percentiles.

Table 6: PopPK results for Enmetazobactam

	Final Combined Cefepime-Enmetazobactam PK Model
Fixed effects	
Clearance (L/h)	7.68 (15, 37)
Central volume of distribution, V1 (L)	13.2 (5, 50)
Intercompartmental clearance (L/h)	7.16 (-)
Peripheral volume of distribution, V2 (L)	5.28 (6)
Correlations	
V1, Cl in healthy	0.983
V1, Cl in cUTI	0.438
Covariate coefficients	
De-indexed eGFR on Cl	0.898
cUTI infection on V1	0.145
Age on V1	0.146
Body weight on V1	0.618
Gender on V2	0.198
cUTI infection on V2	-
Body weight on V2	-
De-indexed eGFR on V2	0.259
Observational error	
Constant error	40.9
Proportional error	0.236

Estimates for a reference individual with a body weight of 70 kg, de-indexed eGFR of 100 mL/min, Sex = Female, and Infection = healthy
cUTI=complicated urinary tract infection; eGFR=estimated glomerular filtration rate; V1=central volume of distribution; V2=peripheral volume of distribution
Source: Study AA101-PK-21-01, Table 20.

Updated model

The PopPK model was updated using equal allometric parameters for central and peripheral compartments. The newly estimated BW-covariate coefficient on the central and peripheral volume of distribution is 0.407 for cefepime and 0.553 for enmetazobactam. These estimates are slightly smaller than the previous covariate coefficients on the central volumes of distribution of 0.618 for cefepime and 0.802 for enmetazobactam. The -2-log likelihood (-2LL) was better by 16 points for the new model despite the same number of parameters for the two models. Diagnostic plots indicated that the models fit the data well.

PopPK analysis of cefepime and enmetazobactam in ELF

The objectives of the PopPK analyses included the following:

1. Model the PopPK of enmetazobactam and cefepime in the plasma and the ELF in study AT103
2. Derive the ELF-to-plasma ratio of enmetazobactam and cefepime

PopPK modelling included data from Study AT-103, a Phase 1, open-label, single-centre, multiple-dose study to assess the concentration of enmetazobactam and cefepime in bronchial ELF in healthy volunteers. The study investigated 1000 mg enmetazobactam and 2000 mg cefepime given every 8 hours as 2-hour IV infusions over a period of 72 hours. Plasma samples were obtained at Day 4 pre-dose and at times 1, 2, 2.5, 3, 4, 6, 8, 12, 16 and 24 h. A single bronchoalveolar lavage fluid (BALF) supernatant sample per subject was obtained at day 4 at times 2, 4, 6 or 8 h.

The plasma and ELF PopPK models of enmetazobactam and cefepime (Table 7) were established using observations from 19 healthy volunteers from study AT-103, with a total of 207 enmetazobactam observations and 225 cefepime observations. The enmetazobactam and cefepime plasma

concentrations were described with a two-compartmental, linear PK model. No covariate model was developed. The visual predictive checks showed that the model predicted the observations satisfactory.

Table 7: Cefepime and enmetazobactam ELF PopPK model parameter estimates

Description	Parameter	Enmetazobactam		Cefepime	
		Estimation (%CV)[a]	RSE[b]	Estimation (%CV)	RSE
Clearance	Cl (L/day)	7.77 (12)	3.02	7.2 (15)	3.39
Central volume of distribution	V1 (L)	13.5 (15)	7.21	12.2 (18)	8.21
Exchange rate	Q2 (L/day)	4.76	27.1	6.45	23
Peripheral volume of distribution	V2 (L)	4.8 (19)	14.6	6.11 (18)	12.2
Offset of the linear biodistribution model	BC_0 (-)	0.301	20.4	0.185	35
Rate of increase of the biodistribution	α_{BC} (1/h)	0.0395	38.3	0.0687	25.7

[a]%CV computed as 100*sqrt(exp(SD^2)-1).
[b]Linearization used for estimation of relative standard error (RSE).
Source: Study AAI101-PK-19-08.

Enmetazobactam and cefepime distribution kinetics into ELF were similar for both compounds.

At Day 4, when the plasma and ELF PKs are expected to be at steady-state, the biodistribution increased during the 2 to 8 hours observation period from 38% to 62% and from 32% to 73% for enmetazobactam and cefepime, respectively. In order to simulate ELF concentrations from 0 to 2 hours during which time no observations were available, the biodistribution coefficients were linearly interpolated using the biodistribution values at the end of the previous dosing interval and after the end of infusion at 2 hours.

Using the ratio of the ELF-to-plasma fAUCs over the entire 8h dosing interval biodistribution coefficients of 46% for enmetazobactam and 46.8% for cefepime (Table 8) were obtained.

Table 8: Biodistribution Coefficients from fAUC ratios at day 4

Analyte	Parameter	Time period	
		0 – 8 hours	2 – 8 hours
Enmetazobactam	*f*AUC Plasma (µg.h/mL)	129	77.1
	*f*AUC ELF (µg.h/mL)	59.2	35.0
	Biodistribution coefficient	46.0%	45.4%
Cefepime	*f*AUC Plasma (µg.h/mL)	222	134
	*f*AUC ELF (µg.h/mL)	104	61.2
	Biodistribution coefficient	46.8%	45.7%

ELF=epithelial lining fluid; fAUC=area under the free concentration curve
Source: Study AAI101-PK-19-08.

In conclusion, the enmetazobactam and cefepime plasma concentrations were well described with a two-compartmental, linear PK model. Both compounds, enmetazobactam and cefepime penetrated in a similar way in the lung of healthy subjects with a biodistribution coefficient ELF to plasma above 45%.

Simulation methods

4,000 subject per patient group (*normal, mild, moderate renal impairment and augmented renal clearance were simulated*). Simulations used resampling of all covariates (absolute eGFR, body weight, age, sex) from the phase 3 population for all renal function groups except for the group with absolute

eGFR <15 mL/min. For this group, covariates were resampled from the renal impairment study AT-102. For the group of absolute eGFR 15 - <30 mL/min, there were 8 subjects in the phase 3 study AT-301 and 7 subjects in AT-102. For this reason, resampling from the phase 3 population was chosen. The sensitivity of the predictions on this sampling approach was investigated (uniform distribution of eGFR with high/low BW/AGE).

Individual PTAs for cefepime and enmetazobactam were defined as the fraction of simulated individuals who attained the PK/PD target for cefepime or for enmetazobactam, respectively, in the respective dosing interval of the first dose on Day 1 or 7. The joint cefepime-enmetazobactam PTA was defined as the fraction of simulated individuals who simultaneously attained the PK/PD target for both cefepime and enmetazobactam. Since the model allowed to simulate enmetazobactam and cefepime PK in the same individuals the simultaneous attainment of both targets could be directly evaluated from the simulations.

Absorption

Absorption is not applicable as this product is for IV infusion only. The clinical studies were performed using separate vials of the two active substances and not with the commercial fixed-dose combination product. The powder formulations used are however dissolved into solutions before administration, and there are no excipients that interact with the drug substance (e.g., complex formation). The reconstituted and diluted solution used during the clinical trials is also very similar to the one obtained following reconstitution and dilution of the commercial formulation.

Distribution

Based on the PopPK analysis, the total volume of distribution was 16.9 L for cefepime and 20.6 L for enmetazobactam.

No *in vitro* binding of enmetazobactam to plasma proteins were observed in the concentration range 1-300 µg/mL. The serum protein binding for cefepime is approximately 20%, independent on concentration.

Based on *in vitro* data, the mean blood/plasma ratio of enmetazobactam in human blood was 0.57 (range 0.491 to 0.660, no indication of concentration dependency) and thus did not indicate preferential partition into blood cells. Blood/plasma ratio from the mass balance study was around 0.8, thus consistent with the *in vitro* data and indicate no extensive portioning into the red blood cells. No blood/plasma ratio data was presented for cefepime.

An epithelial lining fluid (ELF) study in healthy volunteers was performed (Study AT-103). Enmetazobactam and cefepime distributed to ELF to a similar extent. The exposure in this compartment was however lower than that in plasma. The ELF biodistribution coefficient increased during the observation period of 2 to 8 h for both substances. Based on the pop-PK model, ELF penetrations at different time points and a biodistribution coefficient based on the entire dosing interval were obtained. The biodistribution coefficients over the entire dosage interval for the two substances were very similar; 47% for cefepime and 46% for enmetazobactam.

Elimination

Both cefepime and enmetazobactam are primarily excreted as unchanged drug in the urine.

The mean elimination half-life of cefepime at a dose of 2g and administered in combination with enmetazobactam 500 mg in cUTI patients was 2.7 hours (study AT-301). For cefepime, a half-life of approximately 2 hours has previously been reported according to approved SmPCs.

Mean renal clearance for enmetazobactam was 5.4 L/h and mean total clearance for enmetazobactam was 8.1 L/h. (Study AT-104)

Following a single IV dose of 500 mg [^{14}C]-enmetazobactam in healthy subjects, the geometric mean elimination $t_{1/2}$ was 2.3 hours (geometric CV% 13.9; Study AT-104).

The mean elimination half-life of enmetazobactam administered at a dose of 500 mg and administered in combination with cefepime 2g in cUTI patients was 2.6 hours (study AT-301).

Mass balance

Urinary recovery of unchanged cefepime accounts for approximately 85% of the administered dose.

Study AT-104 was a Phase 1, open-label, single-dose, single-centre study in 10 healthy male volunteers to assess the mass balance, PK, and routes of elimination of enmetazobactam and to determine the metabolic profiles for enmetazobactam. A single [^{14}C]-labelled enmetazobactam dose of 500 mg (which is the suggested clinical dose) was administered IV over 2 hours. Approximately 99.1% of the drug related material was recovered in the excreta by 168 hours with 98.9% of the dose excreted in urine (97.4% recovered by 24 hours) and 0.172% excreted in faeces. In urine 90% of the recovered radioactivity during the first 24 hours could be identified as unchanged drug.

Metabolism

No new *in vitro* metabolism data for cefepime has been submitted. Based on previous information, cefepime is primarily excreted as unchanged drug in the urine but is metabolised to a small extent (to N-methylpyrrolidine).

In vitro-metabolism of enmetazobactam was studied in human liver microsomes and human hepatocytes. Data do not indicate significant hepatic metabolism. In the hepatocyte study, one metabolite was detected, named M2 (accounting for 5% of the total radioactivity following 1.5 hours incubation in human hepatocytes). This metabolite was also detected following incubation with rat, dog and rabbit hepatocytes. As turnover was low, the applicant did not continue with additional studies in order to determine which enzyme that was responsible for the metabolism.

Unchanged enmetazobactam represented 94.7% of the total radioactivity in plasma and 90% of the total radioactivity in urine based on plasma and urine samples collected in the first 24 hours after drug administration (study AT-104). Only plasma and urine were profiled as excretion in faeces was extremely low (<1%). The sulfinic acid impurity (formed either through metabolism or extraction process degradation), coming from the cleavage of the thiazolidine ring to form an inactive metabolite, contributed to the remaining drug-related material in both plasma (<6%) and urine (10%).

Pharmacokinetics of metabolites

No major or active metabolites of enmetazobactam are detected. There is one metabolite/degradation product that is inactive and not classified as major.

Interconversion

Enmetazobactam contains three chiral centres and is a pure enantiomer with a 2S, 3S, 5R configuration.

Dose proportionality and time dependencies

Pop-PK analysis indicates linear PK of cefepime across the range 1 g to 2 g. Based on previous data the PK properties of cefepime has been reported to be linear in the range 250 mg to 2 g when given intravenously. There are no indications of accumulation of cefepime when given every 8 hours.

Following single doses in the range 600 mg to 4 g, there were dose-proportional (or very close to dose-proportional) increases in AUC and C_{max} for enmetazobactam. There are no indications of non-linearity when comparing dose-adjusted parameters and in the power analysis the point estimates are

very close to 1 (although the upper limits of the 90% CI were slightly above the pre-defined acceptance criteria). (Study AT-101 SAD part)

Following multiple doses of enmetazobactam, plasma peak concentrations and exposures increased approximately linearly when the enmetazobactam dose was increased from 1 gram to 2 grams. (Study AT-101 MAD part).

With the recommended dose of 500 mg enmetazobactam given three times daily (together with cefepime), no accumulation was observed (study AT-101). There were no signs of time dependency in the clearance of enmetazobactam.

Intra- and inter-individual variability

In the SAD study (study AT-101), variability for enmetazobactam was low to moderate, with CV% for main PK parameters (C_{max} and AUC) in the range of 7.5 to 23.2%.

Pharmacokinetics in target population

Differences between mean enmetazobactam or cefepime exposures for healthy subjects and subjects with a cUTI infection were negligible (PopPK analysis).

Special populations

Renal impairment

A dedicated renal impairment single dose study (study AT-102) was performed in 30 adults with varying degrees of renal insufficiency or normal renal function. Renal function was calculated using the estimated value for creatinine clearance (CLcr) according to the Cockcroft-Gault formula. The effect of renal impairment on $AUC_{0\text{-}inf}$ was large and was similar for both active substances. For cefepime $AUC_{0\text{-}inf}$ was 1.9-fold, 3-fold, 5-fold and 12-fold higher for patients with mild, moderate, severe RI and ESRD (dosed after dialysis), respectively, compared to subjects with normal renal function. For enmetazobactam the corresponding figures were 1.8-fold, 3-fold, 5-fold and 11-fold increase compared to normal renal function. Dialysis showed a strong effect on $AUC_{0\text{-}inf}$ and a negligible effect on C_{max} of both enmetazobactam and cefepime. $AUC_{0\text{-}inf}$ of enmetazobactam and of cefepime was markedly higher (4-fold and 3-fold, respectively) in ESRD subjects who were dosed after dialysis than in subjects who were dialysed after dosing. The urinary excretion of unmodified enmetazobactam and unmodified cefepime decreased with the degree of RI and was about 14% for enmetazobactam and 22% for cefepime in ESRD patients (when dosed after dialysis).

Based on data from the dedicated RI study as well as on PopPK modelling and PTA simulations, dosage adjustment is proposed in patients who have an estimated glomerular filtration rate (eGFR) less than 60 mL/min (Table 9).

Table 9: Simulation groups and dose adjustments for Exblifep for subjects with different degrees of renal elimination capacity.

Absolute eGFR (mL/min)	Recommended Dosage Regimen for Exblifep (cefepime-enmetazobactam)	Dosing Interval	Infusion Time
ARC (≥150)	cefepime 2 g and enmetazobactam 0.5 g	Every 8 hours	4 hours
Normal (90<150)	cefepime 2 g and enmetazobactam 0.5 g	Every 8 hours	2 hours
Mild (60<90)	cefepime 2 g and enmetazobactam 0.5 g	Every 8 hours	
Moderate (30 to 59)	cefepime 1 g and enmetazobactam 0.25 g	Every 8 hours	
Severe (15< 30)	cefepime 1 g and enmetazobactam 0.25 g	Every 12 hours	

Absolute eGFR (mL/min)	Recommended Dosage Regimen for Exblifep (cefepime-enmetazobactam)	Dosing Interval	Infusion Time
End stage renal disease (<15)	cefepime 1 g and enmetazobactam 0.25 g	Every 24 hours	
Patients requiring haemodialysis	cefepime 1g and enmetazobactam 0.25 g loading dose on the first day of therapy and cefepime 0.5 g and 0.125g thereafter	Every 24 hours	

Monte-Carlo simulations were performed using the updated PopPK model, based on these predictions, the exposure in the different groups is expected to be safe.

Hepatic impairment

No dedicated hepatic impairment study has been submitted for this product. Based on previous data, the PK of cefepime were unaltered in patients with hepatic impairment who received a single 1 g dose. Enmetazobactam does not undergo significant hepatic metabolism. Therefore, the systemic clearance of cefepime and enmetazobactam is not expected to be affected by hepatic impairment. No dose adjustment is proposed in patients with hepatic impairment. Based upon PopPK analyses, no dose adjustment is warranted based on gender, race, age or weight in adults.

The pharmacokinetics of cefepime/enmetazobactam has not yet been evaluated in patients from birth to 18 years old. At the time of submission of the application, the PIP P/0047/2023 was not yet completed as some measures were deferred.

Pharmacokinetic interaction studies

Possible interactions between cefepime and enmetazobactam (and between enmetazobactam and piperacillin) were investigated in a DDI part of the SAD study (AT-101). Apart from this, no *in vivo* interaction studies have been performed. Based on this study, there are no indications of a pharmacokinetic interaction between cefepime and enmetazobactam. Also, there are no indications of an indication of a pharmacokinetic interaction between enmetazobactam and piperacillin.

An in silico PBPK model was created to evaluate the potential for enmetazobactam to in inhibit CYP2E1 activity, due to *in vitro* findings.

Pharmacokinetics using human biomaterials

Cefepime

No *in vitro* data has been submitted regarding cefepime as perpetrator or victim of drug interactions. Cefepime is mainly renally eliminated as unchanged drug and renal elimination occurs mainly via filtration. No pharmacokinetic interactions are described in the SmPC for cefepime.

Enmetazobactam

Enmetazobactam as perpetrator of drug interactions

As the product is used for IV administration the relevant cut-off for assessment of DDI potential *in vivo* is 50*C_{max}, u. Using the input parameters C_{max} = 20 µg/ml (study AT-301, 500 mg dose infused during 2 hours, day 7), fu=1 and Mw=314.318 g/mol, the cut-off used by the assessor is 3182 µM, ie approximately 3 mM.

No direct or time-dependent inhibition of any of the mandatory CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) was observed up to a concentration of 3.5 mM,

which is a sufficiently high concentration to exclude *in vivo*-relevant inhibition (*in vitro* study EMT-002-DDI). In study AAI-PK-17-03 *in vitro*-inhibition of the non-mandatory enzyme CYP2E1 was observed, with an IC_{50} value of 55.8 µM which is well below the systemic cut-off of around 3 mM. In studies AAI101-PK-19-01 and EMT-003-DDI, inhibition was not observed.

Four *in vitro* CYP induction studies were submitted. In study AAI-PK-18-15 (investigating concentrations up to 2.5 mM which is sufficiently high in order to exclude clinically relevant induction effects) more than 2-fold increases in mRNA was not observed, except for one single value marginally above 2-fold increase (2.03) at a single concentration in one donor, and with no concentration dependency observed. In study EMT-002-DDI (investigating concentrations up to 3.5 mM) more than 2-fold increases in mRNA were not observed for any donor or at any concentration tested. Based on these studies, a clinically relevant risk of induction by enmetazobactam is not expected. In study AAI-PK-18-03, based on enzyme activity, there was no signal of induction of CYP3A4 or CYP2B6, but for CYP1A2 there was a slightly more than 2-fold increase in one donor at the two lowest concentrations (no concentration dependency observed). According to the DDI guideline, activity data are not accepted in order to exclude induction, but mRNA data should be used. In study AI-PK-17-02, enmetazobactam caused very large increases of mRNA expression of CYP1A2, CYP2B6 and CYP3A4 in all three tested hepatocyte batches. The effects on CYP2B6 were larger than that of the positive control phenobarbital. Regarding the effects on CYP3A4, in two of the hepatocyte batches the effect of enmetazobactam was similar to that of the positive control inducer rifampicin. A clinically relevant induction effect can be excluded based on the submitted studies (see Discussion).

No inhibition by enmetazobactam of P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1, OATP1B3, MATE1 and MATE2-K was observed in the submitted *in vitro* studies. The concentrations tested were sufficiently high to exclude *in vivo* relevant inhibition.

Enmetazobactam as victim of drug interactions

Enmetazobactam is mainly renally eliminated as unchanged drug and renal elimination occurs mainly via filtration. Thus, pharmacokinetic interactions with enmetazobactam as victim are not expected. Enmetazobactam is also not a substrate of the transporters studied (P-gp, BCRP, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1 and MATE2-K).

Exposure relevant for safety evaluation

The following tables are included in the summary of clinical pharmacology (based on the latest PopPK model):

Table 10: Means and Standard Deviations of the Secondary Enmetazobactam Plasma PK Parameters in Study AT-301 by Day and Last Administered Dose

			Mean (SD)							
Day	Dose (mg)	$N_{subjects}$[a]	C_{max} (µg/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{inf,obs}$ (µg h/mL)	AUC_{last} (µg h/mL)	CL_{ss} (L/h) or CL_{obs} (L/h)[b]	$V_{ss,obs}$ (L)	V_z (L) or $V_{z,obs}$ (L)[c]
Day 1	250	96	10.49 (3.896)	2.005[d] (0.05103)	3.381 (1.682)	47.35 (15.39)	41.42 (16.16)	5.887 (2.120)	20.09 (7.675)	20.87 (7.597)
	500	391	17.37 (5.215)	2 (0)	2.58 (1.051)	71.71 (20.90)	61.55 (18.06)	7.666 (2.649)	24.79 (8.032)	25.88 (7.995)
Day 3	250	67	14.27 (5.257)	49.88 (0.857)	3.862 (1.778)	-	65.65 (29.86)	4.608 (2.057)	22.83 (11.82)	23.28 (11.53)
	500	416	19.38 (5.655)	50.17 (1.049)	2.563 (1.034)	-	73.22 (26.00)	7.735 (2.892)	25.73 (10.55)	26.56 (9.483)
Day 7	250	48	14.12 (4.961)	146 (0.807)	4.103 (1.880)	-	67.13 (29.41)	4.431 (1.931)	23.21 (11.16)	23.64 (10.85)
	500	422	19.82 (6.349)	146.2 (1.061)	2.575 (1.055)	-	75.34 (30.77)	7.64 (2.937)	25.26 (9.969)	26.25 (9.704)

[a]Includes subjects with % AUCinf,obs extrapolated >20%.
[b]CL_{obs} for Day 1, CL_{ss} for Day 3 and Day 7.
[c]$V_{z,obs}$ for Day 1, V_z for Day 3 and Day 7.
[d]A single subject (AT-301-325-007) had a T_{max} of 2.5 hours because of a prolonged infusion duration.
Source: Study AAI101-PK-21-01 Amendment 1

Table 11: Means and Standard Deviations of the Secondary Cefepime Plasma PK Parameters in Study AT-301 by Day and Last Administered Dose

			Mean (SD)							
Day	Dose (g)	$N_{subjects}$[a]	C_{max} (µg/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{inf,obs}$ (µg h/mL)	AUC_{last} (µg h/mL)	CL_{ss} (L/h) or CL_{obs} (L/h)[b]	$V_{ss,obs}$ (L)	V_z (L) or $V_{z,obs}$ (L)[c]
Day 1	**1**	97	49.71 (15.13)	2.005[d] (0.0508)	3.522 (1.643)	220.7 (73.83)	194.4 (59.61)	5.025 (1.596)	17.11 (4.955)	18.17 (5.003)
	2	390	87.09 (22.10)	2 (0)	2.72 (1.059)	364.7 (87.86)	311.0 (76.00)	5.829 (1.565)	19.52 (5.383)	20.61 (5.271)
Day 3	**1**	68	66.87 (19.92)	49.88 (0.850)	4.015 (1.751)	-	307.4 (118.7)	3.75 (1.440)	19.07 (7.490)	19.7 (7.214)
	2	415	98.04 (23.87)	50.17 (1.050)	2.703 (1.038)	-	374.5 (116.3)	5.855 (1.810)	20.41 (7.240)	21.29 (6.089)
Day 7	**1**	49	65.91 (18.12)	146 (0.798)	4.286 (1.821)	-	313.5 (115.4)	3.608 (1.284)	19.66 (6.840)	20.28 (6.532)
	2	421	99.82 (26.43)	146.2 (1.062)	2.712 (1.058)	-	379.5 (123.3)	5.794 (1.852)	20.02 (6.443)	21.05 (6.191)

[a]Includes subjects with % AUCinf,obs extrapolated >20%.
[b]CL_{obs} for Day 1, CL_{ss} for Day 3 and Day 7.
[c]$V_{z,obs}$ for Day 1, V_z for Day 3 and Day 7.
[d]A single subject (AT-301-325-007) had a T_{max} of 2.5 hours because of a prolonged infusion duration.
Source: Study AAI101-PK-21-01 Amendment 1.

2.5.2.3. Pharmacodynamics

This section highlights the studies that describe the *in vitro* activity of enmetazobactam, the potentiation of cefepime activity by the addition of enmetazobactam *in vitro* and *in vivo* and the most important studies and analyses for dose selection of the single components of the fixed-dose-combination.

As laid out in the *Guideline on the evaluation of medicinal products indicated for treatment of bacterial infections (CPMP/EWP/558/95 Rev 3)* for a clinical development programme evaluating a licensed beta-lactam (BL) at the approved dose in combination with a new beta-lactamase inhibitor (BLI) it should be noted that the PK/PD analyses incorporating non-clinical PK/PD data and patient PK data are generally considered pivotal to support the dose regimen of the BLI for the proposed indications.

Mechanism of action

Cefepime is a 4th-generation cephalosporin beta-lactam antibacterial agent that inhibits bacterial cell-wall synthesis by targeting penicillin-binding proteins (PBPs).

Enmetazobactam is a novel penicillanic acid sulfone BLI, structurally related to tazobactam, with inhibitory activity against certain beta-lactamases which prevents the hydrolysis of cefepime.

Primary pharmacology

In vitro

In vitro activity studies

Antimicrobial susceptibility testing

In vitro susceptibility testing was conducted using CLSI methodology with cefepime-enmetazobactam by testing varying concentrations of cefepime in the presence of 8 µg/mL of enmetazobactam.

Antibacterial spectrum

Cefepime exerts antibacterial activity against aerobic gram-negative pathogens that include Enterobacterales, *P. aeruginosa*, *A. baumannii* and the gram-positive pathogens *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*, and Viridans group streptococci. Although cefepime is subject to hydrolysis by class A extended spectrum beta-lactamases (ESBL) and both class A and B carbapenemases, it is generally stable to hydrolysis by class C AmpC and class D OXA-48 beta-lactamases.

The inhibitory activity of enmetazobactam against class A, C and D beta-lactamases, was determined in a nitrocefin chromogen assay with periplasmic beta-lactamase extracts from selected isogenic strains. Across the tested class A beta-lactamases (including non-ESBL variants of SHV and TEM, ESBL, and KPC), enmetazobactam exhibited sub-micromolar IC_{50} values comparable to tazobactam and/or avibactam (Table 12). IC_{50} values were several folds higher for class C beta-lactamases and for class D OXA-enzymes.

Table 12: IC_{50} Values for Enmetazobactam, Tazobactam, and Avibactam Using Periplasmic Extracts Containing Selected beta-lactamases From Isogenic Strains

β-lactamase periplasmic extract	Enmetazobactam IC_{50} (μM)	Tazobactam IC_{50} (μM)	Avibactam IC_{50} (μM)
Class A			
SHV-1 (penicillinase)	0.008 ± 0.001	0.026 ± 0.003	0.035 ± 0.004
SHV (R244S) (IR)	0.56 ± 0.06	0.39 ± 0.04	6 ± 1
SHV-49 (IR)	0.36 ± 0.04	0.73 ± 0.08	0.27 ± 0.03
TEM-1 (penicillinase)	0.007 ± 0.001	0.006 ± 0.001	0.024 ± 0.003
TEM-26 (ESBL)	0.082 ± 0.009	0.023 ± 0.003	0.13 ± 0.01
TEM-30 (IR)	0.29 ± 0.03	0.24 ± 0.02	4.1 ± 0.4
CTX-M-14 (ESBL)	0.006 ± 0.001	0.010 ± 0.001	0.025 ± 0.003
CTX-M-15 (ESBL)	0.007 ± 0.001	0.006 ± 0.001	0.010 ± 0.001
KPC-2 (carbapenemase)	0.36 ± 0.04	3.7 ± 0.4	0.09 ± 0.01
KPC-3 (carbapenemase)	0.52 ± 0.05	4.4 ± 0.5	0.06 ± 0.01
Class C			
CMY-2 (AmpC)	31 ± 3	2.5 ± 0.3	0.07 ± 0.01
E. cloacae NCTC 13406 chromosomal AmpC	25 ± 3	5.1 ± 0.5	0.09 ± 0.01
Class D			
OXA-1 (penicillinase)	8.7 ± 0.9	8.6 ± 0.9	0.9 ± 0.1
OXA-48 (carbapenemase)	11 ± 1	1.5 ± 0.2	1.7 ± 0.2
OXA-51 (carbapenemase)	> 100	> 100	> 100
OXA-58 (carbapenemase)	> 100	26 ± 3	13 ± 1

Abbreviations: ESBL=extended-spectrum β-lactamase; IC_{50}=half maximal inhibitory concentration; IR=inhibitor-resistant (clavulanic acid) β-lactamases.
Source: adapted from Study AAI101-MI-17-01.

The spectrum of activity of cefepime-enmetazobactam have been determined against clinical isolates from surveillance programmes, isolates from clinical trials and challenge sets of isolates enriched in specific resistance mechanisms. In the 5 years up to 2022, the *in vitro* activity was determined against a total of 9,905 isolates of Enterobacterales (Table 13).

Table 13: Summary of *in vitro* activity of cefepime-enmetazobactam and comparator agents against the tested Enterobacterales isolates (n=9,905) from 2017-2021

Antibacterial agent	MIC (µg/ml)			CLSI			EUCAST		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Cefepime[a]	0.06	16	≤0.008->64	86.4	3.4	10.2	84.1	4.2	11.8
Cefepime-enmetazobactam[b]	0.03	0.25	≤0.008->64	*98.2*	*0.5*	*1.3*	*97.6*	*0.9*	*1.5*
Meropenem	0.03	0.06	≤0.004->32	97.6	0.3	2.0	98.0	1.3	0.7
Piperacillin-tazobactam[c]	2	32	≤0.25->256	83.3	4.2	12.5	83.3	-	16.7
Ceftriaxone	0.06	>4	≤0.015->64	78.6	1.1	20.3	78.6	1.1	20.3
Ceftazidime	0.25	>16	≤0.03->64	82.1	1.6	16.3	78.8	3.3	17.9
Ceftazidime-avibactam	≤0.12	0.5	≤0.03->64	99.5	0.0	0.5	99.5	-	0.5
Ciprofloxacin	0.03	2	≤0.002->2	77.6	2.6	19.8	77.6	2.6	19.8

Abbreviations: CLSI= Clinical Laboratory Standards Institute; EUCAST= European Committee on Antimicrobial Susceptibility Testing; I=Intermediate; MIC=Minimum inhibitory concentration; MIC_{50}=Minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}= Minimum inhibitory concentration required to inhibit the growth of 90% of organisms; R=resistant; S=Susceptible.

[a]The CLSI cefepime breakpoints are susceptible ≤2 µg/ml, susceptible-dose dependent ≤8 µg/ml, and resistant ≥16 µg/ml [Clinical and Laboratory Standards Institute, 2022]. The EUCAST cefepime breakpoints are susceptible ≤1 µg/ml, susceptible-increased exposure ≤4 µg/ml, and resistant ≥8 µg/ml [The European Committee on Antimicrobial Susceptibility Testing, 2022]. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.

[b]For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at either the CLSI or EUCAST cefepime breakpoints in the %S, %I and %R columns. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.

[c]The CLSI breakpoints for piperacillin-tazobactam were updated in 2022 and are defined as susceptible ≤8 µg/ml, susceptible-dose dependent = 16 µg/ml (shown in the %I column), and resistant ≥32 µg/ml.

Source: adapted from Studies AAI101-MI-22-11 and AAI101-MI-22-12.

ESBL-producing Enterobacterales with confirmed genotypes were analysed from surveillance studies performed 2016 to 2018 demonstrating the utility of enmetazobactam against strains harbouring these enzymes (Table 14). The most prominent ESBL type was CTX-M-15. The cefepime-enmetazobactam MIC_{90} values against the different ESBL types ranged from 0.06 µg/mL to 1 µg/mL.

Table 14: *In vitro* activity of cefepime-enmetazobactam and comparator agents against Enterobacterales species with an ESBL genotype

ESBL organism group[a]/antibacterial agent	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	CLSI			EUCAST		
				%S	%I	%R	%S	%I	%R
Enterobacterales, All (n=722)									
Cefepime[b]	64	>64	0.03->64	11.9	14.5	73.5	6.4	12.5	81.2
Cefepime-enmetazobactam[c]	0.06	0.25	0.015-32	*99.2*	*0.7*	*0.1*	*98.8*	*1.0*	*0.3*
Meropenem	0.03	0.06	≤0.015-16	98.8	0.3	1.0	99.0	0.8	0.1
Piperacillin/tazobactam[d]	4	128	≤0.25->256	75.1	12.6	12.3	63.6	11.5	24.9
E. coli (n=410)									
Cefepime	32	>64	0.03->64	11.7	20.0	68.3	3.9	17.3	78.8
Cefepime-enmetazobactam	0.06	0.25	0.015-8	*99.0*	*1.0*	*0.0*	*99.0*	*0.7*	*0.2*
Meropenem	0.03	0.06	≤0.015-2	99.8	0.2	0.0	100	0.0	0.0
Piperacillin/tazobactam	4	32	0.5->256	84.4	9.0	6.6	73.4	11.0	15.6
K. pneumoniae (n=265)									
Cefepime	>64	>64	0.12->64	9.4	6.0	84.5	7.2	4.5	88.3
Cefepime-enmetazobactam	0.06	0.5	0.015-32	*99.2*	*0.4*	*0.4*	*98.1*	*1.5*	*0.4*
Meropenem	0.03	0.12	≤0.015-16	97.0	0.4	2.6	97.4	2.3	0.4
Piperacillin/tazobactam	16	>256	1->256	61.1	18.1	20.8	48.7	12.5	38.9
E. cloacae (n=17)									
Cefepime	>64	>64	16 – >64	0.0	0.0	100	0.0	0.0	100
Cefepime-enmetazobactam	0.25	1	0.06-1	100	0.0	0.0	100	0.0	0.0
Meropenem	0.06	0.12	≤0.015-0.25	100	0.0	0.0	100	0.0	0.0
Piperacillin/tazobactam	32	256	4->256	35.3	29.4	35.3	17.6	17.6	64.7

Abbreviations: CLSI=Clinical Laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=Intermediate; ESBL=extended spectrum β-lactamases; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}=minimum inhibitory concentration required to inhibit the growth of 90% of organisms; R=resistant; S=Susceptible.

[a]Includes only isolates encoding an ESBL and excludes those co-encoding AmpC, OXA, KPC, or metallo-β-lacatamases.

[b]The CLSI cefepime breakpoints are susceptible ≤2 µg/ml, susceptible-dose dependent ≤8 µg/ml, and resistant ≥16 µg/ml [Clinical and Laboratory Standards Institute, 2019]. The EUCAST cefepime breakpoints are susceptible ≤1 µg/ml, susceptible-increased exposure ≤4 µg/ml, and resistant ≥8 µg/ml [The European Committee on Antimicrobial Susceptibility Testing, 2019]. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.

[c]For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at either the CLSI or EUCAST cefepime breakpoints in the %S, %I and %R columns. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.

[d]The CLSI breakpoints shown for piperacillin-tazobactam are susceptible ≤16 µg/ml, intermediate = 32-64 µg/ml and resistant ≥128 µg/ml whereas the EUCAST breakpoints are susceptible ≤8 µg/ml, intermediate = 16 µg/ml and resistant ≥32 µg/ml. The CLSI breakpoints for piperacillin-tazobactam were updated in 2022 to susceptible ≤8 µg/ml, susceptible-dose dependent = 16 µg/ml, and resistant ≥32 µg/ml. The EUCAST breakpoints for piperacillin-tazobactam were updated in 2021 to susceptible ≤8 µg/ml and resistant ≥16 µg/ml.

Source: adapted from AAI101-MI-21-10 and AAI101-MI-21-14.

Cefepime-enmetazobactam activity was only minorly affected against isolates co-encoding ESBL+AmpC related to the inherent stability of cefepime against hydrolysis caused by AmpC enzymes. MIC_{90} increased minimally (2-fold) to 0.5 µg/mL relative to isolates only encoding ESBL. Similarly, due to the inherent stability of cefepime against hydrolysis by OXA-48 carbapenemase, isolates co-encoding ESBL+OXA-48, the MIC_{90} increased only moderately (8-fold) to 2 µg/mL.

Table 15 depicts the cumulative MIC-distribution for cefepime-enmetazobactam and comparators from a collection of 801 isolates of OXA-48 carbapenemase producing Enterobacterales. The cefepime-enmetazobactam MIC_{90} was essentially comparable to that of ceftazidime-avibactam, meropenem, meropenem-vaborbactam, imipenem alone, and imipenem-relebactam.

Table 15: Cumulative MIC-distribution for cefepime-enmetazobactam and comparators from 801 isolates of OXA-48 carbapenemase-producing Enterobacterales

	MIC in mg/L							
	<=0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**>16**
FEN	61.7	74.8	85	92.5	96	99	99	100
FEP	29.8	36.8	42	51.8	59	66	75	100
CAZ	23.8	31.1	35	37.3	42	49	58	100
CZA	61.0	81.5	96	99.1	100	100	100	100
MEM	27.5	47.4	73	90.5	95	96	97	100
MEV	31.7	53.2	77	91.9	96	97	97	100
IMP	17.2	40.9	78	92.9	97	98	99	100
IMR	24.9	52.3	85	94.3	97	98	99	100
ETP	5.7	14.5	33	58.1	76	91	95	100

FEN: cefepime-enmetazobactam, FEP: cefepime, CAZ: ceftazidime; CZA: ceftazidime-avibactam, MEM: meropenem, MEV: meropenem-vaborbactam; IMP: imipenem; IMR: imipenem-relebactam, ETP: ertapenem.

The *in vitro* activity of cefepime-enmetazobactam was tested against AmpC-producing only and OXA-producing (including different OXA-genotypes, not only OXA-48) Enterobacterales, a shown in Table 16 and Table 17. The EUCAST susceptibility rate was comparable to that of meropenem against the tested isolates.

Table 16: *In vitro* activity of cefepime-enmetazobactam against Enterobacterales clinical isolates encoding AmpC cephalosporinases[a] (n=381)

Antibacterial agent	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	CLSI			EUCAST		
				%S	%I	%R	%S	%I	%R
Cefepime[b]	0.5	4	0.015-64	86.9	11.3	1.8	72.2	23.1	4.7
Cefepime-enmetazobactam[c]	0.25	1	≤0.008-16	*98.4*	*1.3*	*0.3*	*94.5*	*4.5*	*1.0*
Meropenem	0.06	0.12	≤0.015->32	98.4	0.5	1.0	99.0	0.8	0.3
Piperacillin-tazobactam[d]	16	128	≤0.25->256	53.5	23.9	22.6	46.2	7.3	46.5

Abbreviations: CLSI=Clinical Laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC_{50}=minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}=minimum inhibitory concentration required to inhibit the growth of 90% of organisms; R=resistant; S=susceptible.
[a]Consists of ACC-4 (n=2), ACT-type (n=226), CMY-2 (n=16), CMY-4 (n=1), CMY-16 (n=11), CMY-42 (n=2), CMY-type (n=96), DHA-1 (n=8), DHA-type (n=1), FOX-5 (n=3), FOX-new variant (n=1), MIR-type (n=12) or ACT-type+DHA-1 (n=2).
[b]The CLSI cefepime breakpoints are susceptible ≤2 µg/ml, susceptible-dose dependent ≤8 µg/ml, and resistant ≥16 µg/ml [Clinical and Laboratory Standards Institute, 2019]. The EUCAST cefepime breakpoints are susceptible ≤1 µg/ml, susceptible-increased exposure ≤4 µg/ml, and resistant ≥8 µg/ml [The European Committee on Antimicrobial Susceptibility Testing, 2019]. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.
[c]For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at either the CLSI or EUCAST cefepime breakpoints in the %S, %I and %R columns. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.
[d]The CLSI breakpoints shown for piperacillin-tazobactam are susceptible ≤16 µg/ml, intermediate = 32-64 µg/ml and resistant ≥128 µg/ml whereas the EUCAST breakpoints are susceptible ≤8 µg/ml, intermediate = 16 µg/ml and resistant ≥32 µg/ml. The CLSI breakpoints for piperacillin-tazobactam were updated in 2022 to susceptible ≤8 µg/ml, susceptible-dose dependent = 16 µg/ml (shown in the %I column), and resistant ≥32 µg/ml. The EUCAST breakpoints for piperacillin-tazobactam were updated in 2021 to susceptible ≤8 µg/ml and resistant ≥16 µg/ml.
Source: adapted from AAI101-MI-21-10 and AAI101-MI-21-14.

Table 17: *In vitro* activity of cefepime-enmetazobactam and comparator agents tested against OXA-beta-lactamase-producing Enterobacterales (n=34)

Antimicrobial agent	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	CLSI[a]			EUCAST[a]		
				%S	%I	%R	%S	%I	%R
Cefepime[b]	16	>64	0.06->64	29.4	11.8	58.8	20.6	14.7	64.7
Cefepime-enmetazobactam[c]	0.12	4	0.03-32	*88.2*	*5.9*	*5.9*	*79.4*	*14.7*	*5.9*
Meropenem	0.06	8	0.015-32	73.5	3.0	23.5	76.5	14.7	8.8
Piperacillin-tazobactam[d]	64	>512	≤0.25->512	35.3	26.5	38.2	20.6	14.7	64.7

Abbreviations: CLSI=Clinical Laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC_{50}=minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}=minimum inhibitory concentration required to inhibit the growth of 90% of organisms; R=resistant; S=susceptible.
Organisms include: *Enterobacter cloacae* species complex (n=2), *Escherichia coli* (n=7), *Klebsiella oxytoca* (n=1), *K. pneumoniae* (n=22), *Proteus mirabilis* (n=1), *Serratia marcescens* (n=1). Genotypes consist of OXA-1/30 (n=24), OXA-2 (n=1), OXA-10 (n=1), OXA-48 (n=3), OXA-1/30+OXA-9 (n=1), OXA-1/30+OXA-10 (n=2), OXA-1/30+OXA-48 (n=2).
[a]Criteria as published by CLSI 2018 and EUCAST 2018.
[b]The CLSI cefepime breakpoints are susceptible ≤2 µg/ml, susceptible-dose dependent ≤8 µg/ml, and resistant ≥16 µg/ml [Clinical and Laboratory Standards Institute, 2019]. The EUCAST cefepime breakpoints are susceptible ≤1 µg/ml, susceptible-increased exposure ≤4 µg/ml, and resistant ≥8 µg/ml [The European Committee on Antimicrobial Susceptibility Testing, 2019]. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.
[c]For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at either the CLSI or EUCAST cefepime breakpoints in the %S, %I and %R columns. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.
[d]The CLSI breakpoints shown for piperacillin-tazobactam are susceptible ≤16 µg/ml, intermediate = 32-64 µg/ml and resistant ≥128 µg/ml whereas the EUCAST breakpoints are susceptible ≤8 µg/ml, intermediate = 16 µg/ml and resistant ≥32 µg/ml. The CLSI breakpoints for piperacillin-tazobactam were updated in 2022 to susceptible ≤8 µg/ml, susceptible-dose dependent = 16 µg/ml (shown in the %I column), and resistant ≥32 µg/ml. The EUCAST breakpoints for piperacillin-tazobactam were updated in 2021 to susceptible ≤8 µg/ml and resistant ≥16 µg/ml.
Source: adapted from AAI101-MI-18-04.

Although enmetazobactam has been shown to exhibit some inhibitory activity against KPC-2 and KPC-3 enzymes it is comparatively less potent than avibactam. As shown in Table 18, all agents except ceftazidime-avibactam and meropenem-vaborbactam exhibited high MIC_{90} values against Enterobacterales with either the KPC or KPC+ESBL genotypes.

Table 18: *In vitro* activity of cefepime-enmetazobactam and comparator agents against Enterobacterales clinical isolates with a KPC genotype

Antibacterial agent	MIC (µg/ml)			CLSI			EUCAST		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
KPC[a] (n=54)									
Cefepime[b]	>64	>64	4->64	0.0	3.7	96.3	0.0	1.9	98.1
Cefepime-enmetazobactam[c]	64	>64	0.06->64	16.7	14.8	68.5	11.1	13.0	75.9
Meropenem	>32	>32	4->32	0.0	0.0	100	0.0	9.3	90.7
Piperacillin-tazobactam[d]	>256	>256	64->256	0.0	1.9	98.1	0.0	0.0	100
Ceftazidime-avibactam	1	4	0.06-16	98.1	-	1.9	98.1	-	1.9
Meropenem-vaborbactam	0.25	2	0.015-8	98.1	1.9	0.0	100	-	0.0
KPC+ESBL[e] (n=25)									
Cefepime	>64	>64	16->64	0.0	0.0	100	0.0	0.0	100
Cefepime-enmetazobactam	8	>64	0.12->64	36.0	16.0	48.0	32.0	12.0	56.0
Meropenem	32	>32	2->32	0.0	4.0	96.0	4.0	4.0	92.0
Piperacillin-tazobactam	>256	>256	128->256	0.0	0.0	100	0.0	0.0	100
Ceftazidime-avibactam	1	2	0.25-4	100	-	0.0	100	-	0.0
Meropenem-vaborbactam	0.03	1	0.015->4	96.0	4.0	0.0	100	-	0.0

Abbreviations: CLSI=Clinical Laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC_{50}=minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}=minimum inhibitory concentration required to inhibit the growth of 90% of organisms; R=resistant; S=susceptible.
[a]Consists of KPC-2 (n=5), KPC-3 (n=48) and KPC-29 (n=1).
[b]The CLSI cefepime breakpoints are susceptible ≤2 µg/ml, susceptible-dose dependent ≤8 µg/ml, and resistant ≥16 µg/ml [Clinical and Laboratory Standards Institute, 2019]. The EUCAST cefepime breakpoints are susceptible ≤1 µg/ml, susceptible-increased exposure ≤4 µg/ml, and resistant ≥8 µg/ml [The European Committee on Antimicrobial Susceptibility Testing, 2019]. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.
[c]For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at either the CLSI or EUCAST cefepime breakpoints in the %S, %I and %R columns. The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml and the EUCAST cefepime susceptible-increased exposure breakpoint of ≤4 µg/ml is calculated by adding the values for %S and %I.
[d]The CLSI breakpoints for piperacillin-tazobactam were updated in 2022 and are defined as susceptible ≤8 µg/ml, susceptible-dose dependent = 16 µg/ml (shown in the %I column), and resistant ≥32 µg/ml.
[e]Consists of KPC-2 (n=2) and KPC-3 (n=23)
Source: adapted from AAI101-MI-21-10 and AAI101-MI-21-14.

Of 30 isolates of Enterobacterales that encoded metallo-beta-lactamases, all exhibited cefepime-enmetazobactam MIC values ≥16 µg/ml.

The *in vitro* activity of cefepime-enmetazobactam against the *P. aeruginosa* surveillance isolates obtained between 2017 and 2021 is shown in Table 19. The addition of enmetazobactam to cefepime did not lower the MIC_{90} relative to cefepime alone.

Table 19: *In vitro* activity of cefepime-enmetazobactam and comparator agents against Pseudomonas aeruginosa (n=1,165) from 2017-2021

Antibacterial agent	MIC (µg/ml)			CLSI			EUCAST		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Cefepime[a]	4	16	0.06->64	82.8	8.8	8.4	0.0	82.8	17.2
Cefepime-enmetazobactam[b]	2	16	≤0.008->64	*88.4*	*6.6*	*5.0*	*0.0*	*88.4*	*11.6*
Meropenem	0.5	8	≤0.015->32	78.5	6.8	14.8	78.5	13.3	8.2
Piperacillin-tazobactam	4	128	≤0.25->256	77.3	10.1	12.6	0.0	77.3	22.8
Ceftazidime	2	32	0.12->64	79.0	4.9	16.1	0.0	79.0	21.0
Ceftazidime-avibactam	2	8	0.06->64	94.5	0.0	5.5	94.5	-	5.5
Ciprofloxacin	0.12	>2	0.004->4	79.4	4.9	15.7	0.0	79.4	20.6
Ceftolozane-tazobactam[c]	0.5	2	0.06->64	96.1	1.2	2.7	96.1	0.0	3.9

Abbreviations: CLSI=Clinical Laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC_{50}=minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}=minimum inhibitory concentration required to inhibit the growth of 90% of organisms; R=resistant; S=susceptible.
[a] The CLSI cefepime breakpoints are susceptible ≤8 µg/ml, intermediate = 16 µg/ml, and resistant ≥32 µg/ml [Clinical and Laboratory Standards Institute, 2022]. The EUCAST cefepime breakpoints are susceptible ≤1 µg/ml, susceptible-increased exposure ≤4 µg/ml, and resistant ≥8 µg/ml [The European Committee on Antimicrobial Susceptibility Testing, 2022].
[b] For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at the either the CLSI cefepime breakpoints of susceptible ≤8 µg/ml, intermediate = 16 µg/ml, and resistant ≥32 µg/ml, or the EUCAST cefepime breakpoints of susceptible ≤0.001 µg/ml, intermediate 0.002-8 µg/ml, and resistant ≥16 µg/ml, in the %S, %I and %R columns.
[c] Results reported for ceftolozane-tazobactam are for 567 isolates tested in 2017 and 2018.
Source: adapted from Studies AAI101-MI-21-06, AAI101-MI-22-11, and AAI101-MI-22-12.

The MIC_{90} of cefepime-enmetazobactam against 583 isolates of *A. baumannii* was >64 µg/ml. However, when only the 347 carbapenem-susceptible isolates (meropenem ≤2 µg/ml) were examined the MIC_{90} of cefepime-enmetazobactam was 0.015 µg/ml demonstrating that the combination could constitute a possible carbapenem-sparing alternative against *A. baumannii*.

Addition of enmetazobactam to cefepime is not expected to have an impact on cefepime *in vitro* activity against gram-positive pathogens. The applicant does not make a claim for utility of the combination against anaerobic pathogens and has presented very limited data of the activity against this group of pathogens to draw any conclusions on the value of the combination.

Resistance to cefepime-enmetazobactam

Mechanisms of resistance to cefepime-enmetazobactam include production of beta-lactamases that can hydrolyse cefepime and which are not inhibited by enmetazobactam, porin mutations which affect outer membrane permeability, overexpression of efflux pumps and mutations in the penicillin-binding protein genes.

Laboratory selection of resistance

The frequency of spontaneous mutation (single-step) and stepwise selection of resistance to cefepime-enmetazobactam have been evaluated. Overall, spontaneous mutation frequencies to cefepime-enmetazobactam were in general comparable in magnitude to those of relevant comparators (meropenem and ceftazidime-avibactam). In serial passage studies combinations of cefepime-enmetazobactam completed more serial passages compared to cefepime alone before reaching the experimental MIC endpoint.

Whole genome sequencing was used to determine if mutations from either single-step or stepwise selection occurred in beta-lactamase, porin, efflux pump or PBP genes. Of the ten mutants with terminal cefepime-enmetazobactam MIC values ≥16 µg/mL that were selected for resistance to cefepime-enmetazobactam, six had disruptions to porin genes and another had an alteration to an efflux pump gene. No mutations were detected in the remaining three mutants.

Emergence of resistance in the clinical studies

Changes in cefepime-enmetazobactam MIC of ≥4-fold relative to the baseline MIC for isolates obtained at either test of cure or late follow up in the cefepime-enmetazobactam treatment group of the Phase III study AT-301 are shown in Table 20. A single clinical isolate of *P. aeruginosa* exhibited a change in cefepime-enmetazobactam MIC that resulted in a categorical shift from the provisional susceptibility breakpoint of ≤8 µg/ml to the resistant category of ≥16 µg/ml used in the Phase 3 study. The cefepime-enmetazobactam MIC value determined for this isolate at the test of cure visit was 32 µg/ml, a 4-fold increase relative to its baseline MIC of 8 µg/ml. At the late follow-up visit, an MIC value of 8 µg/ml was obtained for the isolate, possibly indicating that the change in MIC at test of cure was transient. No other isolate exhibited a categorical change in susceptibility. No molecular characterisation was provided.

Table 20: Cefepime-enmetazobactam MIC Changes in the Phase 3 Study AT-301

Organism	Cefepime-enmetazobactam MIC (µg/ml) and fold change of isolate from patient visit relative to baseline pathogen				
	Baseline	Test of cure		Late follow up	
	MIC	MIC	Fold change[a]	MIC	Fold change[a]
Escherichia coli	0.12	4	32	0.5	4
Escherichia coli	0.015	0.12	8	0.06	4
Pseudomonas aeruginosa	8	32	4	8	1
Pseudomonas aeruginosa	2	8	4	ND	-
Serratia marcescens	0.25	1	4	1	4
Klebsiella pneumoniae	0.03	0.12	4	0.12	4
Escherichia coli	0.06	0.25	4	0.12	2

Abbreviations: MIC=minimum inhibitory concentration; ND=not determined.
[a]Fold change in MIC at either test of cure or late follow up relative to the baseline MIC value.
Source: Listings 16.2.6.5 and 16.2.6.6.

Effects of human body fluids on susceptibility to cefepime-enmetazobactam

The *in vitro* activity of cefepime-enmetazobactam was generally unaffected by the presence of human urine, human serum and lung surfactant.

In vivo

Cefepime-enmetazobactam evaluated in animal models of infection

The therapeutic effect of cefepime-enmetazobactam has been evaluated in various murine infection models (thigh, lung, urinary tract and sepsis) of which the majority of studies were performed in the neutropenic mouse thigh infection model which was mainly used to establish preclinical PK/PD targets (PDT). In brief, enmetazobactam restored the antibacterial activity of cefepime in a dose-dependent manner in these models against cefepime-resistant isolates of ESBL-producing Enterobacterales some of which encoded AmpC and OXA-beta-lactamases. Results from the thigh and lung models to support dose selection is further described in the following section.

Summary of support for dose selection

Cefepime

The cefepime dose of 2 g administered every 8 hours as a 2-hour infusion was selected to optimise cefepime exposure. As with other cephalosporins, cefepime *f*T>MIC of 30% to 40% is associated with bacterial stasis whereas higher exposures of 50 to 60% *f*T>MIC are associated ≥1-$\log_{10}$ reduction in bacterial burden. In a retrospective study of cefepime dosing in patients with gram-negative pathogen bloodstream infections, exposures of 68 to 74% of *f*T>MIC were shown to optimise survival [Rhodes et al, 2015].

Enmetazobactam

Selection of a 0.5 g dose of enmetazobactam administered every 8 hours (q8h) as a 2-hour infusion was mainly based on the following findings (further described and discussed below):

- Suppression of resistance development and restoration of cefepime antibacterial activity against ESBL-producing Enterobacterales with an enmetazobactam C_T of 2 µg/ml in the hollow-fibre infection model.
- Decreases in bioburden of ≥1-$\log_{10}$ CFU in the mouse neutropenic thigh infection model with ESBL-producing Enterobacterales when achieving a PD target of 45% *f*T >C_T of 2 µg/ml of enmetazobactam.
- Monte Carlo simulations demonstrating a high probability of target attainment of achieving the non-clinical PD target of 45% *f*T >2µg/ml associated with a 1-log kill in the mouse neutropenic thigh infection model.

To identify the pharmacodynamic index and non-clinical pharmacodynamic targets (PDTs) associated with efficacy for enmetazobactam, PK/PD analyses were performed on a series of experiments in the hollow-fibre infection model (HFIM) and murine infection models.

HFIM

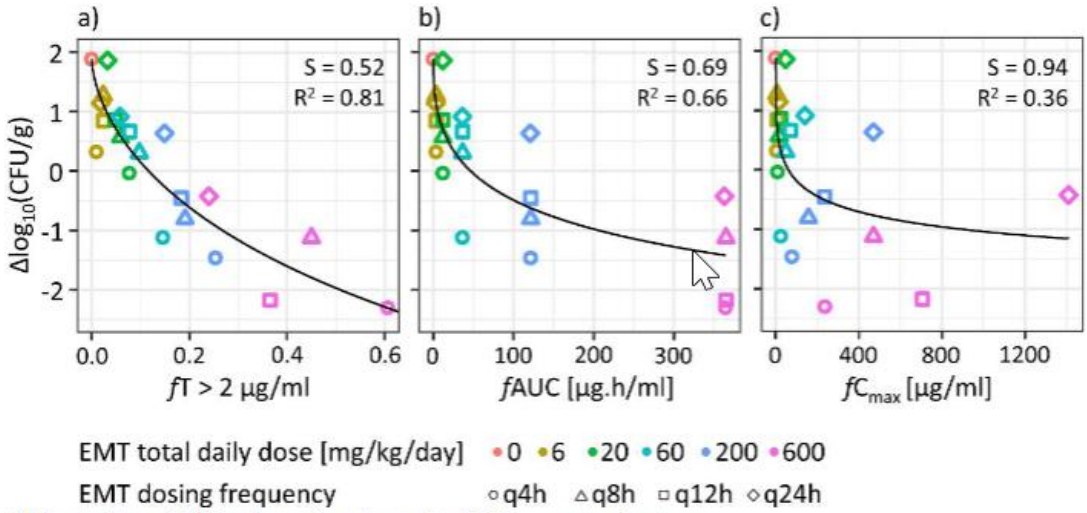
Initial dose ranging studies in a hollow-fibre infection model were performed over 10 days to identify a threshold concentration (C_T) of enmetazobactam associated with bacterial eradication and suppression of resistance emergence when combined with cefepime at a simulated dose regimen of 2 g IV q8h by 2-hour infusion. Enmetazobactam was administered as a continuous infusion at concentrations ranging from 0.5 to 8 µg/ml. These results from this study demonstrated that a C_T ≥2 µg/ml of enmetazobactam in combination with cefepime was required for sustained antibacterial activity and suppression of development of less susceptible populations against two ESBL-producing isolates of *K. pneumoniae* (having a cefepime-enmetazobactam MIC of 0.12 and 0.06, respectively). Against two strains of *K. pneumoniae* harbouring not only ESBLs but also KPC with MICs of 0.06 and 2, respectively, a concentration of 2 µg/ml of enmetazobactam was not able to continuously suppress bacterial growth throughout the experiment. Against the strain with highest MIC, there was a selection of resistant subpopulations that exhibited cefepime-enmetazobactam MIC values of 16-64 µg/ml.

In the HFIM model it was additionally demonstrated that a $fT > C_T$ of 2 µg/m of 31 to 46% of the dosing interval was needed to generate a 1-$\log_{10}$ CFU/ml reduction in the bacterial burden.

Murine infection models

A dose fractionation study was performed to identify the PD index for enmetazobactam that optimises antibacterial activity efficacy when administered in combination with cefepime in the murine neutropenic thigh infection model. A cefepime-resistant, ESBL-producing clinical isolate *K. pneumoniae* was used in these experiments. The highest non-effective dose of cefepime was previously shown to be 100 mg/kg q4h for this isolate and was used in this study. Increasing the total daily dose of enmetazobactam from 6 mg/kg/day up to 600 mg/kg/day administered intravenously concurrently with a fixed dose of 600 mg/kg/day of cefepime caused greater reductions in thigh bioburden, regardless of the dosing frequency used. Furthermore, increasing the dose fractionation of enmetazobactam from once daily (q24h) up to six times daily (q4h) resulted in greater reductions in thigh bioburden. These results indicate that extending the $fT>MIC$ or $fT>C_T$ improves microbiological eradication. For each treatment arm, changes in bioburden (the difference between pre-treatment and treatment groups) were plotted against the respective enmetazobactam exposure expressed as (a) %$fT > C_T$ of 2 µg/ml, (b) fAUC, and (c) fC_{max}, and sigmoid curves were fitted by regression analysis. The PK-PD relationship of enmetazobactam was best described by %$fT > C_T$, followed by fAUC, and fC_{max} (Figure 4).

Figure 4: Enmetazobactam Exposure-Response Relationship in a 26-H Murine Thigh Infection Model Obtained from Dose Fractionation of Enmetazobactam Combined with a Fixed Dose of Cefepime



Abbreviations: CFU=colony-forming units; EMT=enmetazobactam
Source: from Study AAI101-PH-20-01.

The non-clinical PK/PD targets associated with efficacy were determined from the mouse neutropenic thigh infection model using 9 clinical isolates of cefepime-resistant, ESBL-producing *K. pneumoniae*. For the 75th percentile, 8% and 44% fT >2 µg/ml enmetazobactam were identified for stasis and a bioburden reduction of 1-$\log_{10}$ CFU/g, respectively (Table 21). From these findings, an enmetazobactam non-clinical target of 45% $fT>CT$ was selected.

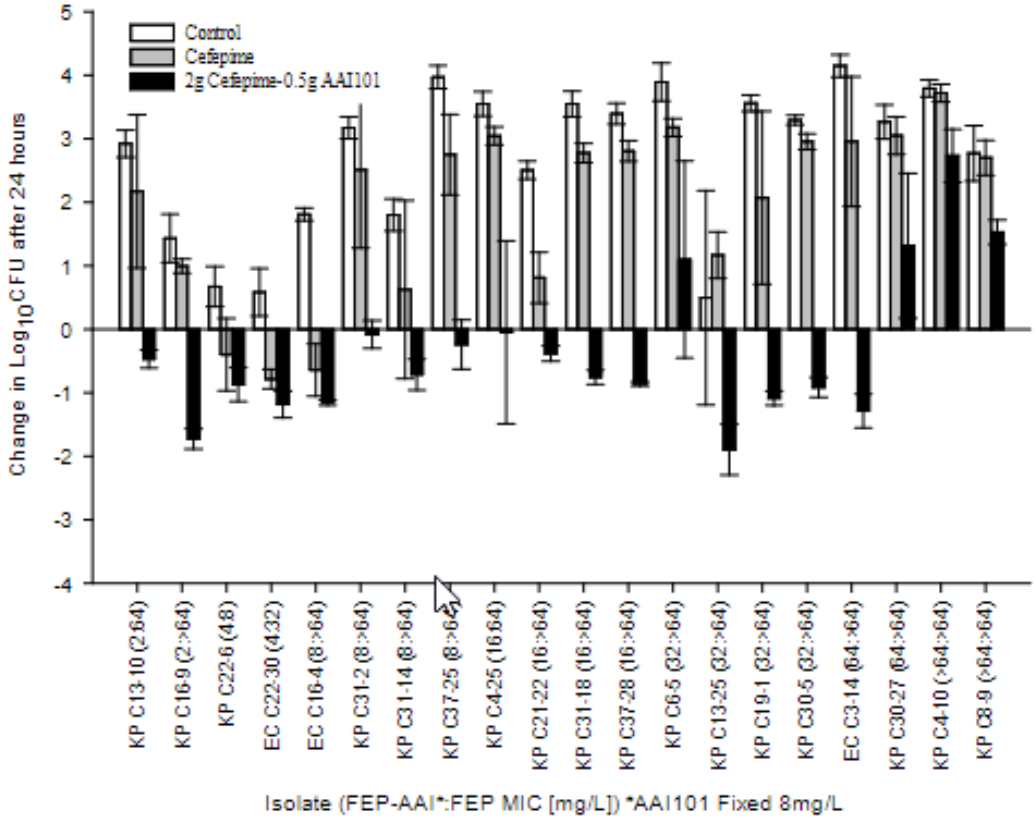
Table 21: Calculated Cefepime-Enmetazobactam PK/PD Targets Resulting in Stasis or 1-log_{10} kill in the Mouse Neutropenic Thigh Infection Model

K. pneumoniae isolate	Calculated enmetazobactam *f*T >2 µg/ml required for		Calculated cefepime *f*T > MIC[b]
	stasis	1-log_{10} kill[a]	
1280740	<1%	3%	79%
B454	1%	1%	53%
1093554[b]	0%	8%	43%
1237221	<1%	1%	79%
1061285	8%	14%	53%
900679	15%	46%	43%
1273479	10%	>100%	43%
1077711	10%	20%	43%
1046194[a]	0%	1%	43%
mean	5%	22%	53%
global fit	2%	16%	-
75th percentile	8%	44%	-
90th percentile	24%	>100%	-

Abbreviations: *f*T=free time; FPE=cefepime-enmetazobactam; MIC=minimum inhibitory concentration.
[a]1-log_{10} decrease (CFU/g) = bioburden as log_{10}(CFU/g) difference between the pre-treatment group and treatment groups.
[b]Stasis achieved with cefepime-only.
Source: from Study AAI101-PH-20-01.

The effect of a dosing regimen that simulated humanised exposures (resulting in the observed fT>MIC) from 2 g cefepime and 0.5 g enmetazobactam every 8 hours as a 30-minute infusion was determined in the mouse neutropenic thigh infection model. A challenge panel of 20 Enterobacterales isolates with either ESBL or KPC phenotypes and MIC from 2 to >64 µg/ml was tested. Treatment with the humanised dosing regimen of cefepime-enmetazobactam caused bacterial reductions of ≥0.5 log_{10} CFU for 12 of the 20 isolates tested, and reductions of ≥1 log_{10} CFU for 6 of these. Increases in bacterial burden were observed for four isolates (KPC6-5, KPC30-27, K C4-10 and KPC8-9), all having cefepime-enmetazobactam MICs ≥32 µg/ml.

Figure 5: Reduction in Mouse Thigh Bioburden from a Humanised Dosing Regimen of Cefepime-Enmetazobactam



Abbreviations: AAI1010, enmetazobactam; FEP, cefepime; EC, *E. coli*; KP, *K. pneumoniae*.
Source: from Study AAI101-PH-14-03.

The PK-PD was furthermore evaluated in the murine neutropenic lung infection model. The AUC_{ELF}:AUC_{plasma} ratio was 73.4% and 61.5% for cefepime and enmetazobactam, respectively. Protein binding in ELF was assumed to be negligible. Dose fractionation studies suggested that both *f*T≥threshold and *f*AUC:MIC are relevant PK/PD indices. The PDTs of the combination to achieve various orders of logarithmic killing against three clinical isolates of cefepime-resistant, ESBL-producing *Klebsiella pneumoniae* was evaluated (Table 22).

Table 22: Cefepime and Enmetazobactam Plasma and ELF Exposures Associated with Bioburden Reductions in Infected Mouse Lungs

Log_{10} kill in lungs based on tissue exposures	Fraction of dosing interval	
	Cefepime *f*T >MIC	Enmetazobactam *f*T >2 µg/ml
Plasma		
≥2	20%	20%
≥3	30%	50%
Epithelial Lining Fluid		
≥2	20% (40%)[a]	20% (10%)[a]
≥3	30%	40%

Abbreviations: *f*T=free time.
[a]Values in brackets indicate an alternate cefepime and enmetazobactam exposure associated with 2-log_{10} killing.
Source: adapted from Study AAI101-PH-21-01.

Probability of target attainment analyses

Monte Carlo simulations for enmetazobactam and cefepime were performed using a PopPK model derived from healthy volunteers in Phase 1 studies, cUTI patients in the Phase 2 study, and cUTI patients in the Phase 3 study. For enmetazobactam, the non-clinical pharmacodynamic target of 45% *f*T >C_T of 2 µg/ml was used as it is associated with ≥1-log_{10} CFU reduction in the mouse neutropenic model of infection and suppression of resistance development in the hollow-fibre infection model. Two pharmacodynamic (PD) targets for cefepime were used: 60% *f*T >MIC, which is associated with maximal bacterial killing by cephalosporins [Andes and Craig, 2005]; and 68% *f*T >MIC, which is associated with higher odds of survival for patients with gram-negative bloodstream infections [Rhodes et al, 2015].

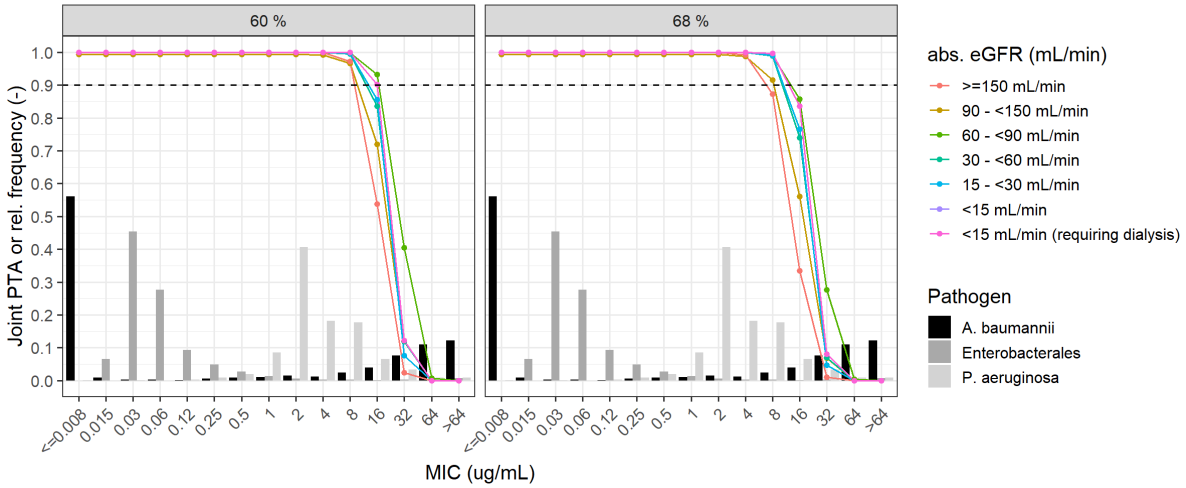
Simulations were conducted using the updated PopPK model for the renal function groups dosed according to the proposal in the SmPC (Table 23).

Table 23: Simulation groups and dose adjustments for Exblifep for subjects with different degrees of renal elimination capacity.

Absolute eGFR (mL/min)	**Recommended Dosage Regimen for Exblifep (cefepime-enmetazobactam)**	**Dosing Interval**	**Infusion Time**
ARC (≥150)	cefepime 2 g and enmetazobactam 0.5 g	Every 8 hours	4 hours
Normal (90<150)	cefepime 2 g and enmetazobactam 0.5 g	Every 8 hours	2 hours
Mild (60<90)	cefepime 2 g and enmetazobactam 0.5 g	Every 8 hours	
Moderate (30 to 59)	cefepime 1 g and enmetazobactam 0.25 g	Every 8 hours	
Severe (15< 30)	cefepime 1 g and enmetazobactam 0.25 g	Every 12 hours	
End stage renal disease (<15)	cefepime 1 g and enmetazobactam 0.25 g	Every 24 hours	
Patients requiring haemodialysis	cefepime 1g and enmetazobactam 0.25 g loading dose on the first day of therapy and cefepime 0.5 g and 0.125g thereafter	Every 24 hours	

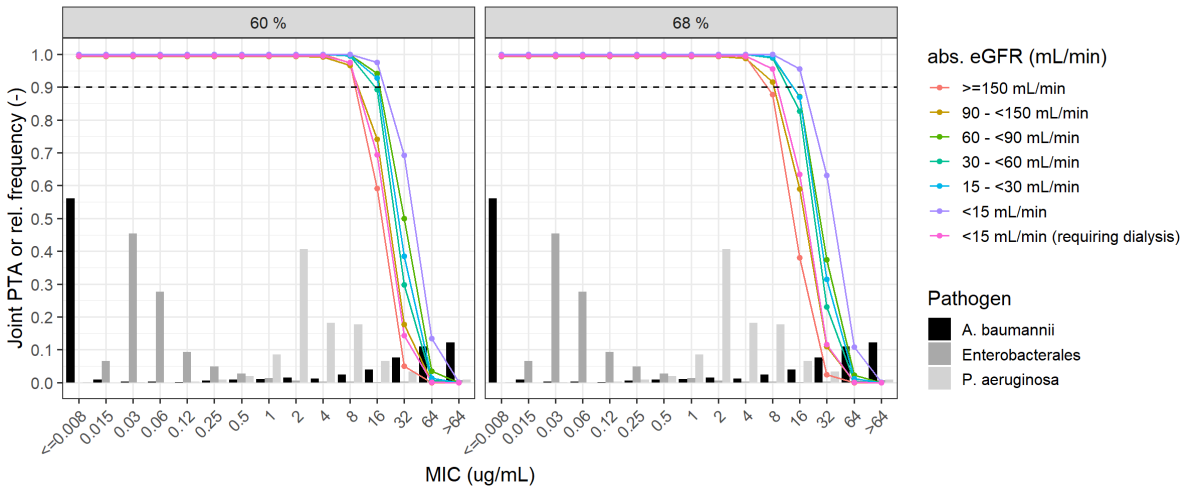
Figure 6 and Figure 7 show the predicted joint cefepime-enmetazobactam PTA on Day 1 and Day 7 for the preclinical targets as a function of the MIC.

Figure 6: Joint cefepime-enmetazobactam Day-1 PTA for subjects with different degrees of renal elimination capacity dosed according to Table 23.



PTA as a function of the MIC for the targets of 45% fT(ENM) > CT of 2 µg/mL and 60% fT (FEP) > MIC (left) or 68% fT (FEP) > MIC (right). Overlaid (bars) are the relative MIC distributions of 9,905 Enterobacterales, 1,165 P. aeruginosa and 583 A. baumannii isolates tested against cefepime-enmetazobactam with enmetazobactam fixed at 8 µg/mL. Horizontal dashed line indicates a PTA of 0.9. 4,000 subjects per group.

Figure 7: Joint cefepime-enmetazobactam Day-7 PTA for subjects with different degrees of renal elimination capacity dosed according to Table 23**.**



PTA as a function of the MIC for the targets of 45% fT(ENM) > CT of 2 µg/mL and 60% fT (FEP) > MIC (left) or 68% fT (FEP) > MIC (right). Overlaid (bars) are the relative MIC distributions of 9,905 Enterobacterales, 1,165 *P. aeruginosa* and 583 *A. baumannii* isolates tested against cefepime-enmetazobactam with enmetazobactam fixed at 8 µg/mL. Horizontal dashed line indicates a PTA of 0.9. 4,000 subjects per group.

Table 24 and Table 25 list the joint cefepime-enmetazobactam PTA on Day 1 and 7 for the PK/PD targets of 45% *f*T(ENM) > C_T=2 µg/mL in combination with 60% *f*T (FEP) > MIC.

Table 24: Joint cefepime-enmetazobactam day-1 PTA by virtual population group and MIC and CFR for PK/PD targets of 45% *f*T(ENM) > C_T=2 µg/mL in combination with 60% *f*T (FEP) > MIC.

	≥150 mL/min	90 - <150 mL/min	60 - <90 mL/min	30 - <60 mL/min	15 - <30 mL/min	<15 mL/min	<15 mL/min (requiring dialysis)
MIC (µg/mL)							
≤0.5	1.000	0.993	1.000	1.000	1.000	1.000	1.000
1	1.000	0.993	1.000	1.000	1.000	1.000	1.000
2	1.000	0.993	1.000	1.000	1.000	1.000	1.000
4	0.999	0.993	1.000	1.000	1.000	1.000	1.000
8	0.972	0.966	0.998	0.996	0.996	1.000	1.000
16	0.538	0.720	0.933	0.836	0.857	0.901	0.901
32	0.024	0.120	0.406	0.119	0.077	0.123	0.123
64	0.000	0.000	0.008	0.000	0.000	0.000	0.000
>64[†]	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CFR							
Enterobacterales	0.987	0.982	0.989	0.988	0.988	0.988	0.988
P. aeruginosa	0.915	0.925	0.959	0.942	0.942	0.948	0.948
A. baumannii	0.676	0.686	0.722	0.695	0.693	0.698	0.698

[†]Used MIC = 128 µg/mL for simulations.

Table 25: Joint cefepime-enmetazobactam day-7 PTA by virtual population group and MIC and CFR for PK/PD targets of 45% *f*T(ENM) > C_T=2 µg/mL in combination with 60% *f*T (FEP) > MIC.

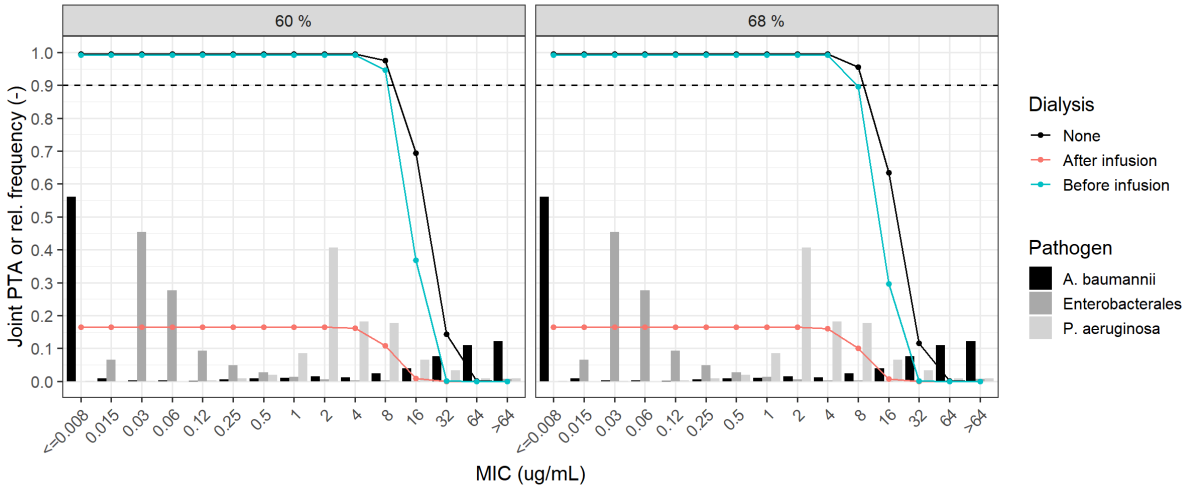
	≥150 mL/min	90 - <150 mL/min	60 - <90 mL/min	30 - <60 mL/min	15 - <30 mL/min	<15 mL/min	<15 mL/min (requiring dialysis)
MIC (µg/mL)							
≤0.5	1.000	0.993	1.000	1.000	1.000	1.000	0.996
1	1.000	0.993	1.000	1.000	1.000	1.000	0.996
2	1.000	0.993	1.000	1.000	1.000	1.000	0.996
4	0.999	0.993	1.000	1.000	1.000	1.000	0.996
8	0.973	0.967	0.998	0.996	0.996	1.000	0.976
16	0.591	0.741	0.942	0.893	0.928	0.976	0.695

32	0.050	0.177	0.500	0.298	0.385	0.692	0.144
64	0.000	0.003	0.035	0.007	0.014	0.134	0.001
>64[†]	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CFR							
Enterobacterales	0.987	0.982	0.989	0.988	0.989	0.990	0.984
P. aeruginosa	0.920	0.928	0.962	0.952	0.957	0.972	0.927
A. baumannii	0.680	0.691	0.732	0.712	0.720	0.759	0.688

[†]Used MIC = 128 µg/mL for simulations.

The figure below shows the predicted effect of a 4-hour haemodialysis directly after end of infusion or before the start of infusion on the joint cefepime-enmetazobactam PTA on Day 7 (the day of haemodialysis). As before it is proposed that on dialysis days, cefepime-enmetazobactam should be administered following dialysis.

Figure 8: Joint cefepime-enmetazobactam Day-7 PTA for subjects with absolute eGFR of <15 mL/min undergoing concomitant haemodialysis before or after cefepime-enmetazobactam dose administration as a function of the MIC for the targets of 45% *f*T(ENM) > C_T of 2 µg/mL and 60% *f*T (FEP) > MIC (left) or 68% *f*T (FEP) > MIC (right).



Overlaid (bars) are the relative MIC distributions of 9905 Enterobacterales, 1165 P. aeruginosa and 583 A. baumannii isolates tested against cefepime-enmetazobactam with enmetazobactam fixed at 8 µg/mL. Horizontal dashed line indicates a PTA of 0.9. 4,000 subjects per group. For comparison the PTA for the same group without concomitant haemodialysis (black) is plotted.

PTA ELF

PTA ELF simulations were performed using the updated Phase 3 PopPK model with equal allometric parameters for central and peripheral compartments, the biodistribution coefficient into the ELF as determined in the healthy volunteers and the PD targets from the thigh mice model. These simulations indicated that a 2-hour infusion might not be sufficient to ensure adequate exposure to maximise the efficacy in the lung. Simulations performed using a 4-hours infusion predict PTA above 0.9 in the different renal function groups except for the patients with augmented renal clearance or the one with severe renal insufficiency and requiring dialysis. A 4-hour infusion is therefore recommended for patients with pneumonia.

Table 26: Joint cefepime-enmetazobactam day-7 ELF PTA by virtual population group and MIC for PK/PD targets of 45% *f*T(ENM) > C_T=2 µg/mL in combination with 60% *f*T (FEP) > MIC for the dosing.

MIC (µg/mL)	≥150 mL/min	90 - <150 mL/min	60 - <90 mL/min	30 - <60 mL/min	15 - <30 mL/min	<15 mL/min	<15 mL/min (requiring dialysis)
≤0.5	0.815	0.871	0.984	0.951	0.989	0.999	0.945
1	0.815	0.871	0.984	0.951	0.989	0.999	0.945
2	0.815	0.871	0.984	0.951	0.989	0.999	0.945
4	0.811	0.870	0.984	0.951	0.989	0.999	0.945
8	0.664	0.776	0.953	0.915	0.981	0.997	0.894
16	0.068	0.231	0.591	0.380	0.680	0.899	0.362
32	0.000	0.006	0.056	0.012	0.051	0.354	0.020
64	0.000	0.000	0.000	0.000	0.000	0.016	0.000
>64[†]	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[†]Used MIC = 128 µg/mL for simulations.

Table 27: Joint cefepime-enmetazobactam day-7 ELF PTA by virtual population group and MIC for PK/PD targets of 45% *f*T(ENM) > C_T=2 µg/mL in combination with 60% *f*T (FEP) > MIC for the dosing but with increased infusion duration to 4 hours for all groups.

MIC (µg/mL)	≥150 mL/min	90 - <150 mL/min	60 - <90 mL/min	30 - <60 mL/min	15 - <30 mL/min	<15 mL/min	<15 mL/min (requiring dialysis)
≤0.5	0.815	0.971	0.998	0.958	0.992	0.999	0.935
1	0.815	0.971	0.998	0.958	0.992	0.999	0.935
2	0.815	0.971	0.998	0.958	0.992	0.999	0.935
4	0.811	0.971	0.998	0.958	0.992	0.999	0.935
8	0.664	0.944	0.996	0.949	0.987	0.998	0.872
16	0.068	0.378	0.743	0.451	0.626	0.877	0.374
32	0.000	0.009	0.083	0.022	0.056	0.370	0.021
64	0.000	0.000	0.000	0.000	0.000	0.019	0.000
>64[†]	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[†]Used MIC = 128 µg/mL for simulations.

Susceptibility testing breakpoints

Susceptibility breakpoints are pending EUCAST interaction. The applicant proposes a susceptibility breakpoint for Enterobacterales and *P. aeruginosa* of ≤8 mg/L and resistance >8 mg/L.

Pharmacodynamic interactions

Checkerboard methodology was used to determine drug interactions of between cefepime-enmetazobactam and representative agents (azithromycin, aztreonam, ceftazidime-avibactam, clindamycin, daptomycin, doxycycline, gentamicin, levofloxacin, linezolid, meropenem, metronidazole, trimethoprim-sulfamethoxazole or vancomycin) from different antibacterial classes. A panel of 11 bacterial pathogens was tested that was composed of 6 gram-negative pathogens, 3 gram-positive pathogens, and 2 anaerobic organisms. A single instance of antagonism was observed in this study, when cefepime enmetazobactam was combined with gentamicin in checkerboard studies against *P. aeruginosa* ATCC 27853. In repeated *in vitro* experiments no antagonism between cefepime-enmetazobactam and gentamicin clinical isolates of *Pseudomonas aeruginosa* was detected.

Relationship between plasma concentration and effect

An analysis was performed to determine whether patients in the Phase 3 study AT-301 achieved the pre-specified pre-clinical PK/PD targets, whether there was a correlation of microbiological outcomes with cefepime and/or enmetazobactam and/or joint target attainment, and whether the pre-clinical PDTs could be adjusted based on the available microbiological outcome data from patients.

There was a high degree of target attainment and therefore no correlation of microbiological outcome with target attainment could be established. Based on the high exposure and the following high fraction of time above the pre-clinical targets, no changes to the PDTs could be recommended.

2.5.3. Discussion on clinical pharmacology

Pharmacokinetics

Methods

Bioanalytical methods

The bioanalytical methods used are considered adequately validated. Overall, the within study validation reports show that the methods performed satisfactorily. Samples were analysed within established long-term stability. ISR was performed for all studies except Study AT-301 (ELF study) and urine samples in Study AT-102 and AT-201.

Pop-PK methods

A combined cefepime-enmetazobactam PopPK model was developed. The applied methods used to develop and evaluate the final model are acceptable. Starting from the previous PopPK models is generally considered a good strategy and is supported.

A total number of 663 subjects and 11,490 observations were collected. Then, 486 and 367 observations were excluded from the analysis since they were classified as outliers and BLQ observations.

PopPK discussion

The company argues that the correlation between age and eGFR was low (-0.35), which is below the commonly applied threshold of 0.5 to 0.6 used for exclusion of correlated covariates. The CHMP notes

that also a threshold of 0.3 is often referred to as the threshold of interest (Marc R. Gastonguay, PAGE 2011). The CHMP generally agrees that the now provided VPCs support the adequacy of the model, both for cefepime and enmetazobactam. In addition, age and eGFR are not covariates on the same parameter (eGFR on clearance and age on V1). Therefore, the inclusion of both age and eGFR is considered acceptable in the current procedure.

Some concerns have been identified regarding the PopPK. The lack of an allometric relationship for disposition parameters of peripheral compartments and the correlation between age and eGFR should still be handled appropriately in the simulation.

An updated version of the PopPK model of cefepime and enmetazobactam has been provided incorporating allometric body weight exponents on apparent volume parameters of both central and peripheral compartments. The estimated values were lower (0.407 for ENM and 0.553 for FEP) than the estimated values considering the central volume of distribution (0.618 for ENM and0.802 for FEP), which indicates less influence of BW on apparent volume of distribution of both compartments for both drugs. The updated model showed statistical improvement in terms of -2LL and better performance of the longitudinal PK profiles of ENM and FEP through the pcVPC. Overall, a more accurate description of the data is observed, which endorses the updated PopPK model.

Simulations used resampling of all covariates (absolute eGFR, body weight, age, sex) from the phase 3 population for all renal function groups except for the group with absolute eGFR <15 mL/min. For this group, covariates were resampled from the renal impairment study AT-102. The simulation settings are considered adequate, with no eGFR <15 mL/min patients available from the phase 3 study, sampling from the renal impairment study is considered appropriate to ensure that the correlation between covariates is kept. The applicant has also conducted a sensitivity analysis exploring a uniform distribution.

ELF PopPK models

ELF PopPK models of enmetazobactam and of cefepime were developed using plasma and ELF observations from 19 healthy volunteers in study AT-103. One BAL sample were available per subject but were not all taken at same time point. It is not feasible to take more than one BAL sample per subject and the data is considered sufficient to conduct a PopPK analysis for cefepime and enmetazobactam ELF. The enmetazobactam and cefepime plasma concentrations were described with two-compartment, linear PK models. The visual predictive checks indicate that the models adequately predict ELF data for both cefepime and enmetazobactam. The ELF PopPK models are deemed satisfactory. Both enmetazobactam and cefepime distribution to the lung were similar with an ELF to plasma ratio above 45% for both compounds.

Absorption

No PK comparison between formulations used in the clinical studies and commercial formulation is needed.

Elimination

It can be concluded that both cefepime and enmetazobactam are primarily excreted as unchanged drug in the urine. The elimination pathways of enmetazobactam are considered sufficiently well characterised. The role of renal elimination in the elimination of both active substances has also been confirmed in a dedicated study investigating the effects of renal impairment on enmetazobactam and cefepime exposure (Study AT-102).

Regarding cefepime, information is in accordance with approved EU SmPCs and is acceptable.

Regarding enmetazobactam, in the mass balance study, the total recovery was 99.1%, which is above the 90% limit recommended according to the Guideline on interactions and thus sufficient. More than 80% of the excreted dose has been identified as recommended.

Mean renal clearance for enmetazobactam was 5.4 l/h and filtration (fu*GFR) is expected to be around 6-7 l/h; thus active renal secretion is not expected to be involved in the renal elimination. Renal transporters are not expected to be involved in the elimination of enmetazobactam and there are no indications of enmetazobactam being a substrate of renal transporters.

One metabolite/degradation product has been identified for enmetazobactam (called "sulfinic acid impurity region" in the mass balance study). The sulfinic acid impurity is not considered a major metabolite and thus does not need to be investigated for DDI potential. It has not been further investigated how this metabolite/degradation product is formed but this is not considered critical considering that it is a minor route of elimination and that the metabolite is inactive.

The applicant discussed possible interconversion of enmetazobactam *in vivo*. Conversion to the enantiomer (ie inversion of all three chiral carbon atoms) is considered unlikely based on the molecular structure. Formation of diastereomers (epimers) could be possible and could potentially be differentiated using an achiral method, however no data is available.

As enmetazobactam is mainly eliminated by passive filtration in the kidney, any genetic polymorphisms in enzymes or transport proteins are not expected to be of clinical relevance.

Pharmacokinetics in target population

Note that the PopPK analysis is currently pending but the conclusion that the differences in enmetazobactam or cefepime exposures for healthy subjects and subjects with a cUTI infection were negligible is not expected to change.

Special populations

Renal impairment

The dedicated renal impairment study confirmed the role of renal elimination in the clearance of enmetazobactam as well as cefepime. The study was performed in accordance with guideline recommendations and classification of renal impairment was performed using absolute eGFR as recommended in the EMA RI guideline. As plasma protein binding is low for both substances, there is no need to measure unbound concentrations. The effect of renal impairment on AUC_{inf} was large, as expected for substances that are almost exclusively eliminated by renal excretion of unchanged drug and was similar for both active substances.

In mild RI (approximately 2-fold higher AUC for both active substances compared to normal renal function), no dose adjustment is proposed. This is similar to what has been previously approved for cefepime, although the cut-off then was 50 ml/min instead of 60 ml/min.

In moderate RI (approximately 3-fold higher AUC for both active substances compared to normal renal function), the dose is proposed to be halved compared to the normal dose without changing the dosage interval, i.e. the normal daily dose is halved. For cefepime, it has previously been approved to give the normal dose but with a longer dosage interval (every 12 hours instead of every 8 hours) in patients with a creatinine clearance of 30-50 ml/min, i.e., corresponding to decreasing the daily dose by one third. Thus, a lower total daily dose of cefepime in moderate RI is proposed than previously approved for the mono-component, and with a different dosage schedule (decreased dose instead of increased dosage interval).

In severe RI, 15-30 ml/min, (approximately 5-fold higher AUC for both active substances compared to normal renal function), the dose given on each occasion is proposed to be halved and the dosage interval increased from 8 to 12 hours, i e the normal daily dose is divided by 3. For cefepime, it has previously been approved to give the normal dose with a longer dosage interval (every 24 hours instead of every 8 hours, ie the normal daily dose divided by 3) at 11-29 ml/min. For subjects with ESRD (<15 ml/min) a dose of 1g/0.25g with a dosage interval of 24 hours is proposed (i.e., the normal daily dose divided by 6), similar to what has been approved for cefepime.

In patients requiring haemodialysis (approximately 11-12-fold higher AUC for both active substances compared to normal renal function when dosed after haemodialysis) a loading dose is proposed (same as in moderate and severe RI) followed by a lower dose (1/4 of the normal dose) given every 24 hours. The following doses thus correspond to the normal daily dose divided by 12. This is similar to what has previously been approved for cefepime.

The dose proposed in CAPD is the one approved for cefepime.

In the phase 3 study AT-301, patients with moderate renal impairment (eGFR ≥30 and <60 ml/min/m^2) were dosed as recommended in the dosage table proposed in the SmPC section 4.2. Patients with severe renal impairment (eGFR <30 mL/min/1.73 m^2) were excluded from study participation. The study included a large proportion of patients with mild RI and also a rather large group of patients with moderate RI. The MITT population receiving cefepime/enmetazobactam had 99 patients with moderate RI at baseline, 280 with mild RI and 108 with normal renal function. The corresponding numbers for the m-MITT population was 79, 187 and 63 patients, respectively.

From an efficacy perspective, see PD part regarding PTA analyses performed in order to support efficacy of the proposed dosing regime in subjects with renal impairment. The proposed dosing yielded satisfactory PTA for mild, moderate, severe and ESRD.

From a safety perspective, regarding cefepime, it can be concluded that the proposed total daily dose in different renal impairment groups is the same or lower than that approved for cefepime as mono-component. Thus, from a safety perspective, no concern is raised regarding cefepime. Regarding enmetazobactam, based on the results of the dedicated RI study, the following increases in exposure are expected with the proposed doses in different renal impairment groups compared to the exposure following normal dosage in subjects with normal renal function: 2-fold increase in subjects with mild RI, 1.5-fold increase in subjects with moderate RI, 1.7-fold increase in subjects with severe RI and 2-fold increase in subjects with ESRD (not on dialysis). Considering that almost 200 patients with mild RI received Exblifep without dose adjustment in the phase 3 study, with no signs of difference in safety due to renal impairment, a 2-fold increase in enmetazobactam exposure is considered covered by safety data. The expected enmetazobactam exposure increases in the other renal impairment groups can thus be considered safe.

The applicant has also performed simulations and compared C_{trough}, and C_{max} for different renal function groups to phase 3 C_{trough} and C_{max}. Simulated exposure was generally comparable and within the observed exposure in the phase 3 trial AT-301 for all groups for cefepime and for most groups for enmetazobactam. Slightly higher exposure was seen for enmetazobactam for the group with absolute eGFR of 60 - <90 mL/min and <15 mL/min.

For AUC, no comparison with the phase 3 exposure was made, with the Applicant explaining that the observed PK data in the phase 3 study was too sparse to reliably compute the AUC from. For this reason, the exposure comparison was limited to the comparison of C_{max} and C_{trough}.

AUC was generally comparable between renal function groups. For the <15 mL/min groups, the cefepime and enmetazobactam AUC were generally higher than the other groups including the Mild RI (60 - <90 mL/min) group. Exposure increase (AUC and C_{max}) similar to mild RI from the phase 3 study

is expected to be safe based on the phase 3 safety data. Supporting that all the exposure for all RI function groups except <15 mL/min are acceptable from a safety standpoint.

The applicant argues the group of <15 mL/min, the exposure was slightly higher because this group contained subjects that given their low renal function in clinical practice will require and undergo dialysis. This explanation is accepted.

Overall, the applicant has shown that the exposure is generally comparable between RI groups and from a safety standpoint the proposed posology is considered adequate.

The applicant developed a PK model for patients on continuous renal replacement therapy (CRRT). The simulated dose needed to achieve adequate PTA is higher than for haemodialysis and this is considered an important finding. With the assumptions made for the simulations, and considering the different settings of the CRRT and different types of CRRT, the simulations are uncertain and making a firm dosing recommendations in the SmPC based on the simulations is difficult. Instead, a general text regarding dosing in CRRT has been included in the SmPC, which is adequate. In addition, the MAH is recommended to provide data describing the clearance of enmetazobactam and cefepime when treating individuals receiving continuous renal replacement therapy (CRRT), in order to provide more specific dosing recommendations (see letter of Recommendations).

The applicant has provided simulations to support that increasing the infusion duration to 4 h for patients with augmented renal clearance optimises PTA while keeping the same daily dose (see PD part).

Hepatic impairment

As both substances are mainly renally eliminated as unchanged drug, hepatic impairment is not expected to impact the PK to a relevant extent. The applicant´s proposal in section 4.2 of the SmPC that no dose adjustment is needed in patients with impaired hepatic function is supported.

Gender, race, weight, age

Dose adjustment with regards to body weight in adult patients is not necessary and dose adjustments based on only eGFR is satisfactory in adults. The conclusion that dose adjustments are not warranted based on sex/gender is also agreed with. Race is unlikely to play a role for substances that are renally excreted as unchanged drug.

No dose adjustment is proposed based on age alone, but only if renal function is altered. This is agreed.

No data in the paediatric population is available for enmetazobactam or the combination cefepime/enmetazobactam. The PopPK analysis includes some simulations in paediatric patients but they are not part of this MAA and the simulations have not been included in the assessment.

Interactions

The PBPK model that was created to evaluate the potential for enmetazobactam to inhibit CYP2E1 activity has not been assessed in detail as the platform is not considered qualified for this enzyme.

Pharmacokinetic interactions with cefepime as victim are not expected, as cefepime is mainly renally eliminated as unchanged drug via filtration. As cefepime in the same dosing is approved and clinically used for a long time, no further data addressing the risk that cefepime would be an enzyme or transporter inhibitor or inducer are requested.

No direct or time-dependent inhibition of the mandatory CYP enzymes by enmetazobactam was observed (study EMT-002-DDI). The highest concentration tested (3.5 mM) is sufficiently high to exclude *in vivo*-relevant inhibition.

In study AAI-PK-17-03 *in vitro*-inhibition of CYP2E1 was observed, with an IC_{50} value of 55.8 µM which is well below the systemic cut-off of around 3 mM and thus a signal that clinically relevant inhibition cannot be excluded. Two new studies were then performed (AAI101-PK-19-01 and EMT-003-DDI), using a higher concentration range, where neither direct inhibition nor TDI was observed (TDI was only investigated in study AAI101-PK-19-01). There is no clear reason to disregard the first study based on eg technical issues that would make the study unreliable. However, based on the two other *in vitro* studies and considering that CYP2E1 is not a mandatory enzyme to investigate, it can be agreed to not include any warnings in the SmPC.

There is a total of three CYP induction studies investigating the induction potential of enmetazobactam on mRNA level (and a fourth study, AAI-PK-18-03, investigating the effects on enzyme activity). It is concerning that in the *in vitro* induction study AAI-PK-17-02, enmetazobactam caused very large increases of mRNA expression of CYP1A2, CYP2B6 and CYP3A4 in all three tested hepatocyte batches (effects in some cases larger or similar compared to that of the positive controls). According to the DDI guideline, induction results should be evaluated separately for each donor and the donor cells with the most pronounced induction effect on the specific enzyme should then be used as a "worst case" in the subsequent calculations. In this case, it is however considered sufficiently justified that the results of study AAI101-PK-17-02 is likely due to experimental issues and can be regarded as non-conclusive, considering the results of the two later mRNA induction studies that did not demonstrate a clinically relevant risk of induction by enmetazobactam, with some support also from the study investigating induction on activity level, and considering that no concentration-dependency was observed in study AAI101-PK-17-02. If the results of study AAI-PK-17-02 were true indications of an induction effect, the two later clean *in vitro* mRNA induction studies would be very unlikely. Thus, it can be concluded that there is no *in vitro* signal of clinically relevant induction by enmetazobactam.

Exposure relevant for safety evaluation

Regarding AUC, it is assumed that the presented AUC_{last} values in Table 10 and Table 11 (62-75 µg h/ml for enmetazobactam 0.5 g and 311-380 ug h/ml for cefepime 2 g) refer to AUC_{tau} so that the total daily AUC relevant for safety evaluation is 3 times these values, ie (based on the day 7 values which are highest), 225 µg h/ml for enmetazobactam and 1140 µg h/ml for cefepime.
Considering that almost 200 patients with mild RI received Exblifep without dose adjustment in the phase 3 study, with no signs of difference in safety due to renal impairment, a 2-fold increase in enmetazobactam exposure is considered covered by safety data.

SmPC

In the proposed SmPC, dose adjustments are only proposed in patients with renal impairment. As both active substances are primarily eliminated renally as unchanged drug, exposure is significantly affected by reduced renal function and dose adjustment is needed in these patients. See above on Renal impairment.

Pharmacodynamics

Mechanism of action

Cefepime is a 4th-generation cephalosporin beta-lactam (BL) antibacterial agent that inhibits bacterial cell-wall synthesis by targeting penicillin-binding proteins (PBPs).

Enmetazobactam is a novel penicillanic acid sulfone beta-lactamase inhibitor (BLI), structurally related to tazobactam, with inhibitory activity against certain beta-lactamases which prevents the hydrolysis of cefepime.

In vitro activity

Cefepime exerts antibacterial activity against aerobic gram-negative and gram-positive pathogens. Although cefepime is subject to hydrolysis by class A extended spectrum beta-lactamases (ESBL) and both class A and B carbapenemases, it is generally stable to hydrolysis by class C AmpC and class D OXA-48 beta-lactamases.

Based on IC_{50} values the potential value of adding enmetazobactam to cefepime is in strains harbouring class A beta-lactamases (including non-ESBL variants of SHV and TEM, ESBL, and KPC).

When considering the *in vitro* activity of cefepime alone and the combination, the value of the addition of enmetazobactam is demonstrated mainly in Enterobacterales spp. with an ESBL genotype. The combination cannot be expected to be active against Enterobacterales with a KPC genotype although some isolates may have lower MIC-values at a level that would be considered susceptible considering current breakpoints for cefepime. Enmetazobactam doesn't have relevant inhibitory activity against OXA-48 carbapenemases and its main added value to cefepime is in isolates that co-produce ESBLs.

The value of the combination compared with cefepime alone in other aerobic gram-negative bacteria such as *P. aeruginosa* and *A. baumannii,* anaerobic gram-negative bacteria and gram-positive bacteria is none or limited except for a possible carbapenem-sparing potential against carbapenem-susceptible *A. baumannii*. This potential added value would only be clinically relevant in settings with a low prevalence of *A. baumannii* carbapenem-resistance.

In summary, the combination of cefepime and enmetazobactam appears mainly to be of value against ESBL-producing Enterobacterales but may have a utility as a carbapenem-sparing combination also against OXA-48 producing Enterobacterales (when these are co-producing ESBLs) and carbapenem-susceptible *A. baumannii*.

Resistance to cefepime-enmetazobactam

Mechanisms of resistance to cefepime-enmetazobactam include production of beta-lacatamases that can hydrolyse cefepime and which are not inhibited by enmetazobactam, porin mutations which affect outer membrane permeability, overexpression of efflux pumps and mutations in the PBP genes.

Summary of support for dose selection

A cefepime dose of 2 g administered every 8 hours as a 2-hour infusion was selected to optimise cefepime exposure. This is the highest dose recommended for cefepime when used alone and applying a prolonged infusion time (normally 30 minutes) which increases the time the drug concentration reaches above the MIC of the pathogen.

Selection of a 0.5 g dose of enmetazobactam administered every 8 hours as a 2-hour infusion was mainly based on joint PTA analyses using PDTs historically derived for cefepime and PDTs derived from murine neutropenic thigh and lung infection models for enmetazobactam.

In hollow fibre infection models, it was preliminary demonstrated that the PK-PD index best correlated with enmetazobactam activity was the percent of time the unbound drug fraction exceeds a certain threshold. This was confirmed in the neutropenic thigh model of infection. The PK-PD relationship of enmetazobactam was best described by %*f*T >2 µg/ml.

Nine clinical isolates of cefepime-resistant, ESBL-producing *K. pneumoniae* were included in the thigh infection model to derive pharmacodynamic targets (PDTs). For the 75th percentile, 44% *f*T >2 µg/ml enmetazobactam were identified for a bioburden reduction of 1-$\log_{10}$ CFU/g. From these findings, an enmetazobactam non-clinical target of 45% *f*T>CT was selected which is acceptable. AUC also correlated relatively well with enmetazobactam activity (Figure 65), and the AUC studied in the thigh model that provided activity is fairly similar to the AUC in patients, providing some additional support. As mentioned above, historically defined pharmacodynamic targets used for cefepime: 60% *f*T >MIC,

which is associated with maximal bacterial killing by cephalosporins; and 68% *f*T >MIC, which is associated with higher odds of survival for patients with gram-negative bloodstream infections.

The PK-PD was furthermore evaluated in the murine neutropenic lung infection model. The number of isolates tested in the model were very limited and the PDTs derived from this model for cefepime and enmetazobactam in ELF were lower than those historically shown for cefepime and in the thigh infection model for enmetazobactam and resulted in an even higher effect on bacterial bioburden reduction (≥2 $\log_{10}$ compared with ≥1 $\log_{10}$). Because the thigh model is the more established model to derive PDTs and the limitations noted regarding the lung experiments, the PTA analyses based on the more conservative targets derived from the thigh model appears more reasonable to consider in the dose justification for enmetazobactam in lung infections. However, the applicant was asked to provide PTA-analyses using PDTs derived from the thigh model for enmetazobactam and historically PDTs for cefepime and considering the ratio of the respective drugs that reaches the lung/ELF .

The joint PTA was satisfactorily above (or very close to) 90% using a joint target of 45% fT>CT=2 µg/ml for enmetazobactam and 60% fT>MIC or 68% fT>MIC for cefepime up to an MIC of 8 µg/ml for all renal function categories except for those with augmented renal clearance (eGFR >150 ml/min). For subjects with augmented renal clearance the limiting factor for an acceptable PTA appears to be the dose of cefepime. The Applicant has provided simulations to support that increasing the infusion duration to 4 h for patients with augmented renal clearance optimises PTA while keeping the same daily dose (no concerns with regards to safety).

PTA ELF simulations were performed using the updated PopPK model using PDTs derived from the thigh model for enmetazobactam (45% fT>CT of 2 µg/mL) and the historically PDT used for cefepime (60% fT> MIC) and considering the ratio of the respective drugs that reaches the lung/ELF. The simulations indicated that a 2-hour infusion might not be sufficient to ensure adequate exposure to maximise the efficacy in the lung. Simulations performed using a 4-hours infusion predict PTA above 0.9 in the different renal function groups except for the patients with augmented renal clearance or the one with severe renal insufficiency and requiring dialysis. Therefore, the applicant now proposes a 4-hour infusion for patients with pneumonia. This is endorsed.

There is a consensus about the PK/PD targets used in plasma, as well as an adequate evaluation of the PK/PD targets in ELF by assuming different targets. However, the current simulation strategy could be greatly affected if a cefepime free fraction of close to 60% were assumed. However, the applicant has justified the use of 80% as the free fraction of cefepime based on recent literature data and Renapime SmPC. The clarification provided regarding lower protein binding values reported in the literature is considered acceptable. Therefore, the use of 80% protein binding for cefepime as a fixed parameter to derive the unbound concentrations of cefepime is endorsed.

Pharmacodynamic interactions

Antagonism was not demonstrated in drug combinations studies with cefepime-enmetazobactam except for a single instance of antagonism when cefepime enmetazobactam was combined with gentamicin against a strain of *P. aeruginosa.* In repeated *in vitro* experiments no antagonism between cefepime-enmetazobactam and gentamicin clinical isolates of *Pseudomonas aeruginosa* was detected.

2.5.4. Conclusions on clinical pharmacology

Regarding pharmacokinetics, the application is approvable.

Regarding pharmacodynamics, it is notable that the efficacy demonstration for enmetazobactam rests on preclinical PK/PD experiments and models, as the design of the pivotal trial does not allow for the isolation of the effect of this new active substance. This has been accepted for previous, analogous

applications. The applicant has provided relevant data that describe the *in vitro* activity of enmetazobactam, the potentiation of cefepime activity by the addition of enmetazobactam *in vitro* and *in vivo* and studies and analyses for dose selection of the single components of the fixed-dose-combination.

The MAH is recommended to provide data describing the clearance of enmetazobactam and cefepime when treating individuals receiving continuous renal replacement therapy (CRRT), in order to provide more specific dosing recommendations (see letter of Recommendations).

2.5.5. Clinical efficacy

The applicant has applied for the following indications:

Exblifep is indicated for the treatment of the following infections in adults (see sections 4.4 and 5.1):

- Complicated urinary tract infections (cUTI), including pyelonephritis

- Hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)

Treatment of patients with bacteraemia that occurs in association with, or is suspected to be associated with, any of the infections listed above.

Exblifep is also indicated for the treatment of infections due to aerobic Gram-negative organisms in adults with limited treatment options (see sections 4.2, 4.4 and 5.1)

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

The clinical development programme for cefepime-enmetazobactam relies partly on previous findings of safety and efficacy of cefepime when used alone. Cefepime, a 4th-generation cephalosporin, was first authorised in Sweden in 1993 (Maxipime). Generics of cefepime is, or has been, marketed by different MAHs in the EU. The indications for cefepime when used alone includes (among other indications) treatment of complicated urinary tract infections, including pyelonephritis, treatment of pneumonia (without a restriction to either community acquired pneumonia or hospital acquired pneumonia, including ventilator associated pneumonia) and bacteraemia when associated with these types of infections.

The clinical development program for cefepime-enmetazobactam included 6 clinical studies:

- Study AT-101, a Phase 1 study with both single-ascending and multiple-ascending doses of enmetazobactam alone and in combination with cefepime in healthy adult male subjects
- Study AT-102, a Phase 1 study of cefepime-enmetazobactam in adults with varying degrees of renal impairment or normal renal function. The effect of dialysis in subjects with end-stage renal disease (ESRD) was also assessed in this study
- Study AT-103, a Phase 1 study evaluating the concentrations of cefepime-enmetazobactam in plasma and epithelial lining fluid (ELF)
- Study AT-104, a mass balance and metabolite identification study in healthy adult male subjects
- Study AT-201, a Phase 2 study to determine the optimal cefepime-enmetazobactam combination dose and the probability of target attainment (PTA) in subjects with cUTI, including AP
- Study AT-301, a Phase 3 study to assess the efficacy of cefepime-enmetazobactam compared with piperacillin-tazobactam in the treatment of cUTI, including AP

The clinical studies mainly relevant to the applied indications is study AT-301 to provide comparative safety and efficacy data and PK-data from patients to update the PopPK model for PTA analyses and study AT-103 to provide enmetazobactam PK-data from ELF to support treatment of lung infections.

It should be noted that phase 3 studies evaluating a beta-lactam (BL) at its approved dose combined with a new beta-lactamase inhibitor (BLI) may not provide stand-alone efficacy data to support the dose regimen for the BLI. This is because it is not a strict requirement to confine such studies to infections caused by pathogens resistant to the BL but susceptible to the BL-BLI combination. Therefore, the PK/PD analyses incorporating the non-clinical PK/PD data and patient PK data are generally considered pivotal to support the dose regimen of the BLI for the proposed indications.

The applicant has received CHMP scientific advice (2018 and 2019) regarding the completeness and robustness of the non-clinical animal PK/PD package, the proposed Phase 3 trial design and the adequacy of the Phase 1 ELF penetration study results in support of the use of cefepime-enmetazobactam in HAP and VAP indications. In general, the CHMP considered the proposed non-clinical package and clinical programme appropriate to support the proposed indications. However, the applicant was informed that cefepime-enmetazobactam would not be eligible for a pathogen-specific indication in patients with limited treatment options as currently proposed.

2.5.5.1. Dose response study

Study AT-201 – A randomized, double-blind, multi-centre study of cefepime/AAI101 (enmetazobactam) in hospitalized adults with complicated urinary tract infections, including acute pyelonephritis

This was a phase 2 study with the primary objective to determine the optimal cefepime-enmetazobactam combination dose to be used in future Phase 3 studies via PK/PD modelling to assess the probability of target attainment (PTA) and the effect of treatment on liver enzymes (see Clinical safety section). The secondary objective was to assess the safety and tolerability. Efficacy was an exploratory objective.

Hospitalised adults with complicated urinary tract infection, including acute pyelonephritis were randomised in a 2:1 ratio in 2 cohorts. Cohort 1 received cefepime 1 g and enmetazobactam 0.5 g or cefepime 1 g infused over 2 h q8h. Cohort 2 received cefepime 2 g and enmetazobactam 0.75 g or cefepime 2 g infused over 2 h q8h. Treatment duration for each cohort was 7 to 10 days. No oral switch was allowed.

The study population (m-MITT population) consisted of 66.7% females at a mean age of 50.0 years. The majority had acute pyelonephritis (66.7%). *E. coli* and *K. pneumoniae* were the most cultured pathogens (66.7% and 23.1%, respectively). Among the 39 patients in the m-MITT Population, 11 of the baseline pathogens identified were ESBL-producers.

Table 28: Per-Patient Microbiological Response Based on EMA Colony Forming Units/mL Criteria – Microbiological Modified Intent-to-Treat Population

Time Point Microbiological Response	Cohort 1 Cefepime 1 g/ AAI101 500 mg (N=13) n (%)	Cohort 1 Cefepime 1 g (N=7) n (%)	Cohort 2 Cefepime 2 g/ AAI101 750 mg (N=11) n (%)	Cohort 2 Cefepime 2 g (N=8) n (%)
Day 3				
Eradication	12 (92.3)	7 (100.0)	10 (90.9)	7 (87.5)
Persistence	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)
Indeterminate	0 (0.0)	0 (0.0)	1 (9.1)	1 (12.5)
End of Treatment				
Eradication	11 (84.6)	5 (71.4)	10 (90.9)	7 (87.5)
Persistence	1 (7.7)	1 (14.3)	0 (0.0)	0 (0.0)
Indeterminate	1 (7.7)	1 (14.3)	1 (9.1)	1 (12.5)
Test of Cure				
Eradication	10 (76.9)	7 (100.0)	10 (90.9)	4 (50.0)
Persistence	3 (23.1)	0 (0.0)	0 (0.0)	3 (37.5)
Indeterminate	0 (0.0)	0 (0.0)	1 (9.1)	1 (12.5)
Late Follow-up				
Eradication	6 (46.2)	6 (85.7)	9 (81.8)	4 (50.0)
Persistence	5 (38.5)	1 (14.3)	1 (9.1)	3 (37.5)
Indeterminate	2 (15.4)	0 (0.0)	1 (9.1)	1 (12.5)

Percentage was calculated using the number of patients in the column heading as the denominator.
EMA = European Medicines Agency.
Source: Post-text Table 14.2.1.2

In patients with an ESBL-producing organism for the micro-MITT Population at TOC, most patients had a microbiological response of eradication except for 1/3 (33.3%) patients in the cefepime 1 g/enmetazobactam 500 mg treatment group (Cohort 1) and 1/1 (100.0%) patient in the cefepime 2 g treatment group (Cohort 2) who had a microbiological response of persistence.

Table 29: Clinical Response – Microbiological Modified Intent-to-Treat Population

Time Point Clinical Response	Cohort 1 Cefepime 1 g/ AAI101 500 mg (N=13) n (%)	Cohort 1 Cefepime 1 g (N=7) n (%)	Cohort 2 Cefepime 2 g/ AAI101 750 mg (N=11) n (%)	Cohort 2 Cefepime 2 g (N=8) n (%)
Day 3				
Improvement	13 (100.0)	7 (100.0)	10 (90.9)	7 (87.5)
Failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Indeterminate	0 (0.0)	0 (0.0)	1 (9.1)	1 (12.5)
End of Treatment				
Cure	13 (100.0)	7 (100.0)	10 (90.9)	7 (87.5)
Failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Indeterminate	0 (0.0)	0 (0.0)	1 (9.1)	1 (12.5)
Test of Cure				
Cure	13 (100.0)	7 (100.0)	10 (90.9)	7 (87.5)
Failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Indeterminate	0 (0.0)	0 (0.0)	1 (9.1)	1 (12.5)
Late Follow-up				
Cure	12 (92.3)	7 (100.0)	10 (90.9)	6 (75.0)
Failure	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)
Indeterminate	1 (7.7)	0 (0.0)	1 (9.1)	1 (12.5)

Percentage was calculated using the number of patients in the column heading as the denominator.
Source: Post-text Table 14.2.2

2.5.5.2. Main study

AT-301 - A phase 3, randomized, double-blind, multi-centre study to evaluate the efficacy, safety, and tolerability of cefepime-AAI101 (enmetazobactam) compared to piperacillin/tazobactam in the treatment of complicated urinary tract infections, including acute pyelonephritis, in adults

This was a phase 3, randomised, double -blind, multicentre study of cefepime-enmetazobactam compared with piperacillin-tazobactam in hospitalised adults with cUTI/acute pyelonephritis.

Methods

Study Participants

Main inclusion Criteria

1. Male or female patients ≥18 years of age at the time of signing of informed consent.

2. Expectation that the patient's cUTI or AP would require hospitalisation and initial treatment with at least 7 days of IV antibiotics.

3. Pyuria, defined as:

    a. White blood cell count >10 cells/mm^3 in unspun urine or ≥10 cells/high power field in spun urine sediment; or

    b. Urinalysis/dipstick analysis positive for leukocyte esterase

4. Clinical signs and/or symptoms of cUTI or AP, as defined in table below.

Table 30: Definition of Complicated Urinary Tract Infection and Acute Pyelonephritis

cUTI	AP
At least 2 of the following new or worsening symptoms: • Dysuria, increased urinary frequency, or urinary urgency; • Fever (oral/tympanic ≥38°C [≥100.4°F] or rectal temperature ≥38.3°C [≥100.9°F]) observed and documented by a health care provider within 24 hours of Screening; • Lower abdominal pain or pelvic pain; • Suprapubic tenderness on physical examination; or • Nausea or vomiting within 24 hours of Screening as reported by the patient; **AND** At least 1 of the following complicating factors: • Male patients with documented history of urinary retention (*e.g.*, benign prostatic hypertrophy); • Use of intermittent bladder catheterization or presence of an indwelling bladder catheter; NOTE: Indwelling bladder catheters that had been in place for >24 hours prior to Screening must have been removed or replaced prior to collection of the Screening visit urine for urinalysis and culture unless removal or replacement was considered unsafe or contraindicated. • Current obstructive uropathy that was scheduled to be medically or surgically relieved during i.v. study therapy and before EOT; • Any functional or anatomical abnormality of the urogenital tract (including anatomic malformations or neurogenic bladder) with voiding disturbance resulting in at least 100 mL residual urine; or • Azotemia, defined as blood urea nitrogen >20 mg/dL, blood urea >42.8 mg/dL, or serum creatinine >1.4 mg/dL, due to known prior intrinsic renal disease.	At least 2 of the following new or worsening symptoms: • Dysuria, increased urinary frequency, or urinary urgency; • Fever (oral/tympanic ≥38°C [≥100.4°F] or rectal temperature ≥38.3°C [≥100.9°F]) observed and documented by a health care provider within 24 hours of Screening; • Flank pain (onset within 7 days prior to randomization); • Costo-vertebral angle tenderness on physical examination; or • Nausea or vomiting within 24 hours of Screening, as reported by the patient.

AP = acute pyelonephritis; cUTI = complicated urinary tract infection; EOT = End of Treatment; i.v. = intravenous(ly).
Source: Study Protocol AT-301 (Appendix 16.1.1)

Main exclusion Criteria

1. Known urine culture with Gram-positive primary pathogen at $\geq 10^5$ colony-forming units (CFU)/mL (not contaminant) or suspected Gram-positive pathogen by Gram staining.

2. History of significant hypersensitivity or allergic reaction to cefepime, piperacillin/tazobactam, any of the excipients used in the respective formulations, any beta-lactam antibiotics, or any BLIs.

3. Concurrent infection that would have interfered with evaluation of response to the study antibiotics.

4. Need for or receipt of concomitant systemic antimicrobial agents after signing of informed consent, in addition to those designated in the study-treatment groups, except for a single oral dose of any antifungal treatment for vaginal candidiasis.

5. Receipt of potentially effective systemic antibacterial therapy for a continuous duration of >24 hours during the previous 72 hours before the study-qualifying baseline urine was obtained.

6. cUTI known at study entry to be caused by pathogens resistant to the study antibiotics.

7. Suspected or confirmed acute bacterial prostatitis, orchitis, epididymitis, or chronic bacterial prostatitis as determined by history and/or physical examination.

8. Impairment of renal function with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m2 calculated by the 4-variable Modification of Diet in Renal Disease (MDRD) study equation.

The study was conducted at 90 sites in Europe, North America, South America, and South Africa.

Treatments

Adult patients were randomised in a 1:1 ratio to receive either 2 g cefepime plus 500 mg AAI101 or 4.5 g piperacillin/tazobactam (4 g piperacillin/0.5 g tazobactam) q8h IV as follows:

- 2 g cefepime plus 500 mg AAI101 infused over a period of 2 hours q8h for 7 days (up to 14 days in patients with a positive blood culture at baseline); or
- 4.5 g piperacillin/tazobactam infused over a period of 2 hours q8h for 7 days (up to 14 days in patients with a positive blood culture at baseline).

No switch to oral antibiotic therapy was permitted. Dose adjustments were made in patients with renal impairment.

Objectives

The primary objective of this study was to assess the efficacy of cefepime-enmetazobactam compared to piperacillin/tazobactam (non-inferiority) in the treatment of cUTI, including acute pyelonephritis (AP).

The secondary objectives of this study were the following:

- To assess the safety and tolerability of cefepime-enmetazobactam in hospitalised patients with cUTI or AP; and
- To characterise the PK of cefepime-AAI101 in patients with cUTI or AP.

Outcomes/endpoints

Primary efficacy endpoint

The primary efficacy endpoint was the proportion of subjects in the microbiological Modified Intent-to-Treat (m-MITT) population who achieved overall success at TOC, determined as a composite of clinical cure and microbiological eradication.

Secondary efficacy endpoints included the following:

- Proportion of subjects in the m-MITT population with overall success at Day 3, EOT, and LFU
- Proportion of subjects in the m-MITT and Microbiologically Evaluable populations with a microbiological outcome of Eradication at Day 3, EOT, TOC, and LFU
- The proportion of subjects in the m-MITT Population, including subjects with resistant isolates (m-MITT+R) and ME Population, including subjects with resistant isolates (ME+R), with a microbiological outcome of Eradication at Day 3, EOT, TOC, and LFU
- The proportion of subjects with a clinical outcome of Cure or Improvement (Day 3 only) in the m-MITT, m-MITT+R, Clinically Evaluable (CE), ME, and ME+R Populations at Day 3, EOT, TOC, and LFU
- Per-pathogen overall treatment success, clinical outcome of Cure, and microbiological outcome of Eradication in the m-MITT, m-MITT+R, ME, and ME+R Populations at Day 3, EOT, TOC, and LFU

Additional secondary efficacy endpoints included an analysis of overall success, clinical cure, and eradication at TOC for each discrete cefepime-enmetazobactam and piperacillin/tazobactam baseline MIC value.

The clinical and microbiological outcome criteria are tabled below:

Table 31: Clinical Outcome Criteria

Category	Criteria
Cure	The complete resolution (or return to premorbid state) of the baseline signs and symptoms of cUTI or AP that were present at Screening (and no new urinary symptoms or worsening of symptoms), such that no further antimicrobial therapy to treat the cUTI/AP was warranted. Symptom resolution did not necessarily include baseline symptoms associated with anatomic abnormalities that predispose to cUTI, such as symptoms associated with the presence of an indwelling urinary catheter. **This outcome category could have been used at Day 3, EOT, TOC, LFU, and ET.**
Improvement	Lessening, incomplete resolution, or no worsening of baseline clinical signs and symptoms of cUTI or AP, but continued i.v. therapy for management of cUTI/AP was warranted. **This outcome category could only have been used at Day 3.**
Failure	Patients who experienced any 1 of the following: • At Day 3 and EOT, worsening of baseline clinical signs and symptoms of cUTI or AP or the development of new clinical signs and symptoms of infection, sufficient to stop study drug and initiate non-study antimicrobial; • At TOC and LFU Visits, persistence, incomplete resolution of baseline clinical signs and symptoms of infection, requiring additional antibiotic therapy; • Withdrawal from the study drug due to an adverse event or due to lack of clinical improvement; or • Death of the patient during the study. Note: Withdrawal from the study drug due to an adverse event or due to lack of clinical improvement or death of the patient during the study led to clinical failure for the subsequent time points but did not change the previous time point assessment. **This outcome category could have been used at Day 3, EOT, TOC, LFU, and ET.**
Indeterminate	Clinical outcome could not be determined. **This outcome category could have been used at Day 3, EOT, TOC, LFU, and ET.**

AP = acute pyelonephritis; cUTI = complicated urinary tract infection; EOT = End of Treatment; ET = Early Termination; i.v. = intravenous(ly); LFU = Late Follow-up; TOC = Test of Cure.
Source: Statistical Analysis Plan version 2.0 (Appendix 16.1.9)

Table 32: Microbiological Outcome Criteria

Category	Criteria
Eradication	• The baseline qualifying Gram-negative pathogen(s) was reduced to $<10^3$ CFU/mL in urine culture; AND • A negative blood culture for a Gram-negative pathogen that was identified as a uropathogen (if repeated after positive baseline blood culture).
Persistence	• Demonstration that 1 or more of the baseline Gram-negative pathogen(s) remained continuously present in urine culture at $\geq 10^3$ CFU/mL; OR • A continuously positive blood culture with an organism that was identified as a Gram-negative uropathogen.
Recurrence	• Isolation of the same baseline Gram-negative pathogen(s) from urine culture after a response of Eradication; OR • A positive blood culture with the same baseline Gram-negative pathogen that was identified as a uropathogen after a response of Eradication.
Indeterminate	No urine culture was available, or the culture could not be interpreted for any reason.

NOTE: The baseline qualifying pathogen was defined as a non-contaminated baseline Gram-negative organism $\geq 10^5$ CFU/mL for urine or growth in blood culture concurrently.
CFU = colony-forming unit(s).
Source: Statistical Analysis Plan version 2.0 (Appendix 16.1.9)

The following analysis sets were used for statistical analysis and presentation of data.

- *Intent-to-Treat (ITT) Population*: All patients who were randomised.
- *PK Population*: All patients in the ITT Population who had at least 1 PK sample taken. PK analyses were based on actual treatment received.
- *Modified Intent-to-Treat (MITT) Population*: All patients who met ITT criteria and received any amount of study drug.
- *Microbiological Modified Intent-to-Treat Population (m-MITT) Population*: All randomised patients who met MITT criteria and who had a baseline Gram-negative pathogen $\geq 10^5$ CFU/mL in urine culture or the same pathogen present in concurrent blood and urine cultures that caused the cUTI that was not resistant to cefepime-AAI101 (MIC determined with AAI101 at a fixed concentration of 8 μg/mL) or piperacillin/tazobactam (defined as MIC ≤8 μg/mL or MIC ≤64 μg/mL, respectively). If ≥3 bacterial isolates were identified, the culture was considered contaminated regardless of colony count unless 1 of the isolates that grew in the urine, even if $\leq 10^5$ CFU/mL, was also isolated from a blood culture obtained within 48 hours prior to randomisation. Any patient with only a Gram-positive pathogen or a bacterial species typically not expected to respond to both study drugs at $\geq 10^5$ CFU/mL, was excluded from the m-MITT Population. Patients with contaminated Screening and baseline samples were also excluded from the m-MITT Population. The m-MITT Population was the primary efficacy population. Efficacy analyses were based on the treatment as randomised.
- *Microbiological Modified Intent-to-Treat Population Including Patients with Resistant Isolate (m-MITT+R) Population*: All randomised patients who met MITT criteria and who had a baseline Gram-negative pathogen $\geq 10^5$ CFU/mL in urine culture or the same pathogen present in concurrent blood and urine cultures that caused the cUTI, including isolates resistant to cefepime-AAI101 (MIC determined with AAI101 at a fixed concentration of 8 μg/mL) or piperacillin/tazobactam (defined as MIC >8 μg/mL or MIC >64 μg/mL, respectively). If ≥3 bacterial isolates were identified, the culture was considered contaminated regardless of colony count unless 1 of the isolates that grew in the urine, even if $\leq 10^5$ CFU/mL, was also isolated from a blood culture obtained within 48 hours prior to randomisation. Any patient with only a Gram-positive pathogen at Baseline was excluded from the m-MITT+R Population. Patients with contaminated Screening and baseline samples were excluded from the m-MITT+R Population.

- *Clinically Evaluable (CE) Population or Per-protocol (PP) population*: All patients who met the definition for the MITT Population and who met the following important components of the study as specified in the Protocol:
    - Received a total duration of antibacterial therapy of at least 15 consecutive doses of study drug or were classified as clinical failures after completing at least 9 doses of IV study drug therapy
    - Had a clinical assessment at TOC, unless criteria for clinical failure were met at an earlier time point
    - Did not receive concomitant antibacterial therapy with a non-study antibacterial drug to which the uropathogen was susceptible between the time of the baseline culture and the TOC culture, unless criteria for clinical failure were met
    - Did not have any other major protocol violations that would have affected assessment of efficacy
- *Microbiologically Evaluable (ME Population)*: All patients who met the definition for both the m-MITT and CE Populations. In addition, to be included in the ME Population, patients must not have had a microbiological outcome at TOC of Indeterminate.

  Concomitant administration of narrow-spectrum Gram-positive active agents to patients who had Gram-positive and Gram-negative co-infection did not affect patient evaluability in the CE or ME Populations.

- *Microbiologically Evaluable Population Including Patients with Resistant Isolates (ME+R) Population*: All patients who met the definition for both the m-MITT+R and CE Populations. In addition, to be included in the ME+R Population, patients must not have had a microbiological outcome at TOC of Indeterminate

  Concomitant administration of narrow-spectrum Gram-positive active agents to patients who had Gram-positive and Gram-negative co-infection did not affect patient evaluability in the CE or ME+R Populations.

- *Safety Population*: All patients who received at least 1 dose of study drug during the study. All safety analyses were based on actual treatment received.

Sample size

A study enrolling 810 patients who were evaluable in the m-MITT Population provided 90% power to demonstrate the non-inferiority of cefepime-AAI101 to piperacillin/tazobactam in the m-MITT population, assuming the overall success rate was 74% in both groups and the non-inferiority margin was 10 percentage points. It was planned that the study would continue until 810 patients were evaluable in the m-MITT Population. It was estimated that approximately 1,040 patients would be recruited to achieve 810 evaluable patients, assuming an evaluability rate of 78%.

Randomisation and blinding (masking)

Randomisation was coordinated through a centralised Interactive Response Technology (IRT) system.

It was planned that at least 50% of randomised patients would have cUTI and at least 30% would have AP.

To ensure balance among the treatment groups, randomisation was stratified by the following factors:

- Type of infection (AP versus cUTI with removable source of infection [e.g., Foley catheter] versus cUTI without removable source of infection, but with other risk factors [e.g., anatomical abnormality, neurogenic bladder, or azotemia])
- Prior antibiotic therapy (short-acting antibiotic up to 24 hours versus no prior antibiotic therapy); and
- Region: Eastern Europe versus Americas (Latin America and United States) versus other countries (including Western Europe, Baltics, and South Africa).

Study patients, CROs and vendors, the sponsor, investigators, and site personnel carrying out study procedures, evaluating patients, entering study data, and/or evaluating study data were blinded to treatment assignment until database lock. Study site personnel involved in the preparation of the study drug (i.e., pharmacist or designated staff member) were unblinded to the patient's randomised treatment.

A total of 8 patients were unblinded during the study: 4 of the unblinding events occurred erroneously or accidentally by site staff and 4 unblinding events occurred as a result of SAEs. One patient was randomised according to the Cenduit IRT system but identified as a screen failure in the eCRF database. The patient was erroneously randomised. The patient was discontinued from the study. Three patients had all completed treatment when the accidental unblinding events occurred. Four patients experienced SAEs and were unblinded to determine suspected unexpected serious adverse reaction (SUSAR) reporting requirements.

Statistical methods

Analysis of primary efficacy endpoint

The primary efficacy endpoint was the proportion of patients in the m-MITT Population who achieved overall treatment success (clinical cure and microbiological eradication) at the TOC Visit. Overall treatment success was defined as the composite of clinical outcome of Cure and the microbiological outcome of Eradication ($<10^3$ CFU/mL in urine culture). Patients were programmatically categorised as a success, failure, or indeterminate response.

Clinical Outcome	Microbiological Outcome			
	Eradication	**Persistence**	**Recurrence[a]**	**Indeterminate**
Cure	Success	Failure	Failure	Indeterminate
Failure	Failure	Failure	Failure	Failure
Indeterminate	Failure if clinical outcome at any prior visit was Failure, otherwise Indeterminate	Failure	Failure	Failure if clinical outcome at any prior visit was Failure, otherwise Indeterminate
a. For an outcome of Recurrence, patients had to have documented prior Eradication				

The difference in proportion of patients with overall treatment successes was calculated as the rate in the cefepime-AAI101 group minus that of the piperacillin/tazobactam group. A 2-sided 95% confidence interval (CI) was calculated using the stratified Newcombe method. Stratification was applied for type of infection, prior therapy category, and region.

Non-inferiority was concluded if the lower limit of the stratified Newcombe 2-sided 95% CI was >-10. The non-inferiority margin was a difference of 10 percentage points.

If non-inferiority was demonstrated, an assessment for superiority on the primary endpoint was to be performed as a secondary objective without the need for type I error alpha correction. Superiority was shown if the treatment difference was positive and the lower bound of the 95% CI around this difference was greater than zero.

Sensitivity analyses

Additional analyses of the primary efficacy endpoint were performed for the Modified Intent-to-treat (MITT), CE, and ME Populations. A 95% CI was computed for the treatment difference in the overall success rates based on the stratified Newcombe method.

In addition, the primary efficacy endpoint was performed for m-MITT+R and ME+R Populations.

Multiplicity

The difference in proportion of patients with overall treatment successes, as well as the stratified Newcombe 2-sided 95% CI for the difference in the proportion were the only calculations presented for the primary endpoint. No multiplicity occurred since there was no multiple testing for different analyses of the primary endpoint. Superiority was tested after the non-inferiority was concluded. Type I error alpha correction was not needed in this case.

Missing data

Patients with missing data or who were lost to follow-up were defined as indeterminate for the primary analysis and were included in the denominator for the calculation of overall success rate. Thus, patients with an indeterminate outcome were considered failures for the primary analysis and secondary efficacy analyses. A clinical failure occurring at an earlier time point was carried forward to the subsequent visits.

Results

Participant flow

Table 7 below summarises patient disposition by treatment for the ITT Population. A total of 81 patients were screen failures. The main reason for screen failure was not satisfying the inclusion and/or exclusion criteria for a total of 77 patients. Three patients withdrew consent after screening and prior to randomisation, and 1 patient screen failed due to another reason, i.e. the fact that IV drug administration was not feasible in this patient and therefore the patient would not have been able to comply with the protocol.

Table 33: Patient Disposition – ITT Population

	Cefepime-AAI101 (N=520) n (%)	Piperacillin/ Tazobactam (N=521) n (%)	Total (N=1041) n (%)
Patients who were randomized	520 (100.0)	521 (100.0)	1041 (100.0)
Patients who received study drug	516 (99.2)	518 (99.4)	1034 (99.3)
Patients who did not receive study drug	4 (0.8)	3 (0.6)	7 (0.7)
Patients who completed the study treatment	489 (94.0)	497 (95.4)	986 (94.7)
Patients who did not complete the study treatment	27 (5.2)	21 (4.0)	48 (4.6)
Adverse event	10 (1.9)	7 (1.3)	17 (1.6)
Death	0 (0.0)	2 (0.4)	2 (0.2)
Lost to follow-up	0 (0.0)	0 (0.0)	0 (0.0)
Physician decision	1 (0.2)	3 (0.6)	4 (0.4)
Pregnancy	0 (0.0)	0 (0.0)	0 (0.0)
Prohibited medication	0 (0.0)	0 (0.0)	0 (0.0)
Protocol deviation	0 (0.0)	0 (0.0)	0 (0.0)
Site terminated by Sponsor	0 (0.0)	0 (0.0)	0 (0.0)
Study terminated by Sponsor	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal of consent	7 (1.3)	6 (1.2)	13 (1.2)
Other	9 (1.7)	3 (0.6)	12 (1.2)
Patients who completed the study	494 (95.0)	501 (96.2)	995 (95.6)
Patients who did not complete the study	26 (5.0)	20 (3.8)	46 (4.4)
Adverse event	3 (0.6)	2 (0.4)	5 (0.5)
Death	0 (0.0)	3 (0.6)	3 (0.3)
Lack of efficacy	1 (0.2)	0 (0.0)	1 (0.1)
Lost to follow-up	3 (0.6)	2 (0.4)	5 (0.5)
Physician decision	0 (0.0)	1 (0.2)	1 (0.1)
Pregnancy	0 (0.0)	0 (0.0)	0 (0.0)
Prohibited medication	0 (0.0)	0 (0.0)	0 (0.0)
Protocol deviation	0 (0.0)	0 (0.0)	0 (0.0)
Site terminated by Sponsor	0 (0.0)	0 (0.0)	0 (0.0)
Study terminated by Sponsor	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal of consent	7 (1.3)	6 (1.2)	13 (1.2)
Other	12 (2.3)	6 (1.2)	18 (1.7)

Percentages were calculated using the number of patients in the column heading as the denominator.
ITT = Intent-to-Treat.
Source: Post-text Table 14.1.1.1

For patient disposition for the MITT Population, see Post-text Table 14.1.1.2.

The study numbers per analysis populations is shown in the following table.

Table 34: Analysis Populations - ITT Population

Population	Cefepime-AAI101 (N=520) n (%)	Piperacillin/ Tazobactam (N=521) n (%)	Total (N=1041) n (%)
ITT Population [1]	520 (100.0)	521 (100.0)	1041 (100.0)
MITT Population [2]	516 (99.2)	518 (99.4)	1034 (99.3)
m-MITT Population [3]	345 (66.3)	333 (63.9)	678 (65.1)
m-MITT+R Population [4]	388 (74.6)	383 (73.5)	771 (74.1)
CE Population [5]	479 (92.1)	471 (90.4)	950 (91.3)
ME Population [6]	308 (59.2)	298 (57.2)	606 (58.2)
ME+R Population [7]	347 (66.7)	337 (64.7)	684 (65.7)
Safety Population [8]	516 (99.2)	518 (99.4)	1034 (99.3)

The reason for exclusion of patients from the m-MITT population (compared with the MITT population) were "no qualifying baseline gram-negative pathogen (128 and 135, for cefepime-enmetazobactam and piperacillin-tazobactam groups, respectively), "baseline culture considered contaminated" (10 and 5, respectively), "baseline gram-positive pathogen only" (16 and 25, respectively) and "qualifying baseline pathogen resistant to either study drug" (43 and 50, respectively).

Recruitment

Initiation date: 24 September 2018

Completion date: 26 November 2019

Conduct of the study

The original Study Protocol version 1.0 was dated 04 December 2017. There have been 4 Protocol Amendments, all made before study initiation, and 1 Protocol Administrative Change Letter was issued since the release of Protocol version 5.0.

The original SAP (version 1.0) was dated 03 May 2019. There was 1 amendment to the original SAP (version 2.0) dated 19 November 2019.

There were 209 and 185 patients with at least 1 CSR-reportable protocol deviation in the cefepime-enmetazobactam and piperacillin-tazobactam treatment groups, respectively. The categories of deviations were evenly distributed between the treatment groups and mostly related to study procedures, investigational product and stratification error.

Baseline data

Table 35: Summary of Demographics and Baseline Characteristics – m-MITT Population

Demographic/Characteristic Category/Statistic	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Total (N=678) n (%)
Age (years)			
n	345	333	678
Mean (SD)	55.4 (18.67)	52.4 (19.68)	53.9 (19.22)
Age group, n (%)			
<65 years	209 (60.6)	220 (66.1)	429 (63.3)
65 to <75 years	85 (24.6)	70 (21.0)	155 (22.9)
≥75 years	51 (14.8)	43 (12.9)	94 (13.9)
Gender, n (%)			
Male	144 (41.7)	127 (38.1)	271 (40.0)
Female	201 (58.3)	206 (61.9)	407 (60.0)
Race, n (%)			
Black or African American	1 (0.3)	0 (0.0)	1 (0.1)
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)
Asian	3 (0.9)	1 (0.3)	4 (0.6)
White	327 (94.8)	316 (94.9)	643 (94.8)
Native Hawaiian or Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)
Other	14 (4.1)	16 (4.8)	30 (4.4)
Multiple	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity, n (%)			
Hispanic or Latino	26 (7.5)	27 (8.1)	53 (7.8)
Not Hispanic or Latino	318 (92.2)	305 (91.6)	623 (91.9)
Not reported	1 (0.3)	1 (0.3)	2 (0.3)
Weight (kg)			
n	344	333	677
Mean (SD)	75.65 (16.902)	74.33 (17.640)	75.00 (17.269)
Height (cm)			
n	345	333	678
Mean (SD)	168.2 (9.60)	167.9 (9.00)	168.0 (9.31)
BMI (kg/m^2)			
n	344	333	677
Mean (SD)	26.739 (5.573)	26.284 (5.500)	26.515 (5.538)
eGFR at baseline ($mL/min/1.73\ m^2$)			
n	331	312	643
Mean (SD)	71.25 (22.295)	71.52 (24.624)	71.38 (23.436)
eGFR group at baseline, n (%)			
Severe: <30 $mL/min/1.73\ m^2$	2 (0.6)	5 (1.5)	7 (1.0)
Moderate: 30-59 $mL/min/1.73\ m^2$	79 (22.9)	75 (22.5)	154 (22.7)
Mild: 60-89 $mL/min/1.73\ m^2$	187 (54.2)	166 (49.8)	353 (52.1)
Normal: ≥90 $mL/min/1.73\ m^2$	63 (18.3)	66 (19.8)	129 (19.0)

Demographic/Characteristic Category/Statistic	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Total (N=678) n (%)
Type of infection, n (%)			
AP	171 (49.6)	178 (53.5)	349 (51.5)
cUTI with removable source of infection	75 (21.7)	73 (21.9)	148 (21.8)
cUTI without removable source of infection but with other risk factors	99 (28.7)	82 (24.6)	181 (26.7)
Prior antibiotic therapy, n (%)			
Short-acting antibiotic up to 24 hours	25 (7.2)	23 (6.9)	48 (7.1)
No prior antibiotic therapy	320 (92.8)	310 (93.1)	630 (92.9)
Region, n (%)			
Eastern Europe	238 (69.0)	240 (72.1)	478 (70.5)
Americas	22 (6.4)	22 (6.6)	44 (6.5)
Other countries	85 (24.6)	71 (21.3)	156 (23.0)
Charlson Comorbidity Index as baseline, n (%)			
<3	198 (57.4)	205 (61.6)	403 (59.4)
≥3	145 (42.0)	125 (37.5)	270 (39.8)
Presence of concurrent bacteremia at baseline, n (%)			
Yes	38 (11.0)	28 (8.4)	66 (9.7)
No	307 (89.0)	305 (91.6)	612 (90.3)
Country category, n (%)			
US	1 (0.3)	0 (0.0)	1 (0.1)
Non-US	344 (99.7)	333 (100.0)	677 (99.9)
Baseline diabetic status, n (%)			
Yes	55 (15.9)	41 (12.3)	96 (14.2)
No	290 (84.1)	292 (87.7)	582 (85.8)
ESBL co-producing (ESBL-genotype, combined with or without any other non-ESBL genotype)	76 (22.0)	66 (19.8)	142 (20.9)
ESBL-only producing	72 (20.9)	65 (19.5)	137 (20.2)
ESBL co-producing (CTX-M-type)	76 (22.0)	65 (19.5)	141 (20.8)
ESBL-only producing (CTX-M-type)	70 (20.3)	63 (18.9)	133 (19.6)
ESBL co-producing (non-CTX-M-type)	2 (0.6)	2 (0.6)	4 (0.6)
ESBL-only producing (non-CTX-M-type)	0 (0.0)	1 (0.3)	1 (0.1)
Carbapenemase (Class A) co-producing	0 (0.0)	0 (0.0)	0 (0.0)
Carbapenemase (Class A)-only producing	0 (0.0)	0 (0.0)	0 (0.0)
Carbapenemase (Class B) co-producing	0 (0.0)	0 (0.0)	0 (0.0)
Carbapenemase (Class B)-only producing	0 (0.0)	0 (0.0)	0 (0.0)
Carbapenemase (Class D) co-producing	0 (0.0)	0 (0.0)	0 (0.0)
Carbapenemase (Class D)-only producing	0 (0.0)	0 (0.0)	0 (0.0)
AmpC co-producing	7 (2.0)	6 (1.8)	13 (1.9)
AmpC-only producing	3 (0.9)	5 (1.5)	8 (1.2)
Other beta-lactamase non-ESBL-, non-carbapenemase-, and non-AmpC-producing	14 (4.1)	12 (3.6)	26 (3.8)

Percentages were calculated using the number of patients in the column heading as the denominator.
Baseline was defined as the last measurement or assessment prior to the first dose of study drug.
Note: There were 58 patients with baseline eGFR missing due to Day 1 central laboratory sample collection time being reported after the first dose date and time of study drug.
AP = acute pyelonephritis; BMI = body mass index; cUTI = complicated urinary tract infection; eGFR = estimated glomerular filtration rate; ESBL = extended-spectrum β-lactamase; m-MITT = Microbiological Modified Intent-to-Treat; SD = standard deviation; US = United States.
Source: Post-text Table 14.1.2.3

Table 36: Summary of Baseline Infection Characteristics – m-MITT Population

Characteristic Category	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Total (N=678) n (%)
Primary infection type [1]			
AP	171 (49.6)	178 (53.5)	349 (51.5)
cUTI with removable source of infection	75 (21.7)	73 (21.9)	148 (21.8)
cUTI without removable source of infection but with other risk factors	99 (28.7)	82 (24.6)	181 (26.7)
Both conditions, cUTI and AP	50 (14.5)	60 (18.0)	110 (16.2)
Signs and symptoms [2]			
Patients with cUTI, N*	174	155	329
Dysuria, increased urinary frequency, or urinary urgency	156 (89.7)	137 (88.4)	293 (89.1)
Fever within 24 hours of screening	55 (31.6)	68 (43.9)	123 (37.4)
Lower abdominal pain or pelvic pain	147 (84.5)	117 (75.5)	264 (80.2)
Suprapubic tenderness on physical examination	145 (83.3)	116 (74.8)	261 (79.3)
Nausea or vomiting within 24 hours of screening	49 (28.2)	55 (35.5)	104 (31.6)
Patients with AP, N*	171	178	349
Dysuria, increased urinary frequency, or urinary urgency	140 (81.9)	148 (83.1)	288 (82.5)
Fever within 24 hours of screening	130 (76.0)	127 (71.3)	257 (73.6)
Flank pain (onset within 7 days prior to randomization)	165 (96.5)	171 (96.1)	336 (96.3)
Costo-vertebral angle tenderness on physical examination	161 (94.2)	164 (92.1)	325 (93.1)
Nausea or vomiting within 24 hours of screening	93 (54.4)	96 (53.9)	189 (54.2)
Evidence of pyuria criteria [2]			
Patients with cUTI, N*	174	155	329
WBC >10 cells/mm^3 in unspun urine or ≥10 cells/hpf in spun urine sediment	155 (89.1)	138 (89.0)	293 (89.1)
Urinalysis/dipstick analysis positive for leukocyte esterase	154 (88.5)	135 (87.1)	289 (87.8)

Characteristic Category	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Total (N=678) n (%)
Evidence of pyuria criteria (continued) [2]			
Patients with AP, N'	171	178	349
WBC >10 cells/mm^3 in unspun urine or ≥10 cells/hpf in spun urine sediment	161 (94.2)	167 (93.8)	328 (94.0)
Urinalysis/dipstick analysis positive for leukocyte esterase	139 (81.3)	137 (77.0)	276 (79.1)
Additional cUTI complicating factors for patients with cUTI, N' [2]	174	155	329
Male patients with documented history of urinary retention	74 (42.5)	59 (38.1)	133 (40.4)
Use of intermittent bladder catheterization or presence of an indwelling bladder catheter	42 (24.1)	42 (27.1)	84 (25.5)
Current obstructive uropathy	73 (42.0)	64 (41.3)	137 (41.6)
Any functional or anatomical abnormality of the urogenital tract with voiding disturbance	88 (50.6)	71 (45.8)	159 (48.3)
Azotemia due to known prior intrinsic renal disease	13 (7.5)	15 (9.7)	28 (8.5)

1. Percentages were calculated using N, the number of patients in the column heading as the denominator.
2. Percentages were calculated using N', the number of patients with cUTI or AP.

Note: The same patient may have had both conditions, cUTI and AP.

AP = acute pyelonephritis; cUTI = complicated urinary tract infection; m-MITT = Microbiological Modified Intent-to-Treat; WBC = white blood cells.

Source: Post-text Table 14.1.2.10

Table 37: Summary of Baseline Pathogens – m-MITT Population

Baseline Pathogen	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Total (N=678) n (%)
Escherichia coli	264 (76.5)	254 (76.3)	518 (76.4)
Klebsiella pneumoniae	34 (9.9)	32 (9.6)	66 (9.7)
Proteus mirabilis	19 (5.5)	19 (5.7)	38 (5.6)
Pseudomonas aeruginosa	13 (3.8)	11 (3.3)	24 (3.5)
Enterobacter cloacae	7 (2.0)	3 (0.9)	10 (1.5)
Serratia marcescens	4 (1.2)	3 (0.9)	7 (1.0)
Klebsiella oxytoca	3 (0.9)	3 (0.9)	6 (0.9)
Citrobacter koseri	1 (0.3)	2 (0.6)	3 (0.4)
Acinetobacter ursingii	0 (0.0)	2 (0.6)	2 (0.3)
Citrobacter freundii	1 (0.3)	1 (0.3)	2 (0.3)
Enterobacter bugandensis	1 (0.3)	1 (0.3)	2 (0.3)
Klebsiella variicola	0 (0.0)	2 (0.6)	2 (0.3)
Morganella morganii	1 (0.3)	1 (0.3)	2 (0.3)
Providencia rettgeri	1 (0.3)	1 (0.3)	2 (0.3)
Citrobacter amalonaticus	1 (0.3)	0 (0.0)	1 (0.1)
Citrobacter braakii	1 (0.3)	0 (0.0)	1 (0.1)
Enterobacter, non-speciated	1 (0.3)	0 (0.0)	1 (0.1)
Proteus vulgaris	0 (0.0)	1 (0.3)	1 (0.1)
Serratia liquefaciens	1 (0.3)	0 (0.0)	1 (0.1)
Stenotrophomonas maltophilia	0 (0.0)	1 (0.3)	1 (0.1)

Percentages were calculated using N, the number of patients in the column heading as the denominator.
m-MITT = Microbiological Modified Intent-to-Treat.
Source: Post-text Table 14.1.2.22

The susceptibility of the most isolated baseline pathogens to test and comparator agents is shown in the table below.

Table 38: Susceptibility of Baseline Pathogens in m-MITT

Organism group/antibacterial agent	MIC (µg/ml)			CLSI			EUCAST	
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%R
Enterobacterales (n=663)								
Cefepime[a]	0.06	64	≤0.008->64	79.5	4.7	15.8	77.7/81.6	18.4
Cefepime-enmetazobactam[b]	0.03	0.12	≤0.008-4	*99.7*	*0.3*	*0.0*	*99.2/100*	*0.0*
Meropenem	≤0.015	0.03	≤0.015-0.5	100	0.0	0.0	100	0.0
Piperacillin-tazobactam[c]	1	8	≤0.25-64	96.1	3.9	0.0	92.5	7.5
E. coli (n=518)								
Cefepime	0.03	64	≤0.008->64	80.1	5.0	14.9	78.4/82.2	17.8
Cefepime-enmetazobactam	0.03	0.06	≤0.008-4	*99.6*	*0.4*	*0.0*	*99.0/100*	*0.0*
Meropenem	≤0.015	0.03	≤0.015-0.06	100	0.0	0.0	100	0.0
Piperacillin-tazobactam	1	8	≤0.25-64	97.3	2.7	0.0	93.6	6.4
K. pneumoniae (n=66)								
Cefepime	0.06	>64	0.03->64	69.7	3.0	27.3	69.7/71.2	28.8
Cefepime-enmetazobactam	0.03	0.12	0.015-1	*100*	*0.0*	*0.0*	*100/100*	*0.0*
Meropenem	0.03	0.06	≤0.015-0.5	100	0.0	0.0	100	0.0
Piperacillin-tazobactam	2	32	0.5-64	89.4	10.6	0.0	83.3	16.7
P. mirabilis (n=38)								
Cefepime	0.06	16	0.03->64	84.2	2.6	13.2	78.9/86.8	13.2
Cefepime-enmetazobactam	0.06	0.12	≤0.015-0.12	100	0.0	0.0	*100/100*	*0.0*
Meropenem	≤0.25	2	≤0.25-64	97.4	2.6	0.0	97.4	2.6
Piperacillin-tazobactam	0.03	0.12	0.015-0.5	*100*	*0.0*	*0.0*	*100*	*0.0*
P. aeruginosa (n=24)								
Cefepime	4	8	1->64	91.7	4.2	4.2	91.7	8.3
Cefepime-enmetazobactam	4	8	1-8	*100*	*0.0*	*0.0*	*100*	*0.0*
Meropenem	0.5	8	0.12-16	62.5	20.8	16.7	62.5	37.5
Piperacillin-tazobactam	8	64	2-64	75.0	25.0	0.0	75.0	25.0

Abbreviations: CLSI=Clinical Laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC_{50}=minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC_{90}=minimum inhibitory concentration required to inhibit the growth of 90% of organisms; m-MITT=microbiologically modified Intent-to-Treat; R=resistant; S=susceptible.

[a]The percentage at the CLSI cefepime susceptible-dose dependent breakpoint of ≤8 µg/ml is calculated by adding the values for %S and %I. Both values for cefepime in the EUCAST %S column are for susceptible ≤1 µg/ml and susceptible-increased exposure ≤4 µg/ml, respectively.

[b]For cefepime-enmetazobactam, values in italics indicate the percentage of isolates at the CLSI cefepime breakpoints in the %S, %I and %R columns. Both values in italics for cefepime-enmetazobactam in the EUCAST %S column are the percentage of isolates at the EUCAST cefepime breakpoints of ≤1 µg/ml and ≤4 µg/ml, respectively.

[c]The Enterobacterales and *P. aeruginosa* CLSI breakpoints for piperacillin-tazobactam shown are those prior to the 2022 update and are both defined as susceptible ≤16 µg/ml, intermediate = 32-64 µg/ml, and resistant ≥128 µg/ml.

Source: from Study AAI101-MI-22-01

Table 39: Extent of Exposure and Compliance to Study Medication – MITT Population

Category Statistic	Cefepime-AAI101 (N=516) n (%)	Piperacillin/ Tazobactam (N=518) n (%)	Total (N=1034) n (%)
Duration of study drug (days) [1]			
n	516	518	1034
Mean (SD)	8.0 (1.49)	8.0 (1.57)	8.0 (1.53)
1-3 days	11 (2.1)	13 (2.5)	24 (2.3)
4-6 days	12 (2.3)	7 (1.4)	19 (1.8)
7 days	43 (8.3)	54 (10.4)	97 (9.4)
8-10 days	425 (82.4)	421 (81.3)	846 (81.8)
11-14 days	17 (3.3)	13 (2.5)	30 (2.9)
≥15 days	8 (1.6)	10 (1.9)	18 (1.7)
Compliance (%) [2]			
n	516	518	1034
Mean (SD)	100.26 (2.339)	100.20 (0.969)	100.23 (1.789)
<80%	1 (0.2)	0 (0.0)	1 (0.1)
≥80%	515 (99.8)	518 (100.0)	1033 (99.9)
Total number of doses received			
n	516	518	1034
Mean (SD)	21.2 (4.41)	21.2 (4.56)	21.2 (4.48)

1. Duration of study drug (days) = last dose date of study drug – first dose date of study drug + 1.
2. Compliance (%) = (no. of doses taken / no. of doses expected) × 100, where no. of expected doses was derived based on elapsed time between first and last doses of specified study drug and its prescribed frequency.

MITT = Modified Intent-to-Treat; no. = number; SD = standard deviation.
Source: Post-text Table 14.1.5.1

A total of 877 (84.8%) patients in the MITT Population received concomitant medications during the study: 440 (85.3%) patients in the cefepime-enmetazobactam group and 437 (84.4%) patients in the piperacillin/tazobactam group. n. A total of 84 (8.1%) patients in the MITT Population received concomitant antibiotics: 41 (7.9%) patients in the cefepime-enmetazobactam group and 43 (8.3%) patients in the piperacillin/tazobactam group.

Outcomes and estimation

Primary analysis

Overall success was seen in a higher proportion of subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group (79.1% vs. 58.9%), with a treatment difference of 21.2% (95% CI: 14.3%, 27.9%). Because the lower limit of the 95% CI is greater than the prespecified non-inferiority margin of -10%, cefepime-enmetazobactam is non-inferior to piperacillin/tazobactam. Additionally, because the lower limit of the 95% CI of the difference is also greater than 0%, cefepime-enmetazobactam is superior to piperacillin/tazobactam (table below).

Table 40: Primary Efficacy Analysis of Overall Response at TOC Visit – m-MITT Population

Overall Response	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Treatment Comparison [2]	
			Difference (%)	95% CI
Success [1]	273 (79.1)	196 (58.9)	21.2	(14.3, 27.9)
Failure/Indeterminate				
Failure	51 (14.8)	116 (34.8)		
Indeterminate	21 (6.1)	21 (6.3)		

Percentages were calculated using the number of patients in the column heading as the denominator.
1. Overall Success was defined as clinical cure and microbiological eradication.
2. Treatment difference was the difference in the overall success rate between the 2 treatment arms (cefepime-AAI101 – piperacillin/tazobactam). The 95% CIs (2-sided) were computed using the stratified Newcombe method.

CI = confidence interval; m-MITT = Microbiological Modified Intent-to-Treat; TOC = Test of Cure.
Source: Post-text Table 14.2.1.1

Secondary analyses

Similar results were seen at the TOC visit for the m-MITT+R population, i.e. the population without exclusion of baseline pathogens considered resistant to cefepime-enmetazobactam and piperacillin-tazobactam (table below).

Table 41: Overall Response at TOC Visit – m-MITT+R Population

Overall Response	Cefepime-AAI101 (N=388) n (%)	Piperacillin/ Tazobactam (N=383) n (%)	Treatment Comparison [2]	
			Difference (%)	95% CI
Success [1]	305 (78.6)	225 (58.7)	20.7	(14.1, 27.0)
Failure/Indeterminate				
Failure	58 (14.9)	128 (33.4)		
Indeterminate	25 (6.4)	30 (7.8)		

Percentages were calculated using the number of patients in the column heading as the denominator.
1. Overall Success was defined as clinical cure and microbiological eradication.
2. Treatment difference was the difference in the overall success rate between the 2 treatment arms (cefepime-AAI101 – piperacillin/tazobactam). The 95% CIs (2-sided) were computed using the stratified Newcombe method.

CI = confidence interval; m-MITT+R = Microbiological Modified Intent-to-Treat including patients with resistant isolates; TOC = Test of Cure.
Source: Post-text Table 14.2.1.5

The largest difference in overall success was noted at the TOC visit. At LFU, the overall success were 68.4% vs. 58.9% with a somewhat smaller treatment difference of 10.7%.

Table 42: Overall Success, at Day 3, EOT, and LFU in Study AT-301 (m-MITT Population)

Overall success [1]	Cefepime-Enmetazobactam (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Treatment Comparison	
			Difference [2]	95% CI [3]
Day 3	318 (92.2)	293 (88.0)	4.1	-0.6, 8.9
EOT	318 (92.2)	311 (93.4)	-1.3	-5.3, 2.9
LFU	236 (68.4)	196 (58.9)	10.7	3.4, 17.8

[1] Overall success was defined as clinical cure (ie, complete resolution of the baseline signs and symptoms present at screening) AND microbiological eradication (ie, baseline qualifying gram-negative pathogen(s) was reduced to 10^3 CFU/ml in urine AND a negative blood culture for a gram-negative pathogen that was identified as a uropathogen (if repeated after positive baseline blood culture).
[2] Treatment difference was the difference in the overall success rate between the two groups (cefepime-enmetazobactam minus piperacillin/tazobactam).
[3] The 95% CIs (2-sided) were computed using the stratified Newcombe method.
CI=confidence interval; EOT=end of treatment; LFU=late follow-up; m-MITT=microbiological Modified Intent-to-Treat
Source: CSR AT-301 Table 20, Table 22, and Table24

The results in favour of the cefepime-enmetazobactam group was mainly driven by a more favourable microbiological response rate. At TOC, a favourable clinical response rate was noted for 319/345 (93.6%) and 296/333 (88.9%), for the cefepime-enmetazobactam and piperacillin-tazobactam groups, respectively, with a treatment difference of 3.5% (95% CI: -1.0. 8.0), whereas a favourable microbiological response rate was noted for 286/345 (82.9%) and 216/333 (64.9%), respectively, with a treatment difference of 19.0% (95% CI: 12.3, 25.4) (tables below).

Table 43: Clinical Response by Visit – m-MITT Population

Visit Clinical Response	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Treatment Comparison [1]	
			Difference (%)	95% CI
Day 3				
Cure	18 (5.2)	16 (4.8)	0.5	(-3.1, 4.0)
Improvement	317 (91.9)	302 (90.7)		
Failure	5 (1.4)	4 (1.2)		
Indeterminate	5 (1.4)	11 (3.3)		
End of Treatment				
Cure	323 (93.6)	315 (94.6)	-1.1	(-4.8, 2.7)
Improvement	2 (0.6)	1 (0.3)		
Failure	10 (2.9)	9 (2.7)		
Indeterminate	10 (2.9)	8 (2.4)		
Test of Cure				
Cure	319 (92.5)	296 (88.9)	3.5	(-1.0, 8.0)
Failure	17 (4.9)	23 (6.9)		
Indeterminate	9 (2.6)	14 (4.2)		
Late Follow-up				
Cure	299 (86.7)	279 (83.8)	2.8	(-2.7, 8.3)
Failure	25 (7.2)	39 (11.7)		
Indeterminate	21 (6.1)	15 (4.5)		

Percentages were calculated using the number of patients in the column heading as the denominator.

1. Treatment difference was the difference in the clinical cure rate between the 2 treatment arms (cefepime-AAI101 – piperacillin/tazobactam). The 95% CIs (2-sided) were computed using the stratified Newcombe method.

CI = confidence interval; m-MITT = Microbiological Modified Intent-to-Treat.

Source: Post-text Table 14.2.2.1

Table 44: Per-Patient Microbiological Response by Visit – m-MITT Population

Visit Microbiological Response	Cefepime-AAI101 (N=345) n (%)	Piperacillin/ Tazobactam (N=333) n (%)	Treatment Comparison [1]	
			Difference (%)	95% CI
Day 3				
Eradication	323 (93.6)	299 (89.8)	3.8	(-0.6, 8.3)
Persistence	11 (3.2)	21 (6.3)		
Indeterminate	11 (3.2)	13 (3.9)		
End of Treatment				
Eradication	332 (96.2)	322 (96.7)	-0.7	(-3.7, 2.5)
Persistence	1 (0.3)	2 (0.6)		
Recurrence	1 (0.3)	1 (0.3)		
Indeterminate	11 (3.2)	8 (2.4)		
Test of Cure				
Eradication	286 (82.9)	216 (64.9)	19.0	(12.3, 25.4)
Persistence	1 (0.3)	2 (0.6)		
Recurrence	39 (11.3)	98 (29.4)		
Indeterminate	19 (5.5)	17 (5.1)		
Late Follow-up				
Eradication	258 (74.8)	221 (66.4)	9.5	(2.6, 16.3)
Persistence	1 (0.3)	1 (0.3)		
Recurrence	71 (20.6)	90 (27.0)		
Indeterminate	15 (4.3)	21 (6.3)		

Percentages were calculated using the number of patients in the column heading as the denominator.
1. Treatment difference was the difference in the microbiological eradication rate between the 2 treatment arms (cefepime-AAI101 – piperacillin/tazobactam). The 95% CIs (2-sided) were computed using the stratified Newcombe method.

CI = confidence interval; m-MITT = Microbiological Modified Intent-to-Treat.
Source: Post-text Table 14.2.3.1

The results for the m-MITT+R at other timepoints and for the CE, ME and ME+R populations at different timepoint were in line with the overall results (data not shown).

For infections caused by the four most isolated Enterobacterales at baseline *E. coli*, *K. pneumoniae*, *P. mirabilis* and *E. cloacae,* a higher proportion of overall success, generally driven by a more favourable microbiological eradication rate, was noted in the cefepime-enmetazobactam group compared with piperacillin-tazobactam group. An essentially similar overall success rate was noted against *P. aeruginosa* reflected by essentially similar clinical cure rates and microbiological eradication rates (table below).

Table 45: Overall Success, Clinical Cure, and Microbiological Eradication by Baseline Pathogen at TOC in Study AT-301 (m-MITT and m-MITT+R Populations)

Population Baseline Pathogen/Response	Cefepime/Enmetazobactam n (%)	Piperacillin/Tazobactam n (%)
m-MITT Population		
Escherichia coli	n=264	n=254
Overall success	220 (83.3)	151 (59.4)
Clinical cure	249 (94.3)	228 (89.8)
Microbiological eradication	229 (86.7)	165 (65.0)
Klebsiella pneumonia	n=34	n=32
Overall success	23 (67.6)	18 (56.3)
Clinical cure	26 (76.5)	24 (75.0)
Microbiological eradication	26 (76.5)	23 (71.9)
Proteus mirabilis	n=19	n=19
Overall success	15 (78.9)	10 (52.6)
Clinical cure	18 (94.7)	17 (89.5)
Microbiological eradication	15 (78.9)	10 (52.6)
Pseudomonas aeruginosa	n=13	n=11
Overall success	4 (30.8)	4 (36.4)
Clinical cure	12 (92.3)	10 (90.9)
Microbiological eradication	4 (30.8)	5 (45.5)
Enterobacter cloacae	n=7	n=3
Overall success	6 (85.7)	1 (33.3)
Clinical cure	7 (100.0)	3 (100.0)
Microbiological eradication	6 (85.7)	1 (33.3)
m-MITT+R Population		
Escherichia coli	n=281	n=271
Overall success	234 (83.3)	160 (59.0)
Clinical cure	264 (94.0)	241 (88.9)
Microbiological eradication	244 (86.8)	176 (64.9)
Klebsiella pneumonia	n=48	n=48
Overall success	33 (68.8)	26 (54.2)
Clinical cure	39 (81.3)	38 (79.2)
Microbiological eradication	36 (75.0)	32 (66.7)
Proteus mirabilis	n=19	n=22
Overall success	15 (78.9)	12 (54.5)
Clinical cure	18 (94.7)	20 (90.9)
Microbiological eradication	15 (78.9)	13 (59.1)
Pseudomonas aeruginosa	n=21	n=22
Overall success	10 (47.6)	10 (45.5)
Clinical cure	19 (90.5)	21 (95.5)
Microbiological eradication	11 (52.4)	11 (50.0)
Enterobacter cloacae	n=10	n=5
Overall success	8 (80.0)	2 (40.0)
Clinical cure	9 (90.0)	5 (100.0)
Microbiological eradication	8 (80.0)	2 (40.0)

Note: Overall success was determined as a composite of clinical cure (complete resolution of the baseline signs and symptoms present at screening) and microbiological eradication ($<10^3$ CFU/ml of qualifying baseline pathogen in urine).
m-MITT=microbiological Modified Intent-to-Treat; m-MITT+R=microbiological Modified Intent-to-Treat, including subjects with resistant isolates;
Source: CSR AT-301 Table 32, Table 33, Table 34, Table 35, Table 36, Table 37

Overall success at the TOC visit in the m-MITT population by the five most commonly isolated baseline pathogens and the MIC (µg/mL) to Piperacillin/Tazobactam is shown below.

Table 46: Overall Success at TOC Visit by Baseline Pathogen and Minimum Inhibitory Concentration (µg/mL) to Piperacillin/Tazobactam – m-MITT Population

Baseline Pathogen	Piperacillin/ Tazobactam MIC (µg/mL)	Cefepime-AAI101 (N=345) n/N' (%)	Piperacillin/Tazobactam (N=333) n/N' (%)
Escherichia coli	≤0.25	2/3 (66.7)	0/1 (0.0)
	0.5	23/25 (92.0)	14/21 (66.7)
	1	114/132 (86.4)	83/129 (64.3)
	2	49/59 (83.1)	31/54 (57.4)
	4	10/15 (66.7)	9/18 (50.0)
	8	11/16 (68.8)	6/11 (54.5)
	16	8/10 (80.0)	2/10 (20.0)
	32	2/2 (100.0)	5/8 (62.5)
	64	1/2 (50.0)	1/2 (50.0)
Klebsiella pneumonia	0.5	1/2 (50.0)	0/0 (0.0)
	1	2/3 (66.7)	3/5 (60.0)
	2	10/12 (83.3)	9/12 (75.0)
	4	3/6 (50.0)	3/8 (37.5)
	8	2/4 (50.0)	1/3 (33.3)
	16	2/3 (66.7)	0/1 (0.0)
	32	1/2 (50.0)	0/1 (0.0)
	64	2/2 (100.0)	2/2 (100.0)
Proteus mirabilis	≤0.25	11/11 (100.0)	7/10 (70.0)
	0.5	2/4 (50.0)	2/6 (33.3)
	1	1/1 (100.0)	1/2 (50.0)
	2	0/0 (0.0)	0/1 (0.0)
	4	0/2 (0.0)	0/0 (0.0)
	64	1/1 (100.0)	0/0 (0.0)
Pseudomonas aeruginosa	2	1/2 (50.0)	1/1 (100.0)
	4	0/3 (0.0)	0/2 (0.0)
	8	0/4 (0.0)	0/1 (0.0)
	16	1/2 (50.0)	1/3 (33.3)
	32	2/2 (100.0)	0/0 (0.0)
	64	0/0 (0.0)	2/4 (50.0)
Enterobacter cloacae	2	1/1 (100.0)	0/1 (0.0)
	4	2/3 (66.7)	0/0 (0.0)
	8	2/2 (100.0)	1/2 (50.0)
	16	1/1 (100.0)	0/0 (0.0)

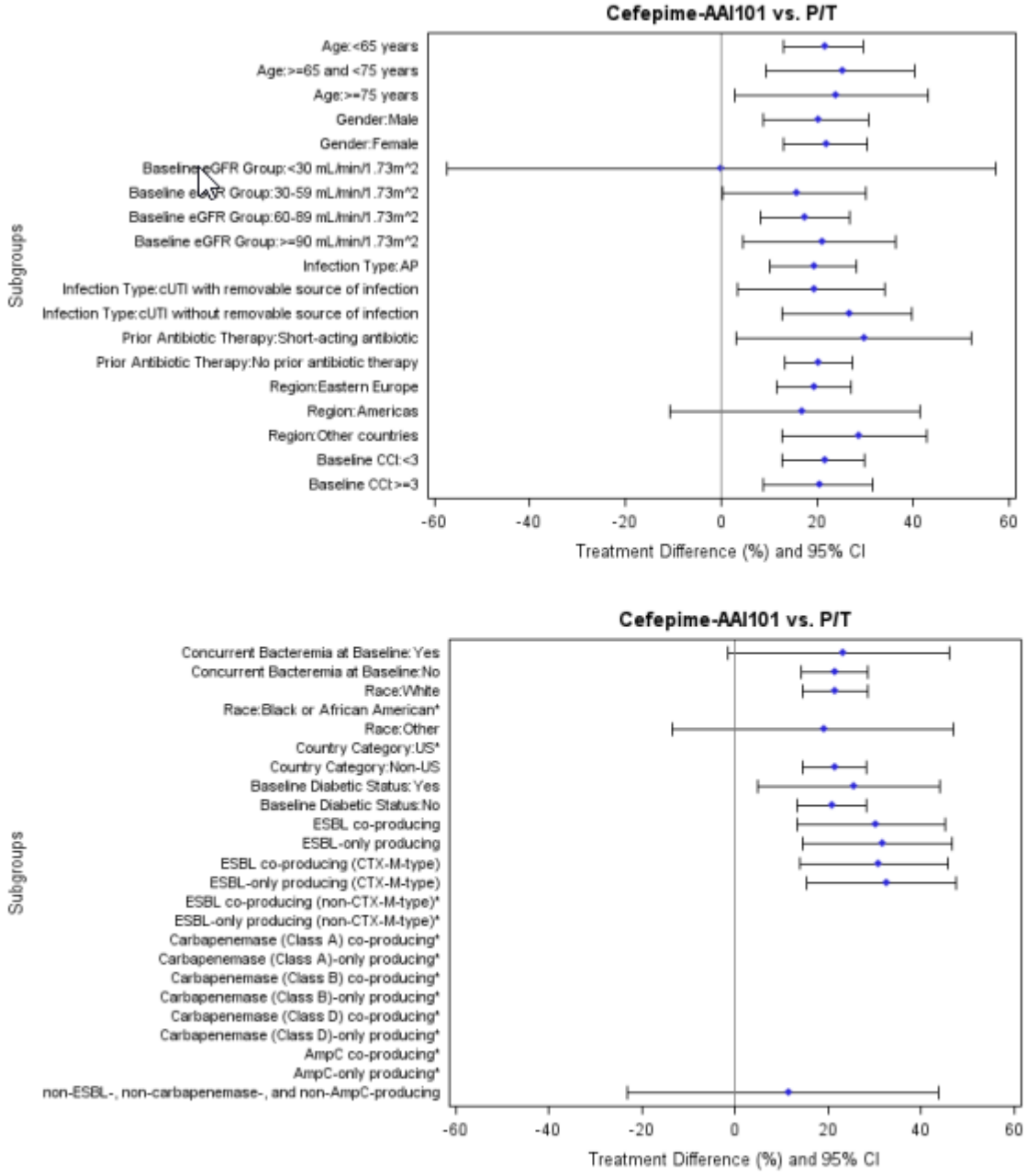
Percentages were calculated using N' as the denominator, where N' was the number of patients with specified baseline pathogen at the specified MIC value.
Note: If same pathogen was isolated from blood and urine, the highest MIC to the specified antimicrobial was used.
MIC = minimal inhibitory concentration; m-MITT = Microbiological Modified Intent-to-Treat; TOC = Test of Cure.
Source: Post-text Table 14.2.6.21

Ancillary analyses

Subgroup analyses

The result for cefepime-enmetazobactam compared with piperacillin-tazobactam regarding the primary endpoint of Overall success at TOC in the m-MITT in subgroup analyses are shown in the figure below.

Figure 9: Forest Plot of Overall Success at TOC Visit by Subgroup – m-MITT Population



*not estimated.
AP = acute pyelonephritis; CCI = Charlson Comorbidity Index; CI = confidence interval; cUTI = complicated urinary tract infection; eGFR = estimated glomerular filtration rate; ESBL = extended-spectrum β-lactamases; m-MITT = Microbiological Modified Intent-to-Treat; P/T = piperacillin/tazobactam; TOC = Test of Cure; US = United States; vs. = versus.
Source: Post-text Figure 14.2.1.1

- **Summary of main efficacy results**

The following table summarise the efficacy results from the main study supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 47: Summary of efficacy for trial AT-301

Title: A Phase 3, Randomized, Double-Blind, Multi-Centre Study to Evaluate the Efficacy, Safety, and Tolerability of Cefepime-AAI101 Compared to Piperacillin/Tazobactam in the Treatment of Complicated Urinary Tract Infections, Including Acute Pyelonephritis, in Adults		
Study identifier	Protocol number: AT-301 EudraCT Number: 2017-004868-35	
Design	A Phase 3, randomized, double-blind, active-controlled, multicentre, non-inferiority study	
	Initiation Date: 24 September 2018 Completion Date: 26 November 2019	
Hypothesis	Non-inferiority	
Treatments groups	Cefepime-Enmetazobactam	2 g cefepime plus 500 mg AAI101 infused over a period of 2 hours once every 8 hours (q8h) for 7 days (up to 14 days in patients with a positive blood culture at baseline)
	Piperacillin/Tazobactam	4.5 g piperacillin/tazobactam infused over a period of 2 hours q8h for 7 days (up to 14 days in patients with a positive blood culture at baseline).
Endpoints and definitions	Primary endpoint	The primary efficacy endpoint was the proportion of subjects in the Microbiological Modified Intent-to-Treat (m-MITT) population who achieved overall success at TOC, determined as a composite of clinical cure and microbiological eradication.
	Secondary endpoints	Proportion of subjects in the m-MITT population with overall success at Day 3, EOT, and LFU Proportion of subjects in the m-MITT and Microbiologically Evaluable populations with a microbiological outcome of Eradication at Day 3, EOT, TOC, and LFU The proportion of subjects in the m-MITT Population, including subjects with resistant isolates (m-MITT+R) and ME Population, including subjects with resistant isolates (ME+R), with a microbiological outcome of Eradication at Day 3, EOT, TOC, and LFU The proportion of subjects with a clinical outcome of Cure or Improvement (Day 3 only) in the m-MITT, m-MITT+R, Clinically Evaluable (CE), ME, and ME+R Populations at Day 3, EOT, TOC, and LFU Per-pathogen overall treatment success, clinical outcome of Cure, and microbiological outcome of Eradication in the m-MITT, m-MITT+R, ME, and ME+R Populations at Day 3, EOT, TOC, and LFU
	Additional secondary endpoints	Additional secondary efficacy endpoints included an analysis of overall success, clinical cure, and eradication at TOC for each discrete cefepime-enmetazobactam and piperacillin/tazobactam baseline MIC value.
Database lock	30 January 2020	

Title: A Phase 3, Randomized, Double-Blind, Multi-Centre Study to Evaluate the Efficacy, Safety, and Tolerability of Cefepime-AAI101 Compared to Piperacillin/Tazobactam in the Treatment of Complicated Urinary Tract Infections, Including Acute Pyelonephritis, in Adults				
Study identifier	Protocol number: AT-301 EudraCT Number: 2017-004868-35			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Microbiological modified intent-to-treat (m-MITT)			
Descriptive statistics and estimate variability	Treatment group	Cefepime-Enmetazobactam	Piperacillin/ Tazobactam	
	Number of subjects	345	333	
	Primary endpoint Proportion of subjects in the Microbiological Modified Intent-to-Treat (m-MITT) population who achieved overall success at TOC	273/345 (79.1 %)	196/333 (58.9 %)	difference (95% CI) 21.2 (14.3, 27.9)
	Secondary endpoints			
	Overall Success at TOC in m-MITT+R Population	305/388 (78.6 %)	225/383 (58.7%)	20.7 (14.1, 27.0)
	Overall Success, at Day 3, EOT, and LFU (m-MITT Population)	Day 3 318/345 (92.2%) EOT 318/345 (92.2%) LFU 236/345 (68.4%)	Day 3 293/333 (88)) EOT 311/333 (93.4%) LFU 196/333 (58.9%)	 4.1 (-0.6, 8.9) -1.3 (-5.3, 2.9) 10.7 (3.4, 17.8)

Title: A Phase 3, Randomized, Double-Blind, Multi-Centre Study to Evaluate the Efficacy, Safety, and Tolerability of Cefepime-AAI101 Compared to Piperacillin/Tazobactam in the Treatment of Complicated Urinary Tract Infections, Including Acute Pyelonephritis, in Adults				
Study identifier	Protocol number: AT-301 EudraCT Number: 2017-004868-35			
	Microbiological Eradication at Day 3, EOT, TOC, and LFU in (m-MITT)	Day 3 323 (93.6%) EOT 332 (96.2%) TOC 286 (82.9%) LFU 258 (74.8%)	Day 3 299 (89.8%) EOT 322 (96.7%) TOC 216 (64.9%) LFU 221 (66.4%)	3.8 (0.6, 8.3) -0.7 (-3.7, 2.5) 19.0 (12.3, 25.4) 9.5 (2.6, 16.3)
	Clinical cures m-MITT	Day 3 18 (5.2%) EOT 323 (93.6%) TOC 319 (92.5%) LFU 299 (86.7%)	Day 3 16 (4.8%) EOT 315 (94.6%) TOC 296 (88.9%) LFU 279 (83.8%)	0.5 (3.1, 4.0) -1.1 (-4.8, 2.7) 3.5 (1.0, 8.0) 2.8 (-2.7, 8.3)

2.5.5.3. Clinical studies in special populations

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age ≥85 (Older subjects number /total number)**
Controlled Trials	259/1192	137/1192	22/1192
AT-101	0/106	0/106	0/106
AT-201	12/45	4/45	0/45
AT-301	247/1041	133/1041	22/1041
Non-Controlled Trials	8/60	0/60	0/60
AT-102	8/30	0/30	0/30
AT-103	0/20	0/20	0/20
AT-104	0/10	0/10	0/10

2.5.5.4. Supportive study

The dose-finding phase 2 study AT-201 is described above. The ELF PK study AT-103 in healthy volunteers is assessed in the report for Clinical pharmacokinetics.

2.5.6. Discussion on clinical efficacy

The applicant has applied for the following indications:

Exblifep is indicated for the treatment of the following infections in adults (see sections 4.4 and 5.1):

- Complicated urinary tract infections (cUTI), including pyelonephritis

- Hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)

Treatment of patients with bacteraemia that occurs in association with, or is suspected to be associated with, any of the infections listed above.

Exblifep is also indicated for the treatment of infections due to aerobic Gram-negative organisms in adults with limited treatment options (see sections 4.2, 4.4 and 5.1)

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

The clinical development programme for cefepime-enmetazobactam relies partly on previous findings of safety and efficacy of cefepime when used alone. Cefepime, a 4th-generation cephalosporin, was first authorised in Sweden in 1993 (Maxipime). Generics of cefepime is, or has been, marketed by different MAHs in EU-countries but none centrally approved. The indications for cefepime when used alone include (among others) treatment of complicated urinary tract infections, including pyelonephritis, treatment of pneumonia (without a restriction to either community acquired pneumonia or hospital acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)) and bacteraemia when associated with these types of infections.

The clinical studies mainly relevant to the applied indications is study AT-301 to provide comparative safety and efficacy data in cUTI/acute pyelonephritis and PK-data from infected patients to update the PopPK model for PTA analyses and study AT-103 to provide enmetazobactam PK-data from ELF to support treatment of lung infections.

It should be noted that phase 3 studies evaluating a beta-lactam (BL) at its approved dose combined with a new beta-lactamase inhibitor (BLI) generally do not provide stand-alone efficacy data to support the dose regimen for the BLI. This is because it is not a strict requirement to confine such studies to infections caused by pathogens resistant to the BL but susceptible to the BL-BLI combination. Therefore, the PK/PD analyses incorporating the non-clinical PK/PD data and patient PK data are generally considered pivotal to support the dose regimen of the BLI for the proposed indications.

The applicant has received CHMP scientific advice (2018 and 2019) regarding the completeness and robustness of the non-clinical animal PK/PD package, the proposed Phase 3 trial design and the adequacy of the Phase 1 ELF penetration study to support the use of cefepime-enmetazobactam in HAP, including VAP.

Overall, in combination with a robust PK/PD package to support the adequacy of the enmetazobactam dose, the clinical programme is considered acceptable to support the proposed indications except for the pathogen-specific indication in patients with limited treatment options. The current thinking of the CHMP is that BL-BLI combinations not addressing carbapenem-resistant pathogens are not eligible for such an indication. *The in vitro* data suggests that cefepime-enmetazobactam could constitute a carbapenem-sparing alternative which is welcomed but not enough for this indication.

Design and conduct of clinical studies

The main study AT-301 was a phase 3, randomised, double-blind, multicentre study of cefepime-enmetazobactam compared with piperacillin-tazobactam in hospitalised adults with cUTI/acute pyelonephritis. The study included adult patients with cUTI and/or acute pyelonephritis. The inclusion and exclusion criteria were in consistency with the recommendations laid out in CPMP/EWP/558/95 Rev 3.

Patients were randomised to receive 2 g/0.5 g cefepime/enmetazobactam or 4 g/0.5 g piperacillin/tazobactam q8h. This is the highest approved dose for cefepime when the product is used alone. Both combinations were administered using 2 h infusions. The choice of piperacillin-tazobactam as comparator and its dose regimen is acceptable. It is the generally recommended dose to treat

cUTI/acute pyelonephritis. It should however be noted that the EU SmPCs for piperacillin-tazobactam no longer recommend its use for treatment of bacteraemia due to extended-beta-lactamase (ESBL) producing *E. coli* and *K. pneumoniae*. This is due to findings of a mortality imbalance disfavouring piperacillin-tazobactam in the Merino trial (JAMA, 2018) in which treatment with piperacillin-tazobactam compared with meropenem did not result in a non-inferior 30-day mortality rate in adults with blood-stream infections caused by ESBL-producing *E. coli* or *K. pneumoniae*.

The primary efficacy endpoint was the composite of microbiological eradication and clinical response outcomes at TOC in the m-MITT population. The primary and secondary efficacy endpoints and outcome criteria are acceptable. The recommended primary analysis population in registrational studies in cUTI/pyelonephritis is the microbiological ITT population. In this case the m-MITT population was used, excluding those not receiving any amount of study drug, which is acceptable.

According to CHMP expectations, it is required that patients with baseline pathogens that are resistant to the comparative regimen should be removed from the primary analysis population before unblinding the database to treatment assignment. If susceptibility testing criteria relevant to the test agent dose regimen being used in a trial have already been established patients with baseline pathogens that are resistant to the test agent should also be removed. Susceptibility criteria have not yet been established for cefepime-enmetazobactam but are established for cefepime alone. The applicant has removed patients with pathogens resistant to cefepime-enmetazobactam defined as a MIC >8 µg/mL. This is acceptable because cefepime breakpoints are available. Furthermore, patients with pathogens resistant to piperacillin-tazobactam has also been removed. The criteria for removal were however based on former CLSI criteria which differ several dilution steps compared with current EUCAST breakpoints, see below.

Approximately 1040 patients would be recruited to achieve 810 evaluable patients. The study recruited 1041 patients, however only 678 were evaluable in the m-MITT population.

The non-inferiority margin of 10 percent points is standard per CHMP guideline; however, demonstrating NI (or superiority) does not isolate the effect of enmetazobactam, due to the overall study design. This has been deemed acceptable for this sort of compounds.

Randomisation was coordinated through a IRT system and was stratified by type of infection, prior antibiotic therapy and region. It was planned that at least 50% of randomised patients would have cUTI and at least 30% would have AP. These planned proportions were observed in the actual study population. This is acceptable.

Study treatment appears to have been adequately blinded. The few cases unblinded before database lock (correctly unblinded due to SAEs or erroneously unblinded) are unlikely to have had any impact on the interpretation of the study.

The difference in the proportions of patients with overall treatment successes was calculated as the rate in the cefepime-AAI101 group minus that of the piperacillin/tazobactam group. A 2-sided 95% confidence interval (CI) was calculated using the stratified Newcombe method. Stratification was applied for type of infection, prior therapy category, and region. This is acceptable.

It is not clear whether stratification was applied for the calculation of the point estimate of the Difference in proportion between the treatment groups. The Statistical Analysis Plan states that treatment difference was the difference in the overall success rate between the two treatment arms (cefepime-AAI101 – piperacillin/tazobactam) however, there is a discrepancy between the percentage point estimate and the actual difference between the two treatment groups. This discrepancy is particularly large in subgroups with fewer patients. The applicant has acknowledged that the statistical analysis plan did not clearly specify the methodology. The calculation of the point estimate of the difference in proportion between treatment groups was not simply a subtraction, as the stratified

Newcombe method was used. The stratification was done based on the randomisation stratification factors, i.e., type of infection, prior antibiotic therapy and region.

For the primary endpoint, superiority was tested after non-inferiority was concluded. This was pre-specified in the Statistical Analysis Plan and hence, is acceptable. The applicant did not have any other secondary endpoint where type 1 error was controlled.

Missing data were generally imputed as failures.

Efficacy data and additional analyses

A total of 1122 patients were screened of which 1041 were randomised. Approximately 95% of patients in both treatment arms completed treatment and completed the study. Reasons for not completing treatment or the study were well-balanced between the treatment groups.

The primary analysis population (m-MITT) consisted of ~65 % of the ITT population, the number of patients excluded were balanced between the two groups.

Study subjects were mainly white (95%) females (60%) with a mean age of 54 years. Approximately 50/50 of the population had acute pyelonephritis or cUTI, respectively. The majority had no concomitant antibacterial therapy (93%). The most common baseline pathogen was *E. coli* (76.4%) followed by *K. pneumoniae* (9.7%), *Proteus mirabilis* (5.6%), *Pseudomonas aeruginosa* (3.5%), and *E. cloacae* (1.5%). Notable is the difference in susceptibility rate between cefepime and cefepime-enmetazobactam with an approximately 20% lower susceptibility rate for cefepime alone against Enterobacterales. Treatment compliance was high across treatment groups with a mean treatment duration of 8 days.

Overall, there were no major differences in baseline or disease characteristics between the cefepime-enmetazobactam and piperacillin-tazobactam groups.

Overall success was seen in a higher proportion of subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group (273/345 [79.1%] vs 196/333 [58.9%]), with a treatment difference of 21.2% (95% CI: 14.3%, 27.9%). Non-inferiority and superiority were met for the primary endpoint.

Similar results were seen at the TOC visit for the m-MITT+R population, i.e. the population without exclusion of baseline pathogens considered resistant to cefepime-enmetazobactam and piperacillin-tazobactam.

The largest difference in overall success was noted at the TOC visit. At LFU, the overall success were 68.4% vs. 58.9% with a somewhat smaller treatment difference of 10.7% but still with a lower bound of the 95% CI above zero.

The results in favour of the cefepime-enmetazobactam group was mainly driven by a more favourable microbiological response rate. At TOC, a favourable clinical response rate was noted for 319/345 (93.6%) and 296/333 (88.9%) for the cefepime-enmetazobactam and piperacillin-tazobactam groups, respectively, with a treatment difference of 3.5% (95% CI: -1.0. 8.0), whereas a favourable microbiological response rate was noted for 286/345 (82.9%) and 216/333 (64.9%) for the respective treatment groups, with a treatment difference of 19.0% (95% CI: 12.3, 25.4) in favour of cefepime-enmetazobactam.

For infections caused by the four most isolated Enterobacterales at baseline *E. coli*, *K. pneumoniae*, *P. mirabilis* and *E. cloacae,* a higher proportion of overall success, generally driven by a more favourable microbiological eradication rate, was noted in the cefepime-enmetazobactam group compared with

piperacillin-tazobactam group. An essentially similar overall success rate was noted against *P. aeruginosa* reflected by essentially similar clinical cure rates and microbiological eradication rates.

As commented above, the m-MITT population included pathogens resistant to piperacillin-tazobactam considering EUCAST interpretive criteria (>8 µg/mL for Enterobacterales and >16 µg/mL for *P. aeruginosa*). In the EU, piperacillin-tazobactam would not be recommended for treatment of these infections when caused by pathogens interpreted to be resistant to piperacillin-tazobactam and thus the applicant was asked to remove subjects from the m-MITT population based on EUCAST criteria and provide a complete set of updated efficacy analyses. Importantly, these analyses confirm that cefepime-enmetazobactam was non-inferior compared to piperacillin-tazobactam and also met the criteria for superiority, as originally observed in the initial m-MITT population.

Additionally, the applicant was asked to provide analyses of overall success, clinical cure and microbiological eradication at TOC including only subjects from the m-MITT population infected with baseline pathogens considered susceptible to cefepime-enmetazobactam but resistant to cefepime (using EUCAST interpretive criteria for cefepime and piperacillin-tazobactam). Overall success was higher in the cefepime-enmetazobactam group compared with the piperacillin-tazobactam group in the subpopulation having infections caused by pathogens resistant to cefepime. The microbiological eradication was numerically higher in the cefepime-enmetazobactam treatment group than in the piperacillin-tazobactam treatment group. Clinical cure was essentially comparable between the treatment groups. These analyses support the usefulness of the combination against infections caused by cefepime-resistant pathogens.

The favourable result for cefepime-enmetazobactam compared with piperacillin-tazobactam regarding the primary endpoint of Overall success at TOC in the m-MITT population was generally reflected in subgroup analyses. The forest-plots provided displays subsets of patients with a baseline isolate of Enterobacterales with minimal inhibitory concentrations (MICs) ≥1 µg/mL for ceftazidime, ceftriaxone, cefepime, meropenem, or cefepime-AAI101, and genotyped as having different production of different beta-lactamases and combinations thereof. However, a subgroup analysis including patients with infections with baseline Enterobacterales without production of beta-lactamases was initially missing and was provided. Overall success was higher in the cefepime-enmetazobactam group compared with the piperacillin-tazobactam group in the subpopulation having infections caused by pathogens without production of beta-lactamases. The microbiological eradication was higher in the cefepime-enmetazobactam treatment group than in the piperacillin-tazobactam treatment group. Clinical cure was essentially comparable between the treatment groups.

The numbers of patients having pathogens harbouring a carbapenemase or AmpC were too low to analyse any differences.

2.5.7. Conclusions on the clinical efficacy

Non-inferiority as well as superiority was met for the primary endpoint of the Phase 3 study AT-301 comparing cefepime-enmetazobactam with piperacillin-tazobactam for the treatment of cUTI including acute pyelonephritis. This finding constitutes supportive evidence of efficacy of cefepime-enmetazobactam and the adequacy of the enmetazobactam dose when combined with the highest approved dose for cefepime when used alone.

As this application relies on a limited clinical programme in line with CHMP expectations for products combining an approved beta-lactam with a new beta-lactamase inhibitor it should be noted that the clinical pharmacology programme, including non-clinical PK/PD analyses and PTA simulations using clinical PK data, is pivotal to support the adequacy of the enmetazobactam dose when combined with

cefepime. Moreover, enmetazobactam PK-data from ELF is essential to support the use of the combination in the treatment of lung infections.

Regarding the pathogen-specific indication in patients with limited treatment options, the current thinking of the CHMP is that BL-BLI combinations not addressing carbapenem-resistant pathogens are not eligible for such an indication. The *in vitro* data suggests that cefepime-enmetazobactam could constitute a carbapenem-sparing alternative which is welcomed but not enough for this indication.

2.5.8. Clinical safety

The safety database includes the following studies:

- AT-101, a Phase I study with both single-ascending (dose range 160 mg – 4 g) and multiple-ascending (dose range 500 mg – 2g q6h) doses of enmetazobactam alone and in combination with cefepime or piperacillin in healthy adult male subjects.
- AT-102, a Phase I study of cefepime-enmetazobactam in adults with varying degrees of renal impairment or normal renal function (1 g + 500 mg or 500 mg + 250 mg with severe renal impairment or and-stage renal disease). The effect of dialysis in subjects with end-stage renal disease (ESRD) was also assessed in this study.
- AT-103, a Phase I study evaluating the concentrations of cefepime-enmetzobactam (2 g + 1 g, IV over 2h, q8h) in plasma and epithelial lining fluid (ELF) in healthy subjects.
- AT-104, a mass balance and metabolite identification study in healthy adult male subjects (enmetazobactam only, 500 mg).
- AT-201, a Phase II study to determine the optimal cefepime-enmetazobactam combination dose (1 g + 500 mg IV over 2 hours q8h for 7-10 days or 2 g + 750 mg IV over 2 hours q8h for 7-10 days) and the probability of target attainment (PTA) in subjects with cUTI, including acute pyelonephritis.
- AT-301, a Phase III study to assess the efficacy of cefepime-enmetazobactam (2 g + 500 mg IV over 2 hours q8h for 7-14 days) compared with piperacillin-tazobactam (4 g + tazobactam 0.5 g IV over 2 hours q8h for 7-14 days) in the treatment of cUTI, including acute pyelonephritis.

Safety parameters across the clinical development program included:

- adverse events (AEs),
- serious adverse events (SAEs),
- AEs leading to study drug discontinuation,
- laboratory tests (clinical chemistry, haematology, and urinalysis),
- vital signs, and
- 12-lead electrocardiograms (ECGs).

In the Phase III study AT-301, adverse events of special interest (AESIs) were also recorded, based on known issues with cefepime:

Table 48: Adverse Events of Special Interest in Study AT-301

Event of interest	Identification
Hypersensitivity reactions	identified with the SMQs of anaphylactic reactions, angioedema, and hypersensitivity
Symptoms of encephalopathy and seizures	identified with SMQs of convulsions, noninfectious encephalopathy/delirium
Hepatic disorders	SMQ of hepatic failure, fibrosis and cirrhosis, and other liver damage-related conditions
Pseudomembranous colitis	SMQ for pseudomembranous colitis/Clostridium difficile infection
LFT elevation AEs	Any subject with an ALT, AST, or total bilirubin elevations from normal at baseline to postbaseline >1×ULN or any shift from baseline >n×ULN to post-baseline >n+1×ULN

AE=adverse event; ALT=alanine aminotransferase; AST=aspartate aminotransferase; LFT=liver function test; SMQ=Standardized Medical Dictionary for Regulatory Activities Query; ULN=upper limit of normal

For each study, the analysed safety population included all subjects who received at least 1 dose of study drug.

No safety data were pooled due to different dose used across the development program.

Primary analysis of safety is based in AT-301, safety data are summarised separately for phase 1, phase 2 and phase 3 studies. The available data from each data was reviewed separately.

2.5.8.1. Patient exposure

Phase I trials

The four Phase I trials included 166 subjects; three of these were conducted in healthy subjects (Study AT-101, Study AT-103, and Study AT-104) and one study included subjects with renal impairment (Study AT-102). Subjects were adult males and females in ages ranging from 19 through 70.

Phase II trial

Study AT-201 was a Phase II, randomised, double-blind, 2-cohort study in hospitalised adults with cUTI, including acute pyelonephritis. All study cohorts were randomised in a 2:1 ratio. Treatment duration for each cohort was 7 to 10 days.

Cohort 1:

- 15 subjects were treated with cefepime 1 g/enmetazobactam 500 mg IV infusion over 2 hours q8h, and
- 7 subjects were treated with cefepime 1 g IV infusion over 2 hours q8h.

Following the completion of Cohort 1, PK modelling and simulation for both PTA and ALT elevations were performed.

Cohort 2:

- 15 subjects were treated with cefepime 2 g/enmetazobactam 750 mg IV infusion over 2 hours q8h, and
- 8 subjects were treated with cefepime 2 g IV infusion over 2 hours q8h.

45 subjects were randomised, and all received the study drugs. The majority of subjects were female <65 years of age and had acute pyelonephritis. One subject withdrew due to an adverse event, one

withdrew for other reasons. Median duration of exposure was 8.0 days in the cefepime-enmetazobactam and cefepime alone groups.

Phase III trial

Study AT-301 was a randomised, double-blind, active-controlled, multi-centre, non-inferiority study to evaluate the efficacy, safety, and tolerability of cefepime-enmetazobactam compared to piperacillin/tazobactam for the treatment of cUTI, including AP.

A total of 1041 subjects were randomised in a 1:1 ratio to 1 of the following groups:

- Cefepime 2 g-enmetazobactam 0.5 mg infused over 2 hours every 8 hours (q8h) for 7 days. In patients with moderate renal impairment (estimated glomerular filtration rate [eGFR]<60 mL/min/1.73 m^2 and ≥30 mL/min/1.73 m^2) the dose of cefepime-AAI101 was adjusted to 1 g cefepime plus 250 mg AAI101, infused over a period of 2 hours q8h. In patients with moderate renal impairment at baseline, dose adjustment was applicable from Day 1 of dosing.
- Piperacillin/tazobactam 4.5 g (piperacillin 4 g/tazobactam 0.5 g) infused over 2 hours q8h for 7 days. Dosing of piperacillin/tazobactam followed the recommendations as per the respective summary of product characteristics, which did not require adjustment of the 4.5 g dose in patients with mild or moderate renal impairment.

Patients with severe renal impairment (eGFR <30 mL/min/1.73 m^2) were excluded from study participation.

At the discretion of the Investigator, treatment may have continued for up to 14 days in subjects with a positive blood culture at baseline. No switch to oral antibiotic therapy was permitted.

Table 49: Extent of Exposure in Study AT-301

Category Statistic	Cefepime 2g-enmetazobactam 0.5 g (N=516) n (%)	Piperacillin4g/ Tazobactam 0.5 g (N=518) n (%)	Total (N=1034) n (%)
Duration of study drug (days) [1]			
n	516	518	1034
Mean (SD)	8.0 (1.49)	8.0 (1.57)	8.0 (1.53)
1-3 days	11 (2.1)	13 (2.5)	24 (2.3)
4-6 days	12 (2.3)	7 (1.4)	19 (1.8)
7 days	43 (8.3)	54 (10.4)	97 (9.4)
8-10 days	425 (82.4)	421 (81.3)	846 (81.8)
11-14 days	17 (3.3)	13 (2.5)	30 (2.9)
≥15 days	8 (1.6)	10 (1.9)	18 (1.7)
Total number of doses received			
n	516	518	1034
Mean (SD)	21.2 (4.41)	21.2 (4.56)	21.2(4.48)

[1] Duration of study drug (days) = last dose date of study drug – first dose date of study drug + 1.
n= number; SD = standard deviation.
Source: CSR AT-301, Table 15

2.5.8.2. Adverse events

Phase I trials

Safety database includes only 70 subjects who received at least 1 dose of enmetazobactam alone. Across the Phase 1 Studies, 36.7% of subjects experienced at least one AE. The most frequently

reported AEs (≥5% of subjects overall) across these studies were infusion site erythema, infusion site extravasation, headache, diarrhoea, and nausea. None of the reported AEs among those subjects were considered as serious. Only two subjects in AT-101 MAD discontinued the study due to an AE.

Hypertension was the only AE that occurred in more than 1 subject within the renal impairment study AT-102 (1 subject with mild RI and 2 subjects with severe renal impairment). All these events were considered severe.

Severe AEs were reported in Study AT-101 MAD and Study AT-102; no severe AEs were reported in either Studies AT-101 SAD/DDI, AT-103, or AT-104.

Table 50: Severe (Grade 3) Adverse Events in Studies AT-101 and AT-102

Preferred Term, n (%)	AT-101 MAD		AT-102	
	Enmetaz 2 g (N=8)	Enmetaz 500 mg-Cefepime 1 g (N=8)	Moderate RI (N=6)	Severe RI (N=6)
Abdominal pain	1 (12.5)	0	0	0
Alanine aminotransferase increased	1 (12.5)	0	0	0
Pyrexia	0	1 (12.5)	0	0
Hypertension	0	0	1 (16.7)	2 (33.3)

Enmetaz=enmetazobactam; MAD=multiple ascending dose; RI=renal impairment

In AT-102 study there were 3 treatment-emergent Adverse Events of worsening of hypertension, one in 1 subject in the group of moderate RI and 2 subjects in the group of severe RI. All were transient and resolved without sequelae.

No AEs definitely related to study drug were reported in either Study AT-101 SAD/DDI or Study AT-101 MAD. In Study AT-101 MAD, 27 subjects experienced AEs that were considered possibly or probably related.

No AEs related to study drug were reported in Studies AT-102 and AT-104.

In Study AT-103, related AEs reported in more than 1 subject included ALT increased, catheter site pain, dizziness, nausea, and thrombophlebitis (2 subjects each).

Phase II trial

In the Phase 2 Study AT-201, the percentage of subjects with at least one AE was lower in the cefepime 2 g-enmetazobactam 0.75 g group compared with the cefepime 2 g group (26.7% vs 37.5%) and higher in the cefepime 1 g-enmetazobactam 0.5 g group compared with the cefepime 1 g group (60.0% vs 42.9%).

Headache, dermatitis allergic, and nephrolithiasis were the only AEs reported by more than 1 subject across the groups. All AEs were either mild or moderate with the exception of one severe AE of dermatitis allergic in the cohort of cefepime 2g/enmetazobactam.

Phase III trial

Table 51: Overview of AEs in Study AT-301

	Cefepime-Enmetazobactam (N=516) n (%)	Piperacillin/ Tazobactam (N=518) n (%)	Total (N=1034) n (%)
Any AEs	258 (50.0)	228 (44.0)	486 (47.0)
Drug-related AEs	102 (19.8)	75 (14.5)	177 (17.1)
AEs by maximum severity			
Mild (CTCAE Grade 1)	174 (33.7)	153 (29.5)	327 (31.6)
Moderate (CTCAE Grade 2)	58 (11.2)	49 (9.5)	107 (10.3)
Severe (CTCAE Grade 3)	20 (3.9)	22 (4.2)	42 (4.1)
Life-threatening (CTCAE Grade 4)	3 (0.6)	1 (0.2)	4 (0.4)
Death (CTCAE Grade 5)	3 (0.6)	3 (0.6)	6 (0.6)
AEs with a fatal outcome	3 (0.6)	3 (0.6)	6 (0.6)
Serious AEs	22 (4.3)	19 (3.7)	41 (4.0)
Discontinuation of study drug due to AEs	9 (1.7)	4 (0.8)	13 (1.3)

Percentages were calculated using the number of subjects in the column heading as the denominator.
AE=adverse event; CTCAE=Common Terminology Criteria for Adverse Events
Source: CSR AT-301 Table 62

The percentage of subjects with at least one AE was somewhat higher in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group (50.0% vs 44.0%). The most frequently reported AEs in both groups were elevations in liver function tests, each of which was reported in a similar percentage of subjects in the cefepime-enmetazobactam and piperacillin/tazobactam groups.

The only AEs that were reported in a higher percentage (>2%) of subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group were headache and phlebitis.

Table 52: Frequent Adverse Events (≥2% of Subjects in Either Group) in Phase 3 Study AT-301

Preferred Term	Cefepime-Enmetazobactam (N=516) n (%)	Piperacillin/ Tazobactam (N=518) n (%)	Total (N=1034) n (%)
Subjects with any AEs	258 (50.0)	228 (44.0)	486 (47.0)
Alanine aminotransferase increased	59 (11.4)	60 (11.6)	119 (11.5)
Aspartate aminotransferase increased	47 (9.1)	46 (8.9)	93 (9.0)
Blood bilirubin increased	30 (5.8)	20 (3.9)	50 (4.8)
Diarrhea	21 (4.1)	26 (5.0)	47 (4.5)
Headache	25 (4.8)	12 (2.3)	37 (3.6)
Transaminases increased	13 (2.5)	19 (3.7)	32 (3.1)
Anemia	13 (2.5)	15 (2.9)	28 (2.7)
Hypokalemia	11 (2.1)	8 (1.5)	19 (1.8)
Urinary tract infection	7 (1.4)	11 (2.1)	18 (1.7)
Phlebitis	14 (2.7)	1 (0.2)	15 (1.5)

AE=adverse event
Coding was based on MedDRA Version 21.0.
Percentages were calculated using the number of subjects in the column heading as the denominator.
MedDRA=Medical Dictionary for Regulatory Activities.

2.5.8.3. Serious adverse event, deaths and other significant events

Serious Adverse Events

The sample size is considered too limited to allow any conclusion regarding SAE. SAEs were reported in Studies AT-301 and AT-201; no SAEs were reported in any of the Phase 1 studies.

The percentage of subjects with at least 1 serious AE (SAE) in Study AT-301 was similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups (4.3% vs 3.7%; Table 13). SAEs reported by ≥ 2 subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group included pyelonephritis acute (4 subjects [0.8%] vs no subjects) and hypertensive crisis (2 subjects [0.4%] vs no subjects).

Treatment-related SAEs in Study AT-301 included *Clostridioides difficile* colitis in 1 subject in the cefepime-enmetazobactam group and atrial fibrillation, pseudomembranous colitis, and glomerular filtration rate decreased, each in 1 subject, in the piperacillin/tazobactam group. All of the treatment-related SAEs resolved spontaneously after drug discontinuation (GFR decreased) or following specific treatment (other SAEs).

In Study AT-201, 2 subjects, both in the cefepime 2 g-enmetazobactam 0.75 g group, had an SAE: metastatic colorectal cancer and nephrolithiasis, respectively. Neither of these events were assessed as treatment-related by the investigator. Metastatic colorectal cancer was treated with best supportive care and was ongoing at study discontinuation; nephrolithiasis resolved following a percutaneous nephrolithotripsy and ureterorenoscopy.

Table 53: Serious Adverse Events in Study AT-301

Preferred Term	Cefepime-enmetazobactam (N=516) n (%)	Piperacillin/ Tazobactam (N=518) n (%)
Subjects with any SAE	22 (4.3)	19 (3.7)
Pyelonephritis acute	4 (0.8)	0 (0.0)
Hypertensive crisis	2 (0.4)	0 (0.0)
Pneumonia	2 (0.4)	2 (0.4)
Urinary tract infection	2 (0.4)	3 (0.6)
Bladder neck obstruction	1 (0.2)	0 (0.0)
Cardiac arrest	1 (0.2)	0 (0.0)
Cerebrovascular insufficiency	1 (0.2)	0 (0.0)
Chest pain	1 (0.2)	0 (0.0)
Chronic obstructive pulmonary disease	1 (0.2)	0 (0.0)
Clostridioides difficile colitis	1 (0.2)	0 (0.0)
Groin pain	1 (0.2)	0 (0.0)
Haematuria	1 (0.2)	0 (0.0)
Lung cancer metastatic	1 (0.2)	0 (0.0)
Neck pain	1 (0.2)	0 (0.0)
Renal colic	1 (0.2)	0 (0.0)
Staphylococcal infection	1 (0.2)	0 (0.0)
Toxic shock syndrome	1 (0.2)	0 (0.0)
Urticaria	1 (0.2)	0 (0.0)
Atrial fibrillation	0 (0.0)	1 (0.2)
Cardiac failure	0 (0.0)	1 (0.2)
Duodenal ulcer	0 (0.0)	1 (0.2)
Epididymitis	0 (0.0)	1 (0.2)
Lung carcinoma cell type unspecified recurrent	0 (0.0)	1 (0.2)
Nephrolithiasis	0 (0.0)	1 (0.2)
Nephrectomy	0 (0.0)	1 (0.2)
Pseudomembranous colitis	0 (0.0)	1 (0.2)
Renal abscess	0 (0.0)	2 (0.4)
Renal haematoma	0 (0.0)	1 (0.2)
Septic shock	0 (0.0)	1 (0.2)
Sudden death	0 (0.0)	1 (0.2)
Urethral stenosis	0 (0.0)	1 (0.2)

SAE=serious adverse event
Coding is based on MedDRA Version 21.0.
Percentages are calculated using the number of subjects in the column heading as the denominator.
MedDRA=Medical Dictionary for Regulatory Activities

Deaths

Across the clinical development program, 6 subjects had a fatal AE. All 6 fatal AEs were reported in Study AT-301: 3 in the cefepime-enmetazobactam group and 3 in the piperacillin/tazobactam group (Table 12). None of the fatal AEs were reported for more than 1 subject and none were considered treatment-related by the investigator, which can be agreed. Except for septic shock which occurred on Day 1, all of the fatal AEs occurred in the Follow-up period (i.e., after the completion of study drug).

Table 54: Fatal AEs in Study AT-301

Group Number of Individual events	Fatal Adverse Event (Preferred Term)
Cefepime-enmetazobactam (N=3)	
N=1	Cerebrovascular insufficiency
	Cardiac arrest
	Lung cancer metastatic
Piperacillin/tazobactam (N=3)	
N=1	Lung carcinoma cell type unspecified recurrent
N=1	Sudden death
N=1	Septic shock

Patient A: Cerebrovascular insufficiency (cerebral insufficiency)

Patient A, an [90-99]-year-old white female with cUTI (Study Day -01), experienced a SAE of cerebrovascular insufficiency. The patient signed informed consent on Study Day-01 and was randomised to cefepime-AAI101 on Day 01.

The first dose of study medication was administered on Study Day 01. The last dose prior to the onset of the event was administered on Study Day 08.

The patient's daughter informed the Investigator that on Study Day 32, the patient died due to cerebrovascular insufficiency. An autopsy was not performed, and additional details were not available.

The Investigator considered the event of cerebral insufficiency as Grade 5 in severity and unrelated to study medication.

B: Cardiac arrest (cardiac arrest)

Patient B, an [60-69] year-old white male with cUTI (Study Day -01) and a history of chronic cardiac insufficiency and aortic valve stenosis, experienced a SAE of cardiac arrest, and in addition, an AESI of alanine aminotransferase increased. The patient signed informed consent on Study Day -01 and was randomised to cefepime-AAI101 on Study Day 01.

The first dose of study drug was administered on Study Day 01. The last dose of study drug prior to the onset of the events was administered on Day 12.

On Study Day 01, baseline laboratory testing revealed ALT 7 U/L (NR 0.10-1.10 mg/dL), AST 68 U/L (NR 9-34 U/L), ALP 73 U/L (NR 37 116 U/L), and total bilirubin 0.85 mg/dL (NR 0.10-1.10 mg/dL).

On Study Day 28, the patient experienced an AESI of alanine aminotransferase increased. Laboratory testing revealed ALT 72 U/L, AST 171 U/L, ALP 102 U/L, and total bilirubin 0.87 mg/dL.

The event alanine aminotransferase increased was considered ongoing.

On Study Day 30, the patient died due to the event cardiac arrest. The patient collapsed at home and was found unresponsive following respiratory arrest and suspected stroke. No autopsy was performed.

No treatment was administered.

No action was taken with the study drug due to the events as the patient was in the follow-up phase at the time.

The Investigator considered the event of alanine aminotransferase as Grade 1 in severity and related to study medication and the event of cardiac arrest as Grade 5 in severity and unrelated to study medication. The Investigator considered the patient's complicated cardiac history of chronic cardiac insufficiency and stenosis of aortic valve as alternative aetiologies for cardiac arrest.

Patient C: Lung cancer metastatic (metastatic right lung cancer)

Patient C, an [70-79] year-old white male with cUTI (Study Day -01) and history of smoking experienced a SAE lung cancer metastatic. The patient signed informed consent on Study Day -01 and randomised to cefepime-AAI101 on the same date.

The first dose of study drug was administered on Study Day 01. The last dose of study drug prior to event onset was administered on Study Day 08.

On Study Day 19, the patient experienced weakness, lack of appetite, and asthenia that had worsened over the preceding few weeks. On 16 April 2019, the patient presented to the emergency department with difficulty breathing and recurring falls. The patient was admitted to the hospital for lung cancer metastatic.

On Study Day 27, the patient died due to the event lung cancer metastatic.

The death certificate identified the cause of death as acute respiratory failure as a result of chronic respiratory failure caused by the event lung cancer metastatic. An autopsy was not performed.

No action was taken with the study drug due to the event as the patient was in the follow-up phase at the time of the event.

The Investigator considered the event lung cancer metastatic as Grade 5 in severity and unrelated to study medication.

Patient D: Lung carcinoma cell type unspecified recurrent (recurrence of lung cancer)

Patient D, an [60-69] year-old white male with cUTI (Study Day -01) and a medical history of lung cancer 15 years prior with chemotherapy and radiation, experienced the SAE of lung carcinoma cell type unspecified recurrent. The patient signed informed consent on Study Day 01 and was randomised to piperacillin/tazobactam on the same date.

The first dose of study medication was administered on Study Day 01. The last dose of study medication prior to event onset was administered on Study Day 06.

On Study Day 06 , during hospitalisation, the patient began to experience breathing difficulty with subsequent tachypnoea and hypoxia requiring large doses of oxygen. A chest X-ray revealed an infiltrate of the right lung and pleural effusion.

A bronchoscopy revealed a recurrence of lung cancer in the right upper lobe. No other treatment was reported.

On the same date, the patient died due to the event lung carcinoma cell type unspecified recurrent. An autopsy was not performed.

Study medication was discontinued, and the patient was discontinued from the study due to the event lung carcinoma cell type unspecified recurrent.

The Investigator considered the event of recurrence of lung cancer as Grade 5 in severity and unrelated to study medication.

Patient E: Sudden death (sudden death)

Patient E, an [80 -89] year-old white female with cUTI (Study Day -01), experienced a SAE of sudden death. The patient signed informed consent on Study Day -01and was randomised to piperacillin/tazobactam on Study Day 01. The patient was in the follow-up phase at the time of the event.

The first dose of study drug was administered on Study Day 01. The last dose of study drug prior to event onset was administered on Study Day 08.

On Study Day 16, emergency services were contacted and responded to find the patient unresponsive. The patient was reportedly last seen alive approximately 1 hour prior and died due to an unknown cause.

No action was taken with the study medication due to the event as the patient was in the follow-up phase at the time of the event.

An autopsy was not performed. The Investigator reported the death certificate was permanently unavailable.

The Investigator considered the event of sudden death as Grade 5 in severity and unrelated to study medication.

Patient F: Septic shock (septic shock)

Patient F, an [80-89] year-old white female with AP (Study Day -01), experienced a SAE of septic shock. The patient signed informed consent on Study Day 01 and randomised to piperacillin/tazobactam on the same date.

The first dose of study drug was administered on Study Day 01. The last dose of study drug prior to event onset was administered on the same date.

On Study Day 01, the patient experienced septic shock and was admitted to the hospital with suspected left-sided pyelonephritis.

On Study Day 02, the patient was unresponsive to pain stimuli and cardiopulmonary resuscitation was started. Resuscitation attempts were unsuccessful, and the patient died due to the event septic shock. Blood and urine cultures identified the causative agent of septic shock as *Escherichia coli*. An autopsy was not performed.

The study drug was discontinued, and the patient was withdrawn from the study due to the event.

The Investigator considered the event septic shock as Grade 5 in severity and unrelated to study medication. Left ventricular failure and the patient’s pre-existing acute tubulointerstitial nephritis were listed on the death certificate as other causes of death.

Adverse Events Leading to Study Drug Discontinuation

The percentage of subjects with an AE leading to study drug discontinuation was small although more than twice in the cefepime-enmetazobactam group compared to the piperacillin/tazobactam group (1.7% vs 0.8%). None of the AEs leading to discontinuation of study drug occurred in more than 1 subject (Table 14). Discontinuation due to an AE is not considered a major concern.

Table 55: Adverse Events Leading to Study Drug Discontinuation in Study AT-301

Preferred Term	Cefepime-enmetazobactam (N=516) n (%)	Piperacillin/ Tazobactam (N=518) n (%)
Any AE leading to discontinuation of study drug	9 (1.7)	4 (0.8)
Abdominal pain	1 (0.2)	0 (0.0)
Bacterial infection	1 (0.2)	0 (0.0)
Dermatitis allergic	1 (0.2)	0 (0.0)
Eructation	1 (0.2)	0 (0.0)
Headache	1 (0.2)	0 (0.0)
Insomnia	1 (0.2)	0 (0.0)
Nausea	1 (0.2)	0 (0.0)
Pneumonia	1 (0.2)	0 (0.0)
Pyelonephritis acute	1 (0.2)	0 (0.0)
Restlessness	1 (0.2)	0 (0.0)
Transaminases increased	1 (0.2)	0 (0.0)
Urinary retention	1 (0.2)	0 (0.0)
Urinary tract infection	1 (0.2)	0 (0.0)
Abdominal pain upper	0 (0.0)	1 (0.2)
Diarrhoea	0 (0.0)	1 (0.2)
Hypersensitivity	0 (0.0)	1 (0.2)
Lung carcinoma cell type unspecified recurrent	0 (0.0)	1 (0.2)
Septic shock	0 (0.0)	1 (0.2)
Vomiting	0 (0.0)	1 (0.2)

Coding is based on MedDRA Version 21.0.
Percentages are calculated using the number of subjects in the column heading as the denominator.
AE=adverse event; MedDRA=Medical Dictionary for Regulatory Activities

Adverse Events of Special Interest

AEs of special interest based on known issues with cefepime were analysed for Study AT-301.

The percentage of subjects with AEs in either the pseudomembranous colitis or encephalopathy and seizures Standardized Medical Dictionary for Regulatory Activities Query (SMQ) categories was similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups. AEs in the hypersensitivity reaction SMQ category were reported in a higher percentage of subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group, however this difference between the groups was not due to a specific hypersensitivity event.

Table 56: Adverse Events of Special Interest in Study AT-301

Event of interest	Identification
Hypersensitivity reactions	identified with the SMQs of anaphylactic reactions, angioedema, and hypersensitivity
Symptoms of encephalopathy and seizures	identified with SMQs of convulsions, noninfectious encephalopathy/delirium
Hepatic disorders	SMQ of hepatic failure, fibrosis and cirrhosis, and other liver damage-related conditions
Pseudomembranous colitis	SMQ for pseudomembranous colitis/Clostridium difficile infection
LFT elevation AEs	Any subject with an ALT, AST, or total bilirubin elevations from normal at baseline to postbaseline >1×ULN or any shift from baseline >n×ULN to post-baseline >n+1×ULN

AE=adverse event; ALT=alanine aminotransferase; AST=aspartate aminotransferase; LFT=liver function test; SMQ=Standardized Medical Dictionary for Regulatory Activities Query; ULN=upper limit of normal

SMQ Hypersensitivity Reactions

AEs in hypersensitivity reaction SMQ category were reported by a higher percentage of subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group. This difference between the groups was not due to a specific hypersensitivity event.

SMQ Encephalopathy and Seizures

AEs in the encephalopathy and seizures SMQ category were reported in a similar percentage of subjects in the cefepime-enmetazobactam and piperacillin/tazobactam groups.

SMQ Hepatic disorders

AEs in the hepatic disorders SMQ category were reported in a similar percentage of subjects in the cefepime-enmetazobactam and piperacillin/tazobactam groups. Increases in AST and ALT constituted the bulk of events in both groups.

SMQ Pseudomembranous Colitis

AEs in the pseudomembranous colitis SMQ category were reported in a similar percentage of subjects in the cefepime-enmetazobactam and piperacillin/tazobactam groups, with diarrhoea the most frequently reported AE in both groups (Table 25). *Clostridium difficile* colitis was only reported in the cefepime-enmetazobactam group (0.6%) and pseudomembranous colitis in the piperacillin/tazobactam group (0.6%).

Adverse events associated with Liver Function Test Elevations

Abnormalities in LFTs were reported as AEs in a similar percentage of subjects in the cefepime-enmetazobactam and piperacillin/tazobactam groups (Table 27). ALT increased was the most frequently reported AE related to an LFT abnormality. None of AEs related to an LFT abnormality were serious but one event of transaminase increased led to study drug discontinuation.

Table 57: AEs Associated with a Liver Function Test Abnormality

	Cefepime-Enmetazobactam (N=516) n (%)	**Piperacillin/ Tazobactam (N=518) n (%)**
ALT increased	59 (11.4)	60 (11.6)
AST increased	47 (9.1)	46 (8.9)
Blood bilirubin increased	30 (5.8)	20 (3.9)
Liver function test increased	1 (0.2)	4 (0.8)
Transaminase increased	13 (2.5)	19 (3.7)

AEs in subjects with an ALT, AST, or total bilirubin elevations from normal at baseline to post-baseline >1×ULN or any shift from baseline >n×ULN to post-baseline >n+1×ULN.
ALT=alanine aminotransferase increased; AST=asparatate aminotransferase

2.5.8.4. Laboratory findings

Haematology

Phase III trial

Mean changes from baseline over time in haematology parameters were small and similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups in Study AT-301. Shifts in haematology

values over time did not reveal any meaningful differences between the cefepime-enmetazobactam and piperacillin/tazobactam groups.

18 subjects in both treatment groups experienced a treatment emergent adverse event in the SOC Blood and lymphatic system disorders, none of these events were serious or led to study drug discontinuation. Anaemia was the most frequently reported abnormality in a haematology parameter reported as an AE in both the cefepime-enmetazobactam and piperacillin/tazobactam groups (2.5% and 2.9%, respectively).

Liver Function Tests

Phase III trial

Mean changes from baseline over time in LFTs were small and similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups in Study AT-301. A total of 150 adverse events were reported due to an abnormal liver function test, most commonly increases in ALT or AST. The rates of events were similar between both treatment arms.

Table 58: AEs Associated with a Liver Function Test Abnormality

	Cefepime-Enmetazobactam (N=516) n (%)	Piperacillin/Tazobactam (N=518) n (%)
ALT increased	59 (11.4)	60 (11.6)

	Cefepime-Enmetazobactam (N=516) n (%)	Piperacillin/Tazobactam (N=518) n (%)
AST increased	47 (9.1)	46 (8.9)
Blood bilirubin increased	30 (5.8)	20 (3.9)
Liver function test increased	1 (0.2)	4 (0.8)
Transaminase increased	13 (2.5)	19 (3.7)

AEs in subjects with an ALT, AST, or total bilirubin elevations from normal at baseline to postbaseline >1×ULN or any shift from baseline >n×ULN to post-baseline >n+1×ULN.
ALT=alanine aminotransferase increased; AST=asparatate aminotransferase

The percentage of subjects with liver function test abnormalities was similar between the groups (Table 28). There was 1 potential Hy's law case in the cefepime-enmetazobactam group at baseline. At baseline, ALT was 76 U/L (ULN of 41 U/L), AST was 178 U/L (ULN of 34 U/L), and total bilirubin was 38.8 µmol/L (ULN of 18.8 µmol/L). Treatment with cefepime-enmetazobactam was initiated with LFTs returning to normal range on Day 3, with an ALT of 8 U/L, AST of 10 U/L, and total bilirubin of 6.3 µmol/L. All of these LFTs remained in normal range during the study.

No treatment-emergent Hy's law cases were seen in either the cefepime-enmetazobactam or piperacillin/tazobactam groups.

In exposure-safety analyses, the change from baseline for ALT, AST, and total bilirubin was correlated with individual enmetazobactam exposure. A slight positive correlation was observed for ALT and AST with enmetazobactam C_{max}. It should be noted that the range of enmetazobactam C_{max} was relatively small (~10-fold).

Renal Function

Phase III trial

Mean changes from baseline over time in blood urea nitrogen (BUN) and creatinine were small and similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups in Study AT-301.

AEs related to an abnormality in a kidney function test included blood urea increased (1 subject in cefepime-enmetazobactam group), blood creatinine decreased (1 subject in cefepime-enmetazobactam group), and blood creatinine increased (1 subject in each group); none of these events were serious or led to study drug discontinuation.

In exposure-safety analyses, the change from baseline for eGFR was correlated with individual enmetazobactam exposure with an increase of eGFR observed on average indicated by predominantly positive changes from baseline independently of the enmetazobactam C_{max}.

Other Serum Chemistry Parameters

Phase III trial

Mean changes from baseline over time in other serum chemistry parameters were small and similar between the cefepime-enmetazobactam and piperacillin/tazobactam groups.

Drug related abnormalities in serum chemistry parameters such as blood creatine phosphokinase, lipase and lactate dehydrogenase increases have been described as frequent (≥2 subjects in either group) in Study AT-301 and have not been included in 4.8. None of these events were serious or led to study drug discontinuation.

Urinalysis

Phase III trial

Mean changes from baseline over time in urinalysis parameters were similar between the cefepime-enmetazobactam and piperacillin/tazobactam groups in Study AT-301.

Urinary sediment abnormal was the only urinalysis abnormality reported as an AE in more than 1 subject (2 subjects [0.4%] in cefepime-enmetazobactam group and no subjects in piperacillin/tazobactam group).

Vital Signs

Phase I trials

No clinically relevant changes in vital signs were seen in Studies AT-101 SAD/DDI, AT-101 MAD, AT-103, and AT-104. In AT-102, clinically relevant increases in blood pressure (both systolic and diastolic) and decreases in heart rates were noted in the groups with moderate and severe renal impairment, resulting in three adverse events being reported.

Phase II trial

Mean systolic and diastolic blood pressures, heart rate, and temperature showed small fluctuations over time and, for each parameter, the mean change from baseline was similar in the cefepime-enmetazobactam and cefepime alone groups.

Phase III trial

Systolic and diastolic blood pressures, heart rate, and temperature showed small fluctuations over time and, for each parameter, the mean change from baseline was similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups.

Electrocardiograms

Phase III trial

Cefepime-enmetazobactam had no clinically significant effects on cardiac repolarisation or other ECG parameters in Study AT-301.

On Day 4, a small mean increase in ΔQTcF was observed in the cefepime-enmetazobactam (4.3 msec [95% CI: 2.4, 6.3]) and piperacillin/tazobactam groups (4.5 msec [95% CI:2.6, 6.4]) msec).

No subject had a QTcF >500 msec post-baseline and the percentage of subjects with a QTcF or QTcB change from baseline >60 msec was small and similar between the groups (Table **59**).

Table 59: Categorical Analysis of QTc in Study AT-301

Category	Cefepime-Enmetazobactam (N=441)	Piperacillin/Tazobactam (N=449)
QTcF interval, aggregate (msec)		
450<QTcF≤480 ms	7 (1.6%)	13 (2.9%)
480<QTcF≤500 ms	0	0
QTcF>500 ms	0	0
30<ΔQTcF≤60 ms	36 (8.2%)	31 (6.9%)
ΔQTcF >60 msec	5 (1.1)	4 (0.9)
QTcB interval, aggregate (msec)		
450<QTcB≤480 ms	27 (6.1%)	36 (8.0%)
480<QTcB≤500 ms	1 (0.2%)	6 (1.3%)
30<ΔQTcB≤60 ms	19 (4.3%)	29 (6.5%)
ΔQTcB >60 msec	4 (0.9)	1 (0.2)

QTcB= QT interval corrected for heart rate based on Bazett's formula and corrected QT interval; QTcF= QT interval corrected for heart rate based on Fridericia's formula
Δ=Change from baseline

A small post-baseline decrease in heart rate was seen in the cefepime-enmetazobactam (-6.2 bpm [95% CI: -7.5, -5.0]) and piperacillin/tazobactam groups (-8.2 bpm [95% CI: -9.5, -6.9]). However, heart rate was elevated at baseline as expected in subjects with an infection and thus, the observed decrease in heart rate is most likely related to the beginning of infection resolution.

No difference was seen between the cefepime-enmetazobactam and piperacillin/tazobactam groups in either the QRS or PR intervals.

A small percentage of subjects in each group had a treatment-emergent clinically significant morphological abnormality (Table **60**). This morphological abnormality was found after reporting.

Additionally, in exposure-safety analyses, no correlation of ΔQTcF with enmetazobactam plasma concentrations was found.

Table 60: Treatment-Emergent, Clinically Significant ECG Morphological Abnormalities in Study AT-301

Category	Cefepime-Enmetazobactam (N=441) n (%)	Piperacillin/ Tazobactam (N=449) n (%)
T wave inversion, consider ischemia -localised in inferior leads (II, III, aVF)	1 (0.2)	0
ST depression, consider ischemia -localised in precordial Leads (V1-V6)	1 (0.2)	0
ST depression, consider ischemia -localised in inferior leads (II, III, aVF)	1 (0.2)	0
First degree AV block above 240 msec	1 (0.2)	3 (0.7%)
Atrial fibrillation with a slow ventricular response	0	2 (0.4%)

2.5.8.5. Safety in special populations

MedDRA Terms	Age <65		Age 65-74		Age 75-84		Age 85+	
	N	%	N	%	N	%	N	%
	312		**126**		**67**		**11**	
Patients with any TEAEs	148	47.4%	70	55.6%	32	47.8%	8	72.7%
Serious AEs – Total	8	2.6%	9	7.1%	2	3.0%	2	18.2%
- Fatal	0	0.0%	2	1.6%	0	0.0%	1	9.1%
- Hospitalisation/prolong existing hospitalisation	8	2.6%	8	6.3%	2	3.0%	1	9.1%
- Life-threatening	1	0.3%	1	0.8%	0	0.0%	1	9.1%
- Disability/incapacity	0	0.0%	0	0.0%	0	0.0%	0	0.0%
- Other (medically significant)	2	0.6%	3	2.4%	0	0.0%	0	0.0%
AE leading to drop-out	1	0.3%	2	1.6%	0	0.0%	0	0.0%
Psychiatric disorders	2	0.6%	2	1.6%	2	3.0%	1	9.1%
Nervous system disorders	23	7.4%	2	1.6%	2	3.0%	2	18.2%
Accidents and injuries	1	0.3%	2	1.6%	0	0.0%	0	0.0%
Cardiac disorders	0	0.0%	3	2.4%	1	1.5%	1	9.1%
Vascular disorders	14	4.5%	12	9.5%	1	1.5%	3	27.3%
Cerebrovascular disorders	0	0.0%	0	0.0%	0	0.0%	1	9.1%
Infections and infestations	23	7.4%	10	7.9%	11	16.4%	0	0.0%
Anticholinergic syndrome	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Quality of life decreased	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures*	2	0.6%	0	0.0%	0	0.0%	0	0.0%
Hepatic Disorders SMQ	75	24.0%	27	21.4%	17	25.4%	3	27.3%
Gastro-intestinal disorder	32	10.3%	8	6.3%	5	7.5%	1	9.1%
Hypertension	1	0.3%	6	4.8%	2	3.0%	2	18.2%

**There were no cases identified for postural hypotension, however there were 3 cases reported for preferred term "Hypotension", among which 2 were reported in age group <65 years and 1 was reported in the age group 75 to 84 years.*

2.5.8.6. Safety related to drug-drug interactions and other interactions

No new drug-drug interactions were described. The following interactions have been previously described for cefepime.

Drug/Laboratory Test Interactions

The administration of cefepime-enmetazobactam may result in a false-positive reaction for glucose in the urine with certain methods because of cefepime. It is recommended that glucose tests based on enzymatic glucose oxidase reactions be used [Maxipime USPI; Renapime SmPC].

Aminoglycosides

Renal function must be monitored if aminoglycosides are to be administered with cefepime for injection because of the increased potential of nephrotoxicity and ototoxicity of aminoglycoside antibacterial drugs [Maxipime USPI; Renapime SmPC].

Diuretics

Nephrotoxicity has been reported following concomitant administration of other cephalosporins with potent diuretics such as furosemide. Renal function must be monitored when cefepime is concomitantly administered with potent diuretics [Maxipime USPI; Renapime SmPC].

2.5.8.7. Discontinuation due to adverse events

Phase I trials

One subject was withdrawn from AT-101 MAD combo due to pyrexia (severe, possible, recovered, treatment given for AE), one subject was withdrawn from AT-101 MAD solo due to elevated ALT and AST, >3xULN (ALT severe, probable, recovered; AST mild, possible, recovered; no treatment given for either AE).

Phase II trial

Two subjects were withdrawn from the study, both due to event (PT) Dermatitis allergic.

Phase III trial

The percentage of subjects with an AE leading to study drug discontinuation was small and similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups (1.7% vs 0.8%). None of AEs leading to discontinuation of study drug occurred in more than 1 subject (Table 61).

Table 61: Adverse Events Leading to Study Drug Discontinuation in Study AT-301

Preferred Term	Cefepime-enmetazobactam (N=516) n (%)	Piperacillin/ Tazobactam (N=518) n (%)
Any AE leading to discontinuation of study drug	9 (1.7)	4 (0.8)
Abdominal pain	1 (0.2)	0 (0.0)
Bacterial infection	1 (0.2)	0 (0.0)
Dermatitis allergic	1 (0.2)	0 (0.0)
Eructation	1 (0.2)	0 (0.0)
Headache	1 (0.2)	0 (0.0)
Insomnia	1 (0.2)	0 (0.0)
Nausea	1 (0.2)	0 (0.0)
Pneumonia	1 (0.2)	0 (0.0)
Pyelonephritis acute	1 (0.2)	0 (0.0)
Restlessness	1 (0.2)	0 (0.0)
Transaminases increased	1 (0.2)	0 (0.0)
Urinary retention	1 (0.2)	0 (0.0)
Urinary tract infection	1 (0.2)	0 (0.0)
Abdominal pain upper	0 (0.0)	1 (0.2)
Diarrhoea	0 (0.0)	1 (0.2)
Hypersensitivity	0 (0.0)	1 (0.2)
Lung carcinoma cell type unspecified recurrent	0 (0.0)	1 (0.2)
Septic shock	0 (0.0)	1 (0.2)
Vomiting	0 (0.0)	1 (0.2)

Coding is based on MedDRA Version 21.0.
Percentages are calculated using the number of subjects in the column heading as the denominator.
AE=adverse event; MedDRA=Medical Dictionary for Regulatory Activities
Source: CSR AT-301 Table 14.3.1.7

2.5.9. Discussion on clinical safety

The overall safety database for Exblifep is based on 6 completed clinical studies: 4 Phase I studies, 1 Phase II study, and 1 Phase III study.

A total of 690 subjects have received at least one dose of enmetazobactam either alone (70 subjects), in combination with cefepime (612 subjects) or with piperacillin (8 subjects). Of these subjects, 546 subjects were hospitalised for treatment of cUTI, including acute pyelonephritis, and received cefepime-enmetazobactam in either the Phase II or Phase III study.

The applicant's primary analysis of safety is based on comparison of cefepime 2 g-enmetazobactam 0.5 g (516 subjects) with piperacillin 4 g/tazobactam 0.5 g (518 subjects), each administered IV, in the treatment of cUTI, including AP, in the Phase III Study AT-301.

The size of the safety database is considered acceptable, also taking into consideration that cefepime has been in clinical use for decades and has a well-established safety profile.

Overall, Exblifep displayed a somewhat higher number of drug-related adverse events than the comparator, 19.8% vs. 14.5%, in the Phase III study. Most of this difference can be attributed to headache and phlebitis, both known and relatively common adverse reactions to cefepime. Other relatively common adverse reactions reported were elevated liver function tests and diarrhoea. The

overall safety profile is comparable with the active comparator piperacillin/tazobactam and what is known about the safety of cefepime.

During the phase III study, 22 subjects (4.3%) in the Exblifep arm experienced a serious adverse event, compared with 19 subjects (3.7%) in the comparator arm. In the Exblifep arm, serious adverse events in more than one subject included acute pyelonephritis (4 subjects), hypertensive crisis, pneumonia, and urinary tract infection (2 subjects each). However, only one event, *Clostridioides difficile* colitis, in the Exblifep arm was considered treatment related by the investigator, and the patient was reported to have recovered. *Clostridioides difficile* colitis is a known adverse reaction to many antibiotics, including cefepime.

Three subjects experienced a hypertensive crisis during the phase III study, two of them serious. No significant changes were seen in the central tendency of measured blood pressures. The applicant has assessed these events as expected in the study population. However, the CHMP disagrees and does not consider hypertensive crisis to be expected in a cUTI population. Considering also that there were three events of hypertension in conjunction with renal impairment in AT-102, the applicant was requested to discuss these events further. Further analysis and discussion of data provided satisfactory conclusions that hypertension is likely not an adverse reaction to Exblifep.

All fatal adverse events during the clinical development programme occurred during the phase III study, three events in each arm. In the Exblifep arm, events were cerebrovascular insufficiency, cardiac arrest, and lung cancer metastatic. In the control arm, events were recurrent lung carcinoma, sudden death, and septic shock. All events except the septic shock occurred during the follow-up period and were considered unrelated to the study drugs. The septic shock occurred during day 1 and became fatal on day 2. The event was considered not related to the study drugs, but rather the underlying disease of acute tubulointerstitial nephritis and left ventricular failure. It is agreed that the fatal events lack a plausible mechanistic and temporal connection to Exblifep.

During the Phase III study, nine subjects (1.7%) in the Exblifep arm experienced a serious adverse event, compared with four subjects (0.8%) in the comparator arm. Five of the events were on the applicants AESI list: dermatitis allergic, hypersensitivity (AESI hypersensitivity reaction), diarrhoea (AESI Pseudomembranous colitis), transaminases increased (AESI hepatic disorders), and restlessness (AESI Encephalopathy and seizures).

AESIs were predetermined based on known safety concerns with cefepime: hypersensitivity reactions, symptoms of encephalopathy and seizures, hepatic disorders, pseudomembranous colitis, and LFT elevation adverse events and analysed via Standardized Medical Dictionary for Regulatory Activities Query (SMQ).

Hypersensitvity reactions were identified as adverse events of special interest in study AT-301, higher percentage of subjects in cefepime-enmetazobactam group compared to piperacillin-tazobactam (4.3% vs. 1.5%). Of them, dermatitis allergic was presented in one patient and was an AE leading to discontinuation (severe grade 3 event). This event was also presented in phase 2 study A-201, in two patients in the the cefepime 2g-enmetazobactam 0.75 g group. This ADR has been added to the list of drug reactions in SmPC section 4.8, under immune system disorders.

The percentage of subjects with AEs in the pseudomembranous colitis, encephalopathy and seizures, and hepatic disorders SMQ categories was similar in the cefepime-enmetazobactam and piperacillin/tazobactam groups. AEs in the hypersensitivity reaction SMQ category were reported in a higher percentage of subjects in the cefepime-enmetazobactam group compared with the piperacillin/tazobactam group, however this difference between the groups was not due to a specific hypersensitivity event.

Laboratory findings for categories haematology, serum chemistry, urinalysis, electrocardiograms, revealed no clinically significant or otherwise notable findings or trends. In vital signs, mean changes were small and transient. In the phase I study AT-102, increases in blood pressure were noted among those with moderate and severe renal impairment, resulting in three reports of adverse events. This was however not seen in the later studies. Renal function tests revealed abnormal values in three subjects: blood urea increase, blood creatinine increase, and blood creatinine decrease. Two of the subjects recovered and one was recovering at the end of study. Most concerning was the liver function tests; 11.4% of subjects reported increased ALT, 9.1% reported increased AST, and 5.8% reported increased bilirubin as adverse events. However, no subjects fulfilled the criteria for Hy's law.

The proportion of elevated liver function tests was comparable with the active control group as well as with what is known about cefepime in clinical experience. One subject was withdrawn from the study due to liver function tests, but all subjects had recovered by the end of study. The elevated liver function tests are included in the SmPC section 4.8.

Available data from published observational studies and case reports indicate that cefepime use is not associated with risks of major birth defects, miscarriage or adverse maternal or foetal outcomes. No such data is however available for enmetazobactam exposure in humans. Animal studies indicate reproductive toxicity at relevant clinical exposure of enmetazobactam but without signs of teratogenicity. Amendments to the wordings in SmPC sections 4.6 and 5.3 are proposed, see Discussion on non-clinical aspects, LoQ and SmPC.

No new pharmacodynamic drug-drug interactions have been described other than those already described for cefepime.

The number of discontinuations is relatively low and does not give cause for concern.

2.5.10. Conclusions on the clinical safety

The overall safety profile of Exblifep appears favourable and essentially similar to that of piperacillin/tazobactam or cefepime when used alone, with the exception for the increased frequency of headaches and phlebitis. Notable adverse reactions are elevations of AST, ALT and bilirubin in laboratory tests, but these occur at similar frequency as cefepime and are duly described in the SmPC.

2.6. Risk Management Plan

2.6.1. Safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	Use in pregnant women Use in lactating women

2.6.1.1. Discussion on safety specification

The applicant has not identified any important identified or important potential risks for inclusion in the list of safety concerns. Use in pregnant and lactating women has been identified by the applicant as missing information for inclusion in the list of safety concerns.

It is agreed with the applicant that there are no important identified risks or important potential risks that warrant inclusion in the list of safety concerns.

It is agreed that use in pregnant and lactating women should be included as missing information based on the findings in animal studies that indicate reproductive toxicity at relevant clinical exposure of enmetazobactam but without signs of teratogenicity.

2.6.1.2. Conclusions on the safety specification

Having considered the data in the safety specification, the Committee agree that the safety concerns listed by the applicant are appropriate.

2.6.2. Pharmacovigilance plan

The applicant did not propose routine pharmacovigilance activities beyond adverse reactions reporting and signal detection. The Applicant did not propose additional pharmacovigilance activities.

The Committee agreed that routine pharmacovigilance is sufficient to identify and characterise the risks of the product, and to monitor the effectiveness of the risk minimisation measures.

2.6.3. Risk minimisation measures

Table 62: Routine Risk Minimisation Measures

Safety concern	Routine risk minimisation activities
Missing Information	
Use in pregnant women	Routine risk communication: SmPC section 4.6 PL section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: Recommendations on cautious use of cefepime-enmetazobactam during pregnancy are included in SmPC section 4.6. Advice in PL section 2 Other routine risk minimisation measures beyond the Product Information: Medicinal product subject to medical prescription.
Use in lactating women	Routine risk communication: SmPC section 4.6 PL section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: Advise to either discontinue breast-feeding or to discontinue/abstain from cefepime-enmetazobactam therapy in lactating females are included in SmPC section 4.6. Advice in PL section 2 Other routine risk minimisation measures beyond the Product Information: Medicinal product subject to medical prescription.

Table Part V.3: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Use in pregnant women	Routine risk minimisation measures: *Recommendations on cautious use of cefepime-enmetazobactam during pregnancy are included in SmPC section 4.6.* *Advice in PL section 2* Additional risk minimisation measures: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None
Use in lactating women	Routine risk minimisation measures: *Advise to either discontinue breast-feeding or to discontinue/abstain from cefepime-enmetazobactam therapy in lactating females are included in SmPC section 4.6.* *Advice in PL section 2* Additional risk minimisation measures: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None

The Committee considered the proposed risk minimisation measures sufficient to minimise the risks of the product in the proposed indications.

2.6.4. Conclusion

The CHMP considers that the risk management plan version 1.3 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR

cycle with the international birth date (IBD) for cefepime. The IBD is 29.06.1993. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.8. Product information

2.8.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Exblifep (cefepime / enmetazobactam) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Exblifep is proposed by the applicant to be indicated for the treatment of the following infections in adults:

Exblifep is indicated for the treatment of the following infections in adults (see sections 4.4 and 5.1):

- Complicated urinary tract infections (cUTI), including pyelonephritis

- Hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)

Treatment of patients with bacteraemia that occurs in association with, or is suspected to be associated with, any of the infections listed above.

Exblifep is also indicated for the treatment of infections due to aerobic Gram-negative organisms in adults with limited treatment options (see sections 4.2, 4.4 and 5.1)

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

3.1.2. Available therapies and unmet medical need

Complicated urinary tract infections (cUTI)/acute pyelonephritis and HAP/VAP is in general treated with empirical antibacterial therapy based on the knowledge of the main causative pathogens and their likelihood of carrying resistance mechanisms. The selection of antibacterial agents is further guided by results of pathogen identification and susceptibility testing.

Beta-lactam antibacterial agents including 3rd- and 4th-generation cephalosporins are commonly used as empirical treatment. However, treatment has become increasingly more challenging because of the rising rates of antimicrobial resistance among pathogens causing these infections including pathogens harbouring ESBLs. Treatment options for infections caused by ESBL-producing pathogens generally require the use of carbapenems. Treatment options for infections caused by carbapenem-producing pathogens are even more limited although there have been some additions to the treatment armamentarium during the recent years including new BL/BLI combinations such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam and cefiderocol. Despite this, there is still a need for additional antibacterial agents addressing infections caused by resistant pathogens including resistance to cephalosporins and carbapenems.

3.1.3. Main clinical studies

The clinical development programme for cefepime-enmetazobactam relies partly on previous findings of safety and efficacy of cefepime when used alone. Cefepime, a 4th-generation cephalosporin, was first authorised in Sweden in 1993 (Maxipime). Generics of cefepime is, or has been, marketed by different MAHs in the EU. The indications for cefepime when used alone includes (among other indications) treatment of complicated urinary tract infections, including pyelonephritis, treatment of pneumonia (without a restriction to either community acquired pneumonia or hospital acquired pneumonia, including ventilator associated pneumonia) and bacteraemia when associated with these types of infections.

It should be noted that phase 3 studies evaluating a beta-lactam (BL) at its approved dose combined with a new beta-lactamase inhibitor (BLI) are generally not designed to provide stand-alone efficacy data to support the dose regimen for the BLI, which is acceptable by precedent and by CHMP guidelines. Therefore, the PK/PD analyses incorporating the non-clinical PK/PD data and patient PK data are pivotal to support the efficacy and dose regimen of the BLI for the proposed indications.

Study AT-301 was a phase 3, randomised, double-blind, multicentre study of cefepime-enmetazobactam compared with piperacillin-tazobactam in hospitalised adults with cUTI/acute pyelonephritis to provide comparative safety and efficacy data in cUTI/acute pyelonephritis and PK-data from infected patients to update the PopPK model for PTA analyses. Patients were randomised to receive 2 g/0.5 g cefepime/enmetazobactam or 4 g/0.5 g piperacillin/tazobactam q8h. This is the highest approved dose for cefepime when the product is used alone. Both combinations were administered using 2 h infusions. The primary efficacy endpoint was the composite of microbiological eradication and clinical response outcomes at TOC in the m-MITT population.

Study AT-103 was a phase 1 study evaluating the concentrations of cefepime-enmetazobactam in plasma and epithelial lining fluid (ELF) in healthy volunteers to support treatment of lung infections.

3.2. Favourable effects

The efficacy of enmetazobactam to protect cefepime from hydrolysis by relevant beta-lactamases, was demonstrated in pre-clinical models, including the neutropenic murine thigh infection model. Nine clinical isolates of cefepime-resistant, ESBL-producing *K. pneumoniae* were evaluated in this model to derive pharmacodynamic targets (PDTs) for enmetazobactam. For the 75th percentile, 44% *f*T >2 µg/ml enmetazobactam were identified for a bioburden reduction of 1-$\log_{10}$ CFU/g. From these findings, an enmetazobactam PDT of 45% *f*T>CT was selected. Historically defined PDTs for were used for cefepime: 60% *f*T >MIC, which has been associated with maximal bacterial killing by cephalosporins; and 68% *f*T >MIC, which had been associated with higher odds of survival for patients with gram-negative bloodstream infections. In simulations of probability of target attainment (PTA),

the joint PTA was satisfactorily above (or very close to) 90% using a joint target of 45% fT>CT=2 µg/ml for enmetazobactam and 60% fT>MIC or 68% fT>MIC for cefepime up to an MIC of 8 µg/ml for all renal function categories included in the analysis except for those with augmented renal clearance (eGFR ≥150 ml/min). The Applicant has provided simulations to support that increasing the infusion duration to 4 h for patients with HAP/VAP (taking the biodistribution coefficients to ELF into account) and augmented renal clearance optimises PTA while keeping the same daily dose (no concerns with regards to safety). This proposed dosing is supported.

In Study AT-301, the composite of microbiological eradication and clinical cure at TOC in the m-MITT population was demonstrated in a higher proportion of subjects in the cefepime-enmetazobactam group compared with the piperacillin-tazobactam group (273/345 [79.1%] vs 196/333 [58.9%]), with a treatment difference of 21.2% (95% CI: 14.3%, 27.9%).

Non-inferiority as well as superiority of cefepime-enmetazobactam compared with piperacillin-tazobactam was met for the primary endpoint. The results in favour of the cefepime-enmetazobactam group was mainly driven by a more favourable microbiological response rate. At TOC, a favourable clinical response rate was noted for 319/345 (93.6%) and 296/333 (88.9%) for the cefepime-enmetazobactam and piperacillin-tazobactam groups, respectively, with a treatment difference of 3.5% (95% CI: -1.0. 8.0), whereas a favourable microbiological response rate was noted for 286/345 (82.9%) and 216/333 (64.9%) for the respective treatment groups, with a treatment difference of 19.0% (95% CI: 12.3, 25.4) in favour of cefepime-enmetazobactam.

As mentioned above, cefepime-enmetazobactam demonstrated superior efficacy compared with piperacillin-tazobactam. According to CHMP expectations, it is required that patients with baseline pathogens that are resistant to the comparative regimen should be removed from the primary analysis population before unblinding the database to treatment assignment. In the initial analyses, the applicant used former CLSI (an American organisation setting clinical breakpoints) MIC criteria for this purpose, which differ several dilution steps compared with current EUCAST (setting clinical breakpoints in the EU) breakpoints. Thus, the m-MITT population included pathogens resistant to piperacillin-tazobactam considering EUCAST interpretive criteria (>8 µg/mL for Enterobacterales and >16 µg/mL for *P. aeruginosa*). The applicant was asked to remove subjects from the m-MITT population based on EUCAST criteria and provide a complete set of updated efficacy analyses. Importantly, these analyses confirm that cefepime-enmetazobactam was non-inferior compared to piperacillin-tazobactam and also met the criteria for superiority, as originally observed in the initial m-MITT population.

In study AT-103 it was demonstrated that cefepime and enmetazobactam distributed to ELF to a similar extent. The exposure in this compartment was as expected lower than that in plasma. The ELF biodistribution coefficient increased during the observation period of 2 to 8 h for both substances. The biodistribution coefficients over the entire dosage interval for the two substances were 47% for cefepime and 46% for enmetazobactam.

3.3. Uncertainties and limitations about favourable effects

As this application relies on a clinical programme that was not designed to isolate the effects of enmetazobactam, the PK/PD package, including *in vitro* data, determination of non-clinical pharmacokinetic/pharmacodynamic targets and PTA simulations using clinical PK data, is pivotal to the application.

Overall, the PK/PD and clinical programmes are considered acceptable to support the proposed indications which are already approved for cefepime when used alone. However, the current thinking of the CHMP is that BL-BLI combinations not addressing carbapenem-resistant pathogens are non-eligible for a pathogen-specific indication in patients with limited treatment options. The *in vitro* data suggests

that cefepime-enmetazobactam has the main utility in infections caused by ESBL-producing Enterobacterales and could therefore constitute a carbapenem-sparing alternative. This is welcomed but not sufficient to grant a pathogen-specific indication in patients with limited treatment options.

3.4. Unfavourable effects

A total of 516 subjects received at least one dose of cefepime-enmetazobactam with the final dosage. Nine subjects experienced adverse events that led to discontinuation of the study drug, but all of these discontinuations were caused by different events.

The most common adverse events were elevated liver function tests, AST, ALT, and blood bilirubin, all of which had an incidence comparable to the active control, piperacillin/tazobactam. Diarrhoea was reported by 4.1% of the subjects in Study AT-301, and one subject experienced *Clostridioides difficile* colitis. These events are known and expected events related to cephalosporin antibiotics.

Overall, Exblifep displayed a somewhat higher number of drug-related adverse events than the comparator, 19.8% vs. 14.5%, in the phase III study. Most of this difference can be attributed to headache and phlebitis, both known and relatively common adverse reactions to cefepime.

Three subjects in each treatment group experienced a fatal adverse event, none of these was however related to the study treatments.

3.5. Uncertainties and limitations about unfavourable effects

The main residual uncertainty relates to the size of the safety database, which, while sufficient for registration, is not sufficient to capture potential rare side effects of enmetazobactam.

3.6. Effects Table

Table 63: Effects Table for Exblifep.

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Dose justification	Probability of target attainment of cefepime and enmetazobactam against target pathogens at the proposed susceptibility breakpoint based on PTA simulations using a non-clinical PK/PD target and clinical PK data				**SoE:** Joint PTA above (or very close to) 90% using a joint target of 45% fT>CT=2 µg/ml for enmetazobactam and 60% fT>MIC or 68% fT>MIC for cefepime up to MIC 8 µg/ml.	Section of Clinical pharmacodynamics
Efficacy	Composite of microbiological eradication and clinical cure per subject at TOC in the Micro-ITT population	n/N %	CEF/ENM 273/345 79.1	PIP/TAZ 196/333 58.9	**SoE:** Non-inferiority and superiority demonstrated: Treatment difference 21.2% (95% CI: 14.3%, 27.9%)	Study AT-301
Distribution to ELF	ELF biodistribution coefficient for cefepime and enmetazobactam				**SoE:** Biodistribution coefficient over the entire dosage interval 47% for cefepime and 46% for enmetazobactam. **UoE:** Effects of the plasma/ELF ratio related to PTA to support the adequacy of the enmetazobactam dose for the treatment of lung infections.	Study AT-103
Unfavourable Effects						
Injection site reactions	Incidence of phlebitis	N %	14/516 2.7	1/518 0.2		Study AT-301
Headache	Incidence of headache	%	4.8	2.3		Study AT-301

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Diarrhoea	Incidence of diarrhoea	%	4.1	5.0		Study AT-301
Liver function tests	Incidence of elevated ALT	%	11.4	11.6		Study AT-301
Liver function tests	Incidence of elevated AST	%	9.1	8.9		Study AT-301
Liver function tests	Incidence of elevated bilirubin	%	5.8	3.9		Study AT-301

Abbreviations: SoE, strength of evidence; UoE, uncertainties of evidence; CEF/ENM, cefepime/enmetazobactam; PIP/TAZ, piperacillin/tazobactam

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Despite some recent approvals of antibacterial agents for multi-resistant pathogens, there is still an unmet need of antibacterial agents with an acceptable safety profile that are active against bacteria resistant to available agents. The microbiology data indicate that cefepime in combination with enmetazobactam may have its main utility in infections caused by ESBL-producing Enterobacterales and could therefore constitute a carbapenem-sparing alternative.

Because the clinical development program for a beta-lactamase inhibitor is generally not designed to isolate the effect of this, a robust PK/PD package is paramount to support the inference that enmetazobactam at the selected dose protect cefepime from hydrolysis by beta-lactamases within the inhibitory range of enmetazobactam. The ELF study supports sufficient lung penetration for activity in pneumonia.

The safety profile of cefepime/enmetazobactam appears overall favourable and generally comparable to piperacillin/tazobactam and what is known for cefepime when used alone. The events that do differ between groups, phlebitis and headache, are generally mild and related to study drug administration.

3.7.2. Balance of benefits and risks

Overall, the PK/PD package and clinical programme are considered acceptable to support the proposed indications which are already approved for cefepime when used alone. However, the current thinking of the CHMP is that BL-BLI combinations not addressing carbapenem-resistant pathogens are not eligible for a pathogen-specific indication in patients with limited treatment options.

The *in vitro* data suggests that cefepime-enmetazobactam has the main utility in infections caused by ESBL-producing Enterobacterales and could therefore constitute a carbapenem-sparing alternative. This is welcomed but not sufficient to grant a pathogen-specific indication in patients with limited treatment options. The potential minimal increase in susceptibility rates of cefepime-enmetazobactam compared with meropenem against OXA-48 producing Enterobacterales is not sufficient to justify a pathogen-specific indication.

3.8. Conclusions

The overall benefit-risk balance of Exblifep is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Exblifep is favourable in the following indications:

Exblifep is indicated for the treatment of the following infections in adults:

- Complicated urinary tract infections (cUTI), including pyelonephritis
- Hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP)

Treatment of patients with bacteraemia that occurs in association with, or is suspected to be associated with, any of the infections listed above.

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that cefepime / enmetazobactam is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

Refer to Appendix 1 on new active substance (NAS).

5. Appendix

1. CHMP assessment report on New Active Substance (NAS) dated 25 January 2024