



Public Assessment Report

Decentralised Procedure

Zevtera 500 mg powder for concentrate for solution for infusion

(Ceftobiprole medocaril sodium)

PL 32205/0003

Applicant: Basilea Medical Ltd

Zevtera 500 mg powder for concentrate for solution for infusion

PL 32205/0003

LAY SUMMARY

Zevtera 500 mg powder for concentrate for solution for infusion
(Ceftobiprole medocaril sodium)

This is a summary of the public assessment report (PAR) for Zevtera 500 mg powder for concentrate for solution for infusion. It explains how Zevtera 500 mg powder for concentrate for solution for infusion was assessed and the authorisation recommended, as well as the condition of use. It is not intended to provide practical advice on how to use Zevtera 500 mg powder for concentrate for solution for infusion.

For practical information about using Zevtera 500 mg powder for concentrate for solution for infusion, patients should read the package leaflet or contact their doctor or pharmacist.

What is Zevtera 500 mg powder for concentrate for solution for infusion and what is it used for?

Zevtera is an antibiotic medicine that contains the active substance ceftobiprole medocaril sodium. It belongs to a group of medicines called ‘cephalosporin antibiotics’.

Zevtera is used to treat adults with infections of the lungs called ‘pneumonia’. Zevtera works by killing certain bacteria, which can cause serious lung infections.

How is Zevtera 500 mg powder for concentrate for solution for infusion used?

This medicine can only be obtained with a prescription.

Zevtera will be given by a doctor or nurse. The recommended dose is 500 mg ceftobiprole every eight hours given as a drip into a vein over a period of two hours. The dose of Zevtera may need to be lowered if the patient has kidney problems.

How does Zevtera 500 mg powder for concentrate for solution for infusion work?

Ceftobiprole exerts bactericidal activity through binding to important penicillin-binding proteins (PBPs) in susceptible species. In Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), Ceftobiprole binds to PBP2a. Ceftobiprole has demonstrated *in vitro* activity against strains with divergent *mecA* homolog (*mecC* or *mecALGA251*). Ceftobiprole also binds to PBP2b in *Streptococcus pneumoniae* (penicillin-intermediate), PBP2x in *S. pneumoniae* (penicillin resistant), and to PBP5 in *Enterococcus faecalis*.

What benefit has Zevtera 500 mg powder for concentrate for solution for infusion shown during studies?

Studies have shown that Zevtera 500 mg powder for concentrate for solution for infusion is effective in treating Hospital-acquired pneumonia (HAP), excluding ventilator-associated pneumonia (VAP) and Community-acquired pneumonia (CAP).

What is the risk associated with Zevtera 500 mg powder for concentrate for solution for infusion?

The most common drug-related treatment emergent adverse reactions were nausea, infusion site reactions, vomiting, diarrhoea, and dysgeusia. There were also a few cases of anaphylactic reaction after ceftobiprole use.). For a full list of all side effects reported with Zevtera 500 mg powder for concentrate for solution for infusion, please see the package leaflet.

Zevtera 500 mg powder for concentrate for solution for infusion should not be used in people who are hypersensitive (allergic) to the active substance or to any of the excipients listed in section 6.1. of the Summary of Product Characteristics (SmPC), to the cephalosporin class of antibacterials or show immediate and severe hypersensitivity (e.g. anaphylactic reaction) to any other type of beta-lactam antibacterial agent (e.g. penicillins or carbapenems).

Why is Zevtera 500 mg powder for concentrate for solution for infusion approved?

It was concluded that the use of Zevtera 500 mg powder for concentrate for solution for infusion for the treatment Hospital-acquired pneumonia (HAP), excluding ventilator-associated pneumonia (VAP) and Community-acquired pneumonia (CAP) in adults was effective.

It was considered that the benefits of Zevtera 500 mg powder for concentrate for solution for infusion outweigh the risks and the grant of Marketing Authorisation was recommended.

What measures are being taken to ensure the safe and effective use of Zevtera 500 mg powder for concentrate for solution for infusion?

A risk management plan has been developed to ensure that Zevtera 500 mg powder for concentrate for solution for infusion is used as safely as possible. Based on this plan, safety information has been included in the SmPC and the package leaflet for Zevtera 500 mg powder for concentrate for solution for infusion, including the appropriate precautions to be followed by healthcare professionals and patients.

This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions via Yellow Card Scheme, web site: www.mhra.gov.uk/yellowcard.

The safety and efficacy of Zevtera in children aged birth to < 18 years have not yet been established. Zevtera is not recommended for use in children or adolescents below 18 years of age.

Other information about Zevtera 500 mg powder for concentrate for solution for infusion

Austria, Belgium, Denmark, Finland, France, Germany, Italy, Luxemburg, Norway, Spain, Sweden and the UK agreed to grant a Marketing Authorisation for Zevtera 500 mg powder for concentrate for solution for infusion on 20th October 2013. A Marketing Authorisation was granted in the UK on 20th November 2013.

For more information about treatment with Zevtera 500 mg powder for concentrate for solution for infusion, read the package leaflet, or contact your doctor or pharmacist.

This summary was last updated in 01-2014.

The full PAR for Zevtera 500 mg powder for concentrate for solution for infusion follows this summary.

TABLE OF CONTENTS

Module 1: Information about initial procedure	Page 6
Module 2: Summary of Product Characteristics	Page 7
Module 3: Patient Information Leaflet	Page 8
Module 4: Labelling	Page 9
Module 5: Scientific Discussion	Page 10
I. Introduction	
II. About the Product	
III. Scientific Overview and Discussion	
III.1. Quality aspects	
III.2. Non-clinical aspects	
III.3. Clinical aspects	
IV. Overall conclusion and Benefit-Risk Assessment	
Module 6	Steps taken after initial procedure
	Page 144

Module 1

Information about initial procedure

Product Name	Zevtera 500 mg powder concentrate for solution for infusion
Type of Application	Article 8.3, New Active Substance
Active Substance	Ceftobiprole medocaril sodium
Form	Powder for solution for infusion
Strength	500 mg
MA Holder	Basilea Medical Ltd. (c/o Cox Costello & Horne Limited) Langwood House 63-81 High Street Rickmansworth Hertfordshire WD3 1EQ United Kingdom
RMS	UK
CMSs	Austria, Belgium, Denmark, Finland, France, Germany, Italy, Luxemburg, Norway, Spain and Sweden
Procedure Number	UK/H/5304/001/DC
Timetable	Day 210: 20 th October 2013

Module 2

Summary of Product Characteristics

In accordance with Directive 2010/84/EU the Summaries of Product Characteristics (SmPCs) for products that are granted Marketing Authorisations at a national level are available on the MHRA website.

▼ This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. See section 4.8 of the SmPC for how to report adverse reactions.

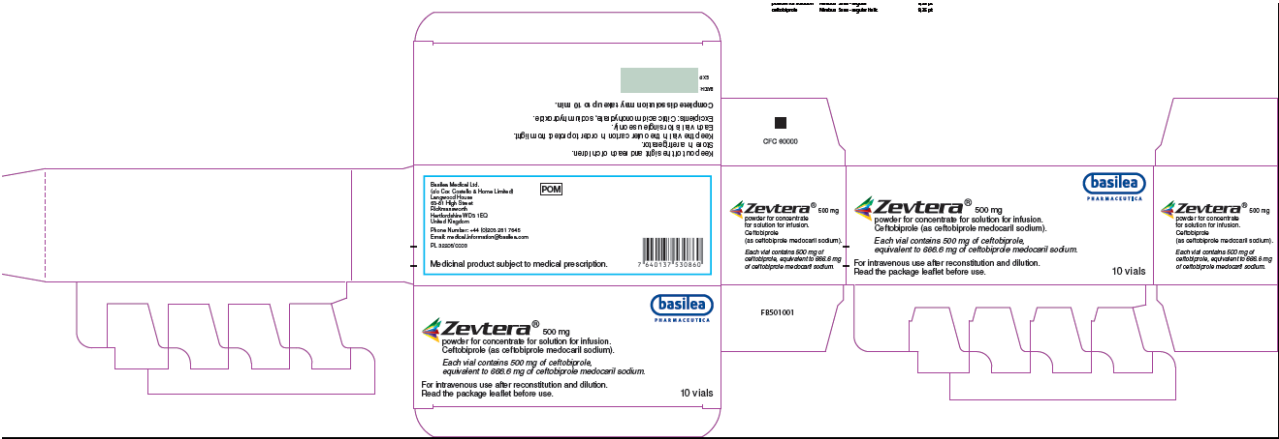
Module 3


Patient Information Leaflet

In accordance with Directive 2010/84/EU the Patient Information Leaflets (PILs) for products that are granted Marketing Authorisations at a national level are available on the MHRA website.

Module 4

Labelling



**Zevtera**[®] 500 mg

powder for concentrate for solution for infusion.
Ceftobiprole.

Each vial contains 500 mg of ceftobiprole, equivalent to 666.6 mg of ceftobiprole medocartil sodium.

POM

For IV use after reconstitution and dilution.
Reconstitution solution: 1 hour at 25°C and up to 24 hours at 2-8°C.
Complete dissolution may take up to 10 min.

Read the package leaflet before use.
Store in a refrigerator. For single use.
Keep out of the sight and reach of children. Excipients: Citric acid monohydrate, sodium hydroxide.
Keep the vial in the outer carton in order to protect from light. Medicinal product subject to medical prescription.
Basilea Medical Ltd. (c/o Cox Costello & Horne Limited) Langwood House, 63-81 High Street, Rickmansworth UK - Hertfordshire WD3 1EQ
PL 32205/0003

501201

EXP

Batch

Module 5

Scientific discussion during initial procedure

I INTRODUCTION

Based on the review of the data on quality, safety and efficacy, the Reference Member State (RMS) and Concerned Member States (CMSs) considered that the application for Zevtera 500 mg powder for concentrate for solution for infusion indicated for the treatment of the following infections in adults, could be approved.

- Hospital-acquired pneumonia (HAP), excluding ventilator-associated pneumonia (VAP)
- Community-acquired pneumonia (CAP)

This decentralised application concerns a full application in accordance with Article 8(3) of Directive 2001/83/EC, as amended, for the new active substance ceftobiprole medocaryl sodium under the trade name Zevtera 500 mg Powder for concentrate for solution for infusion.

Ceftobiprole is a cephalosporin for intravenous administration after reconstitution and dilution. The medocaryl sodium pro-drug form is used due to its better solubility; the active component is ceftobiprole. Like other β -lactam antibiotics, ceftobiprole has a bactericidal mode of action related to tight binding to penicillin binding proteins (PBPs); the membrane associated bacterial enzymes involved in the last steps of peptidoglycan (cell wall) biosynthesis. It is indicated for the treatment of nosocomial pneumonia (NP) and community acquired bacterial pneumonia (CABP) in adults. The usual dose is 500 mg every 8 hours via a slow infusion over 2 hours. Treatment is continued for 4-14 days depending on disease severity and patient response. Dispensing of the product is subject to a medical prescription which may be renewed.

A Marketing Authorisation Application for the treatment of complicated skin and soft tissue infections (cSSTI) was previously submitted by Janssen Cilag via the centralized route (EMA/H/C/0883) in 2007. A licence was refused on the basis of GCP issues for the cSSTI studies conducted. Following a negative opinion of the CHMP and FDA, the product was withdrawn from Canada, Switzerland, Ukraine, Russia, Azerbaijan and Hong Kong (for the treatment of cSSTI).

With UK as the RMS in this decentralised Procedure (UK/H/5304/001/DC), Basilea Medical Ltd applied for Marketing Authorisations for Zevtera 500 mg powder for concentrate for solution for infusion in Austria, Belgium, Denmark, Finland, France, Germany, Italy, Luxemburg, Norway, Spain and Sweden.

A Paediatric Investigation Plan (PIP) was provided with this application.

Scientific advice was given by the EMA (2006, 2007), UK (2005, 2006 and 2011), Sweden (2005, 2006 and 2012) and Germany (2012). National scientific advice given in 2011/2012 relates to indications applied for in the decentralised procedure whereas previous advice was for cSSTI which is not applied for.

The RMS has been assured that acceptable standards of GMP are in place for these product types at all sites responsible for the manufacture and assembly of this product. For manufacturing sites within the Community, the RMS has accepted copies of current manufacturer authorisations issued by inspection services of the competent authorities as certification that acceptable standards of GMP are in place at those sites.

The majority of the pivotal toxicology and safety pharmacology studies conducted by the applicant were reported to be GLP compliant. The safety studies that were not conducted to GLP were conducted to an appropriate scientific standard.

For this current application, according to the clinical study reports, the studies were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and applicable regulatory requirements. Known instances of non-conformance were documented.

Safety pharmacology effects of the β -lactam, ceftobiprole medocartil (BAL5788) and its active metabolite ceftobiprole (BAL9141) were studied in various *in vitro* and *in vivo* studies focussing on potential undesirable pharmacodynamic effects on physiological functions of the cardiovascular, central nervous and respiratory systems.

A series of *in vitro* and *in vivo* test systems were utilised in order to evaluate the pharmacokinetics of BAL5788. *In vitro* studies utilized subcellular liver fractions from humans and hepatocytes from mice, rats, dogs, marmosets, cynomolgus monkeys and humans, as well as blood and plasma samples from mice, rats, marmosets, cynomolgus monkeys and humans. *In vivo* studies were conducted in mice, rats, rabbits, Beagle dogs, marmosets (*Callithrix jacchus*) and Cynomolgus monkeys.

The majority of the pharmacokinetic and toxicokinetic data were generated following intravenous (i.v.) administration (bolus or infusion) with the proposed clinical formulation.

The toxicology of BAL5788 (ceftobiprole medocartil) was characterized in repeated-dose toxicity, genotoxicity and reproductive (fertility, developmental toxicity, pre- and post-natal) toxicity studies, and in studies assessing local tolerability, potential antigenic and hemolytic effects, phototoxicity and nephrotoxicity. Additional information was obtained from several bridging studies with formulations manufactured via different synthesis routes. Since BAL5788 undergoes spontaneous conversion to the active compound BAL9141 (ceftobiprole) after reconstitution, several studies compared local and systemic toxicity after the administration of “fresh” (i.e. immediately prepared) and “aged” (i.e. stored more than 24 hours after reconstitution) solutions, and also addressed the local tolerance of accidental subcutaneous, intramuscular, paravenous or intra-arterial administration. Juvenile toxicity was investigated in neonatal and juvenile rats. *In vivo* studies were conducted in mice, rats, rabbits, Beagle dogs, marmosets (*Callithrix jacchus*) and Cynomolgus monkeys.

Twenty-one clinical pharmacology studies and one exploratory study in healthy subjects and patients were conducted. In addition, pharmacokinetic and pharmacokinetic/pharmacodynamic assessments were conducted in subjects with cSSTI in one Phase 2 study and in two Phase 3 studies (subjects with nosocomial pneumonia and in subjects with CAP). Six pharmacokinetic studies in healthy subjects and six studies in special populations (renally impaired, ESRD, gender, obese, ICU patients and paediatric subjects) were also conducted.

All member states agreed to grant a licence for the above product at the end of the procedure (Day 210 – 20th October 2013). After a subsequent national phase, the UK granted a licence for this product on 20th November 2013 (PL 32205/0003).

II. ABOUT THE PRODUCT

Name of the product in the Reference Member State	Zevtera 500 mg powder for concentrate for solution for infusion
Name(s) of the active substance(s) (USAN)	Ceftobiprole medocaril sodium
Pharmacotherapeutic classification (ATC code)	J01DI01 (other cephalosporins)
Pharmaceutical form and strength(s)	Powder for concentrate for solution for infusion, 500 mg
Reference numbers for the Decentralised Procedure	UK/H/5304/001/DC
Reference Member State	United Kingdom
Concerned Member States	Austria, Belgium, Denmark, Finland, France, Germany, Italy, Luxemburg, Norway, Spain and Sweden.
Marketing Authorisation Number(s)	PL 32205/0003
Name and address of the authorisation holder	Basilea Medical Ltd. (c/o Cox Costello & Horne Limited) Langwood House 63-81 High Street Rickmansworth Hertfordshire WD3 1EQ United Kingdom

III SCIENTIFIC OVERVIEW AND DISCUSSION

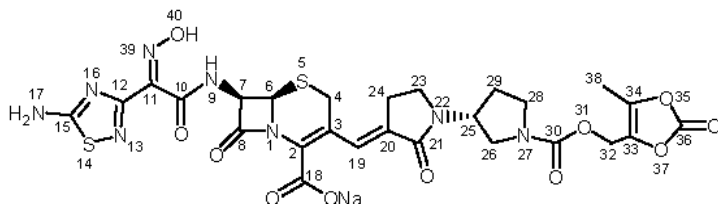
III.1 QUALITY ASPECTS

DRUG SUBSTANCE

rINN: Ceftobiprole medocartil sodium

Chemical Names: (6R,7R)-7-[[[(2Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(hydroxyimino)acetyl]amino]-3-[(E)-[(3'R)-1'-[[[(5-methyl-2-oxo-1,3-dioxol-4-yl)methoxy]carbonyl]-2-oxo[1,3'-bipyrrolidin]-3-ylidene]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt

Structure:



Molecular formula: $C_{26}H_{25}N_8NaO_{11}S_2$

Molecular weight: 712.64 g/mol

Physical form: white to yellowish or slightly brownish amorphous powder with lumps

Solubility (of prodrug) in water: freely soluble (>250 mg/ml at 25 °C).

Solubility (of prodrug) in other solvents: freely soluble in formamide, DMA, n-methyl pyrrolidine, pyridine, DMSO. Sparingly soluble (23.64 mg/ml) in propylene glycol. Insoluble in 2-propanone, tert butyl methyl ether, ethyl formate, tetrahydrofuran, acetonitrile.

Full details of ceftobiprole medocartil sodium synthesis, control of materials and process validation are provided in the dossier.

Satisfactory controls of materials are in place. The routes of synthesis are adequately described and characterised, and the structure of ceftobiprole medocartil sodium has been confirmed by analytical evidence by both the active pharmaceutical ingredient manufacturers.

The proposed drug substance specification is satisfactory.

Stability studies have been performed with the drug substance. No significant changes in any parameters were observed and the proposed retest period of 48 months is justified and a storage statement in line with CHMP guidelines has been proposed.

DRUG PRODUCT

Other Ingredients

Other ingredients consist of the pharmaceutical excipients citric acid monohydrate and sodium hydroxide.

All excipients comply with the relevant European Pharmacopoeia monographs. Satisfactory Certificates of Analysis have been provided for these excipients.

The above excipients do not contain materials of animal or human origin. No genetically modified organisms (GMO) have been used in the preparation of this product.

Pharmaceutical Development

Details of the pharmaceutical development of the medicinal product have been supplied and are satisfactory.

Satisfactory product development data were submitted.

Manufacture

Satisfactory batch formula has been provided for the manufacture of the product, along with an appropriate account of the manufacturing process. The manufacturing process has been validated and has shown satisfactory results. Process validation data on commercial batches have been provided. The results are satisfactory.

Finished Product Specifications

The finished product specifications are satisfactory. Test methods have been described and adequately validated. Batch data have been provided and comply with the release specifications. Certificates of Analysis have been provided for any working standards used.

Container Closure System

The finished product is supplied in clear type I glass vials fitted with a grey bromobutyl elastomeric closure and an aluminium seal with a blue plastic flip-off cap. The pack size is 10 vials.

Specifications and Certificates of Analysis for the primary packaging material have been provided. These are satisfactory. All primary packaging is controlled to European Pharmacopoeia standards and complies with relevant guidelines.

Stability

Finished product stability studies have been conducted in accordance with current guidelines, using batches of the finished product stored in the packaging proposed for marketing.

Based on the results, a shelf-life of 3 years for the powder vial with storage conditions 'Store in a refrigerator (2°C–8°C)' and 'Keep the vial in the outer carton in order to protect from light' is set.

After reconstitution

Chemical, and physical in-use stability of the reconstituted solution (50 mg/mL) have been demonstrated for 1 hour at 25°C and up to 24 hours at 2°C–8°C.

After dilution

Chemical and physical in-use stability data support the total times for reconstitution and infusion (2.67 mg/mL) described in the table below:

Total time by which reconstitution and infusion (including a 2-hour period of infusion) must be completed

Infusion solution diluent	Infusion solutions stored at 25°C		Infusion solutions stored at 2°C–8°C (refrigerator) Protected from light
	Protected from light	NOT protected from light	
Sodium chloride 9 mg/mL (0.9%) solution for injection	24 hours	8 hours	96 hours
Dextrose 50 mg/mL (5%) solution for injection	12 hours	8 hours	96 hours
Lactated Ringer's solution for injection	24 hours	8 hours	Do not refrigerate

From a microbiological point of view, unless the method of reconstitution/dilution precludes the risk of microbiological contamination, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user.

The reconstituted and infusion solutions should not be frozen or exposed to direct sunlight.

If the infusion solution is stored in a refrigerator, it should be equilibrated to room temperature prior to administration. The infusion solution does not need to be protected from light during administration.

The infusion solution should be prepared and used as defined in section 6.6 of the SmPC.

The shelf-life and storage conditions are satisfactory.

Summary of Product Characteristics (SmPC), Patient Information Leaflet (PIL) and Labelling

The SmPC, PIL and labelling are pharmaceutically satisfactory.

A package leaflet has been submitted to the MHRA together with results of consultations with target patient groups ("user testing"), in accordance with Article 59 of Council Directive 2001/83/EC. The results indicate that the package leaflet is well-structured and organised, easy to understand and written in a comprehensive manner. The test shows that the patients/users are able to act upon the information that the package leaflet contains.

Marketing Authorisation Application (MAA) Form

The MAA form is pharmaceutically satisfactory.

Expert Report

A quality overall summary has been written by an appropriately qualified person and is a suitable summary of the pharmaceutical aspects of the dossier.

Conclusion

There are no objections to the approval of this product from a pharmaceutical point of view.

III.2 NON-CLINICAL ASPECTS

PHARMACOLOGY

Safety pharmacology

Safety pharmacology effects of the β -lactam, ceftobiprole medocaril (BAL5788) and its active metabolite ceftobiprole (BAL9141) were studied in various *in vitro* and *in vivo* studies focussing

on potential undesirable pharmacodynamic effects on physiological functions of the cardiovascular, central nervous and respiratory systems.

The pivotal toxicology and the remainder of the safety pharmacology studies conducted by the applicant were reported to be GLP compliant. The safety studies that were not conducted to GLP were conducted to an appropriate scientific standard.

Effects on the cardiovascular system

In vitro studies

The effects of BAL9141 on the membrane currents were assessed in human embryonic kidney (HEK293) cells expressing the human ether-à-go-go-related gene (hERG). The effect of BAL9141 (5 µM, the highest concentration possible under the conditions of the assay due to the limited solubility of BAL9141) was compared to the vehicle (0.2% DMSO) and the reference substance E-4031 (100 nM).

BAL9141 at 5 µM decreased hERG tail current by approximately 10% relative to a 15% reduction seen in cells treated with vehicle alone. In contrast, inhibition was 96.7% with the positive reference substance E-4031 at 100 nM.

In conclusion, BAL9141 at 5 µM did not produce a statistically significant inhibition of hERG tail current in stably transfected HEK293 cells.

In Vivo Studies

Effects of BAL5788 on Blood Pressure and Heart Rate: The effect of BAL5788 on cardiovascular parameters was assessed in conscious spontaneously hypertensive rats and normotensive marmosets (*Callithrix jacchus*).

In rats BAL5788 was administered as a single i.v. bolus injection at doses of either 100 mg/kg or of 30 and 100 mg/kg (re. BAL9141) or the reference compound ceftazidime (FortamTM) at 100 mg/kg. Mean arterial pressure (MAP) and heart rate (HR) were monitored up to 24 h post-dose. No effect on MAP was noted at 30 mg/kg. At 100 mg/kg, a slight, persistent increase in MAP was observed, which was delayed in onset. No change in heart rate (HR) was observed at any dose. The reference compound ceftazidime had no effect on MAP or HR.

Normotensive marmosets (received single slow i.v. bolus injections of BAL5788 at 35 or 100 mg/kg (re. BAL9141) or 1 h infusions of 50 or 100 mg/kg, and MAP and HR were monitored for 24 h after drug administration. After bolus administration a slight, persistent increase in MAP and HR was noted at 100 mg/kg, but not at 35 mg/kg. No effects on HR were observed following i.v. infusion of 50 or 100 mg/kg of BAL5788. Whereas a minimal increase in MAP was seen at the dose of 100 mg/kg, the effect was much less prominent than after i.v. bolus administration and occurred with a delayed onset of approximately 12 h. No effect was noted at 50 mg/kg. Mean plasma concentrations of BAL9141 after 1 h infusion of 100 mg/kg were 54.2 and 3.50 µg/mL at 2 and 6 h post-dose, respectively. It was concluded that increased MAP and HR were related to high C_{max} values after bolus administration.

Effects of BAL5788 on Electrocardiogram, Blood Pressure and Heart Rate: Conscious beagle dogs (n=1/sex) were given BAL5788 at single i.v. doses of 50 or 100 mg/kg (re. BAL9141) via infusion (4 h). The interval between dosings was approximately 1 week. Electrocardiograms (ECGs), mean blood pressure (BP) and HR were recorded prior to infusion, at 20 minute intervals during the 4-h infusion, and at 1 and 2 h post-infusion. No drug-related effects on cardiovascular parameters were noted at 50 or 100 mg/kg.

Exposures to both BAL9141 and the pro-drug BAL5788 increased in a dose-related manner. At the end of the 4-h infusion, average maximum plasma concentration (C_{\max}) values for BAL9141 were 45.0 $\mu\text{g/mL}$ and 89.5 $\mu\text{g/mL}$ at 50 and 100 mg/kg, respectively. As BAL5788 undergoes a relatively slow cleavage in dogs compared to other test species and humans, animals were also exposed to relevant levels of the pro-drug, with average BAL5788 C_{\max} values at the end of the 4-h infusion of 30.1 and 46.0 $\mu\text{g/mL}$ at 50 and 100 mg/kg, respectively.

Effects on the respiratory system

The effects of BAL5788 on airway resistance (R_L) and dynamic lung compliance (C_{dyn}) were investigated in anaesthetised, ventilated male rats the vehicle (reconstitution buffer) or BAL5788 at 125, 250 and 500 mg/kg (re. BAL9141) via an i.v. infusion for 4 h. Acetylcholine (100 $\mu\text{g/kg}$) was used as a positive reference substance. No statistically significant effects on R_L and C_{dyn} were seen at any dose. Acetylcholine caused a substantial increase in R_L and C_{dyn} , which was not enhanced by BAL5788 at any dose.

In conclusion, BAL5788 administered intravenously over a 4-h infusion period at doses of 125, 250 and 500 mg/kg did not affect either R_L or C_{dyn} when compared to vehicle-treated animals.

Effects on the central nervous systems

Several studies were performed in mice in order to evaluate the potential of BAL9141 or BAL5788 to cause convulsant activity and affect behaviour.

In the first study, male mice received single intracerebroventricular (i.c.v.) injections of BAL9141 (microsuspension) at doses of 0.3 to 30 μg . For comparative purposes, imipenem (0.3-30 μg) and meropenem (10 - 1000 μg) were also evaluated. The ED_{50} for the occurrence of convulsions calculated after a 30-minute observation period was 2.55 μg for BAL9141, 3.16 μg for imipenem, and 548.42 μg for meropenem.

In a second study, single slow i.v. bolus injections of BAL5788 (62.5, 125, 250, 500, or 1000 mg/kg re. BAL9141) were given to male mice. At the maximum dose of 1000 mg/kg i.v., clonic and tonic convulsions and mortality were observed within approximately 30 minutes after dosing. At 250 and 500 mg/kg, tremors (tonic and clonic convulsions and mortality occurred with a delay in onset; approximately 0.75 to 4.75 h post-dose. Furthermore, at ≥ 250 mg/kg, vocalization, Straub tail and twitches were observed. Doses ≤ 125 mg/kg were well tolerated and without effect.

In a separate study, BAL5788 was administered to mice as a single, slow i.v. bolus dose at 250 or 500 mg/kg (re. BAL9141) to investigate behavioural effects versus plasma and brain tissue concentrations and renal toxicity. Mice were sacrificed at different time intervals to evaluate plasma and brain concentrations of BAL9141 and to monitor the development of nephrotoxicity upon assessment of blood urea nitrogen (BUN) and creatinine levels and kidney histopathology. Impaired renal function was confirmed by high BUN levels at 250 and 500 mg/kg in all animals evaluated for nephrotoxicity, and by an increase in plasma creatinine levels in a few animals. Dose-related nephrotoxicity (renal tubular dilation and degeneration and congestion, related to drug precipitation in distal tubules and collecting tubules) was noted microscopically as early as 30 minutes post-dose at 250 and 500 mg/kg. Overall, nephrotoxicity was slight to moderate at 250 mg/kg, and moderate to marked at 500 mg/kg. Based on the rapid manifestation of significant kidney toxicity at 250 and 500 mg/kg in mice, it was suggested that marked nephrotoxicity leads to reduced renal drug clearance and, thus, accounts for the observed sustained elevated plasma and brain levels of BAL9141 that were considered to result in delayed convulsions. Brain concentrations at 250 mg/kg were below 3 $\mu\text{g/head}$, corresponding to the approximate ED_{50} after i.c.v. administration and resulting in only one animal with convulsions. At 500 mg/kg concentrations were above the ED_{50} and resulted in a high incidence of convulsions.

In conclusion, results from these studies in mice suggest that the convulsive potential of BAL9141 following i.c.v. administration was comparable to that of imipenem. Delayed convulsions after i.v. administration of high doses of BAL5788 were attributed to high systemic and brain exposures which may be due to reduced renal excretion.

Overall conclusions on pharmacology

In the hERG assay, the maximum concentration tested was 5 μ M (approximately 2.7 μ g/ml), which is considerably lower than the observed clinical C_{max} of 33 μ g/ml. The Applicant has indicated that the maximum concentration was limited by the solubility of BAL9141 and that a number of alternative solvents were investigated but that these were not compatible with the hERG assay. No effects on QT interval were observed in the dog where the C_{max} for the active component, BAL9141 was 2.7-fold higher than that observed clinically. It is noted that the number of animals used in this study was fairly low and that the maximum dose tested should in theory have been higher. However, no effects on QT interval were observed during a thorough QT/QTc study at up to 1000 mg (plasma C_{max} approximately 46 μ g/mL). Given the short duration of treatment and that cephalosporins are not associated with prolongation of QT interval, the data provided are deemed sufficient to support the cardiovascular safety of ceftobiprole medocartil.

In the rat and marmoset, slight increases in blood pressure and heart rate were observed following an intravenous bolus injection of BAL5788 at 100 mg/kg (where C_{max} in the marmoset was slightly higher than that observed clinically), but not at 35 mg/kg. However, the observed effects were less prominent when administered via intravenous infusion. No effects on respiration were observed in the species evaluated (rat).

In the mouse, intracerebroventricular administration of BAL9141, caused convulsions and the ED_{50} was comparable to that of imipenem. Following intravenous administration of BAL5788, convulsions, tremors, behavioural changes, mortality and nephrotoxicity were observed at ≥ 250 mg/kg. At the no-effect level of 125 mg/kg, estimated exposures were similar to those observed clinically. A number of cephalosporins have been associated with epileptic seizures in man and convulsions have been noted during clinical use; hence, appropriate warnings have been included in Section 4.4 of the SmPC, which is acceptable.

Pharmacokinetics

A series of *in vitro* and *in vivo* test systems were utilised in order to evaluate the pharmacokinetics of BAL5788. *In vitro* studies utilized subcellular liver fractions from humans and hepatocytes from mice, rats, dogs, marmosets, cynomolgus monkeys and humans, as well as blood and plasma samples from mice, rats, marmosets, cynomolgus monkeys and humans. *In vivo* studies were conducted in *In vivo* studies were conducted in mice, rats, rabbits, Beagle dogs, marmosets (*Callithrix jacchus*) and Cynomolgus monkeys.

The majority of the pharmacokinetic and toxicokinetic data were generated following intravenous (i.v.) administration (bolus or infusion) with the proposed clinical formulation.

Methods of analysis

A specific and sensitive assay based on gradient reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection was developed and validated for simultaneous quantification of BAL9141 and BAL5788 in plasma (mouse, rat, marmoset, dog, cynomolgus monkey and human) and urine (marmoset and human). The calibration ranges were 0.1-100 μ g/mL for BAL9141 and 0.25–100 μ g/mL for BAL5788 in plasma, and 2-2500 μ g/mL for BAL9141 in urine. In plasma, the quantification limits were 0.1 μ g/mL and 0.25 μ g/mL for BAL9141 and BAL5788, respectively, using a 20 μ L aliquot. Inter-assay precision and accuracy were usually below 5% for either compound. In urine, the quantification limit of BAL9141 was 2 μ g/mL; using a 20 μ L urine aliquot. The main urinary metabolite, BAL1029, was determined semi-

quantitatively at a concentration range of 5-100 µg/mL. Average inter-assay precision for the determination of BAL9141 in urine was 4.6%.

For the more recent toxicokinetic studies (4 and 13-week toxicity studies in rats and marmosets, fertility studies in rats, teratogenicity in rats and cynomolgus monkeys and 2-week toxicity in dogs) validated HPLC/UV methods were also used. The lower limits of quantification (LLOQ) for dog, rat, marmoset and cynomolgus monkey plasma were 0.1 µg/mL and 0.5 µg/mL for BAL9141 or BAL5788, respectively. The LLOQ values in dog, rat and marmoset urine were 5 µg/mL and 100 µg/mL for BAL9141 or BAL5788, respectively. More recently, a liquid chromatographic-triple quadrupole mass spectrometry (LC-MS/MS) assay was developed and validated to quantify the concentration of BAL9141 and BAL5788 in dog plasma samples from the 13-week dog toxicity study.

The pro-drug, BAL5788 undergoes enzymatic degradation by esterases, the rate of which is dependent on temperature, pH, and the presence of esterase inhibitors used. In several species, including man, conversion of BAL5788 to BAL9141 occurs rapidly at 37 °C, with $t_{1/2}$ below 1 minute. Anticoagulation with ethylenediaminetetra-acetic acid (EDTA) inhibited enzymatic degradation and acidification of plasma samples with citric acid reduced the chemical degradation. The applicant has indicated that BAL5788 should be protected from sunlight.

The stability of BAL9141 and BAL5788 in acidified plasma using EDTA as anticoagulant and as esterase inhibitor was limited to about four and two months at –80 °C, respectively, and to about three months for BAL9141 in acidified urine samples. No relevant change of analyte concentration was observed after three freeze/thaw cycles for spiked plasma and urine samples. At room temperature, both the pro-drug and active principle were sufficiently stable in plasma for about 3 hours.

Due to the rapid conversion of BAL5788 to BAL9141 in plasma and other media (most likely caused by esterases), the applicant has taken the relevant precautions to preserve the integrity of the samples collected.

The analytical methods used represent well-established procedures and are considered to be acceptable.

Absorption

The exposure to ceftobiprole was investigated *in vitro* and after single and repeated administration to mice, rats, rabbits, dogs and monkeys *in vivo*. These studies demonstrate that the oral bioavailability of ceftobiprole was low. Absorption is not a crucial factor as the medicinal product is to be administered by the intravenous route. All non-clinical studies in support of the proposed clinical indication have used i.v. administration via a bolus injection or short infusion.

Non-clinical studies that have utilised the oral (p.o.), intramuscular (i.m.), subcutaneous (s.c) and intraperitoneal (i.p.) routes are considered non-pivotal and supplementary in terms of their support of the proposed clinical indication.

Table 2: Pharmacokinetics of BAL9141 analyzed in plasma after single dose administration of BAL9141

Study No.	Species (strain)	Gender (n)	Vehicle	Dose (mg/kg)	Route	C _{max} (µg/ml)	T _{max} (h)	T _{1/2} (h)	AUC _∞ (µg×h/ml)	Cl (ml/min/kg)	Vz/F (l/kg)	Rel bioav (% vs IV)
B-166'154	Mouse (Moro)	M (2)	DMSO and 0.9% NaCl; 25/75 v/v	10	IV	33	0.08	∞=0.29 Z=0.48	14 (0-∞)	12	0.50	
		M (2)	DMSO and 0.9% NaCl; 25/75 v/v.	10 ^A		39	0.08	∞=N.C Z=0.27	17	9.9	0.23	
BAP00057	Mouse (NMRI)	F (2)	Oil microsuspension	50	IM	49	0.08	2.5	41			110
			Water microsuspension	50	IM	12	0.75	1.9	47			130
BAP00071	Rat (Wistar)	M (2)	DMSO/0.9 NaCl (10/90 v/v)	10	IV	41	0.08	0.61	20	8.2	0.43	
		F (2)	With probenecid (50 mg/kg)			41	0.08	0.57	24	7.1	0.35	
BAP00078	Rat (RoRo)	M (4)	DMSO/0.9% NaCl; 10/90 v/v	7.3	IV	23	0.07	0.5	19	8.8	0.38	
BAP00057	Rat (Wistar)	M (2)	Microsuspension	50 ^B	IM	19	1/0.08 ^C	21	67 ^B			105
					IM	0.76	25/0.5 ^C	28	9.7 ^B			15
					SC	1.0	4/0.08 ^C	68	11 ^B			17
BAP00517	Rat (wistar)	M (2)	Aqueous Gelatin (7.5%)	500 ^B	Oral	0.02	2	NC	0.09			<1
				50 ^B		BLQ	NC	NC	NC			<1
			Bioavailability enhancer	420 ^B		0.49	0.25	NC	3.5			<1
BAP00079	Rabbit (NZ-white)	F (4)	DMSO/0.9% NaCl (10/90 v/v)	3.7	IV	0.85	0.07	0.82	20	4.2	0.30	
BAP00057	Dog (beagle)	M (1)	Physiological saline (0.9%)	4.2	IM	0.21	1.5	>>2 ^D	~2.9 ^D			>70 ^D
BAP00080	Monkey (Cyno)	M (2)	DMSO/0.9% NaCl (20/80 v/v) (30 min infusion)	7.3	IV	23	0.5	1.7	60	2.8	0.41	

A-B = All plasma samples are analyzed by HPLC-UV, except A: Bioassay (B-166'154); B: LC-MS-MS (BAP00057 & BAP00517)

C = T_{max} of both animals is given individually because of large differences

D = According to the applicant, elimination of BAL9141 was, limited by the dissolution/permeation rate; hence, the t_{1/2} was > 20 h. The AUC was only calculated through 24 h and hence; the relative bioavailability may be underestimated.

BLQ = Below Level of Quantification

NC = Not Calculated

IV = Intravenous

IM = Intramuscular

SC = Subcutaneous

Table 3: Pharmacokinetics of BAL5788 analyzed in plasma after single dose administration of BAL5788

Study No.	Species (strain)	Gender (n)	Vehicle	Dose (mg/kg)	Route	C _{max} (µg/ml)	T _{max} (h)	T _{1/2} (h)	AUC _∞ (µg×h/ml)	Cl (ml/min/kg)	Vz/F (l/kg)	Rel bioav (% vs IV)
B-169'919	Mouse (moro)	M (2) F (2)	Citric acid anhydrous, H ₂ O, NaOH and NaCl	72	IV	NC	NC	NS	NC	NC		
				180		NC	NC		NC	NC		
				450		8.1	0.08		NC	NC		
				72		NC	NC		NC	NC		
				180		NC	NC		NC	NC		
				450		4.9	0.08		NC	NC		
BAP00057	Mouse (NS)	F (2)	Physiological Saline (0.9%)	10	IV	8.2	0.08	NC	NC			NC
					IM	5.0	0.08	NC	NC			NC
					SC	3.2	0.08	NC	NC			NC
1003801	Rat (Wistar)	M (2)	Citric acid buffer with H ₂ O for injection, NaOH and NaCl	200	IV	60	0.08	NC	NC	NC		
				500		58	0.08	NC	NC	NC		
BAP00067	Rat (Wistar)	M (4)	Aqueous buffer	20		9.6	0.08	0.21	ND	ND	ND	
BAP00517	Rat (Wistar)	M (2)	Aqueous Gelatin (7.5%) + Saquinavir	500 ^A	Oral	0.40	1	NC	NC			NC
		M (1)		50 ^A		0.05	1	NC	NC			NC
		M (2)		500 ^A		0.81	0.25	NC	NC			NC

A = All samples are analyzed by HPLC-UV, except A: LC-MS-MS (BAP00517)

NC = Not Calculated

ND = Not Detected

NR = Not Reported

NS = Not Specified

IV = Intravenous

IM = Intramuscular

SC = Subcutaneous

Table 4: Pharmacokinetics of BAL9141 analyzed in plasma after single dose administration of BAL5788

Study No.	Species (strain)	Gender (n)	Vehicle	Dose (mg/kg)	Route	C _{max} (µg/ml)	T _{max} (h)	T _{1/2} (h)	AUC _∞ (µg×h/ml)	Cl (ml/min/kg)	Vz/F (l/kg)	Rel bioav (% vs IV)
B-169'919	Mouse (moro)	M:2 F:2	Citric acid anhydrous, H ₂ O, NaOH and NaCl	72	IV	109	0.08	NS	53	17		
				180		285	0.08		228	9.9		
				450		1000	0.08		NR	NR		
				72		132	0.08		84	11		
				180		312	0.08		212	11		
				450		808	0.08		NR	NR		
BAP00057	Mouse (NS)	F:2	Physiological Saline (0.9%)	10	IV	15	0.08	0.30	6.4			100
					IM	13	0.25	0.20	6.1			
					SC	9.6	0.25	0.20	6.2			
BAP00057	Mouse (NMRI)	F:2	DMSO	45	IV	80	0.08	0.30	32			100
					IM	33	0.08	0.20	32			
					IP	44	0.25	0.26	29			
					SC	29	0.25	0.39	32			
BAP00295	Mouse	F:6	NS	25	SC	61	0.25	0.33 ^A	30			
1003801	Rat (Wistar)	M:2	Citric acid buffer with H ₂ O for injection, NaOH and NaCl	200	IV	473	0.08	1.0	473	5.0		
				500		1020	0.08	1.5	1088	4.9		
BAP00067	Rat (Wistar)	M:4	Aqueous buffer	20	IV	44	0.08	0.51	26	13	0.58	
BAP00071	Rat (Wistar)	M:2	20mM Na-citrate/0.63% NaCl (w/v) with probenecid (200 mg/kg)	100	IV	231	0.08	0.76	224	7.7	0.51	
		F:2				292	0.08	0.72	239	7.2	0.45	
		M:2	20mM Na-citrate/0.63% NaCl (w/v) without probenecid	100	IV	291	0.08	0.83	259	6.4	0.46	
		F:2				284	0.08	0.68	229	7.3	0.43	
1003574	Rat (Wistar)	M:3	NS infusion solution 2 hours	250	IV	139	NC	NC	NC	NC		NC
			NS infusion solution 4 hours	250		270						
BAP00057	Rat (Wistar)	M:2	Citrate buffer pH 4.5	50 ^A	IM	33	1/0.5 ^B	0.59	54			84
					SC	31	1/0.5 ^B	0.68	50			
BAP00517	Rat (Wistar)	M:2	Aqueous Gelatin (7.5%)	500 ^A	Oral	0.04	2	NC	0.45			<1
		M:1	Aqueous Gelatin (7.5%)	50 ^A		0.01	1	NC	0.28			
		M:2	+ Saquinavir (P-gp inhibitor)	500 ^A		0.50	0.5	NC	1.2			
1003455	Rabbit Himalayan	M:3	NS	160	IV	468	0.5	NC	621 ^C			
			NS + 160 mg/kg cilastatin			388	0.5	NC	498 ^C			
BAP00057	Dog (Beagle)	M:1	Physiological saline (0.9%)	3.3	IV	2.8	0.08	1.5	4.3			100
				3.8	IM	2.1	0.75	1.4	3.3			
BAP00068	Monkey cynomolgus	M:3	Aqueous buffer (reconstitution)	20	IV	146	0.08	1.3	132	2.6	0.30	
B-170'757	Monkey marmoset	M:4	Isotonic citric buffer with NaCl	20	IV	83	0.08	0.95	70	5.1	0.42	

A = All samples are analyzed by HPLC-UV, except A: LC-MS-MS (BAP00057 and BAP00517)

B = T_{max} of both animals is given individually because of large differences.

C = AUC (0.5-6h)

NC = Not Calculated

NR = Not Reported

NS = Not Specified

IV = Intravenous

IM = Intramuscular

SC = Subcutaneous

Distribution

Binding to plasma proteins *in vitro*

As the pro-drug is rapidly cleaved to produce the active principle, BAL9141, the plasma protein binding of BAL5788 was not determined, as this bears no relevance for the *in vivo* situation.

Pilot plasma protein binding studies conducted with BAL9141 in rats, rabbits, dogs, marmosets, cynomolgus monkeys, and humans indicated that plasma protein binding was low; values across species ranged from approximately 10% to 45%, and were independent of drug concentration (10 or 100 µg/mL). As initial studies utilized a small sample size and showed a high intra- and inter-experimental variability, definitive studies were conducted in pooled plasma samples from male and female healthy normal volunteers (at 0.5 to 100 µg/mL BAL9141) or freshly obtained (i.e., within 72 h), non-pooled human plasma samples (at 3.5 and 35 µg/mL BAL9141). Results confirmed that plasma protein binding was low and concentration independent, with mean percentages of binding of 17.3% and 15.4% in two studies, respectively, resulting in a mean plasma protein binding value of ~16%. At concentrations of 2% to 6%, the percentage of BAL9141 bound to human serum albumin (HSA) was low, ranging from 6.5% to 11.5%. Binding to α_1 -acid glycoprotein (AAG) was also low, ranging from 4.8% to 6.8%. Using a fixed concentration of HSA (4.3%) and various AAG concentrations, binding appeared to increase slightly compared to use of either protein alone, with the percent bound ranging from 8.1% to 13.1% at 0.05% to 0.3% AAG. The combination of HSA and AAG did not account for all of the binding observed when using pooled human plasma, which suggests that other plasma proteins may also be involved.

Given that the extent of plasma protein binding of BAL9141 was generally low, the potential for drug-drug interactions with co-administered products that are extensively bound to plasma proteins is also considered to be low.

Table 5 *In vitro* plasma protein binding of BAL9141

Report No.	Species	Concentration (µg/ml)	% bound
BAP00112	Rat (RoRo)	10	15.8; 34.2
		100	14.9; 19.4
BAP00112	Rabbit (New Zealand White)	10	20.2; 26.3
		100	12.4; 22.8
BAP00112	Cynomolgus monkey	10	39.0; 49.8
		100	47.2; 43.4
BAP00112	Marmoset	10	25.0
		100	45.9; 24.8
BAP00112	Dog (beagle)	10	39.1; 14.7
		100	11.1; 10.5
BAP00566	Mouse	10	16.5; 20.8
		25	10.3
		50	18.5
		100	36.7/13.5
BAP00112	Human	10	21.8; 48.5
		100	47.7; 33.9
BAP00566	Human	10	22.1
		100	33.4
FK6157	Human (6 subjects)	3.5	16.7 ± 4.5
		35	14.1 ± 2.4
FK5789	Human (pooled plasma)	0.5	17.8
		5	19.23
		25	19.01
		100	13.24
FK5789	2% HSA	25	8.02
	3% HSA	25	6.52
	4% HSA	25	8.28

	5% HSA	25	10.20
	6% HSA	25	11.53
	0.05% AAG 0% HSA	25	4.85
	0.1% AAG 0% HSA	25	6.79
	0.3% AAG 0% HSA	25	5.99
	0.05% AAG 4.3% HSA	25	8.13
	0.1% AAG 4.3% HSA	25	11.90
	0.3% AAG 4.3% HSA	25	13.06

In vivo studies

Rodent: Tissue distribution of radioactivity was studied after i.v. bolus administration of ^{14}C -BAL5788 (20 mg/kg) to male mice and rats by quantitative whole body autoradiography (QWBA). Highest levels of radioactivity in the mouse were found in the coagulating gland, the excretory organs of the kidney (especially the kidney cortex) and the liver, and the skin and aorta; tissue to plasma (T/P) ratios for these tissues ranged from 1.05 (aorta) to 3.79 (coagulating gland), whereas T/P ratios in other tissues were below 1. In the rat, highest concentrations of radioactivity were found in the kidney, tooth pulp, liver, skin and lung, whereas the brain contained very low levels of radioactivity (0.38 $\mu\text{g-equiv./g}$) at 0.25 h post-dose, the only time point at which concentrations in the brain were at detectable levels (T/P ratio = 0.01). Results were similar to those obtained in the mouse, where concentrations in the brain were very low at 0.25 h post-dose (0.44 $\mu\text{g-equiv./g}$), with a T/P ratio of 0.02. In both mouse and rat, the elimination of radioactivity from tissues occurred in parallel to that of the blood. Elimination was rapid, except in the rat at low levels in the terminal phase, where slower elimination was observed. There was no evidence of relevant retention in any tissue in the rat, except for the kidney cortex, which showed a T/P ratio of 8.9 at 48 h post-dose. No significant retention of drug-related material was observed in melanin-containing tissues in pigmented rats. The high levels and retention of radioactivity in rat kidney cortex coincided with the cytoplasmic inclusions in proximal tubular cells observed in toxicity studies after administration of BAL5788.

Plasma and lung concentrations of BAL9141 (at up to 4 h post-dose) were also determined in female non-infected mice after a single subcutaneous administration of 25 mg/kg BAL5788. The plasma C_{max} observed at the first time point (0.25 hours post-dose) was 60.7 $\mu\text{g/mL}$, and the corresponding AUC was 29.7 $\mu\text{g}\cdot\text{h/mL}$. The lung concentrations were approximately 6-fold lower than that observed in plasma. In an acute pneumonia murine model, the penetration of BAL9141 into the lung was approximately 25% (estimated by the ratio of free BAL9141 in the lung to the free AUC in plasma and the median percentage penetration of BAL9141 into epithelial lining fluid (ELF) was 68.8% after a single s.c. dose in infected animals.

A series of rat studies were performed in order to simultaneously assess plasma and CSF concentrations of BAL9141 relative to kidney specific clinical chemistry parameters (BUN, creatinine). In the first experiment, rats received a single i.v. bolus injection of BAL5788 at 500 mg/kg. Animals were sacrificed at varying time-points between 10 minutes and 6 h post-dose and plasma and CSF levels of BAL9141 were measured, along with plasma levels of BUN and creatinine. In the second experiment, rats received a single i.v. bolus injection of BAL5788 at 20 or 500 mg/kg. Rats were sacrificed at different time-points between 10 minutes and 6 hours post-dose, and plasma and CSF levels of BAL9141 were determined. In the last experiment, rats received four bolus injections of 20 mg/kg BAL5788 separated by a dosing interval of eight hours. Rats were sacrificed 1, 3 and 5 h after administration of the last dose, and plasma and brain levels of BAL9141 measured. As expected, findings in the rat were similar to those observed in mice; administration of a high i.v. dose resulted in significant kidney toxicity (manifested as increases in BUN and creatinine), which was considered to lead to reduced drug clearance, and, thus, sustained elevated plasma and CSF

levels of BAL9141.

Initial plasma concentrations obtained at 500 mg/kg (714 µg/mL) were approximately 25-fold greater than those obtained after a single-dose of 20 mg/kg (48.0 µg/mL). Similarly, initial CSF concentrations obtained at 500 mg/kg (2.29 µg/mL) were approximately 25-fold greater than those obtained at 20 mg/kg (0.128 µg/mL). However, at the nephrotoxic dose of 500 mg/kg, $t_{1/2}$ in plasma was prolonged by at least a factor of eight ($t_{1/2} > 4$ h), resulting in a prolonged exposure in plasma that in turn resulted in CSF concentrations that progressively increased with time (e.g., 2.29 µg/mL at 0.17 h versus 8.32 µg/mL at 1.5 h), due to reduced elimination of the compound by the kidney.

An additional exploratory study in the rat investigated levels of BAL9141 in tissue cage fluid obtained from a pharmacological model in the rat that mimics deep foreign body infection in humans in order to determine appropriate doses for subsequent pharmacological studies. Male rats (n=6/group) received a Teflon s.c. tissue cage implant, and then, three weeks later, were given BAL5788 at 50 and 150 mg/kg/day via intraperitoneal injection for seven days. The pharmacokinetics of BAL9141 in tissue cage fluid remained roughly proportional to the dose and did not show time dependency. Based on C_{max} and AUC_{last} values, no significant accumulation of BAL9141 was observed in tissue cage fluid or plasma. This observation was further confirmed by the absence of changes to $t_{1/2}$ values between Days 4 and 7. The $t_{1/2}$ of BAL9141 in the tissue cage fluid was significantly longer than in plasma.

Rabbit: The concentrations of BAL9141 in plasma, bone, and bone marrow were determined in order to support dose selection for a rabbit osteomyelitis study. Following administration of single or repeated s.c. dose of BAL5788 at 20 and 80 mg/kg/day, the pro-drug BAL5788 was not measurable in any of the plasma, bone or bone marrow samples, indicating the rapid conversion of the pro-drug to BAL9141 after s.c. administration in the rabbit. By contrast, BAL9141 was measurable in all samples, and rabbits were exposed to BAL9141 in manner that was roughly proportional with dose. There was no evidence of accumulation of BAL9141 in plasma, and concentrations of BAL9141 in bone and bone marrow were above the minimum inhibitory concentration (MIC) of 0.39 µg/mL at the 2 doses examined.

Marmoset: The distribution of BAL5788 and its active moiety, BAL9141, was evaluated upon repeated administration of BAL5788 via i.v. infusion (q.d. for four hours at 2 mL/kg/h) for up to 13 weeks at 0 (5% dextrose), 50, 100 or 200 mg/kg/day (10/sex/group). Samples of brain tissue were collected from all animals at terminal necropsy, and apparent fibrous clots, obtained from the area near the cannula tip, were collected from a subset of ten marmosets. Levels of BAL5788 and BAL9141 detected in the brain tissue of male and female marmosets at approximately 20 hours following the final dose of BAL5788 were exceedingly low; in general, whereby concentrations were below the LOQ for most animals. These findings were consistent with results previously observed in mice and rats, showing limited distribution to the CNS and confirming the absence of accumulation of BAL5788 or BAL9141 in the brain after repeated dosing in marmosets. Although low levels of BAL5788 and BAL9141 were detected in the fibrinous clot material, they comprised a small fraction (less than 1% by weight for all animals) of the clot.

Distribution into red blood cells

Blood/plasma ratios ranged from 0.51 to 0.66, indicating that drug-related material did not penetrate red blood cells. These findings were in agreement with results from single-dose pharmacokinetic studies, indicating that distribution of BAL9141 is restricted to the extracellular compartment.

Transfer across the placenta and into milk

The amounts of BAL9141 and its pro-drug excreted in milk were evaluated in lactating rats on Day 20 of lactation after a once daily (q.d.) 4-h i.v. infusion of BAL5788 at 175, 250 or 360 mg/kg/day to pregnant female rats from implantation (Gestation Day [GD] 6) to weaning of the F1 generation pups on Day 21 of lactation. Milk was analysed at 1 to 2 h post-dose on Day 20 of lactation. The results indicated the pro-drug BAL5788 could only be detected in 1/5 milk samples at the maximum dose of 360 mg/kg. BAL9141 was excreted in all milk samples at levels of approximately 18% to 21% of the maternal plasma concentrations of BAL9141 measured at the end of the infusion. Nursing pups were not exposed systemically to BAL9141, consistent with low absorption via the oral route of administration.

Table 6. Plasma and milk concentrations in pregnant and nursing rats; BAL5788 administered once daily in 5% dextrose during a 4-hour infusion

Species (strain)	Gender (n)	Sampling	Analyte	Dose (mg/kg)	C _{plasma} at final 10 min infusion (µg/ml)	C _{plasma} at 2 h post infusion (µg/ml)	C _{milk} at 1 to 2h after start infusion (µg/ml)	Ratio Milk/Plasma
Rat Crj:CD(SD)IGS	F(5)	GD 6	BAL5788	175	22	1.5	BLQ	NA
		LD 20			19	6.1		
	F(3)	GD 6		250	22	3.3		
		LD 20	BAL9141		19	6.3	ND	NA
	F(5)	GD 6		360	34	7.5		
		LD20			18	20		
	F(5)	GD 6		175	106	10	1.8	0.1
		LD 20			87	10	17	0.19
	F(3)	GD 6		250	151	11		
		LD 20			89	18	16	0.18
	F(5)	GD 6		360	194	27		
		LD 20			93	26	20	0.22

GD 6 = gestation day 6

LD 20 = lactation day 20

Metabolism

In vitro studies

The *in vitro* metabolism of BAL5788 and BAL9141 was studied in hepatocytes from rat, mouse, dog, marmoset, cynomolgus monkey and human. The pro-drug was rapidly converted to the active drug BAL9141 in hepatocyte incubations. The most prominent metabolite in all species was the ring-opened hydrolysis product BAL1029. At least 3 or 4 additional metabolites were present in small amounts. In studies on the metabolism of BAL9141 and its pro-drug BAL5788 in mouse and rat brain homogenate *in vitro*, the pro-drug was cleaved rapidly, but the active drug appeared to be relatively stable (50% degradation within 3 h). *In vitro* incubation of BAL9141 with rat kidney and liver homogenates for 3 h resulted in a complete biotransformation with almost 100% of the drug degraded to the ring-opened product BAL1029 and at least 4 unknown compounds. The limited metabolism of BAL9141 observed in kidney homogenates following exposure *in vivo* is probably due to the very limited cellular uptake of the drug.

The rank order of $t_{1/2}$ for the degradation of BAL5788 in plasma was as follows: marmosets (10 sec) < cynomolgus monkeys (21 sec) < humans (38 sec) < mice (54 sec) < rats (201 sec). The rapid degradation of BAL5788 in plasma is caused by enzymes, most likely esterases, as chemical hydrolysis of BAL5788 was much slower in phosphate buffer pH 7.4 ($t_{1/2}$ = 3.3 h). The rank order of hydrolytic stability observed *in vivo* was marmosets < mice \approx Cynomolgus monkeys \approx humans < rats, which is similar to the rank order observed *in vitro*.

Cleavage of BAL5788 to BAL9141 was not inhibited by acetylcholinesterase inhibitors such as neostigmine at 0.1 µg/mL. EDTA and dichlorvos (DDVP) are known inhibitors of esterase

activity for Type A and Type B esterases, respectively. Inhibition studies with DDVP and EDTA suggested that Type A esterases (paraoxonases) are involved in the cleavage of BAL5788 to BAL9141. The activity of Type A esterase could be efficiently inhibited by addition of EDTA to plasma, whereas only moderate inhibition was achieved by addition of an organophosphate, such as DDVP.

A mechanistic study was conducted to study the involvement of specific esterases and the extent of conversion in several matrices. This study investigated the following:

1. The role of paraoxonase enzymes in the hydrolysis of BAL5788 (60 μ M) by human plasma in the presence of the paraoxonase inhibitors EDTA, metal ions (Cu and Hg) and p-hydroxymercuribenzoate (p-OHMB)
2. The calcium dependence of BAL5788 hydrolysis (10 to 200 μ M) (paraoxonases require calcium for both stability and catalytic activity) was determined in erythrocytes, and S9 subcellular fractions from liver, kidney and small intestine
3. The hydrolysis of BAL5788 (10 to 200 μ M) in plasma from liver impaired patients was compared with plasma from healthy volunteers and
4. The hydrolysis of BAL5788 (10, 60 and 200 μ M) by human plasma was determined in the presence of various diagnostic esterase inhibitors.
5. The hydrolysis of BAL 5788 was also investigated in rat and human plasma from juvenile and neonates

The results demonstrate that the hydrolysis of BAL5788 to BAL9141 is rapid in the presence and absence of diagnostic inhibitors (carboxyl-esterase, paraoxonase, acetylcholine-esterase, cholinesterase, pseudocholinesterase, serine-esterase, amidase- and retinyl- palmitoyl-hydrolase) in multiple matrices (human liver, kidney and intestinal S9, erythrocytes, plasma from healthy volunteers, plasma from liver impaired patients and plasma from neonate and juvenile donors) and provide evidence that the conversion of BAL5788 to BAL9141 is catalyzed by plasma paraoxonases and other hydrolytic enzymes and also occurs non-enzymatically at an appreciable rate. The potential for drug-drug interactions with BAL5788 or reduced exposure to BAL9141 in patients with hepatic impairment is low. The results also suggest that the hydrolysis of BAL5788 to BAL9141 is not age-dependent in human, whereas it is in rat.

In vivo studies

The *in vivo* metabolism of BAL9141 and BAL5788 has been studied in the rat, mouse and marmoset. The main metabolite, BAL1029, seen in plasma, urine, bile, brain and kidney was identified as the β -lactam ring-opened hydrolysis product of BAL9141, by liquid chromatography/mass spectrometry (LC-MS) and by chromatographic comparison with the compound itself.

The pro-drug, BAL5788, undergoes enzymatic hydrolysis by Type A esterases. Metabolic profiles of rat plasma revealed rapid cleavage of the pro-drug with an approximate $t_{1/2}$ of 4 - 5 minutes; BAL9141 was the only predominant component detected at the end of a 2-h i.v. infusion of BAL5788. In rat urine, where about 80% of the dose was excreted, the major drug-related material was unchanged BAL9141, which accounted for about 50% of the dose within 24 h after dosing. The ring-opened metabolite BAL1029 was the main urinary metabolite (~10% of the dose), and at least 3 other minor unknown metabolites were detected, which amounted to less than 5% of the dose each. Traces of intact pro-drug were also found in rat urine (< 1% of the dose). Following repeated i.v. dosing in the marmoset, the major drug-related material in the urine was the active drug BAL9141, together with small amounts of the ring-opened metabolite BAL1029, the pro-drug BAL5788 and traces of

the ring-opened pro-drug. The urine contained sediment which was analyzed by HPLC and LC-MS/MS. The major peak was identified as BAL9141 and accounted for 90% of the sediment, with 5% of BAL1029 and less than 1% of the intact pro-drug, BAL5788.

Biliary metabolite profiles in rats revealed excretion of a small amount of intact pro-drug BAL5788 (0.3% of the dose), similar amounts of active drug BAL9141 and the ring-opened metabolite BAL1029 (~ 3% of the dose each), and 4 other metabolites with less than 4% of the dose each, which were not further investigated.

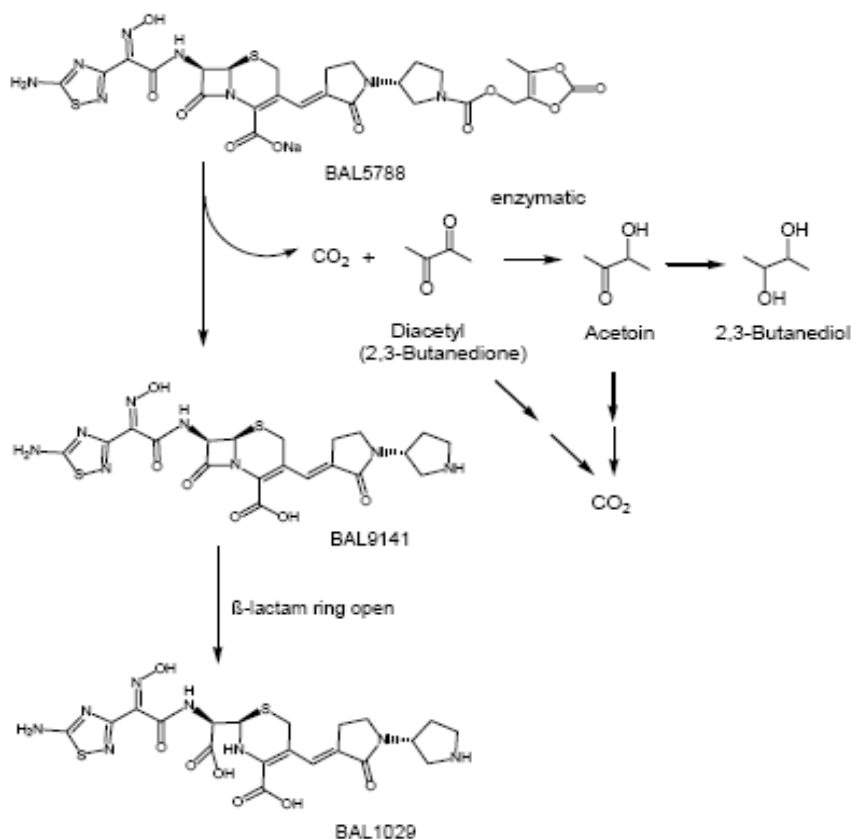
Brain homogenates obtained from *in vivo* studies in mice and rats receiving i.v. administration of BAL5788 contained predominantly the active drug BAL9141, and very few other metabolites in small amounts. In the brains of mice receiving an i.c.v. dose of ^{14}C -BAL5788 at 50 μg , more than 40% of the pro-drug was cleaved to the active drug within a few minutes and no additional metabolites were detected. The brains were obtained when the mice had tonic seizures; hence, the drug itself rather than a potential metabolite appeared to be responsible for the observed effect on the CNS. It is also noted that in rat CSF, BAL9141 was the only component detected following i.v. infusion of either BAL5788 or BAL9141.

Rat kidney homogenates, obtained after i.v. administration of BAL5788 at 20 or 500 mg/kg, contained mainly BAL9141 with less than 10% of the ring-opened product BAL1029. Following administration of BAL5788 at 500 mg/kg, BAL9141 distributed to the kidneys, reaching levels of approximately 16 mg per kidney at 2-4 h post-dose. The results suggest that renal effects observed after administration of BAL5788 were due to the active drug and not to a prominent metabolite.

The assessment of the fate of the carbamate moiety of the pro-drug both *in vitro* and *in vivo* was not deemed necessary as the diacetyl cleavage product is present endogenously in animals and humans, and its metabolic fate has been characterized (Otsuka 1996, EFSA 2004). However, a single exploratory study was conducted in the rat which utilised ^{14}C -BAL5788 labelled on the carbamate moiety of the pro-drug, to assess the main route of elimination of the pro-drug moiety of BAL5788. The results indicated that the majority of the radioactive dose (ca. 70%) was not recovered in urine, feces, fur/carcass and cage wash. These data suggest that the pro-drug cleavage product (diacetyl) was rapidly further metabolized and eventually eliminated as CO_2 via the lung. This result is consistent with published data on the metabolic fate of diacetyl, a generally recognised as safe (GRAS) substance that is used as a food additive and acetoin which shows elimination as CO_2 via lung (Gabriel 1972, EFSA 2004). The same pro-drug moiety has been used for lenampicillin (the pro-drug of ampicillin), which is approved in Japan and South Korea. The degradation products of the pro-drug cleavage moiety for lenampicillin have been identified as the non-toxic natural substance acetoin resulting from the formation of diacetyl and carbon dioxide and further metabolized to 2,3 butanediol (Tsukamoto 1981, Alexander 1996, Sakamoto 1984).

Figure 1 summarises the proposed breakdown of the carbamate moiety of the pro-drug BAL5788 (sodium salt) to its active drug BAL9141, carbon dioxide and 2,3 butanedione (diacetyl), the latter subsequently being cleaved to acetoin and 2,3 butanediol. In addition, diacetyl and acetoin can be further oxidized to yield carbon dioxide.

Figure 1 Proposed Metabolic Pathway for BAL5788



Excretion

The routes and extent of BAL9141 excretion were studied following administration of a single i.v. bolus dose of ¹⁴C-BAL5788 (labelled on the thiadiazole ring) to rats. The results indicated that excretion was rapid with a mean recovery of $88 \pm 1\%$ of the dose at 24 h. Excretion was almost complete within 4 days, with mean total recovery in urine, feces, cage wash and carcass amounting to 95% of the administered dose. The predominant route of excretion was via the urine (73% of the dose) and the fraction in feces accounted for only 17% of the recovered dose. In bile-duct cannulated rats where ¹⁴C-BAL5788 at 13.3 mg/kg was administered as a single i.v. dose, approximately 18% of the radioactive dose was recovered in bile.

An exploratory study was conducted in the male rat in order to assess the elimination pathways of radioactivity following administration of a single i.v. dose of ¹⁴C-BAL5788 labeled in the pro-drug moiety (methyls-¹⁴C). Over the 96 h collection period, the mean total recovery of the radioactive dose in urine, feces, cage wash and fur/carcass amounted to 28%. Elimination was rapid since on average 93% and 43% of the total radioactivity eliminated in urine and feces, respectively, were recovered within the first 24 h after dosing. As the predominant part of the radioactive dose (i.e., ~72%), however, was not recovered in urine, feces, fur/carcass and cage wash, the main elimination pathway of the radioactivity was assumed to be pulmonary.

Pharmacokinetic Drug Interactions

Interactions at the level of cytochrome P450 enzymes

Induction potential: Human hepatocytes were treated with the vehicle (DMSO, 0.1%), BAL9141 (5 μM, the highest concentration that could be attained to treat hepatocytes) or one

of three known human cytochrome P450 enzyme inducers (i.e., omeprazole [100 µM], phenobarbital [750 µM], and rifampin [10 µM]) and subsequently examined for their effects on phenacetin O-dealkylation (CYP1A2), bupropion hydroxylation (CYP2B6), diclofenac 4'-hydroxylation (CYP2C9), S-mephenytoin 4'-hydroxylation (CYP2C19), testosterone 6-β-hydroxylation (CYP3A4), and midazolam 1'-hydroxylation (CYP3A4/5). While the treatment of human hepatocytes with the CYP inducers omeprazole, phenobarbital or rifampin caused anticipated increases in CYP activities, treatment with BAL9141 at 5 µM caused no induction of CYP1A2, CYP2B6, CYP2C9, CYP2C19 or CYP3A4/5.

Due to the limited solubility of BAL9141, experiments with the pro-drug, BAL5788 were also conducted to investigate its induction potential. Cultured human hepatocytes were treated with vehicle (DMSO, 0.1%) or BAL5788 (10, 80 and 200 µM). Using the same design and prototypical inducers and substrates as indicated previously, ceftobiprole medocartil had no effect on the activity of CYP1A2, CYP2B6, CYP2C8 (amodiaquine, 20 µM) CYP2C9, CYP2C19 or CYP3A4/5 up to the maximum concentration tested of 200 µM.

In human liver microsomes, BAL9141 (0.05-10 µM) showed no significant inhibition of the tacrine 1- and 7-hydroxylation (CYP1A2), diclofenac 4'-hydroxylation (CYP2C9), S-mephenytoin 4'-hydroxylation (CYP2C19), bufuralol 1'-hydroxylation (CYP2D6), and testosterone 6-β-hydroxylation (CYP3A4); only slight inhibitory potential was seen at the highest tested concentration range of 50 to 100 µM. The lack of direct or time-dependent inhibition of CYP2C8 was also demonstrated in human liver microsomes using amodiaquine (20 µM) as a specific substrate. As BAL9141 is restricted mainly to the extracellular water compartment, the potential of BAL9141 to affect the cytochrome P450 (CYP450)-dependent metabolic clearance of co-administered drugs is considered to be low.

Overall, the applicant has concluded that BAL5788 is not expected to affect the CYP450-dependent metabolic clearance of co-administered products in the clinical setting.

Interactions at the level of transporter systems

Interactions via P-glycoprotein (P-gp): *In vitro* studies were conducted to determine whether BAL9141 is a substrate or inhibitor of the drug (efflux) transporter P-glycoprotein (P-gp). MDR-MDCK cells were dosed on the apical side (A to B) or basolateral side (B to A) and incubated in the presence and absence of cyclosporin A, a known P-gp inhibitor, or in the presence of digoxin, a known P-gp substrate. Results indicated that the bi-directional permeability of BAL9141 in MDR-MDCK cell monolayers was very low, and BAL9141 does not seem to be a P-gp substrate or inhibitor under the test conditions.

Potential for interaction with probenecid: Rats were administered a single intravenous dose of BAL9141 at 10 mg/kg in combination with probenecid at 50 mg/kg i.v., or BAL5788 at 100 mg/kg in combination with probenecid at 200 mg/kg i.v or of BAL5788 at 100 mg/kg alone, in order to determine the effects of probenecid on pharmacokinetics of BAL9141 and BAL5788.

Plasma clearance (6.0-9.2 mL/min/kg) was in the range of the glomerular filtration rate in rat (8.7 mL/min/kg). Estimated V_{dss} values were generally low (0.26-0.45 L/kg) and in the range of extracellular space for the rat (0.30 L/kg). The apparent terminal $t_{1/2}$ was relatively short (0.54-0.66 h) following co-administration of BAL9141 and probenecid, and agreed with the data from previously conducted studies where BAL9141 was administered alone. Following administration of BAL5788 at 100 mg/kg alone, the apparent terminal $t_{1/2}$ tended to be prolonged (0.59-0.93 h) and probenecid did not affect the V_{dss} or plasma clearance.

In summary, the plasma time-concentration profiles of BAL9141 or the pro-drug BAL5788 were not affected by co-administration with probenecid, indicating a lack of relevant pharmacokinetic interaction. As probenecid is known to inhibit active renal secretion processes, it is unlikely that BAL9141 is significantly excreted by active transport processes in the kidney.

Overall conclusion on pharmacokinetics

The pharmacokinetics of BAL5788 and the active component, BAL9141 were examined in the mouse, rat, rabbit, dog, marmoset and monkey. No major differences in pharmacokinetics of BAL9141 were observed between intravenous administration as BAL9141 or BAL5788, which is due to the rapid conversion of BAL5788 to BAL9141. The bioavailability of BAL5788 or BAL9141 after oral administration of BAL5788 was low (<1%). However, bioavailability of BAL9141 was high (90-100%) after intramuscular, intraperitoneal or subcutaneous administration of BAL5788 when compared to intravenous administration. Elimination of BAL9141 was fast ($t_{1/2}$ approximately 0.3 h, 1 h and 1.5 h for the mouse, rat and dog, respectively) and was generally independent of the route (iv, im, ip or sc). Following intravenous administration of BAL5788, systemic exposures (AUC and C_{max}) to BAL9141 generally increased in proportion with dose. No apparent gender differences were observed; however, it is noted that there was considerable variation between the studies.

The protein binding of BAL9141 appeared to be low (<50% binding), with some binding to albumin, α -acid glycoproteins and other proteins. In vivo studies in mice and rats both showed high levels of drug-related radioactivity within the coagulating gland (tissue:plasma ratios up to respectively 50 and 15, respectively). However, this finding was not considered to be clinically relevant as the coagulating gland has no direct equivalent in man. The blood:plasma ratios were considered to be low. High levels of radioactivity were detected in the kidney cortex, kidney medulla, liver, skin, aorta and tooth pulp. In most tissues radioactivity declined at the same rate as that for the blood. However, in kidney cortex radioactivity decreased in a slower manner in both the mouse and the rat. This slow initial decline caused a relatively high level of radioactivity in the renal cortex even after 24 hours. In the mouse, the liver showed a similar pattern as the renal cortex. These findings indicate that BAL9141 or its metabolites may accumulate in the renal cortex and liver following repeated administration.

A series of other distribution studies of an exploratory nature, were performed. Distribution of BAL9141 was demonstrated in infected and non-infected mice, which supports the proposed indication. In the rat (and mouse); administration of a single i.v. bolus at 500 mg/kg resulted in significant renal toxicity, which was associated with a significant increase in half life and sustained elevated plasma and CSF levels of BAL9141. In a separate study conducted in the mouse, distribution to the extracellular water compartment was considerable as levels of BAL9141 in tissue cage fluid generally exceeded those observed in plasma and the observed rate of elimination was considerably slower in tissue cage fluid than in plasma/blood.

BAL5788 was excreted in milk at low concentrations; higher concentrations of its metabolite, BAL9141 were detected in milk which amounted to approximately 20% of the maternal plasma concentration at 2 h post- infusion. Section 4.6 of the proposed SmPC suggests that caution should be exercised when prescribing to breast-feeding mothers; however, further revision to the text is warranted so that the wording is in line with that proposed by the Guideline on risk assessment of medicinal products on human reproduction and lactation: from data to labelling [EMA/CHMP/203927/2005].

In hepatocytes BAL5788 was rapidly converted (by paraoxonase enzymes, other hydrolytic enzymes and also via non-enzymatic degradation) to BAL9141 and the most predominant metabolite was BAL1029. Some additional metabolites were detected in small amounts. The in vitro metabolic profile was similar in all species investigated. Likewise, in vivo, the pro-drug was rapidly cleaved to BAL9141. BAL9141 was the main component encountered in plasma (at 2 hours post-dose in the rat), urine (24 h post-dose in the rat) and bile (rat). The ring-opened metabolite BAL1029 was the main metabolite in rat urine (10%), and bile (3%). Additional metabolites were found in rat bile (4 other metabolites <4% of dose) and rat urine (3 other metabolites <5% of dose). There is evidence to suggest that the carbamate moiety of the pro-drug BAL5788 is converted to carbon dioxide and 2,3 butanedione (diacetyl); the latter is subsequently cleaved to acetoin and 2,3 butanediol. In addition, diacetyl and acetoin can be further oxidized to yield carbon dioxide.

Following administration of a single intravenous bolus injection of BAL5788, BAL9141 was excreted predominantly via the urine, and a minor proportion was excreted via the feces (17%). Excretion was rapid as 66% and 88% of the 95% were recovered in 7 and 24 h, respectively. In bile-duct cannulated rats, ~ 18% of the intravenous dose was recovered in the bile within 24 h, which suggests that BAL9141 undergoes enterohepatic circulation.

When BAL5788 was labelled in the pro-drug moiety only 28% of the radioactivity was recovered in 96 h, mainly in the urine and the carcass. Considering the metabolic pathway, it is agreed that a large proportion of the radioactivity was excreted via the lung.

In human liver microsomes, BAL9141 (up to 10 μM) did not inhibit CYP1A2, 2B6, 2C9, 2C8, 2C19, 2D6 or 3A4. At the higher concentrations (50 and 100 μM), slight inhibition was observed, where the maximum observed inhibition was 22% inhibition of CYP1A2 at 100 μM , 8% inhibition of CYP2C9 at 100 μM , 28% inhibition of CYP2D6 at 100 μM and 13% inhibition at 50 μM . Effects on enzyme inhibition were therefore noted at concentrations that were 0.8-1.6 fold the observed C_{max} ; however, only slight inhibition was observed which did not facilitate the calculation of an IC_{50} value.

In human hepatocytes, the potential of BAL9141 (5 μM) to induce cytochrome P450 expression was investigated and no induction or suppression of CYP1A2, 2B6, 2C9, 2C19, or 3A4/5 was observed. As the maximum concentration of BAL9141 was severely limited by solubility, a follow-up study was performed where the effects of BAL5788 were evaluated at up to 200 μM . BAL5788 had no effect on the induction of CYP1A2, 2B6, 2C8, 2C9, 2C19, or 3A4/5. The data provided thus far, suggest that ceftobiprole medocaril is unlikely to cause a drug interaction via enzyme inhibition or induction. However, it is noted that the maximum concentrations evaluated were limited by solubility and are not in accordance with the Guideline on the Investigation of Drug Interactions (which suggests that concentrations of up to 50-fold the unbound C_{max} should have been evaluated).

It is noted that BAL5788 is cleaved by plasma esterases such as paraoxanases and other hydrolytic enzymes. It is also noted that the SmPC does not discuss the potential to interact with co-administered esterase inhibitors. There is evidence to suggest that BAL5788 may be cleaved to BAL9141 via non-enzymatic pathways. Hence, if ceftobiprole medocaril is co-administered with an esterase inhibitor, it is likely that the active moiety will still be formed in vivo; so in theory, the potential for a co-administered esterase to interfere with the release of the active component should not be clinically significant.

The bi-directional permeability of BAL9141 across MDR-MDCK cell monolayers was low and the efflux ratio was not decreased in the presence of the Pgp-inhibitor cyclosporine A. In

the rat, co-administration with the P-gp inhibitor saquinavir, slightly increased the oral bioavailability of BAL5788 (AUC doubled); however, the observed increase was small (as bioavailability was very low; <1%). These data suggest that BAL9141 and BAL5788 are not P-gp substrates. The P_{app} of the P-gp substrate, digoxin was not affected by the presence of BAL9141, which would suggest that BAL9141 does not inhibit Pgp-transport.

A separate study was initiated to assess whether ceftobiprole is an inhibitor or a substrate of P-gp, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2. At the concentrations tested (0.3–200 μ M) ceftobiprole inhibited OATP1B1 and OATP1B3 with IC_{50} values of 67.6 and 44.1 μ M, completely inhibited BSEP in a concentration-independent manner, and inhibited MRP2 at the highest concentration tested (200 μ M). However, it should be noted that the observed inhibition of MRP2 and BSEP may be an artefact due to the effect of the solvent/acidic buffer. Ceftobiprole did not inhibit any other transporter (P-gp, BCRP, OAT1, OAT3, OCT1, OCT2) at the concentrations evaluated.

The permeability of ceftobiprole appeared to be very low, based on the permeability in the transwell assays (P-gp and BCRP substrate assays) and the amount that accumulated in cells in the uptake transporter assays. Ceftobiprole was possibly a weak substrate of the renal uptake transporters OAT1 and OCT2. However the results should be interpreted with caution, as the amount of compound that accumulated in the cells was very low, which can have a large impact on the results. Ceftobiprole did not appear to be a substrate of any other transporters.

As the maximum concentrations evaluated were limited by solubility and not in line with those proposed by the regulatory guidelines, the possibility of an interaction at the level of the enzymes and transporters cannot be completely ruled out. Hence, the Applicant has made reference to these limitations within Section 4.5 of the SmPC, which is acceptable.

TOXICOLOGY

The toxicology of BAL5788 was characterized in repeated-dose toxicity (up to 13-week duration in rats, marmosets and dogs), genotoxicity and reproductive (fertility, developmental toxicity, pre- and post-natal) toxicity studies, and in studies assessing local tolerability, potential antigenic and hemolytic effects, phototoxicity and nephrotoxicity. Additional information was obtained from several bridging studies with formulations manufactured via different synthesis routes. Since BAL5788 undergoes spontaneous conversion to the active compound BAL9141 (ceftobiprole) after reconstitution, several studies compared local and systemic toxicity after the administration of “fresh” (i.e. immediately prepared) and “aged” (i.e. stored more than 24 hours after reconstitution) solutions, and also addressed the local tolerance of accidental subcutaneous, intramuscular, paravenous or intra-arterial administration. Juvenile toxicity was investigated in neonatal and juvenile rats.

All studies were conducted in accordance with best scientific principles. Pivotal studies were conducted according to Good Laboratory Practice (GLP) guidelines.

Unless stated otherwise, all dose levels are expressed in BAL9141 equivalents.

Three-day pilot intravenous (i.v.) toxicity studies in rats and marmosets compared effects after BAL5788 administration via bolus injection and infusion. After bolus dosing, degenerative changes were observed in the distal and collecting tubules of the kidney in both rats (≥ 150 mg/kg) and marmosets (≥ 100 mg/kg) administered three times a day (t.i.d.), and was associated with drug precipitation in the distal parts of the nephron. Drug precipitation and renal toxicity were not present after administration by i.v. infusion, indicating that the administration of BAL5788 by infusion was associated with a larger safety margin than

administration by bolus injection.

In a 2-week infusion study in male rats BAL5788 was administered by 4-hour infusion twice daily (b.i.d.). Results indicated that BAL5788 was well tolerated up to a dose of 360 mg/kg/day. Abnormal urine color was seen in all dose groups (observed in the majority of toxicology studies), and was considered to be due to renal excretion of the test article (the primary excretory route) and judged to be toxicologically insignificant. Enlargement of the cecum, seen in some animals at 360 mg/kg, was related to the pharmacological activity of the drug. At doses ≥ 250 mg/kg, pigment deposits and amorphous precipitates were present in distal renal tubules and collecting tubules. At the end of the administration period, hyaline droplets in the proximal renal tubules of almost all treated animals may have reflected facilitated re-uptake of excreted test article, and were considered to be toxicologically insignificant, since no degenerative changes were observed in the tubules. The treatment-related findings exhibited a trend towards complete reversibility following the 4-week recovery period. Mean plasma concentrations were dose-related.

Results from a 4-week infusion study (4-hour) in rats indicated that once daily (q.d.) doses of BAL5788 were well tolerated up to 360 mg/kg/day. Minimally increased levels of urinary N-acetyl-beta-glucosaminidase (NAG) in high dose group males were comparable to levels seen in control animals at the end of the recovery period. Dose-dependent minimal to slight cytoplasmic inclusions in renal proximal tubules at doses ≥ 250 mg/kg/day were not associated with any functional or other morphological changes in the kidneys, and were fully reversible following a 4-week recovery period. Based on these findings, the No Observed Adverse Effect Level (NOAEL) in this study was 360 mg/kg/day. Toxicokinetics were consistent with those seen in the previous 2-week study; no clear indication of plasma or urinary accumulation of BAL9141 was evident, no time dependence was detected in systemic or urinary exposure, nor were there any gender-related differences in systemic or urinary exposures to BAL9141. As expected, BAL5788 plasma and urine concentrations in rats were highly variable, albeit relatively low.

In 13-week infusion toxicity study in rats BAL5788 was administered via q.d. infusion to males at doses up to 750 mg/kg/day and to females at doses up to 376 mg/kg/day. The primary targets of toxicity included the kidneys and the infusion site, where local irritation and the presence of a yellowish-brown fibrinous material at the tip of the catheter were associated with thrombus formation. Signs of vascular irritation at the injection site (thickening of the femoral vein) were likely related to the small size of the test species and use of catheters with large diameter relative to the size of the lumen of the *Vena cava* and femoral veins. Physical irritation of the veins by the catheter, possibly in combination with additional effects due to obstruction of blood flow, was considered to predispose the vessel wall to irritation from the compound; these effects were considered to be exacerbated by the length of the treatment period and by the infusion of high concentrations of test article, and resulted in the release of emboli from thrombi at the injection site. Incidences of mortalities in males at 250, 500 and 750 mg/kg/day were attributed to acute renal failure or thrombo-embolic changes. Renal toxicity at doses ≥ 250 mg/kg was consistent with that observed in previous subchronic studies in rats, and was associated with the precipitation of drug-like material in the distal part of the nephron. Findings in the kidneys were dose-related and trended towards reversibility after a 4-week recovery period. The NOAEL for kidney toxicity was 125 mg/kg/day. Rats were exposed to high plasma and urine concentrations of BAL9141 throughout the study. Systemic exposure was almost dose-related. Urinary exposure was dose-related on the first day of dosing in both male and female rats, and in female rats after repeated administration. Very high plasma levels were obtained in moribund male rats, indicating accumulation of BAL9141 consistent with a reduced urinary elimination of BAL9141 related to impaired kidney function at high doses.

In a 2-week infusion study in Beagle dogs, dose-dependent vomiting and reddening of the skin and mucous membranes (associated with increased histamine release) were noted with a 0.5-hour infusion, but were substantially attenuated by prolongation of the infusion time to two hours. Inflammatory cell infiltration and mild hyperplasia of the tunica intima of the vein at the injection site were observed in all dose groups and resolved in the recovery period. In the kidneys, eosinophilic droplets in the proximal tubular epithelium were seen at doses ≥ 50 mg/kg/day, but no drug precipitation was seen up to the highest dose of 100 mg/kg/day. The NOAEL in this study was determined to be < 25 mg/kg/day due to minor clinical findings. Animals were exposed to BAL9141 in a dose-proportional manner, and to BAL5788 in a dose-related manner. The decreased incidence and severity of vomiting following extension of the infusion time in the mid- and high-dose groups was associated with a 2-fold decrease in the maximum concentration (C_{\max}) for BAL9141 and BAL5788, which occurred without a substantial change in the area under the concentration vs. time curve (AUC) of BAL9141, and with at least a doubling of the AUC of BAL5788.

Clogging of the surgically implanted cannula resulting in an inability to dose was the primary cause of the premature sacrifice of five out of six dogs administered the highest dose of 32 mg/kg in a 13-week i.v. infusion (2-hour) toxicity study. No findings of toxicological importance were observed in these five animals, which had received doses for four to 12 weeks. Clinical observations during the study were similar to those seen in the 2-week study in dogs, and consisted of findings of discolored urine at doses of 8 and 32 mg/kg, and of reddening of the skin and mucous membranes, associated with increased histamine release at 32 mg/kg. No macroscopic or microscopic pathology findings were observed. Plasma concentrations of BAL5788 tended to increase in dose-related manner, with BAL9141 plasma levels exhibiting linear kinetics over the tested dose range. No gender-related differences were observed.

In an exploratory 3-day tolerance study in male marmosets (*Callithrix jacchus*) BAL5788 was administered at doses up to 200 mg/kg t.i.d. as slow bolus injections. Convulsions or tremors were noted at doses ≥ 20 mg/kg, and renal toxicity (distal and collecting tubules) was seen at doses ≥ 100 mg/kg. No signs of local irritation were seen at the injection sites. Based on the results of this pilot study it was decided to perform further studies with an infusion regimen.

Results from two 2-week infusion studies (4-hour, b.i.d.) in male marmosets revealed precipitates and pigment deposits in distal renal tubules and collecting tubules at doses ≥ 175 mg/kg b.i.d., and slight kidney tissue reaction at doses of 250 mg/kg. Incidences of marked impairment of general condition, vomiting, delayed convulsions and mortalities were observed at ≥ 250 mg/kg, and dosing at 360 mg/kg was discontinued after a total of three infusions. After a 4-week recovery period, renal changes noted at 250 mg/kg were essentially reversible, and no signs of kidney tissue reaction were apparent. Based on these findings, the No Observed Effect Level (NOEL) was considered to be 100 mg/kg/day. Toxicokinetic analysis indicated that animals were exposed to BAL9141 in a dose-proportional manner, however, significant inter-individual variability was observed in both studies at various study phases (i.e., at the end of the first or last infusion cycle).

In a 4-week infusion study (4-hour, q.d.) in marmosets, doses up to 200 mg/kg were well tolerated. The NOEL was 100 mg/kg/day, with only minor and reversible changes (i.e., slightly increased blood urea nitrogen (BUN) levels, minimal occurrence of brown pigment in the distal tubular epithelium of the kidneys) noted at 200 mg/kg/day, which was considered to be the NOAEL in this study. Toxicokinetic monitoring indicated dose-proportional exposure, and no gender differences or drug accumulation were apparent.

In a 13-week i.v. infusion study (4-hour, q.d.) in marmosets, mortalities were observed at all doses (50, 100, and 200 mg/kg/day) and were attributed to thrombo-embolic effects

secondary to infusion site reactions, including thrombus formation at the catheter tip. Signs of vascular irritation at the injection site (thickening of the femoral vein) were likely related to the large size of the catheter used relative to that of the lumen of the *Vena cava* and femoral veins. Physical irritation of the veins by the catheter, possibly in combination with additional effects due to the resultant alteration of blood flow caused by the mechanism of dosing, was considered to predispose the vessel wall to irritation from the compound; these effects were considered to be exacerbated by the length of the treatment period and by the infusion of high concentrations of test article. The dose-related venous irritation was similar to that seen in the 13-week study in rats, and was also observed in subchronic studies in rats (2-week bridging studies) and marmosets (4-week study). The effect was more pronounced in the chronic studies due to prolonged exposure. In the 13-week study in marmosets, the thrombo-emboli released at the infusion site lodged primarily in pulmonary vessels and were considered to be responsible for 10 of the 11 early mortalities. Mortalities were preceded by clinical signs indicative of a general deterioration of physical condition (i.e. vomiting and decreased activity), and by convulsions that are considered to be agonal rather than centrally mediated. In surviving animals, vomiting and soft stool were observed in males and females treated with 200 mg/kg/day and in one female treated with 100 mg/kg/day. Findings in the kidneys (i.e. brown pigment in the cytoplasm and lumen of proximal tubules) were reversible and similar to those seen in the previous 4-week study in marmosets, and were not associated with other microscopic changes. With the exception of the infusion site reactions and associated mortalities, the NOAEL in the study was 100 mg/kg/day for males and 50 mg/kg/day for females. Review of individual animal exposure data showed no relationship between plasma or urine exposure and subsequent mortality. Plasma BAL9141 concentrations increased almost dose-proportionally after administration of the first and final doses, and no gender-related differences or signs of accumulation were noted. Urinary concentrations and excretion of BAL9141 tended to increase with increasing doses.

The genotoxic potential of BAL5788 and/or BAL9141 was examined in a battery of *in vitro* and *in vivo* assays. The Ames test was negative up to cytotoxic concentrations. BAL5788 exhibited clastogenic activity in the ML/TK test at cytotoxic concentrations, and BAL9141 induced an equivocal effect at very high (cytotoxic) concentrations. In the HCA assay, BAL5788, but not BAL9141, was clastogenic under the described *in vitro* conditions at cytotoxic concentrations. The positive response was attributed to the cleavage product diacetyl, with no contribution from the active cephalosporin BAL9141, and was considered the consequence of artificial *in vitro* test conditions. No genotoxic activity was seen in the two *in vivo* assays (MNT and UDS assays) even at extremely high and acutely toxic doses of BAL5788.

The *in vitro* genotoxic activity of diacetyl and other dicarbonyl compounds is thought to be mediated by an oxidative mechanism of action due to the formation of oxygen radicals. The presence of multiple anti-oxidative defense mechanisms *in vivo*, which are not present in *in vitro* test systems, helps to account for the absence of genotoxicity under *in vivo* conditions. Diacetyl is rapidly metabolized *in vivo* by oxido-reductases acting on carbonyl compounds, resulting in the formation of non-toxic glycol metabolites. Diacetyl is an endogenous substance and is also present in many items of human food. It is included in the Generally Recognized as Safe (GRAS) list of substances and is used as a food additive. Based on these observations, a genotoxic liability of BAL5788 in man is not likely.

Carcinogenicity studies were not conducted based on the intended short-term clinical duration of therapy, 7-10 days for the majority of patients, and because results of the genotoxicity testing showed a low potential for genotoxicity. Although positive *in vitro* clastogenic results were seen with BAL5788, they were attributable to the diacetyl cleavage product, an endogenous molecule that is listed as GRAS, and that is rapidly metabolized *in vivo*. The likelihood that BAL5788 is carcinogenic is considered to be very low because

there is no retention of BAL5788 in specific tissues and because no precancerous lesions related to BAL5788 administration occurred in the repeated-dose toxicity or reproductive toxicity studies

BAL5788 was neither teratogenic nor embryotoxic in rats and Cynomolgus monkeys after i.v. infusion of doses up to 360 mg/kg/day (4-hour) and 120 mg/kg/day (2-hour), respectively, and had no effects on fertility and early embryonic development in rats after i.v. infusion of doses up to 360 mg/kg/day. In a pre- and post-natal toxicity study in rats, the NOAEL in dams (F0 generation) was 175 mg/kg/day for maternal toxicity and 250 mg/kg/day for reproductive toxicity. Functional and physical development of the F1 and F2 generations were normal in all groups.

Cephalosporin-specific nephrotoxicity was not observed in rabbits.

The potential for a phototoxic skin response in humans is considered to be low, as results from both an *in vitro* test in cultured mouse fibroblasts and from an *in vivo* test in hairless rats revealed that neither BAL9141 nor BAL5788 exhibited phototoxic potential following ultraviolet A (UVA) irradiation.

No signs of skin sensitization were seen in the guinea pig, indicating that cutaneous exposure to BAL5788 would be unlikely to cause skin sensitization in humans. In a guinea pig antigenicity study, signs of potential antigenicity were seen only at high i.v. bolus doses ≥ 20 mg/kg or at subcutaneous (s.c.) doses of 50 mg/kg in combination with adjuvant.

BAL5788 formulations produced with or without mannitol were noted to exhibit comparable toxicological profiles. No differences between BAL5788 manufactured with different synthesis procedures were seen in terms of the incidence or degree of changes observed at the injection site and in the kidneys. Local changes observed at the infusion site after the administration of “aged” (administered 30 hours after preparation) or “fresh” (administered immediately after preparation) BAL5788 solutions were comparable to those seen in previous studies, but very minimally increased effects at the infusion site were noted in rats administered the “aged” BAL5788 solution vs. rats given the “fresh” BAL5788 solution. The irritating potential of BAL5788 after single-dose subcutaneous, intramuscular, perivascular or intra-arterial administration was very low.

The impurity BAL6235 was found to be negative in the Ames assay, positive for *in vitro* chromosomal aberrations in the presence, but not in the absence of S9, and negative in the *in vivo* mouse micronucleus test. No mutagenic potential was seen for the impurities BAL1030 (Ames assay and forward mutation assay using Chinese hamster ovary cells) and BAL30056 (Ames assay), and an *in vivo* mouse micronucleus test for paranitrophenol (PNP) was also negative.

Administration of BAL5788 by subcutaneous injection to neonatal and juvenile rats at doses of 50, 100 and 250 mg/kg/day (corresponding doses of BAL9141 are 37.5, 75 and 187.5 mg/kg/day) for 50 days resulted in effects upon clinical condition, reduced body weight gain and local irritation at the injection sites at the highest dose administered. Effects in the urine at the end of the treatment period were attributed to excretion of the test article or a metabolite, and were no longer evident after a 4-week recovery period. Microscopic changes in the kidney were comparable to those seen in adults and were partially reversed in recovery animals. The NOAEL in juvenile rats was 100 (75) mg/kg/day.

No hemolysis or precipitation was observed in dog plasma at concentrations $\leq 1.25\%$. At higher concentrations, hemolysis, plasma turbidity and precipitation were observed, and were similar to increasingly adverse reactions (precipitation, flocculation and coagulation) seen in human, rat or marmoset plasma at concentrations ≥ 12.5 mg/mL.

In conclusion, the toxicity of BAL5788 in preclinical safety studies included effects on the

central nervous system (CNS) at high doses and rapid administration (i.e., convulsions), local infusion site reactions, renal toxicity related to drug precipitation due to the high concentration and low solubility of BAL9141 in urine, and vomiting, and could be managed by an appropriate infusion regimen. Signs of liver toxicity were not observed. Given the mechanism for the positive clastogenic effect, the absence of an *in vivo* correlate, the short duration of use, and the severity of infections that are targeted, BAL5788 is not considered to have relevant genotoxic potential. BAL5788 is neither phototoxic nor skin sensitizing. Antigenic potential, a characteristic of this class of compounds, was noted in guinea pigs only at high i.v. doses, or with s.c. doses in combination with adjuvant. No adverse effects on reproductive functions were observed.

Safety margins based on plasma exposures noted at the NOAEL in animals are ~1 to 5-fold for C_{max} and 2 to 3-fold for AUC for administration of 500 mg BAL9141 infused to humans over two hours as BAL5788. The safety margin based on plasma concentrations of BAL9141 seen at the NOEL for renal toxicity is 2 to 7-fold, whereas the exposure ratio based on urine concentrations of BAL9141 seen at the NOEL for renal toxicity is 4 to 34-fold. These safety margins are applicable to single- or multiple-dose administration, since drug accumulation is negligible with multiple dosing in humans.

A full environmental risk assessment (Phase II, Tier A) has been performed. BAL9141 was not found to represent a risk for the environment.

Intravenous bolus dosing was limited by high plasma C_{max} . The resultant high concentrations of BAL9141 in the urine, which is excreted mainly in the urine, led to drug precipitation in the distal parts of the nephron, and was associated with renal tissue damage characterized by degenerative changes in the distal and collecting tubules of the kidney in both rats (≥ 150 mg/kg) and marmosets (≥ 100 mg/kg) when BAL5788 was administered TID. Bolus administration of doses sufficiently high to provide a safety margin was therefore deemed inappropriate, and the non-clinical program was conducted using i.v. infusion dosing, which is consistent with the regimen proposed for clinical use. The administration of BAL5788 by infusion was associated with a larger safety margin in both the rat and the marmoset and was thus the chosen dosing method used during subsequent repeated-dose studies.

In the rat, the primary targets of toxicity were at the kidneys and the infusion site where local irritation and the presence of a yellowish-brown fibrinous material at the tip of the catheter were associated with thrombus formation. Abnormal urine colour (orange/red brown) was seen in all treated animals. In the kidneys, cytoplasmic inclusions were seen in the proximal tubules at ≥ 250 mg/kg/day, accompanied by slightly increased levels of urinary N-acetyl-beta-glucosaminidase (NAG). These changes were not associated with functional or other morphological changes and were reversible following a 4-week recovery period.

The urinary concentrations of BAL5788 were significantly lower than those of BAL9141. The urinary concentration of BAL9141 was highly variable in the 4-week study. In the 13-week study, this concentration was dose-related on the first day, but inversely proportional to the dose at week 13. This suggests that in the long term the excretion of BAL9141 is less efficient.

Deposition of drug-like material in the distal and collecting tubules was associated with renal toxicity (tubular dilation and necrosis, and in some animals with dilation of the renal pelvis and ureter). These effects were either partially or completely reversible following cessation of treatment in the 2- and 4-week studies. Complete recovery was not observed during the 13-week study, which is possibly due to the fact that following longer treatment periods, the precipitates will be large enough to obstruct several distal tubules and collecting duct and lead to irreversible damage of nephrons and loss of renal function. The NOAEL for

kidney toxicity in the 13-week study where BAL 5788 was administered once a day via intravenous infusion was 125 mg/kg/day. The exposures associated with this no-effect level corresponded to approximately 2-fold the clinical C_{max} .

Other treatment-related effects noted during the repeat dose studies were soft stool (at ≥ 125 mg/kg/day QD) and enlargement of the cecum (at 360 mg/kg/day BID). These changes most likely reflect pharmacological effects on microbial flora in the intestine. Incidences of mortality were noted in males at ≥ 250 mg/kg/day and were attributed to acute renal failure or thrombo-embolic changes in peripheral organs. Clinical findings of deteriorated physical condition and occasional clonic convulsions were observed in moribund males. At the site of injection, vascular irritation (thickening of the femoral vein), precipitation of drug-like material and associated thrombus formation were observed at ≥ 125 mg/kg QD in the 13-week study. Although these effects were also observed in the controls, this finding was more pronounced in ceftobiprole-treated animals.

In the 13-week dog study where BAL5788 was administered as an i.v. infusion (2-h), clogging of the surgically implanted cannula resulted in an inability to dose and was the primary cause of the premature sacrifice of 5 out of 6 dogs at the 32 mg/kg dose. The exact cause of the clogging and subsequent dosing problems in the dog was not entirely clear. No other findings of toxicological importance were observed in these 5 animals, which were treated for up to 12 weeks. The applicant set out to establish whether there could be a common cause behind the findings at the injection/infusion site in the rat and the marmoset and dosing problems (clogging) in the dog. Thorough evaluations were conducted at the infusion site and in potential target organs for thrombo-embolic effects.

In the 2-and 13-week dog studies, eosinophilic droplets in the proximal tubular epithelium were seen at ≥ 50 mg/kg/day and this was not considered to be toxicologically significant. In both studies, no drug precipitates were seen in the kidneys up to the highest doses of 30 and 100 mg/kg/day, respectively (note: that this was observed in the rat only). This is an unexpected finding in view of the fact that, in the dog, the concentration of BAL9141 in urine (between ± 6000 and ± 11000 $\mu\text{g/ml}$) were within the same range as those measured in the rat (between 4000 and 14000 $\mu\text{g/ml}$). These data suggest that the formation of drug precipitation in the kidney is not only dependent on the urinary concentration of the drug but is also dependent on the species.

In the marmoset following repeated intravenous dosing of BAL5788, brown pigment in the cytoplasm and lumen of cortical tubules was observed and this was reversible upon cessation of treatment. Pigment deposition in the distal and collecting tubules was observed at doses of ≥ 175 mg/kg BID and was accompanied by tubular damage and, on occasion inflammation of the renal interstitium. During the pivotal 13-week study, the reversible tubule pigmentation was not accompanied by microscopic or biochemical correlates of renal toxicity.

Signs of vascular irritation at the injection site (thickening of the femoral vein) were observed in control and treated animals and this finding was consistent with that observed in the rat; however in the treated group it was somewhat dose-dependent and was associated with necrosis. In the marmoset, vascular changes at the injection site in the treated animals were more severe than that observed in controls, and included an increased incidence of thrombosis and necrosis, indicating that the test article was irritating to the eroded vein wall or exacerbated the irritating effect of the catheter on the wall of the vena cava. In marmosets, the released thrombo-emboli at the injection site lodged within vessels distal to the infusion site (predominantly pulmonary) and were considered to be responsible for 10 of 11 early mortalities observed during the 13-week study. The NOEL for the 13-week study is therefore

<50 mg/kg/day, due to the observed necrosis and thrombosis at the infusion site.

BAL9141 is the active compound and main metabolite of BAL5788 in the urine. The administration of BAL5788 was associated with findings in the kidneys including discoloured, enlarged and roughened surface, and multiple yellow foci and by microscopic findings showing the presence of drug-related material in the renal tubular system. The retention of drug-like material in the proximal tubular cells is not considered to be toxicologically relevant, as no remarkable adverse effects were observed, even with very high doses or prolonged exposures.

The precipitation of drug-like material in the distal parts of the nephron was associated with renal tissue damage and may lead to impairment of renal function. The observed effects included dilatation, hyaline casts, and degeneration and/or necrosis of the distal tubules and collecting ducts and/or dilatation of the renal pelvis. These effects were more pronounced for a bolus dose as compared to a 2-hour infusion and showed reversibility after cessation of dosing, indicating a relationship to C_{max} in plasma and urine.

Xenobiotics of low solubility have a tendency to precipitate in the distal nephron, where the urine reaches its greatest concentration. Following administration of BAL5788, the deposition of drug-like material in the distal part of the nephron has been observed in rats and marmosets, but not in dogs. Based on the NOAELs for these kidney findings, safety margins of 2-7 based on plasma exposures and 4-34 based on urine exposures were calculated as compared to the recommended therapeutic dose of 500 mg administered as a 2-h infusion. Given that the safety margins with respect to renal toxicity are somewhat low, the risk of renal toxicity has been specified as an important identified risk within the risk management plan; which is acceptable.

Convulsions in mice were observed following a single dose at 250 mg/kg, where the associated exposure was 6-fold higher than that observed clinically (based on C_{max}). At 125 mg/kg, the NOEL for convulsions in mice, estimated exposure was slightly higher than the human exposure. Convulsions were seen in the marmoset following repeated dosing at low doses i.e. ≥ 50 mg/kg/day for up to 13 weeks and at higher doses where the associated exposures are ~ 10 -fold the clinical C_{max} for up to 2 weeks. Convulsions have also been observed following repeated administration in the rat.

The occurrence of convulsions is an effect that should be considered in man, as the potential for convulsions is comparable to that of imipenem. The applicant has included relevant warnings within the SmPC and convulsions have been specified as an important identified risk within the risk management plan, which is acceptable.

Infusion site-related problems, necrosis and thrombus formation, were observed in rats and marmosets. In dogs, these effects were observed to a lesser extent and not dose-related. There were treatment-related problems in the dog, whereby at the maximum dose evaluated, clogging of the cannula caused a number of dogs to be euthanized (13-week study). However infusion site reactions and the subsequent events were not observed in manner that was comparable to that observed in the smaller non-clinical species. Overall, the dog is a more robust species for chronic i.v. infusion studies than the rat or marmoset and is considered more relevant to humans (as the blood vessels are of a larger diameter), which suggests that these effects should not occur in humans.

Moreover, it is likely that the high concentrations of the solutions used played a significant contribution to all of these findings as lower concentrations of BAL5788 that were similar to

those to be administered clinically did not cause any signs of irritancy at the site of injection. It is therefore agreed that overall, the potential for infusion site reactions in man is considered to be low.

A direct comparison of C_{max} value was not always possible as different dosing regimens have been used (e.g. iv bolus versus iv infusions of variable durations). C_{max} (measured at the same time after dosing) increased generally approximately proportional to dose. In some instances and only at high doses [e.g. 200 mg/kg in marmoset (BAP00045)] the C_{max} did not increase in a manner that was proportional with or related to dose.

In most studies, C_{max} and AUC did not change upon repeated dosing. However, in some studies C_{max} values in the marmoset and dog decreased substantially (factor 2 to 4) after repeated dosing over the course of 2 or 4 weeks. In other studies C_{max} and AUC seemed to increase with repeated dosing by up to a factor of 2. No gender differences in pharmacokinetics were observed for rat, dog, and marmoset.

Although no potential to cause genotoxicity was seen in the Ames assay, cytotoxicity was observed at very low concentrations, which is due to the fact that ceftobiprole is an antibiotic. Hence in this instance, the Ames test is considered to be less suitable. In the mouse lymphoma assays, mainly small colony mutants were observed, but also, at higher concentrations, large colony mutants, which are indicative for point mutations. Although these large colony mutants were only observed at cytotoxic concentrations, the fact remains that even though quite a number of genotoxicity assays has been performed, because of the Ames test being less suitable and the equivocal assay regarding large colonies in the mouse lymphoma assay, a clearcut negative result of a suitable gene mutation test was deemed necessary. Hence, a different in vitro gene mutation assay based on mammalian cells was also provided and BAL5788 was considered to be non-mutagenic in this assay.

The results of the in vitro chromosome aberration assay indicate that the explanation of the Applicant that the clastogenicity of BAL5788 is caused by the diacetyl group which is then split off to form BAL9141, is plausible: BAL5788 and diacetyl were mutagenic and while BAL9141 was considered not be mutagenic. However, the results of a mouse lymphoma assay cast some doubt on this aspect, as BAL9141 increased the frequency of chromosomal aberrations; this was observed mainly at cytotoxic concentrations but also once at a non-cytotoxic concentration. However, the results from two in vivo studies with BAL5788 (micronucleus and unscheduled DNA synthesis tests) suggest that, overall the potential to cause genotoxicity is low. Regarding the diacetyl moiety, data from the literature was provided to demonstrate that in vivo, this molecule is rapidly reduced to non-mutagenic substances; the data and discussion provided is deemed acceptable.

At the doses evaluated, BAL5788 was neither teratogenic nor embryotoxic in rats, nor teratogenic in monkeys. In the monkey, abortions were observed at the high dose which could possibly be treatment-related; however this is not certain an abortion and an embryonic death were also observed in the control group. At the maximum dose evaluated, the C_{max} of BAL9141 was 243.1 µg/ml or 7-fold the proposed clinical C_{max} and it is important to note that the C_{max} at the no-effect level was 4-fold higher than that proposed clinically.

A slight increase in gestation length was observed in F0 animals at 360 mg/kg/day, the maximum dose evaluated. In F1 pups, litter size and survival up to 4 days post partum was decreased at 360 mg/kg/day.

Dilated cecum, observed in F0 and F1 animals is likely due to the effect of an antibiotic on

the intestinal bacteria. Hydronephrosis, as observed in F0 animals at doses ≥ 175 mg/kg is in line with the renal toxicity as observed in the repeated dose studies.

The findings from the juvenile toxicity studies will not be discussed at length as this application is to support administration of the proposed product to adults only. However, it is noted that following repeated subcutaneous administration of BAL5788 the findings were generally comparable to that observed in adult animals and the NOAEL for BAL5788 was considered to be 100 mg/kg/day (i.e. 75 mg/kg/day for BAL9141).

Generally, no irritation was observed after intravenous, subcutaneous, intramuscular, perivascular and intra-arterial administration of solutions containing BAL5788 at concentrations of 2 mg/mL, when compared to vehicle and/or saline. Slightly more pronounced signs of irritation were observed (e.g. inflammatory cell infiltration, erythema and edema (although less pronounced than dextrose vehicle) following administration of 10 mg/mL.

Given that the proposed clinical formulations to be administered to patients will contain 2 mg/mL of the active moiety, despite the findings noted at 10 mg/mL and the findings noted in the repeated-dose studies, where significantly higher concentrations of BAL5788 were administered, the potential to cause irritancy at the injection site is considered to be low.

The positive ASA reaction only being present in the high dose groups receiving BAL5788 intravenously or subcutaneously along with adjuvant, and the absence of passive cutaneous anaphylactic reaction suggest that the antigenicity of BAL5788 was not severe. BAL5788 is a compound of the cephalosporin antibiotic class; antibiotics of this class are known to possess antigenic properties.

The genotoxic evaluation of impurities revealed that the specified impurities have no genotoxic potential.

Based on the maximum dose of 1500 mg per day, the proposed limits for all specified impurities within the drug substance have been qualified at levels above those proposed via the applicant, with respect to their potential to cause general toxicity. The proposed impurity limits for the drug product are also considered to be acceptable from a toxicological point of view.

No hemolysis or precipitation was observed in dog blood or plasma at concentrations ≤ 16.62 mg/mL, however, hemolysis and plasma turbidity and precipitation were noted at concentrations ≥ 33.25 mg/mL. No hemolysis or precipitation was observed in human, rat or marmoset blood or plasma at concentrations ≤ 5 mg/mL, but increasingly adverse reactions (precipitation, flocculation and coagulation) were seen in human, rat and marmoset plasma at concentrations ≥ 12.5 mg/mL. Since human C_{max} is 33 $\mu\text{g/mL}$, the safety margin for blood hemolysis or precipitation is adequate.

No phototoxic potential was demonstrated by BAL9141 in vitro or BAL5788 in vivo.

Overall conclusion on toxicology

No single-dose toxicity studies were conducted. In three-day pilot studies in rats, i.v. bolus administration of ≥ 150 mg/kg (TID) (i.e. 450 mg/kg/day) of BAL5788 led to drug precipitation in the distal parts of the nephron, which was associated with damage in the distal part of the nephron. In contrast, doses up to 250 mg/kg given via intermittent infusion

(4 h, BID) (i.e. 500 mg/kg/day) were well tolerated without signs of renal damage. Administration via intermittent infusion seems therefore less toxic than via bolus injection. Three-day pilot studies in marmosets confirmed these findings.

In repeat-dose toxicity studies (up to 13 weeks in rats, dogs and marmosets), main observed effects were renal toxicity, convulsions and infusion site irritation associated with thrombus formation.

Findings in the kidney indicated the presence of drug-related material in the renal tubular system. The retention of drug-like material in the proximal tubular cells is not considered to be toxicologically relevant, since it did not cause remarkable adverse effects. The precipitation of drug-like material in the distal parts of the nephron was associated with renal tissue damage in rats and marmosets and may lead to impairment of renal function. The observed effects included dilatation, hyaline cast, and degeneration and/or necrosis of the distal tubules and collecting ducts and/or dilatation of the renal pelvis. These effects were more pronounced for a bolus dose as compared to a 2h infusions and showed reversibility after cessation of dosing. Based on the NOAEL's for these kidney findings, safety margins of 2-7 based on plasma exposures and 4-34 based on urine exposures were calculated as compared to the recommended therapeutic exposure of 500 mg dose administered as a 2-h infusion.

Convulsions were observed in marmosets (and in mice, see section on Pharmacology). After 2-week administration, convulsions were observed at the high dose of 360 mg/kg/BID (10-fold higher than the human exposure based on C_{max}). After 13-week administration, convulsions were also observed at the low dose of 50 mg/kg/day, probably due to poor condition of the animals.

Infusion site-related problems, necrosis and thrombus formation, leading to pulmonary thrombosis, were observed in rats and marmosets. Although there were treatment-related problems with dosing in the dog, in this species, infusion site irritation was observed to a lesser extent and not dose-related. The infusion site problems observed in the non-clinical studies are of low clinical relevance as the veins are smaller and the indwelling catheters used for administration in animals are larger than that in man. Moreover, during the repeated dose studies, the test article was administered for up to 13 weeks in animals compared to administration for 7-14 days in humans, and the concentrations of infusate in the non-clinical studies was higher.

The results of the Ames test suggest that BAL5788 is not genotoxic; however, cytotoxicity was observed at very low concentrations, (due to the fact that ceftobiprole is an antibiotic), which in turn limited the maximum concentrations evaluated. In a mouse lymphoma assay, large colony mutants were observed at high, cytotoxic doses. Mouse lymphoma assays and in vitro chromosome aberration assays indicated clastogenicity of BAL5788, which may have been caused by the diacetyl group which is split off from BAL5788 to form BAL9141, since diacetyl also tested positive in these tests. Literature showed that diacetyl, an endogenous molecule, in vivo is rapidly reduced to non-mutagenic substances. BAL9141 scored negative in the in vitro chromosome aberration assay and positive in the mouse lymphoma assay. However, the results of an in vitro gene mutation assay (in mammalian cells) suggest that the BAL5788 is not genotoxic. In addition, two in vivo assays, the mouse micronucleus test and the unscheduled DNA synthesis suggest that BAL5788 is not genotoxic; therefore, overall the result for potential for this molecule to cause genotoxicity is considered to be low.

Carcinogenicity studies were not conducted based on the intended short-term clinical

duration of therapy, 7 - 10 days for the majority of patients, and because results of the genotoxicity testing showed a low potential for genotoxicity. This approach is acceptable.

BAL5788 had no effects on fertility and early embryonic development in rats. BAL5788 was neither teratogenic nor embryotoxic in rats, nor teratogenic in monkeys. In monkeys at the maximum dose tested (360 mg/kg/day), abortions were observed which could be treatment-related; however this is not certain since spontaneous abortion and an embryonic death were observed in the control group. The safety margin for this effect was 4 (based on C_{max}). In a pre-and postnatal toxicity study in rats, a slight increase in gestation length was observed in F0 animals at the high dose. In F1 pups, litter size and survival up to 4 days post partum were decreased at the high dose. No effects were observed in F2 pups. The toxicity profile in juvenile rats was similar to that observed in adult animals.

In local tolerance studies in rabbits, when compared to vehicle, no significant irritation was observed after intravenous administration and administration via other parenteral routes at concentrations of 2 mg/mL. Some signs of irritancy were noted following injection of 10 mg/mL which is in excess of the concentration to be injected clinically. No hemolysis or precipitation in blood occurred at concentrations ≤ 16.62 mg/ml (blood from dogs) or ≤ 5 mg/ml (blood from rats, marmosets and humans); given that the proposed C_{max} is 33 µg/ml, this safety margin is deemed acceptable.

BAL5788 showed potential for skin sensitization in the active systemic anaphylaxis test in guinea pigs. This is consistent with findings for other cephalosporins. A Maximization Test and a passive anaphylactic assay, both in guinea pigs, were negative.

No genotoxic potential was seen for the specific impurities.

BAL9141 absorbs light between 240 and 400 nm. An in vitro test in mouse fibroblasts and an in vivo test in rats revealed no potential to cause phototoxicity.

The non-clinical expert report has been written by an appropriately qualified person and is a suitable summary of the non-clinical aspects of the dossier.

The applicant has conducted a full environmental risk assessment (ERA). This is satisfactory.

There are no objections to the approval of this product from a non-clinical point of view.

III.3 CLINICAL ASPECTS

Ceftobiprole medocaril (BAL5788-001) is the water soluble pro-drug of the active drug moiety, ceftobiprole (BAL9141-000). The drug product is a sterile lyophilised powder for reconstitution for intravenous infusion.

Pharmacokinetics

Two formulations were used in the clinical Phase 1, 2, and 3 studies. Both of these formulations (designated as Phase 2 and Phase 3 formulations) were used in the studies summarised in this submission. A third formulation, for use in studies initiated after September 2006 and identified as the 'Commercial formulation', was not used in any of the studies. Differences between the compositions of these formulations include changes in the amount of citric acid, the pH and the presence of mannitol. All three formulations are solutions for intravenous administration after reconstitution, therefore, according to the applicant no clinically relevant differences in pharmacokinetics between formulations should be expected.

Twenty-one clinical pharmacology studies in healthy subjects and patients in all were conducted by J&JPRD and Basilea, and one exploratory study was conducted by F. Hoffmann-La Roche Ltd. In addition, pharmacokinetic and pharmacokinetic/pharmacodynamic assessments were conducted in subjects with cSSTI in one Phase 2 study and in two Phase 3 studies (subjects with nosocomial pneumonia and in subjects with CAP). Six pharmacokinetic studies in healthy subjects and six studies in special populations (renally impaired, ESRD, gender, obese, ICU patients and paediatric subjects) were conducted.

Table 2 Clinical pharmacology studies of ceftobiprole

Protocol Study Title Section in this Clinical SummaryCTD Chapter 2.7.2 / CSR location	Objectives	Study design	Dose regimen	No. of subjects
Phase 1 Studies in healthy subjects				
NP16104 An Early Viability Test of BAL5788 (Pro-drug of BAL9141, A New Parenteral, Broad Spectrum Cephalosporin Antibiotic For Severe Hospital Infections) in Healthy Male Volunteers. Section 2.1.1 / 5.3.3.1	To assess the PK and safety after single dose administration	Single-center, open-label, intra-subject, single-dose ascending study	30-min i.v. infusion of 250 mg, 500 mg, and 750 mg of BAL9141 (given as BAL5788)	M = 3
BAP00006 * Assessment of the Pharmacokinetics and Safety of BAL9141 After Single Ascending Dose Infusions of BAL5788 in Healthy Male Volunteers Section 2.1.2 / 5.3.3.1 (including Errata)	To assess the PK and safety after single dose administration	Single-center, double-blind, randomized, placebo-controlled, single ascending dose study	30-min i.v. infusion of 125, 250, 500, 750, or 1000 mg of BAL9141 (given as BAL5788) or placebo	M = 40 (6 active and 2 placebo per dose level)
BAP00010 * Assessment of the Pharmacokinetics and Safety of BAL9141 After Multiple Ascending Dose Infusions of Its Pro-drug BAL5788 in Healthy Male Volunteers Section 2.1.3 / 5.3.3.1 (including Errata)	To assess the PK and safety after multiple dose administration	Single-center, double-blind, randomized, placebo-controlled, parallel-group, multiple-dose study of two regimens	30-min i.v. infusion of 500 mg or 750 mg q12h of BAL9141 (given as BAL5788) or placebo for 8 days	M = 16 (6 active and 2 placebo per dose level)
Phase 2 and 3 Studies in healthy subjects and patients				
Protocol Study Title Section in this Clinical SummaryCTD Chapter 2.7.2 / CSR location	Objectives	Study design	Dose regimen	No. of subjects
BAP00210 Assessment of the Tolerability and Pharmacokinetics of Two Different Dosing Regimens of BAL5788 in Healthy Subjects. Section 2.1.4 / 5.3.3.1 (including Errata)	To assess the PK, PD, and safety of BAL5788 with 2 infusion regimens	Single-center, open-label, randomized, single-dose, parallel-group study	i.v. infusions of 500 mg BAL9141 (given as BAL5788) given over 60 min, and 750 mg given over 30 min	M = 18 F = 18
BAP00058 * Safety and Pharmacokinetics of Multiple Infusions of BAL5788 in Healthy Male Volunteers Section 2.1.5 / 5.3.3.1 (including Errata)	To assess the PK and safety after multiple dose administration	Single-center, double-blind, randomized, placebo-controlled, multiple-dose study	30-min i.v. infusion of 750 mg q12h of BAL9141 (given as BAL5788) or placebo for 12 days	M = 12 (6 on active and 6 on placebo)
BAP00393 * Safety and Pharmacokinetics of Multiple Infusions With 1g BAL9141 Administered as BAL5788 in Healthy Male Volunteers Section 2.1.6 / 5.3.3.1 (including Errata)	To investigate tolerability and to assess the PK	Double-blind, randomized, placebo-controlled, multiple-dose study	Day 1 and Day 8: 90-min i.v. infusion 1 g BAL9141 q.d. given as BAL5788; Days 2-7: 90-min infusion 1g BAL9141 q8h	M = 12 (6 active + 6 placebo)
CSI-1004 * An Open-Label, Pharmacokinetic Study of Single and Multiple Intravenous Infusions of Ceftobiprole 500 mg Administered to Healthy, Adult Subjects Section 2.1.7 / 5.3.3.1	To assess the PK and safety after single and multiple dose administration	Single-center, open-label, single- and multiple-dose PK study	Single 2-hour i.v. infusion of 500 mg BAL9141 (given as BAL5788) on Days 1 and 5 and q8h on Days 2-4	M = 14 F = 14

Protocol Study Title Section in this Clinical SummaryCTD Chapter 2.7.2 / CSR location	Objectives	Study design	Dose regimen	No. of subjects
CSI-1005 An Exploratory, Open-Label Study to Assess the Bronchoalveolar Distribution and Pharmacokinetics of Ceftobiprole Administered as 500 mg Every 8 Hours to Healthy, Adult Subjects Section 2.1.8 / 5.3.4.1	To assess the bronchoalveolar distribution and construct the intrapulmonary concentration-time profile of BAL9141	Single-center, open-label, multiple-dose study	2-hour i.v. infusions of 500 mg BAL9141 (given as BAL5788) q8h for 4 doses	M = 16 F = 12 (28 active + 2 control) (25 evaluable for plasma PK 24 evaluable for BAL)
CSI-1002 An exploratory Study to Evaluate the Penetration of Ceftobiprole into Soft Tissue Determined by In Vivo Microdialysis in Healthy subjects Section 2.1.9 / 5.3.4.1	To assess the muscle and adipose distribution of BAL9141	Single-center, open-label, single-dose	2-hour i.v. infusion of 500 mg BAL9141 (given as BAL5788)	Pilot study M=3 Main study M=6, F=6
CEFTO-PED-1001 Open-Label, Pharmacokinetic Study of the Penetration of Ceftobiprole into Bone Section 2.1.10 / 5.3.4.1	To assess the bone distribution of BAL9141 in healthy subject undergoing total hip replacement surgery	Multi-centers, open-label, single-dose	2-hour i.v. infusion of 500 mg BAL9141 (given as BAL5788)	Control: M=1, F=1 Verum: M=10, F=9

In all of the studies presented, the drug was infused as the prodrug ceftobiprole medocartil. The dose amount refers to the active substance ceftobiprole.

Methods

Analytical methods

The plasma calibration curve for the HPLC-UV assay, ranged from 0.1 to 100 µg/mL for ceftobiprole and 0.25 to 100 µg/mL for ceftobiprole medocartil. The urine calibration curve for the HPLC-UV assay, ranged from 2 to 2,500 µg/mL for ceftobiprole and a semi-quantitative approach for the quantification of open-ring metabolite from 5 to 100 µg/mL. Overall the assays performed well within the required precision and accuracy specified in the respective standard operating procedures (SOPs) at the various contract research organisations (CROs) at the time of study conduct.

Pharmacokinetic data analysis

Pharmacokinetic variables, e.g. AUC_{0-t} , AUC_{inf} , AUC_t , C_{max} , $t_{1/2}$, Cl and CL_r were calculated according to standard procedures.

Absorption

Ceftobiprole is for intravenous administration only. Plasma exposure is described below

Study **NP16104** was a single-centre, single-dose, open-label, dose-escalation pilot study in 3 healthy male volunteers to determine the pharmacokinetics of ceftobiprole after 30-minute intravenous infusions of 250 mg, 500 mg, and 750 mg ceftobiprole.

Mean plasma concentrations of ceftobiprole medocartil were very low (0.83 µg/mL, 1.74 µg/mL, and 1.01 µg/mL, at the end of infusion for the 250 mg, 500 mg, and 750 mg doses, respectively). The results for ceftobiprole are displayed below.

Table 3 Mean (SD) ceftobiprole pharmacokinetic parameters following single ascending intravenous 30-minute infusions of ceftobiprole at 250, 500, and 750 mg in healthy subjects (study NP16104; N=3)				
Parameter	250 mg	500 mg	750 mg	
C_{max} ($\mu\text{g/mL}$)	24.1 (3.39)	42.7 (11.6)	62.2 (4.16)	
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$)	48.7 (2.99)	103 (10.8)	135 (8.84)	
$t_{1/2\alpha}$ (h) ^a	0.45 (0.13)	1.07 (0.68)	0.43 (0.20)	
$t_{1/2\beta}$ (h)	2.90 (0.24)	3.16 (0.17)	3.37 (0.35)	
V_{ss} (L)	14.9 (1.98)	15.4 (2.39)	17.6 (1.27)	
CL_S (L/h)	5.14 (0.33)	4.89 (0.52)	5.57 (0.35)	
CL_R (L/h)	3.01 (1.09)	2.57 (0.49)	2.91 (0.36)	
A_e , % of dose	59.6 (24.1)	53.6 (14.5)	52.8 (10.0)	

Study **BAP00006** was a single-center, randomized, double-blind, placebo-controlled, single-ascending-dose pharmacokinetic study in 40 healthy male volunteers. There were 8 subjects per dose group (6 active + 2 placebos). Each subject received a single 30-minute infusion of ceftobiprole 125, 250, 500, 750, 1000 mg, or placebo (5% dextrose). Systemic exposure (C_{max} and $AUC_{0-\infty}$) of ceftobiprole increased in proportion with dose.

Figure 2 Mean plasma ceftobiprole concentration-time profiles following single ascending intravenous 30-minute infusions of ceftobiprole at 125, 250, 500, 750, or 1000 mg in healthy subjects (study BAP00006)

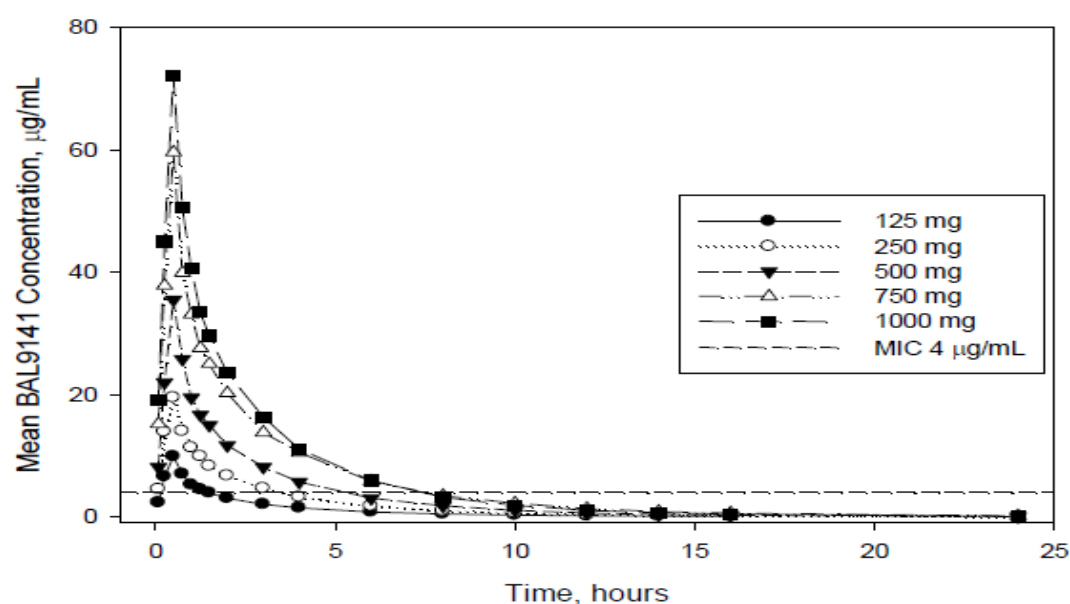


Table 4 Mean (SD) ceftobiprole pharmacokinetic parameters following single ascending intravenous 30-minute infusions of ceftobiprole at 125, 250, 500, 750, or 1000 mg in healthy subjects (study BAP00006; N=6)					
Parameter	125 mg	250 mg	500 mg ^a	750 mg	1000 mg
C_{max} ($\mu\text{g/mL}$)	9.87 (0.78)	19.5 (2.75)	35.5 (6.79)	59.6 (10.7)	72.2 (8.78)
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$)	20.3 (2.82)	43.7 (5.99)	76.6 (3.88)	135 (27.6)	151 (9.04)
$t_{1/2\alpha}$ (h) ^b	0.37 (0.14)	0.47 (0.15)	0.57 (0.22)	0.48 (0.27)	0.56 (0.36)
$t_{1/2\beta}$ (h)	2.84 (0.21)	3.42 (0.29)	3.44 (0.30)	3.47 (0.37)	3.25 (0.20)
V_{ss} (L)	17.9 (2.00)	17.8 (3.11)	19.8 (1.95)	18.4 (2.63)	18.9 (2.31)
CL_S (L/h)	6.27 (0.97)	5.81 (0.84)	6.54 (0.34)	5.74 (1.13)	6.64 (0.41)
CL_R (L/h)	4.80 (0.65) ^c	4.35 (0.57)	5.07 (0.22)	4.08 (0.75)	4.16 (0.57)
A_e , %	77.0 (8.11) ^c	76.1 (14.4)	77.5 (3.27)	71.3 (1.48)	62.5 (6.33)

^a N=5
^b obtained by 2-compartmental analysis
^c Urine volume was incorrect in original study report. These values were recalculated based on corrected volumes

Study **BAP00010** was a single-center, randomized, double-blind, placebo-controlled, multiple-ascending-dose pharmacokinetic study in 16 healthy male volunteers. There were 8 subjects per dose group (6 active + 2 placebos). Each subject received a single 30-minute infusion of ceftobiprole (500 mg or 750 mg) or placebo (5% dextrose) on Days 1 and 8, and multiple infusions (q12h) on Days 2-7. Plasma concentrations of ceftobiprole medocaril were very low and only measurable during the infusion.

Mean plasma ceftobiprole concentration-time profiles following single and multiple 30-minute intravenous infusions of ceftobiprole are presented in the figure below.

Trough concentrations of ceftobiprole collected on Days 3 through 8 (mean ranged from 0.976 µg/mL to 1.27 µg/mL and from 1.61 µg/mL to 1.72 µg/mL during 500 mg and 50 mg dosing, respectively) remained consistent during repeated dose administrations, such that steady-state was achieved by Day 3.

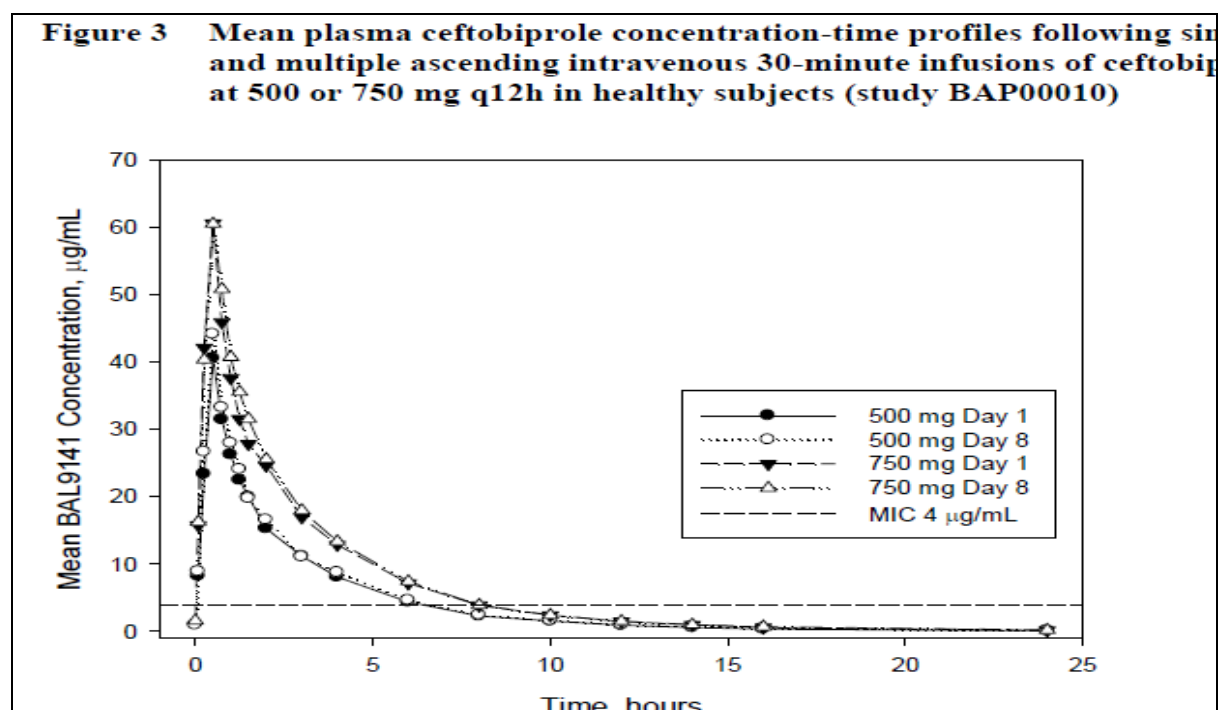


Table 5 Mean (SD) ceftobiprole pharmacokinetic parameters following single and multiple ascending intravenous 30-minute infusions of ceftobiprole at 500 or 750 mg q12h in healthy subjects (study BAP00010; N=6)

Parameter	500 mg Day 1	500 mg Day 8	750 mg Day 1	750 mg Day 8
C_{max} (µg/mL)	40.6 (7.38)	44.2 (10.8)	60.7 (4.55)	60.6 (9.99)
$AUC_{0-\infty}$ (µg·h/mL)	101 (9.04)	108 (22.2)	156 (19.3)	165 (12.8)
AUC_{0-12} (µg·h/mL)	95.9 (7.59)	102 (20.0)	148 (17.6)	156 (11.1)
$t_{1/2\alpha}$ (h) ^a	1.06 (0.46)	1.03 (0.42)	0.96 (0.59)	0.99 (0.50)
$t_{1/2\beta}$ (h)	3.63 (0.48)	4.04 (0.31)	3.64 (0.32)	4.11 (0.41)
V_{SS} (L)	16.4 (2.11)	16.7 (3.58)	16.3 (1.82)	16.1 (2.20)
CL_S (L/h)	4.99 (0.46)	5.06 (0.95)	4.85 (0.57)	4.83 (0.34)
CL_R (L/h)	4.12 (0.75)	4.47 (1.07)	4.05 (0.47)	4.08 (0.59)
accRatio ^b	NA	1.06 (0.17)	NA	1.06 (0.08)
A_e , % ^c	82.2 (9.11)	87.8 (9.30)	83.7 (3.91)	84.4 (9.23)

^a Value obtained by 2-compartmental analysis

^b AUC_{0-12} (Day 8)/ AUC_{0-12} (Day 1)

^c 0-24 h recovery on Day 1 and 0-12 h recovery on Day 8.

NA = not applicable

Definitive studies (FK5789, FK6157)

In the first study, human plasma was spiked with ceftobiprole to provide concentrations of 0.5, 5, 25, and 100 µg/mL (5 replicates per concentration). Plasma samples were incubated at 37°C for 15 minutes, ultra-centrifuged, and then analyzed for ceftobiprole using LC-MS/MS. The mean (SD) percent binding to plasma protein was 17.3 (4.58)% (FK5789). Binding was not significantly affected by altering protein concentrations. This experiment was repeated with similar design, where ceftobiprole concentrations of 3.5 and 35 µg/mL were evaluated as the clinically relevant concentrations in humans associated with the 500 mg dose, and the protein binding was 15.4 (3.7)% (FK6157). Given the reproducibility of these results and rigor of the design of the latter two experiments, the protein binding of ceftobiprole is considered to be 16% and is independent of ceftobiprole concentration across the range of 0.5 to 100 µg/mL.

Based on the definitive studies, ceftobiprole at a concentration of 25 µg/mL (12.5 µM) is primarily bound to albumin (6.5% to 11.5%, i.e. 0.8 to 1.4 µM bound), and also bound to α-1 acid glycoprotein (4.8% to 6.8%, i.e. 0.6 to 0.9 µM bound) (FK5789). Binding of ceftobiprole to both proteins is low compared to their normal molar concentrations (albumin 530-760 µM; α-1 acid glycoprotein 10-40 µM).

When pooled across studies, the median (range) estimated ceftobiprole VSS in healthy subjects from non-compartmental analysis was around 18 L (the average per study ranged from 11.0 to 27.2 L), approximately equal to the extra-cellular fluid volume in humans (range 9 to 21 L).

Distribution volume of ceftobiprole was influenced by sex, where estimates of volume of distribution (Vd) were 29% lower in females compared to males (BAP00036 and CSI-1004) and body weight, where estimates of Vd were 26% higher in morbidly obese compared to non-obese subjects (CSI-1008). These differences correlated with the body weight.

Elimination

Excretion

The main route of elimination of ceftobiprole is by renal excretion, with mean urinary recoveries of 53% to 99% in healthy subjects in various dose groups across the Phase 1 studies with a mean recovery of 76%. Following single dose administration, approximately 89% of the dose was excreted in the urine as the pro-drug, unchanged ceftobiprole, and the open-ring metabolite. The urinary recovery of both ceftobiprole and the open-ring metabolite were confirmed in subsequent Phase 1 studies.

Ceftobiprole is rapidly eliminated from plasma in healthy subjects. Mean $t_{1/2}$ for individual studies ranged from 2.4 to 4.1 hours, with an overall mean of approximately 3.0 hours that is independent of the dose (125 mg, 250 mg, 500 mg, 750 mg, or 1000 mg), infusion duration (30, 60, 90 or 120 minutes) and dosing interval (8 or 12 hours). Mean total systemic clearance for individual studies ranged from 4.18 to 7.50 L/h, covering the typical estimated clearance (5.36 L/h) of a healthy subject weighing 75 kg with a CLCR of 120 ml/min). These pharmacokinetic parameters were similar following single- and multiple-doses.

Across all studies, the mean renal clearance (CLR) corrected for the free fraction (0.84) ranged on average from 2.91 to 5.11 L/h and was less than the typical glomerular filtration rate (125 mL/min), suggesting that ceftobiprole is eliminated predominantly by passive glomerular filtration. The lack of effect of concomitant administration of probenecid on the clearance of ceftobiprole in rats supports the absence of active renal secretion of ceftobiprole.

The elimination parameters of ceftobiprole from healthy subjects were pooled across studies after both single- and multiple-dose administration and are presented in Table 50 and Table

51, respectively.

Table 50 Mean (SD) elimination pharmacokinetic parameters for ceftobiprole and its open-ring metabolite following single intravenous infusions of ceftobiprole

Ceftobiprole							
Dose Regimen Study	Dose (mg)	Infusion Duration	N	t _{1/2} (h)	CL _S (L/h)	CL _R (L/h)	Ae (% , Dose)
Single Dose							
BAP00006	125	0.5h	6	2.84 (0.21)	6.27 (0.97)	4.80 (0.65)	77.0 (8.11)
NP16104	250	0.5h	3	2.90 (0.24)	5.14 (0.33)	3.01 (1.09)	59.6 (24.1)
BAP00006	250	0.5h	6	3.42 (0.29)	5.81 (0.84)	4.35 (0.57)	76.1 (14.1)
BAP00018	250	0.5h	5	3.45 (0.37)	4.80 (0.61)	4.38 (0.51)	91.6 (6.55)
CSI-1007	250	2h	6	3.00 (0.40)	5.62 (0.73)	5.11 (0.81)	88.6 (4.06)
JPN-01 (Japanese)	250	2h	8	2.81 (0.39)	4.08 (0.47)	3.97 (0.69)	97.1 (7.65)
NP16104	500	0.5h	3	3.16 (0.17)	4.89 (0.52)	2.57 (0.49)	53.6 (14.5)
BAP00006	500	0.5h	5	3.44 (0.30)	6.54 (0.34)	5.07 (0.22)	77.5 (3.27)
BAP00010	500	0.5h	6	3.63 (0.48)	4.99 (0.46)	4.12 (0.75)	82.2 (9.11)
BAP00210	500	1h	18	2.85 (0.55)	4.46 (0.84)	NC	NC
CSI-1003	500	2h	57	3.0 (0.5)	5.50 (1.00)	NC	NC
CSI-1004	500	2h	28	3.1 (0.3)	4.89 (0.69)	4.08 (0.72)	83.1 (9.06)
CSI-1002	500	2h	12	2.61 (0.33)	5.15 (0.53)	NC	NC
CSI-1008	500	2h	13	3.21 (0.47)	4.65 (0.65)	4.08 (0.67)	87.3 (7.33)
CSI-1008 (obese)	500	2h	11	3.38 (0.26)	5.58 (0.75)	4.72 (0.74)	82.9 (5.70)
JPN-01 (Japanese)	500	2h	8	3.01 (0.24)	4.10 (0.35)	4.08 (0.50)	99.9 (7.65)
NP16104	750	0.5h	3	3.37 (0.35)	5.57 (0.35)	2.91 (0.36)	52.8 (10.0)
BAP00006	750	0.5h	6	3.47 (0.37)	5.74 (1.13)	4.08 (0.75)	71.3 (1.48)
BAP00010	750	0.5h	6	3.64 (0.32)	4.85 (0.57)	4.05 (0.47)	83.7 (3.91)
BAP00036 (male)	750	0.5h	12	3.42 (0.28)	5.54 (0.58)	5.11 (0.91)	92.3 (12.7)
(female)	750	0.5h	12	2.78 (0.45)	4.87 (0.73)	4.67 (0.75)	96.1 (9.92)
BAP00210	750	0.5h	18	2.89 (0.47)	4.94 (0.62)	NC	NC
BAP00058	750	0.5h	6	3.51 (0.20)	4.18 (0.24)	3.35 (0.21)	80.5 (7.06)
JPN-01 (Japanese)	750	2h	8	2.93 (0.30)	4.05 (0.35)	3.57 (0.52)	88.2 (5.97)
BAP00006	1000	0.5h	6	3.25 (0.20)	6.64 (0.41)	4.16 (0.57)	62.5 (6.33)
BAP00393	1000	1.5h	6	3.98 (0.30)	5.12 (0.70)	4.36 (0.33)	85.6 (11.3)
CSI-1003	1000	2h	56	3.2 (0.5)	5.48 (0.897)	NC	NC
JPN-01 (Japanese)	1000	2h	8	3.00 (0.27)	4.16 (0.42)	3.38 (0.33)	81.8 (7.48)
Single dose studies N			342				
NC = not calculated							

Table 51 Mean (SD) elimination pharmacokinetic parameters for ceftobiprole and its open-ring metabolite following multiple intravenous infusions of ceftobiprole

Ceftobiprole							
Dose Regimen Study	Dose (mg)	Infusion Duration	N	t _{1/2} (h)	CL _S (L/h)	CL _R (L/h)	Ae (% , Dose)
Multiple Dose							
BAP00010	500	0.5h	6	4.04 (0.31)	5.06 (0.95)	4.47 (1.07)	87.8 (9.30)
CSI-1001	500	2h	15	3.4 (0.5)	6.72 (1.44)	NC	NC
CSI-1004	500	2h	27	3.3 (0.3)	4.98 (0.58)	4.28 (0.57)	NR
CSI-1005	500	2h	25	2.4 (0.4)	5.08 (0.66)	NC	NC
JPN-01 (Japanese)							
q12h	500	2h	8	3.55 (0.28)	4.23 (0.48)	3.86 (0.76)	91.6 (13.2)
q8h	500	2h	8	3.34 (0.40)	4.38 (0.46)	4.15 (0.65)	94.4 (8.46)
BAP00010	750	0.5h	6	4.11 (0.41)	4.83 (0.34)	4.08 (0.59)	84.4 (9.23)
BAP00058	750	0.5h	6	3.41 (0.31)	4.35 (0.55)	3.61 (0.53)	82.8 (6.29)
BAP00393	1000	1.5h	5	4.08 (0.40)	4.72 (0.25)	4.21 (0.30)	89.2 (1.93)
CSI-1001	1000	2h	11	3.4 (0.4)	7.50 (1.74)	NC	NC
Multiple dose studies N			117				

NC = not calculated

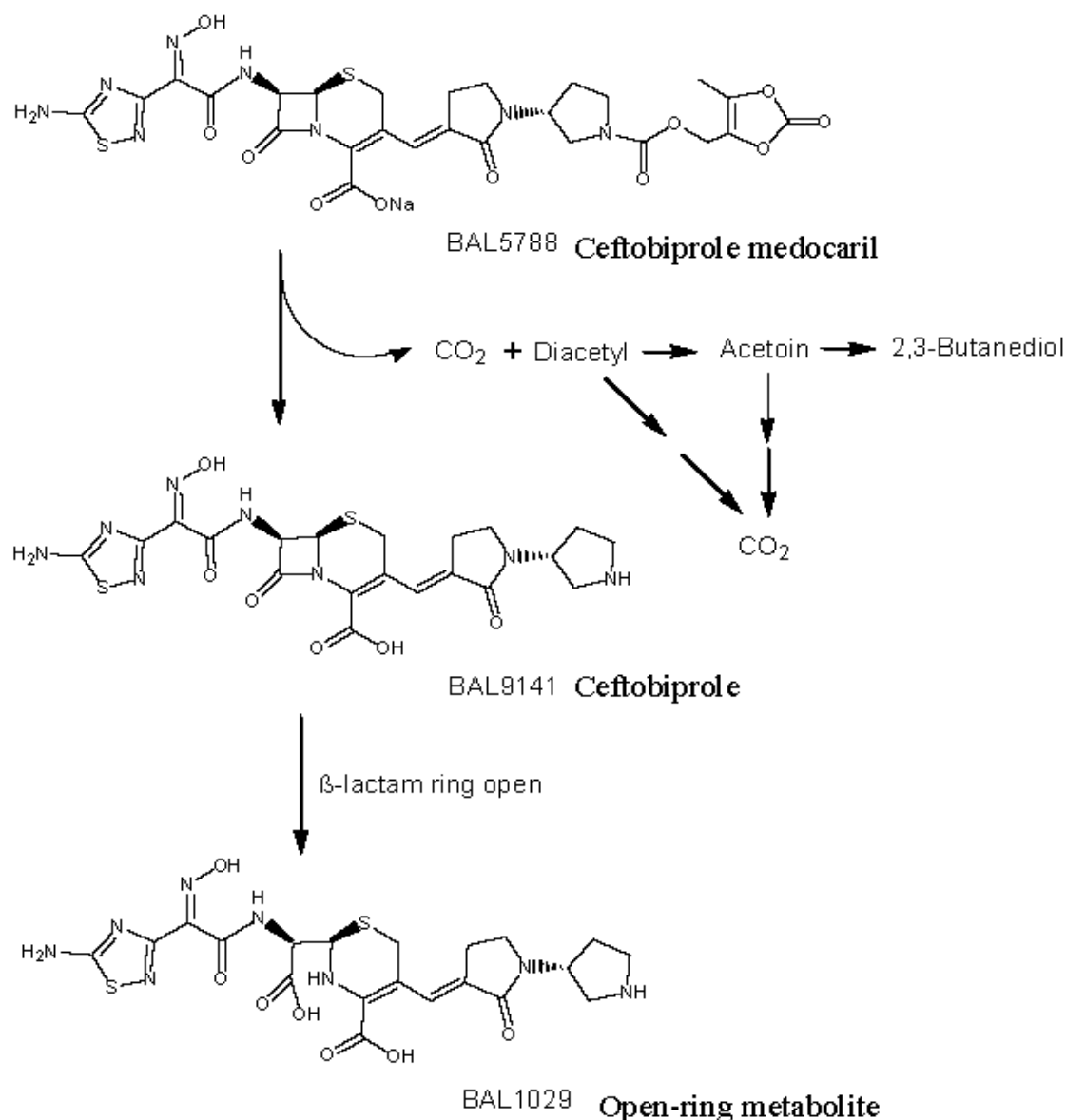
NR = Not reliable. Urine collected over 24 hours rather than dosing interval would overestimate recovery because of carryover from previous dose

Metabolism

In vitro metabolism

Conversion of ceftobiprole medocaril to ceftobiprole is very rapid. In human plasma the conversion time is reported as 38 seconds (BAP00072). Inhibition studies with ethylenediaminetetraacetic acid (EDTA) suggested that type A plasma esterases are involved in the cleavage of ceftobiprole medocaril to ceftobiprole. Hydrolysis of ceftobiprole medocaril to ceftobiprole was not inhibited by acetylcholinesterase inhibitors such as neostigmine at 0.1 mg/mL, and moderately inhibited by dichlorvos, a known Type B esterase inhibitor (BAP00207).

The prominent metabolite in human hepatocytes was the open-ring hydrolysis product BAL1029 [Figure 1].

Figure 1 *In Vitro* and *In Vivo* metabolism of BAL5788 (ceftobiprole medocartil

In vitro experiments were conducted to determine whether ceftobiprole is a substrate or inhibitor for the drug (efflux) transporter P-gp (FK5942), this showed that under the test conditions, ceftobiprole is not a P-gp substrate or inhibitor. These experiments were not performed for ceftobiprole medocartil or the open-ring metabolite because of their very low systemic exposure in man.

Ceftobiprole showed no significant inhibition of the tacrine 1- and 7-hydroxylation (CYP1A2), diclofenac 4'-hydroxylation (CYP2C9), S-mephenytoin 4'-hydroxylation (CYP2C19), bufuralol 1'-hydroxylation (CYP2D6), and testosterone 6- β -hydroxylation (CYP3A4); only a slight (8% to 28%) inhibitory potential was apparent in the highest concentration range tested (50 to 100 μM) (B-109572). The lack of direct or time-dependent inhibition of CYP2C8 was also demonstrated in human liver microsomes using amodiaquine as a specific substrate (less than 6% inhibition at the highest concentration tested of 200 μM). (FK6366).

In another set of experiments (FK5831), cultured human hepatocytes were treated with the vehicle (DMSO, 0.1%), ceftobiprole (5 μM) or 1 of the 3 known human cytochrome P450

enzyme inducers (i.e., omeprazole [100 µM], phenobarbital [750 µM], and rifampin [10 µM]) and subsequently examined for their effects on phenacetin *O*-dealkylation (CYP1A2), bupropion hydroxylation (CYP2B6), diclofenac 4'-hydroxylation (CYP2C9), S-mephenytoin 4'-hydroxylation (CYP2C19), testosterone 6-β-hydroxylation (CYP3A4), and midazolam 1'-hydroxylation (CYP3A4/5). While the treatment of human hepatocytes with the CYP inducers omeprazole, phenobarbital or rifampin caused anticipated increases in CYP activities, treatment with 5 µM ceftobiprole caused no induction of CYP1A2, CYP2B6, CYP2C9, CYP2C19 or CYP3A4/5. Due to the solubility restrictions with ceftobiprole, an additional study was performed with ceftobiprole medocaril to investigate the potential to induce cytochrome P450 in human hepatocytes. Using the same design and prototypical inducers and substrates, ceftobiprole medocaril had no effect on the activity of CYP1A2, CYP2B6, CYP28 (amodiaquine, 20 µM) CYP2C9, CYP2C19 or CYP3A4 up to the highest concentration tested of 200 µM. (FK6603).

***In-vivo* metabolism**

In-vivo studies confirmed the rapid pro-drug cleavage observed *in-vitro*. After intravenous bolus injection of ceftobiprole medocaril to rats, marmosets, and cynomolgus monkeys, no pro-drug was detected in the marmoset and only low levels were detected in cynomolgus monkeys and rats up to 20 minutes and 1 hour after dosing, respectively.

After intravenous administration of ceftobiprole or ceftobiprole medocaril to rat or marmoset, the major metabolite (besides ceftobiprole) in plasma, bile, urine, brain, and kidney was the open-ring product (BAL1029). In these matrices, up to 5 minor unidentified metabolites were also observed, which together accounted for a maximum of 5% of the administered dose of ceftobiprole. Traces of intact pro-drug were also found in rat urine (less than 1% of the dose) and in rat bile (0.3% of the dose).

Inter-conversion

Ceftobiprole is not subject to inter-conversion

Pharmacokinetics of metabolites

The pro-drug ceftobiprole medocaril is completely converted into ceftobiprole. Only low concentrations of pro-drug could be detected in plasma of some subjects during infusion. Ceftobiprole is metabolised to a small extent into the open ring metabolite (BAL1029). In study CSI-1004 Systemic exposure to the open-ring metabolite increased approximately 33% for C_{max} and 48% for AUC_{0-8} from Day 1 to Day 5. Normalised for molecular weight, the AUC ratio for the open-ring metabolite compared with ceftobiprole was approximately 0.04; as such the systemic exposure to the open-ring metabolite accounts for approximately 4% of the parent ceftobiprole exposure following single dose administration. Elimination half-life of the open-ring metabolite was slightly longer, approximately 5 hours compared with ceftobiprole, which was approximately 3 hours. The accumulation ratio was approximately 1.5, indicating that the open-ring metabolite accumulates to a slightly higher degree than ceftobiprole (accRatio of approximately 1.1), which may be attributed to its longer elimination half-life. The observed accRatio was similar to the predicted accIndex (1.44) based on $t_{1/2}$ and τ . Approximately 5% of ceftobiprole was excreted in the urine as the open-ring metabolite following single dose administration.

Table 11 Mean (SD) open-ring metabolite (BAL1029) pharmacokinetic parameters following single and multiple intravenous infusions of ceftobiprole 500 mg q8h administered over 2 hours in healthy adults (study 30982081-CSI-1004; PK Evaluable dataset)

Parameter	Day 1			Day 5		
	All Subjects N=28	Male N=14	Female N=14	All Subjects N = 27	Male N=14	Female N=13
t_{max} (h) ^a	1.97	1.97	1.90	1.97	1.97	1.97
C_{max} (µg/mL)	0.80 (0.21)	0.68 (0.11)	0.93 (0.23)	1.07 (0.25)	0.96 (0.18)	1.19 (0.27)
AUC ₀₋₈ (µg•h/mL)	3.68 (0.88)	3.16 (0.51)	4.20 (0.88)	5.45 (1.35)	5.03 (1.20)	5.91 (1.40)
AUC _{last} (µg•h/mL)	5.01 (1.35)	4.35 (0.85)	5.68 (1.46)	8.13 (2.40)	7.67 (2.29)	8.63 (2.51)
AUC _{0-∞} (µg•h/mL)	5.61 (1.39)	4.97 (1.00)	6.24 (1.47)	NC	NC	NC
$t_{1/2}$ (h)	4.7 (0.8)	4.8 (0.9)	4.5 (0.7)	5.8 (0.9)	6.0 (0.8)	5.6 (1.1)
accRatio	NA	NA	NA	1.48 (0.20)	1.58 (0.19)	1.37 (0.13)
Ae (mg)	23.5 (4.22)	23.1 (3.83)	24.0 (4.67)	NR	NR	NR
A _e , % ^b	4.71 (0.85)	4.61 (0.77)	4.81 (0.94)	NR	NR	NR

^a Median^b Ae normalised by the ratio of molecular weights of ceftobiprole to the open-ring metabolite

NC = Not calculated

NA = Not applicable

NR = Not reliable. Urine collected over 24 hours rather than dosing interval would overestimate recovery because of carryover from previous dose

Consequences of possible genetic polymorphism

No genetic polymorphism testing was done on subjects participating in the clinical studies with a pharmacokinetic component. Since the extent of hepatic metabolism is minimal, differences in the rates of metabolic enzyme activity are not expected to significantly alter the pharmacokinetics of ceftobiprole.

Dose proportionality and time dependency

Dose proportionality

Following administration of ceftobiprole at doses of 125 mg to 1000 mg given over 30 minutes, C_{max} and AUC increased proportionally with dose. Estimates for C_{max} were dependent on the infusion duration; when compared across the same infusion duration, C_{max} also increased proportionally with dose. Estimates of CLs, VSS, and $t_{1/2}$ were consistent across the dose range of 125 mg to 1000 mg, suggesting that the pharmacokinetics of ceftobiprole were linear and predictable.

Time dependency

After repeated administration (q8h or q12h for up to 12 days), the pharmacokinetic properties of ceftobiprole were time independent. Estimates of CLs, VSS, and $t_{1/2}$ were consistent following multiple infusions. The accumulation factor was $\leq 16\%$ across studies, suggesting minimal accumulation from single to multiple doses. Overall the pharmacokinetics of ceftobiprole appeared to be independent of the infusion duration and the dosing interval.

Intra- and inter-individual variability

Intra-subject variability has not been evaluated. There appears to be a certain degree of inter-individual variability as noted in PK assessment performed with the pivotal phase III studies.

Pharmacokinetics in target population

In study **BAP248/307**, pharmacokinetic assessments were conducted using both rich and sparse sampling methods to evaluate the systemic exposure to ceftobiprole in subjects with nosocomial pneumonia in selected centres.

Results

Rich pharmacokinetic sampling

Rich pharmacokinetic sampling was performed in only 3 subjects on ceftobiprole and two subjects on linezolid/ceftazidime. Individual plasma concentrations are presented in Table 24.

Table 24 Plasma concentrations (µg/ml) following a 2-hour intravenous infusion of ceftobiprole 500 mg or ceftazidime 2000 mg to subjects with nosocomial pneumonia (study BAP248/307 – Rich Pharmacokinetic dataset)							
Subject ID	Predose	15 min	1 h	2 h	4 h	6 h	8 h
Ceftobiprole							
120417	6.13	NS	17.6	29.1	16.7	14.3	NS
120418	6.43	NS	11.7	14.3	8.32	5.72	3.98
130719	8.47	12.4	17.8	29.3	22.1	17.1	12.6
Ceftazidime							
5352	1.23	NA	NA	44.2	8.58	3.04	1.32
5353	72.6	NA	NA	114	74.5	64.2	54.7

^a Infusion was 2 hours 20 minutes; NS = no sample; NA = not available

Sparse pharmacokinetic sampling

Sparse pharmacokinetic sampling was performed with 80 subjects in the ceftobiprole group and 71 subjects in the linezolid/ceftazidime group. Descriptive statistics of the plasma concentrations of ceftobiprole and ceftazidime are presented in Table 26.

Table 26 Plasma concentrations (µg/ml) following a 2-hour intravenous infusion of ceftobiprole 500 mg or ceftazidime 2000 mg to subjects with nosocomial pneumonia (study BAP248/307 – Sparse Pharmacokinetic dataset)			
Ceftobiprole			
Statistics	0.5 – 2 h	2 h	6 h
N	58	68	68
Mean	17.8	16.3	12.3
SD	21.3	7.78	46.4
% CV	120	47.6	377
Min	0.18	0.37	0.39
Median	13.2	15.4	4.98
Unbound* Median	11.1	12.9	4.18
Max	158	43.8	387
Ceftazidime			
Statistics	0.5 – 2 h	2 h	6 h
N	66	61	65
Mean	53.9	62.2	22.8
SD	33.3	37.6	14.7
% CV	61.9	60.4	64.8
Min	2.19	BQL	BQL
Median	49.5	54.0	20.4
Unbound* Median	44.6	48.6	18.4
Max	144	168	66.0

BQL=below quantification limit; * free fraction of 0.84 for ceftobiprole and 0.90 for ceftazidime.

The plasma concentrations of ceftobiprole tended to be lower in ventilator-associated pneumonia (VAP) subjects when compared to nosocomial pneumonia excluding VAP subjects. In the VAP subjects, the median levels of unbound ceftobiprole at 6 h post-start of the infusion were 2.79 µg/mL. In the nosocomial pneumonia excluding VAP population the median concentrations of unbound drug at 6 h post-start of the infusion were above 4 µg/mL in the ceftobiprole arm. These results must be interpreted with caution, in view of the limitations of sparse sampling and the high variability of the plasma concentrations in all groups.

In study **CAP3001** rich pharmacokinetic sampling was performed on Day 4 ± 1, in the remaining subjects on ceftobiprole and on ceftriaxone with or without linezolid, sparse pharmacokinetic sampling was conducted.

Results

Only 7 subjects on ceftobiprole participated in the rich pharmacokinetic sampling. Sparse pharmacokinetic sampling was obtained from 212 subjects on ceftobiprole.

Rich pharmacokinetic sampling

Only 4 subjects were evaluable for PK and, of those, 2 had moderate renal impairment.

Table 29 Plasma concentrations (µg/ml) following a 2-hour intravenous infusion of ceftobiprole 500 mg to subjects with community acquired pneumonia (study 30982081-CAP-3001– Rich Pharmacokinetic dataset)							
Total plasma concentrations							
Subject ID	Predose	15 min	1 h	2 h	4 h	6 h	8 h
304304	4.55	4.23	14.4	19.0	12.6	7.37	5.03
312301	2.22	5.33	14.2	15.9	7.26	3.56	2.69
102203 (Mod RI)	6.95	11.4	18.2	22.2	14.3	10.8	9.25
305201 (Mod RI)	4.61	NS	28.8	26.2	NS	NS	9.97
Unbound plasma concentrations*							
Subject ID	Predose	15 min	1 h	2 h	4 h	6 h	8 h
304304	3.82	3.55	12.1	16.0	15.6	6.19	4.22
312301	1.86	4.48	11.9	13.4	6.10	2.99	2.26
102203 (Mod RI)	5.84	9.58	15.3	18.6	12.0	9.10	7.77
305201 (Mod RI)	3.87	NS	24.2	22.0	NS	NS	8.37

NS = no sample; Mod RI = Moderate renal impairment; * free fraction of 0.84

Mean unbound time above MIC ($fT > MIC$), assuming a target of 4 µg/mL, was 81.1% and 100% of an 8-hour dosing interval for subjects with normal renal function and with moderate renal impairment, respectively. Based on the limited number of subjects with rich pharmacokinetic assessments, ceftobiprole exposure in CAP subjects was comparable to that observed in healthy subjects.

Sparse pharmacokinetic sampling

All subjects assigned to ceftobiprole who contributed sparse pharmacokinetic samples had at least one sample with a measurable ceftobiprole concentration, confirming that the subject had been dosed with ceftobiprole. Plasma ceftobiprole concentrations at the end of infusion ranged from 1.60 to 222 µg/mL (mean 21.1 ± 21.5 µg/mL; median 18.9) and from 0.89 to 327 µg/mL at 6 hours (mean 8.79 ± 23.5 µg/mL; median 5.59). These data are consistent with that observed in healthy subjects.

Table 30 Plasma concentrations (µg/ml) following a 2-hour intravenous infusion of ceftobiprole 500 mg to subjects with community acquired pneumonia (study 30982081-CAP-3001 – Sparse Pharmacokinetic dataset)			
Statistics	0.5 – 2 h	2 h	6 h
N	180	197	195
Mean	18.3	21.1	8.79
SD	11.9	21.5	23.5
% CV	64.7	102	267
Min	0.23	1.6	0.89
Median	17.9	18.9	5.59
Unbound* Median	15.0	15.9	4.94
Max	102	222	327

BQL=below quantification limit; * free fraction of 0.84

Special populations

Impaired renal function

The influence of renal dysfunction on ceftobiprole pharmacokinetics was assessed in study

BAP00018, a single-center, open-label, single-dose study with a sequential design in male subjects with normal and various degrees of renal impairment. The study population was comprised of 4 groups with a total of 5 subjects each starting with Group A (normal renal function: CLCR >80 mL/min) and sequentially Group B (mild renal impairment: CLCR = 50 to 80 mL/min), Group C (moderate renal impairment: CLCR = 30 to <50 mL/min), and Group D (severe renal impairment: CLCR <30 mL/min). Each subject received ceftobiprole 250 mg infused over 30 minutes.

Plasma concentrations of ceftobiprole were higher in subjects with renal impairment compared with subjects with normal renal impairment [Figure 13].

Based on geometric mean ratios, the systemic exposure in terms of AUC_{last} was 29% higher in subjects with mild renal impairment, and 2.5- and 3.3-fold higher in subjects with moderate and severe renal impairment, respectively, compared with subjects with normal renal function. Total systemic clearance (CLS) and CLR decreased with decreasing renal function such that the greatest reductions were noted in the moderately (62% for CLS and 78% for CLR) and severely (75% for CLS and 91% for CLR) renally impaired groups. Urinary recovery of ceftobiprole over 24 hours ranged from 74% to 32% in subjects with mild to severe renal impairment. Elimination half-life increased with decreasing renal function, such that subjects with severe renal impairment exhibited, on average, the longest mean $t_{1/2}$ of approximately 11 hours. Estimates of C_{max} and VSS were similar across the varying degrees of renal impairment. Pharmacokinetic parameter estimates for ceftobiprole in subjects with normal renal function were similar to that reported in previous studies.

Figure 13 Mean Plasma Ceftobiprole Concentration-Time Profiles In Healthy Subjects and Subjects with Various Degrees of Renal Impairment after a Single Intravenous Infusion of Ceftobiprole 250 mg for 30 Minutes (study BAP00018)

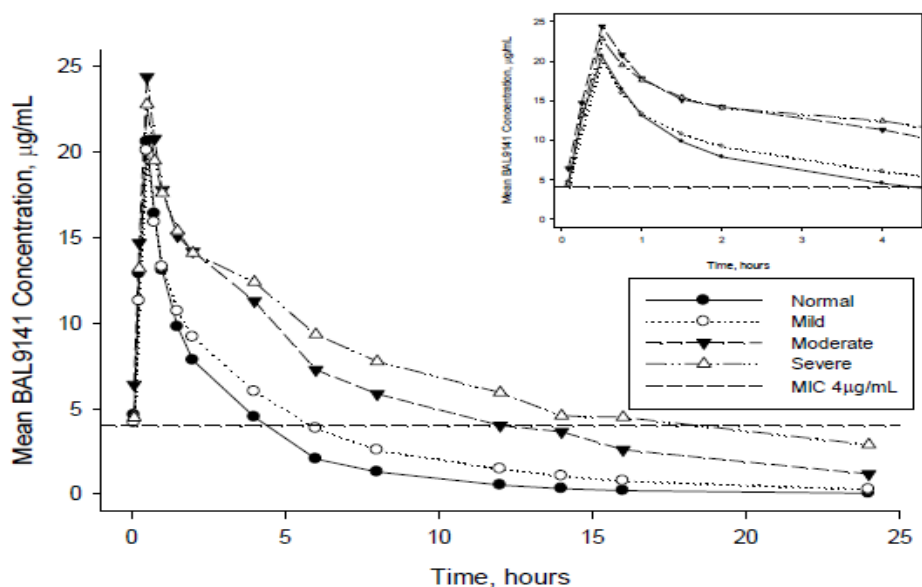


Table 32 Mean (SD) ceftobiprole pharmacokinetic parameters in healthy subjects and subjects with various degrees of renal impairment after a single intravenous infusion of ceftobiprole 250 mg for 30 minutes (study BAP00018; N=20)

Parameter	Renal Function			
	Normal	Mild	Moderate	Severe
	CL _{CR} >80 mL/min N=5	CL _{CR} 50-80 mL/min N=5	CL _{CR} 30-<50 mL/min N=5	CL _{CR} <30 mL/min N=5
C _{max} (µg/ml)	20.6 (2.06)	20.1 (1.45)	24.4 (1.65)	22.8 (3.48)
AUC _{last} (µg•h/ml)	52.4 (6.95)	72.7 (13.9)	139 (15.7)	174 (44.50)
AUC _{0-∞} (µg•h/ml)	52.8 (6.91)	74.8 (15.6)	151 (21.6)	222 (71.00)
t _{1/2α} * (h)	0.82 (0.45)	0.57 (0.20)	0.41 (0.24)	0.55 (0.26)
t _{1/2β} (h)	3.45 (0.37)	4.75 (0.81)	6.87 (1.12)	11.1 (1.96)
V _{SS} (L)	15.8 (1.81)	18.0 (0.76)	14.2 (0.80)	16.9 (2.39)
CL _S (L/h)	4.80 (0.61)	3.46 (0.71)	1.68 (0.25)	1.21 (0.36)
CL _R (L/h)	4.38 (0.51)	2.48 (0.63)	0.88 (0.25)	0.407 (0.24)
A _e , %	91.6 (6.55)	71.1 (7.32)	51.9 (9.93)	31.5 (9.65)

Values are presented as arithmetic means (SD);

* obtained by 2-compartmental analysis

For practical considerations, the following dose and dose interval adjustments are proposed for patients with renal impairment and used in the pivotal studies. They are based on Simulations of the plasma concentration-time profiles using parameter estimates derived from the BAP00018. Based on 16% protein binding, the predicted %fT>MIC for the unbound ceftobiprole concentration on Day 1 was 75% in subjects with normal renal function, 97% for subjects with mild renal impairment, 99% in subjects with moderate renal function, and 97% in subjects with severe renal impairment. The predicted %fT>MIC for the unbound ceftobiprole concentration on Day 3 (steady-state) was 87% for normal subjects and 100% for all of the renally impaired groups.

Renal Function	CL _{CR} (mL/min) ^a	Recommended Dose ^b
Normal	>80	500 mg q8h
Mild	50 - 80	500 mg q8h
Moderate	30 - <50	500 mg q12h
Severe	10 - <30	250 mg q12h

^a the Cockcroft-Gault formula using actual body weight was the recommended method for estimating CL_{CR} in the ceftobiprole clinical studies

^b all doses administered as a 2-hour i.v. infusion

CSI-1007 was an open-label pharmacokinetic study of ceftobiprole in healthy subjects and subjects with ESRD receiving haemodialysis. Healthy subjects were given a single 2-hour infusion of 250 mg ceftobiprole; subjects with ESRD on haemodialysis were given a 2-hour infusion of 250 mg ceftobiprole 3 hours before dialysis in Period 1 and immediately after dialysis in Period 2, with a washout period of at least 14 days between periods. Exposure to ceftobiprole and to the open-ring metabolite was much higher in ESRD subjects dosed pre-dialysis or post-dialysis than in healthy subjects. In pre-dialysis ESRD subjects the AUC was 2 times and 12 times higher than in normal subject for ceftobiprole and the metabolite, respectively. The corresponding increases in post-dialysis ESRD subjects were 6 times and 20 times. In pre-dialysis and post-dialysis ESRD subjects ceftobiprole and the open-ring metabolite were still detectable in plasma on Day 3 post-treatment, in contrast to healthy subjects. These observations were expected as both compounds are predominantly eliminated in urine. Ceftobiprole and the open-ring metabolite were easily extracted during haemodialysis with extraction ratios of 0.69 and 0.64, respectively, consistent with the less marked increases in exposure when ESRD subjects were treated pre-dialysis rather than post-

dialysis.

Figure 15 Mean (SD) plasma ceftobiprole concentration-time profiles following a single 2-h intravenous infusion of ceftobiprole 250 mg administered to healthy subjects and subjects with ESRD before and after haemodialysis (study 30982081-CSI-1007: Pharmacokinetic analysis set)

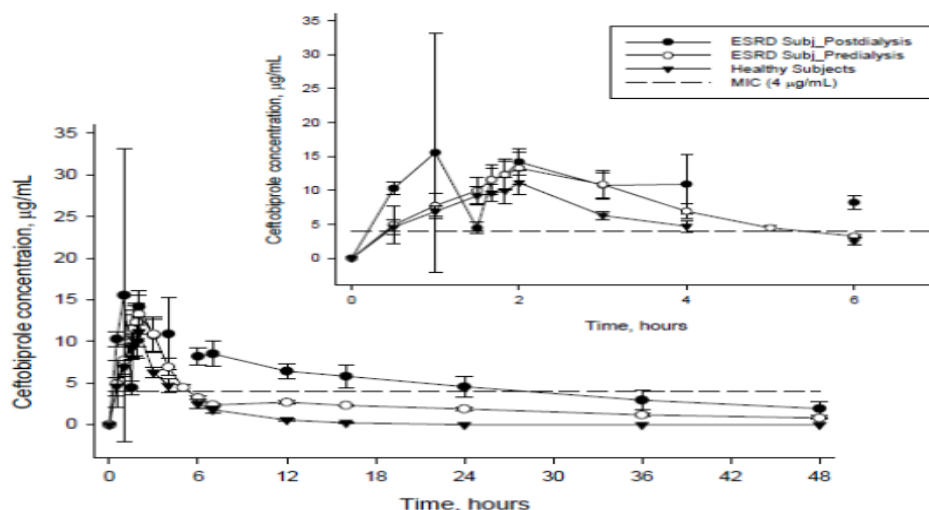


Table 33 Mean (SD) ceftobiprole pharmacokinetic parameters in healthy subjects and ESRD subjects following a single intravenous infusion of ceftobiprole 250 mg administered over 2 hours (study 30982081-CSI-1007: Pharmacokinetic analysis set)

Parameter	Healthy N=6	Pre-dialysis N=5 ^a	Post-dialysis N=5 ^b
C _{max} (µg/mL)	11.1 (1.77)	13.3 (2.33)	21.1 (14.7)
t _{max} (h)	2.00 (2.00, 2.00)	2.00 (2.00, 2.00)	2.13 (1.00, 4.00)
AUC _{last} (h•µg/mL)	44.3 (7.12)	118 (8.73)	249 (49.0)
AUC _{0-∞} (h•µg/mL)	45.2 (6.84)	143 (8.53)	311 (75.1)
t _{1/2} (h)	3.0 (0.4)	20.7 (1.83)	20.5 (5.33)
V _d (L)	24.4 (3.68)	52.5 (5.23)	23.9 (5.14)
CL _S (L/h)	5.62 (0.731)	1.76 (0.102)	0.845 (0.212)
CL _R (L/h)	5.11 (0.814)	NC	NC
Ae, %dose	88.6 (4.06)	NC	NC
Extraction ratio	NC	0.685 (0.125)	NC
CL _{HD} (L/h)	NC	7.91 (1.21)	NC

^a without Subject 100002

^b without Subject 100005

NC = not calculated

Table 34 Mean (SD) plasma open-ring metabolite pharmacokinetic parameters following a single 2 hour intravenous infusion of ceftobiprole 250 mg to healthy subjects and subjects with end-stage renal disease (study 30982081-CSI-1007: Pharmacokinetic Analysis set)

Parameter	Healthy N=6	Pre-dialysis N=5 ^a	Post-dialysis N=5 ^b
C _{max} (µg/mL)	0.39 (0.06)	0.986 (0.091)	1.93 (1.30)
t _{max} (h) ^c	1.97 (1.83, 2.00)	1.97 (1.83, 2.00)	24.4 (1.83, 36.0)
AUC _{last} (h•µg/mL)	2.58 (0.30)	25.5 (2.29)	51.2 (13.2)
AUC _∞ (h•µg/mL)	3.23 (0.54)	NC	NC
t _{1/2} (h)	5.3 (1.2)	NC	NC
Extraction ratio	NC	0.644 (0.179)	NC
CL _{HD} (L/h)	NC	7.48 (2.14)	NC

^a without subject 100002

^b without subject 100005

^c Values presented were median and the minimum/maximum

NC = not calculated

Simulations of ceftobiprole plasma concentrations

The recommended dose of 250 mg q12h (2-hour infusion) ceftobiprole for patients with severe renal impairment would lead to accumulation in ESRD subjects requiring haemodialysis ($t_{1/2}$ of ceftobiprole of approximately 21 hours). Therefore alternative regimens in this population were simulated and after validation of the model with the observed ceftobiprole plasma concentrations from the ESRD subjects requiring haemodialysis (CSI-1007). The extraction ratio during haemodialysis for ceftobiprole was conservatively set to 0.6, haemodialysis time to 4 hours and the CLCR to 10 mL/min. While ceftobiprole is easily haemodialysable, simulations were performed only for post-dialysis treatment.

Simulation and experience in ESRD subjects would suggest that a ceftobiprole dose of 250 mg (2-h) infusion post-dialysis once-daily is an appropriate regimen considering both ceftobiprole and the open-ring metabolite in this patient population.

Impaired hepatic function

The pharmacokinetics of ceftobiprole in patients with hepatic impairment has not been established. Ceftobiprole does not appear to undergo significant hepatic metabolism and its systemic clearance is not expected to be significantly affected by hepatic impairment. As the protein binding of ceftobiprole is low, differences in albumin concentrations associated with hepatic impairment are not expected to significantly affect the free fraction of ceftobiprole..

Gender

Study **BAP00036** compared the single-dose pharmacokinetics of ceftobiprole in male versus female subjects, data showed that systemic exposure to ceftobiprole, in terms of C_{max} and AUC was approximately 21% and 15% higher respectively, in females compared with males and was attributed to the lower body weight in females. Similar results were observed in CSI-1004 study where the pharmacokinetics of ceftobiprole was compared between males and females under single- and multiple-dose conditions. The degree to which systemic exposure to ceftobiprole in females exceeded males was more pronounced following single dose administration (32% for C_{max} and 21% for AUC_{0-8}) compared to multiple-dose administration (16% for C_{max} and 11% for AUC_{0-8}). In both studies, distribution volume was lower in females compared to males (20% to 29%). When pharmacokinetic parameters were corrected for body weight, no sex differences were apparent. In both studies, the %fT>MIC corresponding to an 8-hour dosing interval was similar in both males and females (84% in CSI-1004 and 82% in BAP00036).

Race

Study **JPN-01** investigated the pharmacokinetics and safety of ceftobiprole administered as single and repeated intravenous infusions to healthy Japanese adult males. Part 1 consisted of the following four cohorts who received as verum a 2-hour infusion of ceftobiprole medocaril: Cohort 1 (250 mg), Cohort 2 (500 mg), Cohort 3 (750 mg), and Cohort 4 (1000 mg). Administration was sequential starting with the low-dose Cohort 1 and proceeded in order to Cohort 2, Cohort 3, and Cohort 4 while subject safety was adequately evaluated. Ten healthy adult Japanese males made up one cohort. Eight of these subjects received ceftobiprole and 2 subjects received a 5% glucose vehicle placebo.

Part 2 consisted of the following 2 cohorts: Cohort 5 (500-mg dose of ceftobiprole twice daily every 12 hours during 9 days), and Cohort 6 (500-mg dose 3 times daily every 8 hours during 9 days). Single dose pharmacokinetics was performed on Day 1 and Day 9. Administration started with Cohort 5 and then proceeded to Cohort 6 as subject safety was adequately examined.

This study confirmed that there was no ethnic difference in ceftobiprole disposition after

single or multiple doses in Japanese subjects when compared to historical data obtained in Caucasian subjects. The lack of race-effect was also confirmed in a population analysis.

Weight

Study **CSI-1008** was a single-dose study was conducted to investigate the pharmacokinetics of ceftobiprole in morbidly obese subjects compared with non-obese subjects. 500 mg ceftobiprole as a 2-hour infusion were given to subjects. Exposure to ceftobiprole was lower in morbidly obese subjects attributed to a larger volume of distribution and a higher clearance.

The effects of body weight were also evaluated in the population pharmacokinetic analysis. It was identified as a statistically significant, but clinically not relevant, covariate on central volume of distribution (V1) in the final pharmacokinetic model. When simulations were performed to evaluate the effects of weight on %T>MIC, there was no apparent trend between body weight and %T>MIC in similar ranges of creatinine clearance.

Elderly

The effect of age on ceftobiprole pharmacokinetics was examined through a population pharmacokinetic analysis. Age was not identified as a statistically significant covariate in the final population pharmacokinetic model as CLCR explained the apparently lower clearance in elderly patients. Therefore, no dosage adjustments are recommended based on age alone. Dose adjustment in elderly may be warranted if CLCR is reduced to 50 mL/min or less, i.e., in case of moderate to severe renal impairment

Children

A single-dose pharmacokinetic study was conducted in pediatric subjects with age ranging from ≥ 3 months to < 18 years. Male and female subjects were enrolled sequentially from the oldest age group to the youngest age group. The i.v. 2-hour infusion dose was decreased by increasing age group, i.e. 15 mg/kg for subjects < 6 years, 10 mg/kg for subjects 6 years to < 12 years and 7 mg/kg, up to a maximum of 500 mg in subjects 12 years to < 18 years of age. Ceftobiprole volume of distribution and systemic clearance increased with age and almost reached healthy adult values in the 12 years to < 18 years age group. At the doses administered in this study the single-dose pharmacokinetics of ceftobiprole were generally within the range of what has previously been observed in healthy adult subjects after a single dose of ceftobiprole 500 mg.

Interactions

Clinical drug-drug interaction studies have not been conducted as the overall likelihood for significant interactions is considered minimal based on the pharmacokinetic properties of ceftobiprole. As ceftobiprole does not appear to undergo significant hepatic metabolism, its pharmacokinetics are not expected to be significantly affected known inhibitors and inducers of the CYP450-system. As ceftobiprole is not a substrate for p-glycoprotein *in vitro*, interactions with other agents that are substrates or inhibitors for p-glycoprotein are not anticipated.

Ceftobiprole exhibits low protein binding (16%) and is primarily bound to albumin, a high capacity binding system. Thus the potential for other agents to displace ceftobiprole and increase the unbound concentrations is considered low. Exploratory population pharmacokinetic screening indicated that the administration of the following concomitant medications did not affect the pharmacokinetics of ceftobiprole: fentanyl, lidocaine, diclofenac, paracetamol, acetyl salicylic acid, heparin, diphenhydramine, propofol, hydromorphone hydrochloride, hydrocodone, matamizole sodium, methadone, and furosemide.

Ceftobiprole is excreted predominantly unchanged in the urine and only a small portion is metabolised in the presence of liver microsomes. When using specific catalyzed CYP450 reactions in human microsomes, ceftobiprole showed no inhibitory or induction potential towards CYP450 1A2, -2B6, -2C8, -2C9, -2C19, -2D6, and -3A4/5 at lower concentrations; only a slight (8% to 28%) inhibitory potential was apparent in the highest concentration range tested (up to 200 μ M). Furthermore, ceftobiprole is restricted to the extra-cellular water compartment, the potential of ceftobiprole to affect the CYP450-dependent metabolic clearance of co administered drugs is regarded as very low. Thus, ceftobiprole is not expected to affect the CYP450-dependent metabolic clearance of co administered drugs, and no relevant pharmacokinetic drug-drug interactions are anticipated in the clinical setting.

Overall conclusions on pharmacokinetics

The pharmacokinetics of ceftobiprole appears to be straight forward. Ceftobiprole medocaril (pro-drug) is converted very rapidly to ceftobiprole. There is a dose proportional increase in systemic exposure after single and multiple dose administration. There is low protein binding and the volume of distribution is approximately 18l. One open ring metabolite has been identified which accounts for about 4% of systemic exposure.

Ceftobiprole is eliminated mainly by renal excretion with a half –life of approximately 3 hours which is independent of dose. The total systemic clearance ranged from approximately 4-7L/h and the mean renal clearance was about 3 to 5 L/h covering > 80% of total clearance. Urine recovery including pro-drug, ceftobiprole and the open ring metabolite is about 89%.

PHARAMCODYNAMICS

According to the applicant, the microbiological investigations of the antibacterial activity of ceftobiprole have been studied extensively globally. In total, over 10,000 historical clinical isolates from the Europe, United States and the rest of the world have been tested for their susceptibility to ceftobiprole.

Susceptibility tests following the guidelines of the CLSI (CLSI 2006b) were performed with ceftobiprole at major microbiology laboratories in the Europe and the United States

Mechanism of action

The primary targets of ceftobiprole like other β -lactam antibiotics are membrane-associated bacterial enzymes involved in the last steps of peptidoglycan (cell wall) biosynthesis, known as penicillin-binding proteins (PBPs). Ceftobiprole exerts its antibacterial activity by binding to essential PBPs in both Gram-positive and Gram-negative bacteria. The anti-MRSA activity of ceftobiprole is due to its ability to rapidly and tightly bind to PBP2a, as well as to the normal complement of β -lactam sensitive PBPs.

Primary pharmacology

Gram-positive aerobic bacteria

Activity against Staphylococci

Ceftobiprole was found to be effective against MRSA, displaying MIC₉₀ values of ≤ 2 μ g/mL in most cases although some strains displayed MIC₉₀ values of 4 μ g/mL. Ceftobiprole was shown to have *in vitro* activity against MSSA with MIC₉₀ values of 0.5 - 1 μ g/mL. For MRSA isolates of different SSCmec or clone types the MIC₉₀ value of ceftobiprole was consistently ≤ 2 μ g/mL. However a minority of hospital associated clones exhibited MIC₉₀ values of 4 μ g/mL.

Ceftobiprole exhibited MIC₉₀ values ≤ 2 μ g/mL against *S. epidermidis*, both methicillin-susceptible and –resistant. For most other staphylococci species, ceftobiprole exhibited MIC₉₀ values of 0.25 - 4 μ g/mL. Apart from a small group of 31 isolates of *S. haemolyticus* with a MIC₉₀ value of 8 μ g/mL.

Activity of ceftobiprole in global surveillance studies against *s.aureus* (European data 2010)

Organism (n)	Agent	MIC50 (µg/mL)	MIC90 (µg/mL)	Range (µg/mL)
<i>Staphylococcus aureus</i> , oxacillin-susceptible (1,974)	Ceftobiprole	0.25	0.5	≤0.06 – 1
<i>Staphylococcus aureus</i> , oxacillin-resistant (690)	Ceftobiprole	1	2	0.25 – 4
<i>Coagulase-negative staphylococci</i> , oxacillin-susceptible (170)	Ceftobiprole	0.12	0.25	≤0.06 – 1
<i>Coagulase-negative staphylococci</i> , oxacillin-resistant (488)	Ceftobiprole	1	2	≤0.06 – 4

Activity against streptococci

Ceftobiprole was demonstrated to have low MIC90 values (≤1 µg/mL) against the majority of isolates of *S. pneumoniae*. For PSSP (penicillin-susceptible *S. pneumoniae*) ceftobiprole displayed MIC90 values ≤0.03 µg/mL and for PISP (penicillin-intermediate *S. pneumoniae*) ceftobiprole MIC90 values were <0.5 µg/mL. Against 62 ceftriaxone intermediate (ceftriaxone MIC values ≤2 µg/mL), the ceftobiprole MIC90 value was 1 µg/mL. Ceftobiprole was active against PRSP (penicillin-resistant) or ceftriaxone-resistant *S. pneumoniae* with MIC90 values ≤1 µg/mL for most of the isolate collections tested, with the exception of one set of 20 penicillin-resistant organisms, and two sets of 171 and 33 ceftriaxone-resistant organisms, each of which had a ceftobiprole MIC90 value of 2 µg/mL. Thus, ceftobiprole has potent anti-pneumococcal activity with MIC values ≤2 µg/mL against most isolates of *S. pneumoniae*, whether penicillin-susceptible, -intermediate, or -resistant, including ceftriaxone-resistant strains.

Ceftobiprole exhibited low MIC90 against viridans group streptococci with MIC values of ≤1 µg/mL, even against penicillin-resistant strains, with lower ceftobiprole MIC90 values for penicillin-susceptible strains (0.06 µg/mL) and penicillin-intermediate strains (0.25 µg/mL) compared to penicillin-resistant strains (1 µg/mL).

Ceftobiprole also exhibited low MIC90 values of ≤0.25 µg/mL. Against all other streptococci tested, including *S. agalactiae*, *S. pyogenes*, *Streptococcus* spp. and including erythromycin-resistant isolates.

In summary, ceftobiprole inhibited all streptococci, including penicillin-susceptible, -intermediate, and -resistant isolates with MIC90 values ≤2 µg/mL. A few of the isolates tested exhibited ceftobiprole MIC values >2 µg/mL.

Activity of ceftobiprole in global surveillance studies against streptococci (European data 2010)

Organism (n)	Agent	MIC50 (µg/mL)	MIC90 (µg/mL)	Range (µg/mL)
beta-haemolytic streptococci (734)	Ceftobiprole	≤0.06	≤0.06	≤0.06 – 0.25
<i>Streptococcus pneumoniae</i> (779)	Ceftobiprole	≤0.06	0.5	≤0.06 – 2

Streptococcus pneumoniae, penicillin-nonsusceptible (208)a	Ceftobiprole	0.5	0.5	≤0.06 – 2
Streptococcus pneumoniae, penicillin-resistant (41)b	Ceftobiprole	0.5	0.5	0.5 - 2
viridans group streptococci (293)	Ceftobiprole	≤0.06	0.25	≤0.06 – >8

Activity against Enterococci

For *E. faecalis*, the ceftobiprole MIC₉₀ value was 1 - 4 µg/mL. Ceftobiprole displayed an MIC₉₀ value of 1 µg/mL against vancomycin-resistant and β-lactamase-positive strains. For *E. faecium* the MIC₉₀ value of ceftobiprole ≥8 µg/mL. Against *Enterococcus* spp. (19 isolates from one laboratory (Jones 2002) the MIC₅₀ and MIC₉₀ values of ceftobiprole were 0.5 and >32 µg/mL, respectively.

Activity against other Gram-positive bacteria

Ceftobiprole demonstrated a bimodal distribution of MIC values against 41 isolates of *Corynebacterium*, exhibiting MIC₅₀ values of 0.06 – 0.12 µg/mL and MIC₉₀ values >32 µg/mL.

PBPs from Gram-positive bacteria

Table 77 Binding of ceftobiprole and comparators to PBPs from Gram-positive bacteria

Strain	PBP	IC ₅₀ (µg/mL) ^a						Reference
		BPR ^b	CRO	CAZ	MET	IPM	PEN	
<i>S. aureus</i> ATCC 29213 Methicillin-susceptible	1	0.1	0.5	ND ^c	ND	ND	ND	(Davies 2007a)
	2	0.5	0.1	ND	ND	ND	ND	
	3	0.05	1	ND	ND	ND	ND	
	4	1	10	ND	ND	ND	ND	
<i>S. aureus</i> 3726 (MRSA)	2a	0.9	>50	>50	ND	ND	ND	(Davies 2007a)
<i>S. aureus</i> COL (MRSA)	2a ^d	1.7	ND	ND	320	ND	ND	(Chung 2008)
<i>S. aureus</i> COL Bla+ (MRSA)	2a	0.31	ND	ND	39	ND	ND	(Entenza 2002)
<i>S. aureus</i> P8-Hom (MRSA)	2a	0.47	ND	ND	78	ND	ND	(Entenza 2002)
<i>S. epidermidis</i> (MRSE)	2a ^d	0.47	70	ND	>190	>160	ND	(Hebeisen 2001)
<i>S. pneumoniae</i> 8865 penicillin-susceptible	1a	0.03	0.01	ND	ND	0.015	ND	(Davies 2007a)
	1b	0.05	0.03	ND	ND	0.005	ND	
	2x	0.01	0.03	ND	ND	0.013	ND	
	2a	0.03	0.1	ND	ND	0.015	ND	
	2b	0.06	>1	ND	ND	0.008	≤0.015	
	3	0.02	0.02	ND	ND	0.004	ND	
<i>S. pneumoniae</i>	2x ^d	0.14	0.096	ND	0.019	0.05	ND	(Hebeisen 2001)
<i>S. pneumoniae</i> 8819 penicillin-resistant	1a	0.1	0.02	ND	ND	0.04	ND	(Davies 2006, Davies 2007a)
	1b	>8	0.02	ND	ND	0.06	ND	
	2x	1	8	ND	ND	8	ND	
	2a	0.1	0.5	ND	ND	0.015	ND	
	2b	>8	>8	ND	ND	2	>8	
	3	0.01	0.01	ND	ND	<0.015	ND	
<i>S. pneumoniae</i> 8882 penicillin-intermediate	2b	1.2	>8	ND	ND	ND	2.5	(Davies 2010)
<i>S. pneumoniae</i> 8009 penicillin-intermediate	2b	0.9	>8	ND	ND	ND	2.5	(Davies 2010)
<i>E. faecium</i> D63 penicillin-intermediate	1	0.6	ND	0.9	ND	ND	0.9	(Henry 2010)
	2	0.2	ND	0.5	ND	ND	0.1	
	3	0.6	ND	0.04	ND	ND	1.3	
	4	1.5	ND	>200	ND	ND	10	
	5	0.7	ND	>200	ND	ND	75	
	6	>16	ND	>50	ND	ND	1.5	
Penicillin-resistant <i>E. faecium</i>	5 ^d	>270	>300	ND	>190	350	300	(Hebeisen 2001) (Hujer 2005)

^a Concentration of drug that inhibited binding of Bocillin FL or [³H]-penicillin by 50%. ^b BPR, ceftobiprole; CRO, ceftriaxone; CAZ, ceftazidime; MET, methicillin (oxacillin used in the study of Chung *et al.* 2008); IPM, imipenem; PEN, benzylpenicillin. ^c ND, not determined. ^d Cloned and purified PBP tested: the PBP is a mutant form of PBP5 with several amino acid substitutions (Hujer 2005).

Gram-negative aerobic bacteria

Ceftobiprole was active against ESBL-negative *E. coli* displaying MIC₉₀ values ≤ 0.12 $\mu\text{g/mL}$. Against ESBL-negative *E. coli* isolates of human and animal origin, ceftobiprole exhibited MIC₉₀ values of 2 and 1 $\mu\text{g/mL}$, respectively. Against ESBL-positive *E. coli*, ceftobiprole lacked appreciable activity against most isolates, with MIC₉₀ values ≥ 32 $\mu\text{g/mL}$.

Against ESBL-negative *K. pneumoniae*, ceftobiprole exhibited MIC₉₀ values ≤ 0.25 $\mu\text{g/mL}$. Against ESBL-positive *K. pneumoniae*, ceftobiprole, had MIC₅₀ and MIC₉₀ values usually > 16 $\mu\text{g/mL}$.

Ceftobiprole was generally effective against *Klebsiella oxytoca* and ESBL-negative *Klebsiella* spp. with MIC₉₀ values ≤ 2 $\mu\text{g/mL}$. However, a subset of the *K. oxytoca* isolates had elevated ceftobiprole MICs.

The activity of ceftobiprole against *Citrobacter freundii* was variable, with reported MIC₉₀ values of $0.5 \mu\text{g/mL}$. For *Citrobacter koseri*, the ceftobiprole MIC₉₀ value was 2 $\mu\text{g/mL}$. Against the majority of *Enterobacter cloacae* isolates, ceftobiprole had MIC₉₀ values of ≤ 0.12 $\mu\text{g/mL}$. In some subsets of isolates, ceftobiprole had an MIC₉₀ value of 2 $\mu\text{g/mL}$ or greater (8 $\mu\text{g/mL}$).

Enterobacter aerogenes susceptibility to ceftobiprole was also variable. For a couple of subsets *E. aerogenes* isolates and a subset of *Enterobacter* spp. isolates, the MIC₉₀ values of ceftobiprole were 4, 0.12, and 0.12 $\mu\text{g/mL}$, respectively but for another set *E. aerogenes*, the MIC₅₀ and MIC₉₀ values of ceftobiprole were 0.03 and > 32 $\mu\text{g/mL}$.

Ceftobiprole demonstrated potent antibacterial activity against *Moraxella catarrhalis* with MIC₉₀ values of ≤ 1 $\mu\text{g/mL}$. These data include a group of 40 isolates known to be β -lactamase positive.

Ceftobiprole also exhibited strong antibacterial activity against *H. influenzae*. A large group of isolates from four laboratories exhibited ceftobiprole MIC₉₀ values of ≤ 1 $\mu\text{g/mL}$, with most of these isolates displaying ceftobiprole MIC₉₀ values ≤ 0.25 $\mu\text{g/mL}$.

An additional set of β -lactamase negative, ampicillin-resistant (BLNAR) isolates also responded to ceftobiprole, with MIC₉₀ values ≤ 2 $\mu\text{g/mL}$.

Ceftobiprole had low MIC₉₀ values (0.03 $\mu\text{g/mL}$) against *Salmonella* spp. and *Shigella* spp.. The Ceftobiprole had potent activity against *Neisseria* spp. exhibiting MIC₉₀ values of ≤ 0.12 $\mu\text{g/mL}$.

Ceftobiprole was active (MIC₉₀ value of 0.5 $\mu\text{g/mL}$) against a group of 40 isolates which included *Vibrionaceae*, *Aeromonas* spp., *Plesiomonas shigelloides*, and *Vibrio* spp.

Ceftobiprole displayed MIC₅₀ and MIC₉₀ values of ≤ 1 $\mu\text{g/mL}$ and ≤ 8 $\mu\text{g/mL}$ against *Serratia marcescens* and *Serratia* spp.

Ceftobiprole had limited activity against *Pantoea agglomerans*, MIC₉₀ value of 16 $\mu\text{g/mL}$). In another study of 10 isolates each of *S. marcescens* and *S. liquefaciens*, the ceftobiprole MIC₉₀ values were ≤ 1 $\mu\text{g/mL}$.

In general, ceftobiprole demonstrated good activity against *Proteus mirabilis*, MIC₉₀ ≤ 0.06 $\mu\text{g/mL}$), *Morganella morganii*, MIC₉₀ values of ≤ 0.12 $\mu\text{g/mL}$) and *Providencia* spp. including *P. rettgeri* and *P. stuartii*; MIC₉₀ values of ≤ 0.12 $\mu\text{g/mL}$). However, ceftobiprole demonstrated reduced activity against certain indole-positive Proteae; MIC₅₀ and MIC₉₀ values of ≤ 0.016 and 16 $\mu\text{g/mL}$, respectively) which included some isolates of *P. vulgaris*, *Morganella* spp. and *Providencia* spp., and is consistent with the results of other studies of *P. vulgaris* (10 isolates and of *P. vulgaris*, *P. mirabilis*, and *P. rettgeri*, MIC₅₀ and MIC₉₀ values of 0.06 and > 32 $\mu\text{g/mL}$, respectively. In these studies, high MICs were generally associated with the production of a class A cephalosporinase specific to *P. vulgaris* with hydrolytic activity against ceftobiprole.

In summary, ceftobiprole was effective (based on MIC₉₀ ≤ 4 $\mu\text{g/mL}$) against a wide range of Gram-negative bacteria typically encountered in pneumonia infections, including ESBL-

negative *E. coli*, *K. pneumoniae*, *Enterobacter* spp., *Klebsiella* spp., *K. oxytoca*, *M. catarrhalis*, *H. influenzae* (including β -lactamase positive and BLNAR isolates), *Neisseria* spp., *Salmonella* spp., and *Shigella* spp.. Ceftobiprole also demonstrated good activity against *P. mirabilis* (MIC₉₀ \leq 0.06 μ g/mL), but was poorly active against *P. vulgaris* and ESBL-producing Enterobacteriaceae species (MIC₉₀ > 32 μ g/mL).

Activity of ceftobiprole in global surveillance studies against gram negative bacteria

Organism (n)	Agent	MIC ₅₀ (μ g/mL)	MIC ₉₀ (μ g/mL)	Range (μ g/mL)
<i>Escherichia coli</i> (1,812)	Ceftobiprole	\leq 0.06	>8	\leq 0.06 – >8
<i>Escherichia coli</i> , ESBL-confirmed (275)	Ceftobiprole	>8	>8	\leq 0.06 – >8
<i>Klebsiella</i> (672)	Ceftobiprole	\leq 0.06	>8	\leq 0.06 – >8
<i>Klebsiella</i> , ESBL-confirmed (181)	Ceftobiprole	>8	>8	\leq 0.06 – >8
<i>Enterobacter</i> (334)	Ceftobiprole	\leq 0.06	>8	\leq 0.06 – >8
<i>Citrobacter</i> (86)	Ceftobiprole	\leq 0.06	>8	\leq 0.06 – >8
<i>Proteus mirabilis</i> (156)	Ceftobiprole	\leq 0.06	0.12	\leq 0.06 – >8

PBPs from Gram-negative bacteria

In Gram-negative bacteria the PBPs of primary importance are the high-molecular weight class A PBP 1a and PBP 1b and the high-molecular weight class B PBP 2 and PBP 3. Ceftobiprole binds to PBP1b from *C. freundii* (0.085 μ g/mL). In *E. coli*, ceftobiprole binds to PBP3 with IC₅₀ values \leq 0.2 μ g/mL. It also binds to PBP 2 with IC₅₀s of 0.2 μ g/mL which is 20-fold less than that of imipenem which has IC₅₀ of 0.01 μ g/mL.

Table 73 Bactericidal activities of ceftobiprole and comparators against Gram-negative bacteria

Isolate	N	Compound	MIC ₉₀ (μ g/mL)	MBC ₉₀ (μ g/mL)
<i>H. influenzae</i>	30	Ceftobiprole	\leq 0.12	\leq 0.12
	30	Ceftriaxone	\leq 0.12	\leq 0.12
	30	Cefepime	\leq 0.12	\leq 0.12
<i>K. pneumoniae</i> (ESBL-positive)	30	Ceftobiprole	128	>128
	30	Ceftriaxone	>128	>128
	30	Cefepime	>128	>128
<i>K. pneumoniae</i> (ESBL-negative)	30	Ceftobiprole	\leq 0.12	1
	30	Ceftriaxone	0.25	0.25
	30	Cefepime	\leq 0.12	\leq 0.12
<i>E. cloacae</i> (ESBL-negative)	30	Ceftobiprole	\leq 0.12	\leq 0.12
	30	Ceftriaxone	\leq 0.12	\leq 0.12
	30	Cefepime	\leq 0.12	\leq 0.12
<i>P. aeruginosa</i>	30	Ceftobiprole	8	32
	30	Ceftriaxone	128	128
	30	Cefepime	8	32

Source: (Issa 2004)

Pathogens causing atypical pneumonia and other bacterial respiratory infections

Ceftobiprole was active, when tested by broth microdilution, against the atypical bacterium *Legionella pneumophila*. For 30 isolates, the MIC range of ceftobiprole was 0.03-0.12 μ g/mL, with MIC₅₀ and MIC₉₀ values of 0.03 and 0.06 μ g/mL, respectively. Ceftobiprole, like other cephalosporins, is not active against the atypical bacterial CAP pathogens *M. pneumoniae* or *C. pneumoniae*, towards which ceftobiprole exhibited MICs > 32 μ g/mL against 10 and 12 isolates of each species, respectively. Against *Nocardia* spp. which can cause serious infection including slowly progressing pneumonia, ceftobiprole exhibited *in vitro* activity against *N. cyriacigeorgica*, *N. transvalensis* complex and *Nocardia* spp., but was not active against isolates of *N. brasiliensis*, *N. farcinica*, *N. nova* complex, and *N. otitidiscaviarum* complex. Like other cephalosporins, ceftobiprole was not active against

rapidly growing mycobacteria.

Bactericidal activity

For the majority of isolates tested the ceftobiprole MBC90/MIC90 ranged from 1 to 4. In addition, time-kill experiments were conducted with ceftobiprole and comparators against *S. aureus* (N = 21), coagulase-negative staphylococci (N = 6), *S. pneumoniae* (N = 12) and *P. aeruginosa* (N = 6). In the time-kill studies, ceftobiprole demonstrated bactericidal ($>3 \log_{10}$ decrease in colony forming unit (CFU)/mL) activity at 24 h at 2X the MIC against most tested isolates, including *S. aureus*, coagulase-negative staphylococci, *S. pneumoniae*, *H. influenzae*, and *E. coli*. The *P. aeruginosa* isolates were tested against a more narrow concentration range of ceftobiprole because the main purpose of this study was to examine the bactericidal activity of antibiotic combinations. Ceftobiprole as a single agent reduced viable bacterial counts after 6h against those strains tested at 2X MIC, but with a subsequent increase in viable count evident by 24 h.

In a study evaluating ceftobiprole, cefepime and ceftriaxone against 30 isolates each of *H. influenzae*, *K. pneumoniae* (ESBL positive), *K. pneumoniae* (ESBL negative), *E. cloacae*, and *P. aeruginosa* (Issa 2004) all three drugs maintained consistent bactericidal activity against the *H. influenzae* and *E. cloacae* isolates tested, with MBC90 and MIC90 values of $\leq 0.12 \mu\text{g/mL}$. Bactericidal activity was also observed against ESBL-negative *K. pneumoniae*. Against *P. aeruginosa*, ceftobiprole and cefepime had MIC90 values 16-fold lower than ceftriaxone ($8 \mu\text{g/mL}$ versus $128 \mu\text{g/mL}$); however, the MBC90 values for ceftobiprole and cefepime were 4-fold higher than their MIC90 values.

Time-kill studies

In a study by Bogdanovich *et al.* the bactericidal activity of ceftobiprole was studied using time-kill methodology for 12 staphylococcal isolates including MSCoNS (N = 2), MRCoNS (N = 4), MSSA (N = 2), and MRSA (N = 4; two of which were also vancomycin-intermediate and two were vancomycin-resistant). At the MIC ceftobiprole was bactericidal for six of the isolates by 24 h and at 2X and 4X the MIC, ceftobiprole was bactericidal for 11 of the isolates; for one isolate ceftobiprole was bacteriostatic. Against this VISA isolate, ceftobiprole was bactericidal at the MIC but not at higher concentrations. For the other methicillin-resistant vancomycin-intermediate isolate ceftobiprole was bactericidal at 2X and 4x the MIC.

Another study by Lin *et al* investigated the antistaphylococcal activity of ceftobiprole by using time-kill studies to examine its activity against 10 MRSA strains with different resistance phenotypes, compared with those of vancomycin, tigecycline, linezolid, and quinupristin/dalfopristin. The 10 strains all exhibited ceftobiprole MIC values of $\leq 2 \mu\text{g/mL}$. Ceftobiprole, at 2X and 4X MIC, was bactericidal (99.9% killing) after 24 h against 8 of 10, and 9 of 10 strains, respectively. The one strain not killed by ceftobiprole at $4\times$ MIC after 24 h was a community-associated MRSA with a ceftobiprole MIC of $1.0 \mu\text{g/mL}$ and exhibited 99% killing at 2X MIC after 24 h. Ceftobiprole also yielded significant activity at earlier periods, with 90% killing of all strains at 2X MIC at 6 and 12 h.

A study by Kosowska and colleagues examined the bactericidal activity of ceftobiprole against 12 isolates of *S. pneumoniae* including isolates that were penicillin-intermediate (N = 4) and penicillin-resistant (N=4). Some of these isolates were also macrolide-resistant (N = 10) and fluoroquinolone-resistant (N = 3). At 2X and 4X the MIC, ceftobiprole was bactericidal in 10 of the isolates by 12 h. By 24 h ceftobiprole was bactericidal for 11 of the isolates at 2X the MIC and 12 of the isolates at 4X the MIC.

Bogdanovich and examined the bactericidal activity of ceftobiprole against 10 isolates of *H. influenzae* including β -lactamase positive (N = 4), β -lactamase positive amoxicillin-clavulanate-resistant (BLPACR) (N = 2), and β -lactamase negative ampicillin-resistant (BLNAR) (N = 2) isolates. Two β -lactamase positive *M. catarrhalis* isolates were also tested. At the MIC, ceftobiprole was bactericidal for 7 of the *H. influenzae* isolates by 24 h while at 2X and 4X the MIC ceftobiprole was bactericidal for all 10 isolates. For the two *M. catarrhalis* isolates, ceftobiprole and amoxicillin were bactericidal for one isolate at 4X the MIC by 24 h, and bacteriostatic for the other isolate.

Post antibiotic effect (PAE).

To evaluate the *in vitro* PAE of ceftobiprole, and to investigate the effect of subinhibitory ceftobiprole concentrations on the PAE, a study was performed with ceftobiprole at 10-times the MIC with strains of MSSA (N = 2), MRSA (N = 4), *E. faecalis* (N = 3) and one strain each of penicillin-susceptible, -intermediate, and -resistant *S. pneumoniae* (Pankuch 2006). Two of the MRSA strains were also vancomycin intermediate, and one MRSA strain was vancomycin-resistant. The PAE values for the MSSA strains ranged from 0.0 to 0.8, while those for the MRSA strains ranged from 0.0 to 1.8 h. The PAE observed with the enterococci was similar to that for the MSSA strains, ranging from 0.0 to 0.9 h. The PAE of the pneumococcal isolates appeared to be on average slightly longer than that observed for the other Gram-positive isolates, ranging from 1.4 to 3.1 h.

Evaluation of the *in vivo* PAE was performed in infected thighs of neutropenic mice, with a methicillin-resistant *S. aureus* isolate and a penicillin-resistant pneumococcal strain. Thigh infections with each of the strains were produced prior to starting treatment with ceftobiprole at doses of 40 mg/kg and 160 mg/kg. Escalating doses of ceftobiprole produced *in vivo* PAEs of 3.8 to 4.8 h against *S. aureus* ATCC 33591 (MRSA). In a similar study with *S. pneumoniae* CDC 673, a highly penicillin resistant strain, *in vivo* PAEs of 0 to 0.8 h were demonstrated.

Efficacy in animal models

Ceftobiprole demonstrated activity in several different animal models of infection. In mouse septicemia models, ceftobiprole was efficacious against infections caused by both gram-negative and gram-positive pathogens. Anti-staphylococcal activity of ceftobiprole was examined in experimental endocarditis, osteomyelitis, subcutaneous (s.c.) infections and in foreign body (tissue cage) infection models. In these models, ceftobiprole significantly reduced the CFU found in cardiac vegetations, tissue cage fluid, subcutaneous abscesses and skin tissue. Antibacterial activity was comparable or superior to vancomycin. Overall, ceftobiprole demonstrated equivalent or better activity *in vivo* than vancomycin and extended spectrum cephalosporins, and was more effective than linezolid against the organisms tested in the different *in vivo* models.

The *in vivo* activities of ceftobiprole (dosed as the water soluble prodrug ceftobiprole medocaril) and ceftriaxone were examined in a mouse model of acute pneumococcal pneumonia. Mice were treated with ceftobiprole medocaril twice daily for 3 days. Animals infected with strains P-40422 and P-40984 were treated three times a day for three days for a total daily dose of 51 or 75 mg/kg/day. A second group of mice infected with these two strains was treated twice daily for a total dose of 75 mg/kg/day. Ceftriaxone was dosed every 12 hours for three days. Survival rates, bacterial clearance from blood and lungs, and development of resistance to ceftobiprole following therapy was determined. Against the penicillin-susceptible strain P-52181, and the penicillin-, ceftriaxone-, cefotaxime-resistant strain P-40422, survival rates were similar in mice treated with

ceftobiprole medocartil or ceftriaxone. Ceftobiprole treatment resulted in higher survival rates relative to ceftriaxone in mice infected with the penicillin-resistant strain P-15986 or the penicillin-, ceftriaxone-, cefotaxime-resistant strain P-40984.

In a second study using a mouse lethal pneumonia model, 18 and 20 hours post-infection, mice were dosed subcutaneously with ceftobiprole medocartil, ceftriaxone or penicillin, mortality was monitored for 48h post-infection and the ED50 was calculated. ceftobiprole, ceftriaxone and penicillin had similar MICs against the four tested strains (ranging from 0.008 to 0.015 mg/L), ceftobiprole was 13.5 to 50-fold more efficacious than penicillin against all strains, based on calculated ED50 values and compared with ceftriaxone, ceftobiprole ED50s were similar against the wild-type OC6232 strain. Against the wild-type strain, ATCC 6301, and the two macrolide-resistant isolates, the ceftobiprole ED50 was 2.5 to 4-fold lower than that determined for ceftriaxone.

An immunocompetent murine pneumonia model of *H. influenzae*, *E. cloacae* or *K. pneumoniae* infection was used to compare ceftobiprole, ceftriaxone or cefepime. Experimental pneumonia was established in female Swiss mice by instilling a bacterial suspension into the trachea. There were no significant differences between the cephalosporins. Ceftobiprole and cefepime MICs were equivalent for *H. influenzae* and *E. cloacae* and within 2 dilutions for the non-ESBL-producing *K. pneumoniae* strain. The ceftriaxone MIC was 4- to 32-fold higher for *E. cloacae* and *K. pneumoniae* respectively. For the ESBL-producing *K. pneumoniae* strain where the MICs were all greater than or equal to 128 µg/mL, there was no difference between treated and untreated animals ($P=0.32$).

Secondary pharmacology

Effect of ceftobiprole on ECG

Two studies were conducted to evaluate the effects of ceftobiprole on cardiac parameters. Study **CSI-1001** and study **CSI-1003**. Study **CSI-1001** was discontinued.

Study **CSI-1003**, was a randomized, double-blind, placebo- and positive-controlled, double-dummy, 4-way crossover, single-center study of ceftobiprole at presumed therapeutic and supra-therapeutic doses of 500 and 1000 mg, respectively, intravenously infused over 2-hour. The supra-therapeutic dose of 1000 mg is the highest dose ever administered to humans. Moxifloxacin 400 mg, as a single oral dose, was used as a positive control for the evaluation of QT/QTc. Matching placebos were provided for both the intravenous (i.v.) and oral dosage forms. Subjects were randomized to 1 of 4 treatment sequences. There was a washout of at least 7 days between treatments.

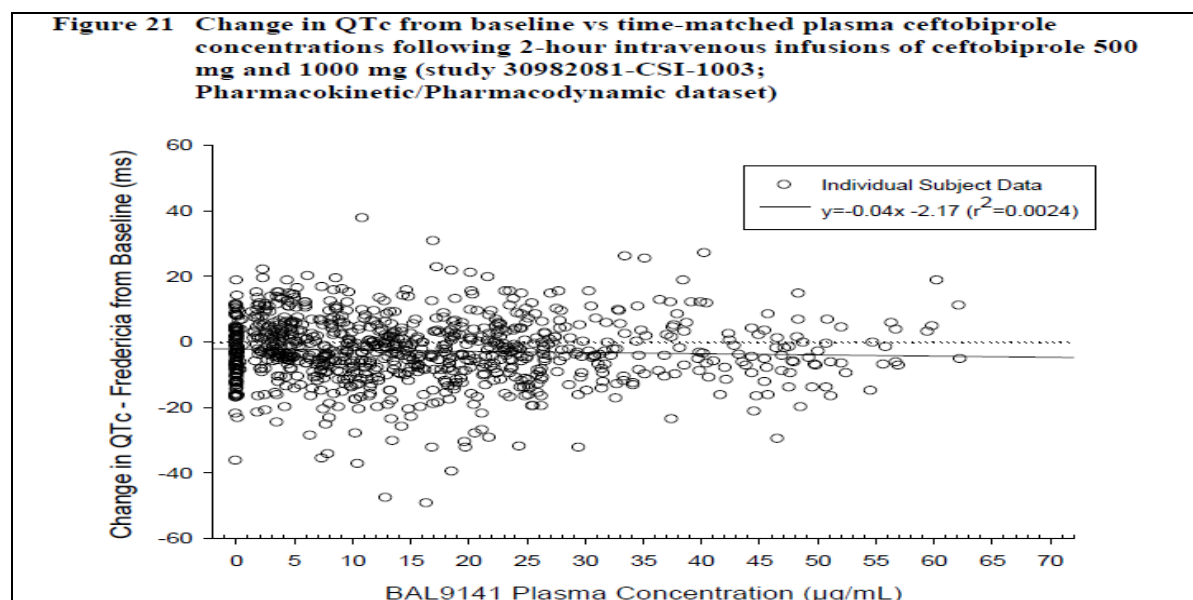
Baseline ECGs were collected for 8 hours on the Day before dosing for each of the 4 treatment periods. Serial time matched 12 lead ECG triplicate readings were recorded on Day -1 and on Day 1 at the following time points for each of the 4 treatment periods: 0, 0.5, 1.25, 2, 3, 4, 6, 8, and 24 hours (Day 2 only). Serial pharmacokinetic samples to measure plasma concentrations of ceftobiprole medocartil and ceftobiprole were obtained either alone or immediately following ECG, heart rate and blood pressure (taken in a supine position) measurements at 0 (pre-dose), 0.25, 0.5, 1.25, 2, 2.5, 3, 4, 6, 8, 12, 16, and 24 hours after the start of the infusion.

Results

The upper limits of the 90% confidence intervals for the difference in mean change from baseline in QT interval corrected with Fridericia correction method ($\Delta QTcF$) between ceftobiprole 1000 mg and placebo were below 10 msec for all time points. The same was true for the difference in means between ceftobiprole 500 mg and placebo at all time points demonstrating that ceftobiprole is non-inferior to, or no worse than, placebo with regard to QTc prolongation. The lower limit of 90% confidence intervals for the difference in mean

Δ QTcF between moxifloxacin and placebo was above zero for all time points between 1.25 and 24 hours, with mean differences ranging from 5.5 msec to 13.1 msec, thereby establishing assay sensitivity.

Individual Δ QTc and time-matched ceftobiprole plasma concentrations following ceftobiprole 500 mg and 1000 mg infusions are presented in Figure 21. Change in QTc was clustered between -20 ms and +20 ms for both ceftobiprole doses, similar to the placebo response. There was no discernible relationship between ceftobiprole plasma concentration and Δ QTc.



Effect of ceftobiprole on the intestinal microflora

Study **CEFTO-BAC-1002** was an open-label, multiple-dose, explorative non-comparative single-center study for the treatment of healthy adult male and female subjects with 500 mg ceftobiprole every 8 hours over 7 days via i.v. infusion over 2 hours. Blood was collected at pre-treatment. Three blood samples were collected each on Day 1, 4 and 7 (after the first infusion of the day immediately after completion of infusion, at 3 and at 6 hours after infusion) and one blood sample was collected in the morning on Day 10, 14 and 21. Fecal samples were collected at pre-dose and on Day 2, 4, 7, 10, 14 and 21. Ceftobiprole plasma concentrations were determined using a validated LC-MS/MS method. Fecal concentrations were determined with a bioassay with *Micrococcus luteus* ATCC 9341 as the test organism (lower limit of quantification of 0.25 mg/L).

Aerobic intestinal microflora:

The mean counts of *E. coli* decreased by approximately 1.5 log cfu/g of feces from Day -1 to Day 14 with recovery to baseline counts on Day 21. The mean values for *Enterobacteriaceae* did not change from Day -1 to Day 21. The mean numbers of enterococci decreased 1.0 log cfu/g of feces from Day -1 to Day 7 and then increased 2 log cfu/g of faeces to Day 14. On Day 21, the numbers of enterococci were recovered to baseline. The numbers of *Candida albicans* were within the normal variation (≤ 2 log cfu/g feces). The changes in the aerobic intestinal microflora were also within the normal variation (≤ 2 log cfu/g feces).

Anaerobic intestinal microflora:

There were no changes in the numbers of lactobacilli and bifidobacteria from Day -1 to Day 21. The counts of clostridia increased from Day 2 to Day 7 with approximately 1.5 log cfu/g feces and then returned to baseline counts. The numbers of bacteroides were only influenced

on Day 2 with a decrease of approximately 0.5 log cfu/g faeces. All alterations were within the normal variation and significance.

Antibiotic susceptibility test:

No new colonising aerobic and anaerobic bacteria resistant to ceftobiprole (MIC >4 µg/mL) were found.

Relationship between plasma concentration and effect

Results from non-clinical animal infection models have shown that the proportion of the dosing interval for which drug concentrations exceed the minimum inhibitory concentration (%T>MIC) to be the principle pharmacokinetic/pharmacodynamic measure predictive of *in vivo* efficacy for the β-lactam class of antibiotics.

According to the applicant a strong correlation between T>MIC and effect was found for ceftobiprole in both *in vitro* and animal studies (Andes 2006).

In a neutropenic mouse thigh infection model, a total of 14 Gram-positive strains were tested to characterize the relationship between dose, dosing interval and microbiologic effect. The eight *S. aureus* strains included three MSSA and five MRSA strains, with ceftobiprole MIC values ranging from 0.5 to 2µg/mL. The ceftobiprole %T>MIC required for microbiologic stasis with *S. aureus* was between 14.1 and 25.4% of the dosing interval. The static doses ranged from 2.03 to 29.2 mg/kg dosed every six hours, and doses that resulted in a 2-log₁₀ kill ranged from 10.3 to 53.9 mg/kg dosed every six hours. The mean %T>MIC value for efficacy against all *S. aureus* strains was 21.1% of the dosing interval. Against *S. pneumoniae* (n=6), microbial stasis resulted when the plasma concentration of ceftobiprole was above the MIC for 15.2% to 22.2% (mean 18.8%) of the dosing interval, similar to the parameters observed for *S. aureus*. Ceftobiprole produced 2-log₁₀ kills against *S. pneumoniae* at doses ranging from 0.45 to 29.0 mg/kg dosed every six hours. The six *S. pneumoniae* strains tested included four that were penicillin-resistant and one that was ciprofloxacin-resistant, with ceftobiprole MICs for these strains ranging from 0.015 - 1µg/mL. Penicillin- or ciprofloxacin-resistance did not alter the magnitude of the %T>MIC necessary for efficacy.

Ceftobiprole was also tested against five strains of Gram-negative bacteria, including *E. coli*, *K. pneumoniae* and *P. aeruginosa* and *E. cloacae*. The ceftobiprole %T>MIC required to produce a static effect was significantly longer for the Gram-negative bacteria than for *S. pneumoniae* and *S. aureus*. The static dose %T>MIC ranged from 41.2 to 46.7 for all tested Gram-negative strains, with the highest value observed for *P. aeruginosa*. The mean value for stasis for the Gram-negative strains, exclusive of *P. aeruginosa*, was 40.8% T>MIC.

Table 137 Pharmacodynamic parameters determined for ceftobiprole in a neutropenic mouse thigh model

Organism ^a	MIC (µg/mL)	Static Dose (mg/kg)	Dosing Interval	Static Dose T>MIC (% dosing interval)
<i>S. aureus</i> ATCC 33591(MRSA)	1	7.91	q. 6 h	19.1
<i>S. aureus</i> WIS MRSA	1	16.1	q. 6 h	25
<i>S. aureus</i> MRSA 11888	2	22.6	q. 6 h	22.5
<i>S. aureus</i> MRSA 12248	2	29.2	q. 6 h	24.5
<i>S. aureus</i> MRSA 22115	1	16.9	q. 6 h	25.4
<i>S. aureus</i> ATCC 22923	0.5	2.03	q. 6 h	14.1
<i>S. aureus</i> ATCC 29213	0.5	3.78	q. 6 h	18.9
<i>S. aureus</i> Smith	0.5	4.21	q. 6 h	19.6
				Mean = 21.1
<i>S. pneumoniae</i> ATCC 10813 (Pen-S)	0.03	0.180	q. 6 h	19.4
		0.307 ^b	q. 6 h	
<i>S. pneumoniae</i> MNO-418 (Cipro-R)	0.015	0.238	q. 6 h	21.3
<i>S. pneumoniae</i> CDC 145 (Pen-R)	0.25	0.956	q. 6 h	15.2
<i>S. pneumoniae</i> CDC 1293 (Pen-R)	0.5	2.72	q. 6 h	16.3
<i>S. pneumoniae</i> CDC 1329 (Pen-R)	0.25	2.93	q. 6 h	22.2
<i>S. pneumoniae</i> CDC 673 (Pen-R)	1	6.68	q. 6 h	18.5
				Mean =18.8
<i>E. coli</i> ATCC 25922	0.06	8.25	q. 6 h	41.9
		10.4 ^b	q. 6 h	
<i>K. pneumoniae</i> ATCC 43816	0.06	6.61	q. 6 h	41.2
		8.57 ^b	q. 6 h	
<i>E. cloacae</i> 2249	2	22.2	q. 3 h	44.6
<i>E. cloacae</i> 4567	0.5	3.28	q. 3 h	35.6
				Mean = 40.8
<i>P. aeruginosa</i> ATCC 27853	2	25.2	q. 3 h	46.7

^a Pen-S, penicillin-susceptible; Cipro-R, ciprofloxacin-resistant; Pen-R, penicillin-resistant.

^b Both static doses are shown for organisms where studies were repeated on different days. The average value for these two studies is shown for T>MIC.

Source: (Craig 2008)

The pharmacokinetic parameters and the pharmacodynamics effects of ceftobiprole were also characterised in *Streptococcus pneumoniae* and *Staphylococcus aureus* murine models (three studies) of acute pneumonia with isolates comprising penicillin-, ceftriaxone- and cefotaxime-resistant *Streptococcus pneumoniae* and CA- and HA-MRSA.

In one study, four strains of *S. pneumoniae* were tested, including one Pen-S strain, one Pen-R strain and two highly penicillin-, ceftriaxone- and cefotaxime-resistant strains. The MRTMIC values in serum were 1.2 h for ceftobiprole medocaril dosed at 25 mg/kg for *S. pneumoniae* P-40422, which displays MICs of 1 for ceftobiprol. For the four tested strains of *S. pneumoniae*, the T>MIC as a percent of the dosing interval ranged from 9 to 18% for ceftobiprole.

Table 138 Activity of ceftobiprole (dosed as ceftobiprole medocaril) and ceftriaxone in a mouse model of acute pneumococcal pneumonia

Strain	Phenotype	Ceftobiprole				Ceftriaxone			
		MIC (µg/mL)	Active Dose ^b (mg/kg)	% Survival	T>MIC ^a	MIC (µg/mL)	Active Dose ^b (mg/kg)	% Survival	T>MIC ^a
P-52181	Pen-S, Cro-S, Ctx-S	0.008	1.05 q12h	91	18	0.03	10 3 × q12h	100	>50
P-15986	Pen-R, Cro-S, Ctx-S	0.25	4.2 q12h	93	9	0.5	50 3 × q12h	75	50
P-40422	Pen-R, Cro-R, Ctx-R	1	17 q8h	80	13	4	100 3 × q12h	85	38
P-40984	Pen-R, Cro-R, Ctx-R	1	25 q8h	80	15	8	200 3 × q12h	83	30

^a Percentage of dosing interval.^b Dose and dosing interval achieving highest observed survival for that strain. Animals were dosed for a total of three days.

Source: (Azoulay-Dupuis 2004)

In the other study, Eight *S. aureus* isolates (two MSSA, three CA-MRSA, and three HA-MRSA isolates) with ceftobiprole MIC values of 0.25 to 2 µg/ml were used for the pharmacodynamic evaluation of ceftobiprole. The pharmacodynamic profiles of ceftobiprole appeared to be similar against the eight *S. aureus* isolates [Table 139], and exerted maximal antibacterial effects when $fT > MIC$ ranged from 6 to 22%, regardless of the phenotypic profile of resistance to beta-lactam.

Table 139 $fT > MIC$ s for corresponding effective doses of ceftobiprole against *S. aureus* isolates in an immunocompromised murine pneumonia model

<i>S. aureus</i> strain	% $fT > MIC$ for:			Maximum log ₁₀ CFU reduction ^d
	ED ₈₀ ^a	ED ₅₀ ^b	Stasis ^c	
MSSA 25923	10.2	8.1	7.6	4.2
MSSA 29213	19.5	14.8	13.3	3.9
HA-MRSA 56	15.5	13.7	13.1	3.9
HA-MRSA 149	13.8	8.6	6.6	4.1
HA-MRSA 152	15.9	14	13.4	3.8
CA-MRSA 144	14.3	13.8	13.6	2.9
CA-MRSA 146	6	5.5	5.6	3.6
CA-MRSA 147	21.6	17.1	14.9	3.2
Mean (SD)	14.6 (4.9)	11.9 (4)	11 (3.7)	3.7 (0.5)

^a80% effective dose^b50% effective dose^cbacterial stasis exposure value^dlog₁₀ change in the numbers of CFU at 24 h versus control

Source: (Laohavaleeson 2008)

In the third study, there was a detailed analysis of the PK/PD relationship correlating both plasma and ELF concentrations of ceftobiprole with the pharmacodynamic antibacterial effect against the eight *S. aureus* isolates in the lung. The median penetration percentage of ceftobiprole into the ELF was 68.8%. The free-drug time>MIC in plasma calculated to achieve a static dose can be seen below.

Table 140 Relationship between ceftobiprole exposure, expressed as free-drug time>MIC in murine plasma or total-drug time>MIC in murine ELF and cell kill for eight strains of *Staphylococcus aureus*

Fraction of dosing interval time>MIC		
	Plasma ^a	ELF ^b
Stasis	8.8%	7.7%
1log ₁₀ kill	13.5%	12.9%
2log ₁₀ kill	23%	24%

^afree-drug time>MIC in murine plasma^btotal-drug time>MIC in murine ELF

Source: (Rodvold 2009)

Simulations were then performed using limited pharmacokinetic data from the multiple-ascending-dose study BAP00010, in which subjects received 30-minute infusions of ceftobiprole at either 500 mg q12h (n=6) or 750 mg q12h (n=6) for 8 days. Probability of target attainment for 3 targets (%fT>MIC 30 to 50%) was estimated for several dosing regimens for MIC-values of 0.5 to 16 [Table 59]. Based on these Monte Carlo simulations, the 500 mg q12h regimen was predicted to have 100% probability of target attainment assuming a 30% T>MIC target and an MIC of up to 4 µg/mL. Regarding the target of 50% T>MIC, the 500 mg q8h regimen was predicted to have 99% probability of target attainment for an MIC of up to 4 µg/mL, while the 750 mg q12h regimen infused over 30 minutes was predicted to have a 78% probability of target attainment. The 750 mg q12h, 30-minute infusion regimen was subsequently selected for evaluation in the Phase 2 study for Gram-positive cSSTI (BAP00034).

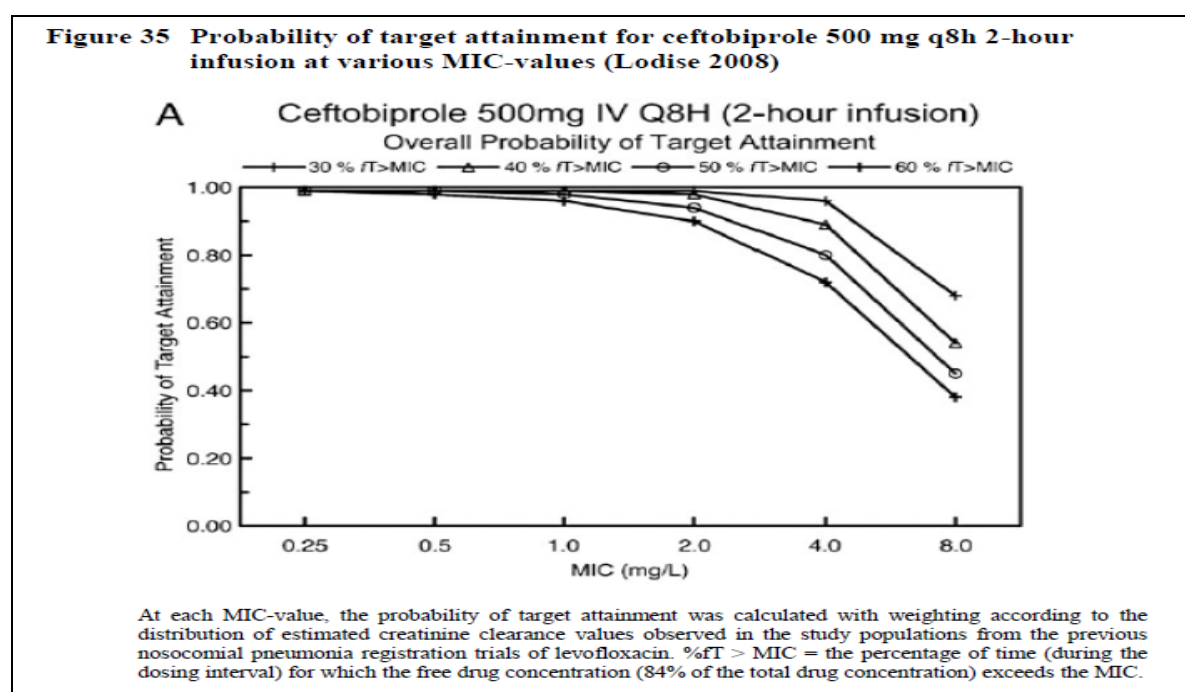
Table 59 Probability of target attainment for ceftobiprole

MIC (µg/mL)	500 mg q12h				500 mg q8h				750 mg q12h			
	30 minute infusion				30 minute infusion				30 minute infusion			
%T>MIC	30	40	50	60	30	40	50	60	30	40	50	60
0.5	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	100	100	100	100	100	100	100	100	100
2	100	100	100	72	100	100	100	100	100	100	100	99
4	100	59	1	0	100	100	99	79	100	100	78	15
8	0	0	0	0	80	13	0	0	69	3	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0

Adapted from (Mouton 2004) using a protein binding correction of 40%.

Another set of Monte Carlo simulations was performed in which aggregate concentration data of 150 subjects from several Phase 1 studies, including the renal impairment study (BAP00018) and concentration data from patients with cSSTI in the Phase 2 study (BAP00034), was applied to describe the pharmacodynamic profile of ceftobiprole. Probability of target attainment for different targets (%fT>MIC 30 to 60%) was estimated for various regimen including 500 mg t.i.d. as a 2-hour infusion investigated in nosocomial pneumonia and CAP. Only the results relevant to the nosocomial pneumonia indication are presented in the following discussion. Since ceftobiprole is essentially eliminated as unchanged drug in urine by the kidney, and the patient population of interest covers a range of renal function, including patients with renal impairment, CLCR was used as a covariate in the population pharmacokinetic model. Of note, among the 150 subjects, subjects with cSSTI had higher CLCR values than did the other subjects included in the population pharmacokinetic analysis. The distribution of renal function among patients was integrated in the population pharmacokinetic model and PTA analysis was based on the distribution of CLCR from a previous registration study of levofloxacin in patients with nosocomial pneumonia.

The overall analyses of the probability of target attainment for ceftobiprole with the nosocomial pneumonia therapeutic dosing regimen are shown in the figure below. For the 500 mg q8h regimen administered as a 2-hour infusion, the probability of target attainment corresponding to 50% fT>MIC for an MIC of 4 µg/mL (probability of adequate patient exposure) was 80% or greater for subjects with normal renal function (CLCR of 80 to 120 mL/min).



The following brief overview provides a summary of the main components of the PK/PD analysis used to calculate the probability of target attainment (PTA) for ceftobiprole in nosocomial pneumonia patients. The PK/PD targets for ceftobiprole have been recalculated based on the exposure (%fT>MIC) of ceftobiprole required to achieve a bactericidal (1 log-kill) effect in pre-clinical infection models. These recalculated bactericidal exposure targets were 30% fT>MIC for Gram-positive organisms, and 60% fT>MIC for Gram-negative organisms. Importantly, these targets correlated closely with the clinical PK/PD analysis of nosocomial pneumonia subjects in study BAP248/307, for whom an exposure of 60% fT>MIC ceftobiprole was shown to be predictive of outcome. Further validation of these exposure targets was provided by the similar target attainment and exposures in the ceftobiprole and ceftazidime arms of this study, at the given doses.

The PTAs were then calculated based on the following parameters: (i) the bactericidal (1 log-kill) target of 60% fT>MIC as defined in preclinical models and confirmed by the PK/PD analysis of nosocomial pneumonia subjects in study BAP248/307 (ii) the measured ceftobiprole exposures in study BAP248/307, and (iii) a ceftobiprole MIC of 4 µg/mL (the highest MIC intended to be covered). Based on these parameters, the PTA of ceftobiprole in nosocomial pneumonia subjects in study BAP248/307 exceeded 96%.

Table 7 Study BAP248/307: Proportion (%) of subjects reaching %fT>MICs of 30% and 60% for target MIC values 0.5 µg/mL – 32 µg/mL (PK/PD ITT population, N=364)

%fT>MIC	MIC						
	0.5 µg/mL	1 µg/mL	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	32 µg/mL
30% ¹	100	100	100	100	96.7	17.6	1.1
60% ²	100	100	99.5	96.7	63.5	4.67	0.824

¹%fT>MIC associated with a 1 log-kill in the neutropenic murine infection model for Gram-positive organisms

²%fT>MIC associated with a 1 log-kill in the neutropenic murine infection model for Gram-negative organisms

Source: Mouton BAP248/307 Pharmacokinetic and pharmacodynamic analysis, Table 2.

The following sections describe a dosing rationale for ceftobiprole in nosocomial pneumonia in which the targeted exposure (%fT>MIC of ceftobiprole) is based on a bactericidal (1 log-kill) effect in pre-clinical animal models of infection. The three sections address:

- The exposure target ($\%fT > MIC$ of ceftobiprole) required to achieve a bactericidal (1 log-kill) effect in pre-clinical animal models of infection, for Gram-positive and Gram-negative bacteria. A justification for utilising these models and extrapolating the data for dose selection in humans is provided.
- PK/PD analysis of nosocomial pneumonia study BAP248/307, which demonstrated that (i) $\%fT > MIC$ is the driver for outcome, with a magnitude similar to the bactericidal (1 log-kill) effect in animal models, and (ii) comparable, adequate, exposures were demonstrated for both ceftobiprole and ceftazidime. Descriptions are provided of the population PK model used, and how inter-subject variability was accounted for in this analysis.
- The dosing rationale for ceftobiprole in nosocomial pneumonia based on the PTA of the bactericidal (1 log-kill) exposure ($\%fT > MIC$) targets in all subjects in study BAP248/307, and in nosocomial pneumonia (excluding VAP) and VAP subjects.

PK/PD targets defined by bactericidal (1 log-kill) activity in pre-clinical animal models of infection

Adequacy of the preclinical models in predicting exposure and penetration of ceftobiprole into the ELF in humans.

Murine lung and thigh infection models were used to derive the PK/PD targets for ceftobiprole which corresponded with bactericidal activity against Gram-positive and Gram-negative pathogens. These are well established models of bacterial infection which have been used to define and quantify PK/PD drivers and targets of different classes of antibacterial agents, including other cephalosporins. A common feature of these models is the incorporation of parameters derived from plasma PK into the PK/PD model.

[Andes 2002, Andes 2006a, Andes 2006b, Andes 2007, Craig 2010, Dudhani 2010] Beta-lactam agents, in particular, exhibit similar and predictable pharmacokinetic properties in these models, and demonstrate a close relationship between plasma PK and the pharmacodynamic effects in different tissues, such as the thigh and lung. Historically, the measured plasma pharmacokinetics have been used in conjunction with thigh and lung infection models as the basis for determining PK/PD targets for other intravenously administered cephalosporins such as cefuroxime, cefotaxime and ceftazidime. The acceptance of this approach and its extrapolation to dosing in humans is demonstrated by EUCAST, which used such data in establishing clinical breakpoints for these cephalosporins [EUCAST 2010a, EUCAST 2010b, EUCAST 2010c].

As shown in detail further below, the PK/PD targets for ceftobiprole in the lung infection model were similar to those derived from the thigh infection model, i.e., 30% and 60% $fT > MIC$ respectively were required for a bactericidal (1log10 kill) effect against Gram-positive and Gram-negative bacteria. This demonstrates that the pharmacokinetics of ceftobiprole in plasma were predictive of the pharmacodynamic effect in both thigh and lung tissue, and is consistent with the effects of members of the cephalosporin class.

Cephalosporins, like most beta-lactams, are confined to the extracellular matrix, and as a class, most exhibit similar pharmacokinetic and plasma protein binding properties in mammals.

The predictable PK/PD properties of ceftobiprole in the same models used to describe PK/PD for other cephalosporins provided a good rationale for utilizing the derived PK/PD targets as an aid to dose selection for the investigational clinical studies of ceftobiprole.

The common pharmacokinetic properties shared by ceftobiprole and other members of the cephalosporin class are further demonstrated by a comparison of epithelial lining fluid (ELF) penetration in healthy human subjects or non-infected patients shown in Table 8.* These data demonstrate that the pharmacokinetic behaviour of ceftobiprole in humans is consistent with

other cephalosporins, and as such justify the use of the same preclinical approaches to the definition of PK/PD targets and the selection of the dose.

It is expected that the human ELF penetration of cephalosporins which are confined to the extracellular matrix, including ceftobiprole, will show a similar pattern to that observed in mice. This is because the main factors thought to influence the entry of antibiotics into the ELF (protein binding, and physiochemical properties such as lipophilicity [Kiem 2008]) are similar among this group of antibiotics and, as described below, ceftobiprole shows very similar PK/PD behaviour to these other cephalosporins in the preclinical models.

Table 8 Cephalosporin literature values for ELF penetration in healthy subjects and non-pneumonia patient populations

Cephalosporin (protein binding)	% ELF penetration by time-points ¹	References
Cefpodoxime (21%)	Non-infected patients (range 8%–14%)	Muller-Serieys 1992
Cefditoren (12%)	Bronchoscopy patients (range 26%–43%)	Lodise 2008
Ceftazidime (21%)	Chronic bronchitis patients (14%)	Cazolla 1995
Cefpirome (10%)	Bronchoscopy patients (range 13%–48%)	Baldwin 1991
Ceftobiprole (16%)	Healthy volunteers (range 19%–43%)	Rodvold 2009

¹Ratio of ELF/unbound plasma or serum concentration at the various times points.

While plasma pharmacokinetics are routinely used to describe the pharmacodynamic effects of beta-lactam antibiotics in the lung, the aspect of lung penetration has been a consideration when analysing these data. Rodvold and colleagues measured the penetration of ceftobiprole into the ELF of infected mice, and reported that the exposure of ceftobiprole in ELF was 68.9% of plasma levels [Rodvold 2009]. However, it should be noted that this point estimate was determined from within a wide interquartile range of 25.1 to 187.3%. While the authors of this study also noted that the degree of ELF penetration in mice with acute pneumonia was greater than the 25.5% reported for healthy human subjects, the validity of this comparison is open to question because it is based on a comparison of pneumonia-infected mice and healthy human subjects.

Inflammation and tissue damage caused by bacterial infection leads to increased tissue permeability [Kiem 2008]. Published observations show that the degree of ELF penetration of beta-lactam antibiotics is greatly influenced by the lung infection status of the host (Table 9).

A comparison of the published ELF penetration properties of beta-lactam antibiotics in uninfected and infected populations (Table 9) shows that ELF penetration of the antibiotic is significantly greater in an infected population.

The conclusions by Rodvold and colleagues about ceftobiprole penetration into the ELF appear to be consistent with the observations from others described in the literature which suggest that ELF measurements in healthy human subjects are likely to be a conservative estimate of the magnitude of drug concentrations that may be observed in patients with infections.

It can be concluded that the murine models used to establish the PK/PD targets for ceftobiprole were adequate for the purpose, because both the pharmacokinetic (plasma PK, ELF penetration, protein binding) and pharmacodynamic (bactericidal activity in thigh and lung tissue) properties of ceftobiprole in this model were as expected for a member of the

cephalosporin class.

The PK/PD targets defined from the preclinical models and described above were used to select the dose of ceftobiprole for the CAP and nosocomial pneumonia studies CAP-3001 and BAP248/307, in which ceftobiprole was shown to be non-inferior to ceftriaxone (\pm linezolid) and ceftazidime (plus linezolid), respectively.

Table 9 Literature values of ELF penetration of beta-lactam antibiotics between infected and non-infected hosts.

Antibiotic (protein binding)	Pneumonia patients (% ELF penetration) ¹	Non-pneumonia population (% ELF penetration) ¹	References
Ertapenem (90%)	Ventilator-associated pneumonia (range 310%–420%)	Non-pneumonia patients (range 62%–94%)	Boselli 2006 Burkhardt 2005
Ceftazidime (21%)	Nosocomial pneumonia (average 27%)	Chronic bronchitis patients (average 14%)	Boselli 2004 Cazzolla 1995
Piperacillin/tazobactam (30%)	Nosocomial pneumonia (average 81%) Ventilator-associated pneumonia (range 41%–92%)	Healthy volunteers (average 37%)	Boselli 2004 Boselli 2008 Chandorkar 2012

¹Ratio of ELF/unbound plasma or serum concentration at the various times points.

Gram-positive organisms

The exposure (%fT>MIC) of ceftobiprole necessary to achieve a bactericidal (1 log-kill) effect in animal models of Gram-positive bacterial infection has been defined in five studies. The %fT>MIC values corresponding to bactericidal (1 log-kill) activity from each of these different studies are summarized in Table 10. In addition, the bacteriostatic targets (which have historically been used to define PK/PD targets for cephalosporins) from each study are provided to allow comparison with the bactericidal targets. Importantly, the measured %fT>MIC values corresponding to a 1 log-kill were in close agreement across different studies and infection sites (thigh and lung). Similarly, the %fT>MIC for a 1 log-kill was similar for *S. aureus* and *S. pneumoniae* when investigated at different inocula, and which included penicillin-, oxacillin-, and cephalosporin-resistant isolates.

Table 10 Exposure targets required for bactericidal activity (1 log-kill) in murine models of Gram-positive infection

Bacteria	Ceftobiprole MIC µg/mL	Model	Ceftobiprole % <i>fT</i> >MIC		Reference
			Stasis	1 log-kill	
<i>S. aureus</i> ¹	0.25–2	Neutropenic murine pneumonia	8.8%	13.5%	Laohavaleeson 2008 Rodvold 2009
<i>S. aureus</i> ²	0.5–2	Neutropenic murine thigh infection	11.4%	15.3%	Lee 2013
<i>S. aureus</i> ³	0.5–2		16.1%	19.8%	
<i>S. pneumoniae</i> ⁴	0.015–0.5		8.4%	13.8%	
<i>S. pneumoniae</i> ⁵	0.015–0.5		16.7%	18.4%	Craig 2008
<i>S. aureus</i> ⁶	0.5–2	Neutropenic murine thigh infection	21.1%	25.8%	
<i>S. pneumoniae</i> ⁷	0.03–1		18.8%	22.3%	
<i>S. pneumoniae</i> ⁸	0.008–1	Murine pneumonia		12.5%	Azoulay-Dupuis 2004

¹ Two MSSA, three HA-MRSA and three CA-MRSA

² Two MSSA and four MRSA. Low inoculum infection 10^{4.5–5.7} CFU

³ Two MSSA and four MRSA. High inoculum infection 10^{6.4–7.2} CFU

⁴ Two penicillin-susceptible and two penicillin-resistant strains. Low inoculum infection 10^{4.5–5.7} CFU

⁵ Two penicillin-susceptible and two penicillin-resistant strains. High inoculum infection 10^{6.4–7.2} CFU

⁶ Eight *S. aureus*, including five MRSA

⁷ Six *S. pneumoniae*, including 4 penicillin-resistant strains

⁸ Two *S. pneumoniae*, including one Penicillin-resistant strain and two Penicillin-, Ceftriaxone- and Cefotaxime-resistant strains

In summary, the %*fT*>MIC of ceftobiprole required to achieve a bactericidal (1 log-kill) effect is independent of the infection model or site (lung or thigh) with *S. aureus* and *S. pneumoniae*. Very concordant %*fT*>MIC values were obtained for bactericidal activity (1 log-kill) of Gram-positive organisms across five studies, with a range of 12.5%–25.8% *fT*>MIC. Consequently, an exposure target based on bactericidal (1 log-kill) activity was selected as 30% *fT*>MIC, corresponding to the upper limit of the range seen in the pre-clinical models with Gram-positive organisms.

Gram-negative organisms

The exposures (%*fT*>MIC) required for bactericidal (1 log-kill) activity against Gram-negative organisms tended to be greater than for the Gram-positive organisms. The Gram-negative exposure targets (%*fT*>MIC) for a 1 log-kill are summarised in Table 11. The bacteriostatic targets are also provided for comparison.

Table 11 Exposure targets required for a 1 log-kill in murine models of Gram-negative infection

Bacteria	Ceftobiprole MIC µg/mL	Model	Ceftobiprole %fT>MIC		Reference
			Stasis	1 log-kill	
<i>E. coli</i>	0.06	Neutropenic murine thigh infection	41.9%	47.4%	Craig 2008
<i>K. pneumoniae</i>	0.06		41.2%	46.5%	
<i>E. cloacae</i>	2.0		44.6%	60.4%	
<i>E. cloacae</i>	0.5		35.6%	35%	
<i>P. aeruginosa</i>	2.0	Murine lung infection	46.7%	56.9%	Rouse 2007
<i>E. cloacae</i> non-ESBL	≤ 0.125			44.3% ¹	
<i>K. pneumoniae</i> non-ESBL	0.5			35.2% ²	

¹ Lung CFU following ceftobiprole treatment was 3.59 log₁₀ CFU/g, compared to untreated control of 6.04 log₁₀ CFU/g.

² Lung CFU following ceftobiprole treatment was <2.50 log₁₀ CFU/g (below limit of detection), compared to untreated control of 6.80 log₁₀ CFU/g.

Taken together, the ceftobiprole exposures required for bactericidal activity (1 log-kill) against these Gram-negative bacilli ranged from 35% to 60.4% fT>MIC. An exposure target based on bactericidal activity (1 log-kill) was selected by taking the upper limit of this observed range, at 60% fT>MIC. Selection of 60% fT>MIC as the Gram-negative target was also based on the PK/PD analysis of study BAP248/307, which showed that exposures above 60% fT>MIC corresponded to positive microbiological and clinical outcomes in subjects with Gram-negative infections.

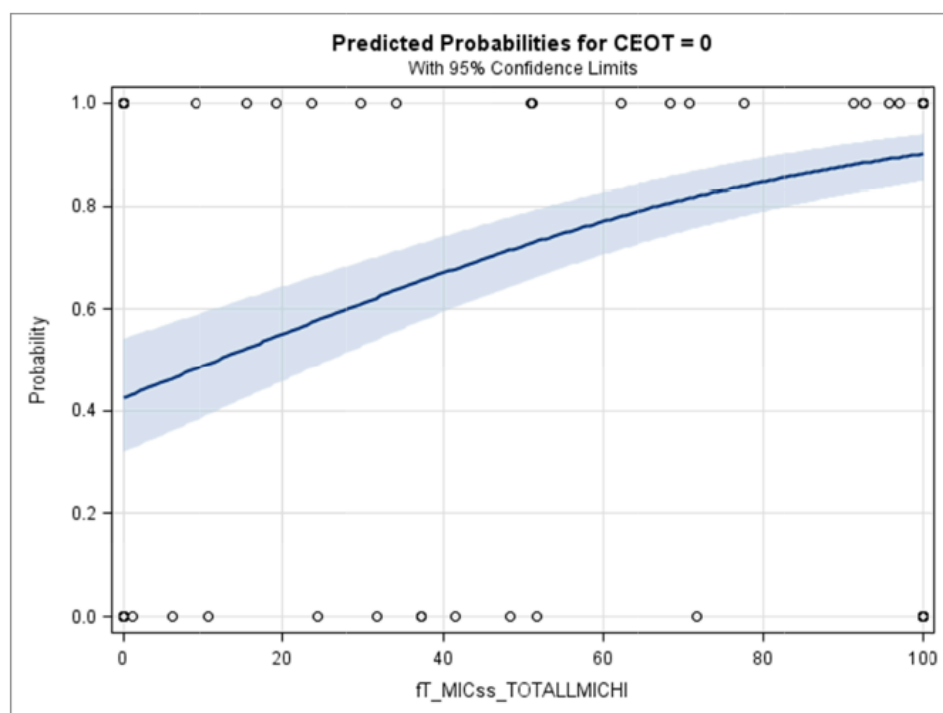
PK/PD analysis of nosocomial pneumonia study BAP248/307

The 500 mg t.i.d. dose administered in study BAP248/307 was selected on the basis of thorough PK/PD analysis consistent with the standard approach for the dose-selection of beta-lactam antibiotics. In order to confirm the adequacy of the ceftobiprole dosing regimen, an additional retrospective analysis was conducted of the PTA for bactericidal activity (1 log-kill) for ceftobiprole using exposure data from study BAP248/307. Further details regarding the adequacy of this analysis, including apparent inter-subject variability, are provided.

PK/PD analysis

The PK/PD analysis of nosocomial pneumonia study BAP248/307 was performed by Mouton and Muller, who demonstrated that %fT>MIC was the PK/PD driver of ceftobiprole for all pathogens in the entire population of the study. This was consistent with the animal model data, and was expected for ceftobiprole as a member of the cephalosporin class. The relationship between the %fT>MIC (of the highest observed MIC at baseline and/or end of treatment [EOT]) and microbiological eradication was investigated by a logistic regression analysis (p<0.0001) as depicted in Figure 1.

Figure 1 Logistic regression analysis for ceftobiprole (estimated individual %fT>MIC vs probability of microbiological eradication)



x-axis: %fT>MIC (of the highest observed MIC at baseline and/or end of treatment [EOT]).

y-axis: probability of microbiological eradication at EOT.

‘CEOT=0’: Subjects with negative culture at EOT.

Further analysis of the PK/PD data from study BAP248/307 showed that the %fT>MIC of ceftobiprole also corresponded to the microbiological outcome at EOT and clinical response at TOC, in NP subjects (excluding VAP) in the ceftobiprole treatment group.

Importantly, the analysis conducted by Mouton and Muller derived for ceftobiprole and Gram-negative pathogens an exposure target 60% or above %fT>MIC (by classification regression analysis [CART], $p < .0001$) that was associated with a high probability of microbiological eradication at EOT in all subjects. This target was very similar to that required for a bactericidal activity (1 log-kill) in the pre-clinical models described above. Further validation of the exposure target selection, and of the utility of the pre-clinical models in dose selection, is provided by a comparison of the PK/PD analyses of ceftobiprole- and ceftazidime-treated subjects in study BAP248/307. Drug exposures in terms of %fT>MIC were comparable in the ceftobiprole and ceftazidime arms, and overall, the proportion of subjects with an adequate exposure (%fT>MIC) during treatment was similar for the two treatment groups.

Further evidence to support the adequacy of the selected ceftobiprole dosing regimen is provided by estimating the probability of target attainment (PTA) in the nosocomial pneumonia population.

As described above, a ceftobiprole exposure target of 60% fT>MIC is sufficient for bactericidal (1 log-kill) activity against both Gram-positive and Gram-negative pathogens in pre-clinical models, and was predictive of microbiological and clinical outcome in the nosocomial pneumonia population in study BAP248/307.

The probabilities of attaining a range of exposure targets (%fT>MIC), at different ceftobiprole MICs, are shown in Table 12, with the bactericidal (1 log-kill) targets for Gram-positive pathogens (30% fT>MIC) and Gram-negative pathogens (60% fT>MIC) highlighted.

Further details regarding the methodology of this analysis are provided.

The ceftobiprole MIC of most relevance is 4 µg/mL, the highest MIC intended to be covered by the current dosing regimen. When a ceftobiprole MIC of 4 µg/mL is considered in the context of the bactericidal (1 log-kill) exposure targets (%fT>MIC), the PTA for Gram-positive organisms is 100%, and the PTA for Gram-negative organisms is 96.7% (Table 12).

Table 12 Study BAP248/307: Proportion (%) of subjects reaching a given %fT>MIC of ceftobiprole for target MIC values 0.5 µg/mL–32 µg/mL for the therapeutic dose, 500 mg t.i.d. (PK/PD ITT population, N=364)

%fT>MIC	MIC						
	0.5 µg/mL	1 µg/mL	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	32 µg/mL
30% ¹	100	100	100	100	96.7	17.6	1.1
40%	100	100	100	99.5	90.1	8.24	0.824
50%	100	100	100	98.9	75	5.22	0.824
60% ²	100	100	99.5	96.7	63.5	4.67	0.824
70%	100	99.7	98.9	92	50.8	4.12	0.824
80%	100	99.5	98.1	83	33.8	2.47	0.824
90%	99.7	99.2	96.4	75	26.6	2.2	0.549
100%	99.5	98.4	93.7	61.5	18.4	2.2	0.549

¹ %fT>MIC associated with a 1 log-kill in the neutropenic murine infection model for Gram-positive organisms

² %fT>MIC associated with a 1 log-kill in the neutropenic murine infection model for Gram-negative organisms

Source: Mouton BAP248/307 Pharmacokinetic and pharmacodynamic analysis.

Furthermore, the PTA for ceftobiprole was similar in the nosocomial pneumonia (excluding VAP), and VAP, subpopulations of study BAP248/307. A target ceftobiprole MIC of 4 µg/mL was used to determine coverage of Gram-negative and Gram-positive organisms with a bactericidal (1 log-kill) exposure target of 60% fT>MIC. In this analysis, more than 94% of both the nosocomial pneumonia (excluding VAP) and VAP subjects were estimated to have been adequately exposed to ceftobiprole, i.e., to have attained 60% fT>MIC (Table 13).

Table 13 Study BAP248/307: Ceftobiprole observed target attainment (% of subjects) for an MIC of 4 µg/mL in nosocomial pneumonia (excluding VAP) and VAP subjects for the given therapeutic dose, 500 mg t.i.d. (PK/PD ITT population)

Target %fT>MIC	NP (excluding VAP) n/N (%)	VAP n/N (%)
≥ 30%	273/273 (100%)	91/91 (100%)
≥ 40%	272/273 (99.6%)	90/91 (98.9%)
≥ 50%	271/273 (99.3%)	89/91 (97.8%)
≥ 60%	266/273 (97.4%)	86/91 (94.5%)

Taken together, the PK/PD analyses of ceftobiprole- and ceftazidime-treated subjects from study BAP248/307 provide several grounds for concluding that ceftobiprole was adequately dosed in this study:

- The ceftobiprole analysis demonstrated a high (> 96%) PTA for a bactericidal (1 log-kill) exposure target, and that attainment of this target was associated with a high probability of a favourable microbiological and clinical response.
- Further analysis revealed that the investigated dosing regimen resulted in a high PTA of

ceftobiprole in both NP (excluding VAP) and VAP subjects.

- Analysis of the subgroup of subjects with Gram-negative infections showed that ceftobiprole achieved similar exposures to ceftazidime in this study, resulting in comparable clinical cure at TOC in nosocomial pneumonia (excluding VAP) subjects.

In summary, the bactericidal (1 log-kill) exposure targets for ceftobiprole were derived from a robust dataset across a series of animal infection models. These models demonstrated that the plasma pharmacokinetics of ceftobiprole were able to predict the bactericidal response independently of infection site and, importantly, that the observed PK/PD targets and parameters from these models were also predictive of microbiological and clinical response in subjects in study BAP248/307. Furthermore, attainment of the bactericidal (1 log-kill) exposure targets for ceftobiprole in all subjects in this study was high (> 96%), regardless of whether subjects had NP (excluding VAP), or VAP.

Taken together, these data describe a robust and consistent PK/PD profile of ceftobiprole in which parameters derived in pre-clinical animal models were predictive of responses in nosocomial pneumonia subjects in study BAP248/307, and formed the basis of a dosing rationale which resulted in adequate exposure (> 96% PTA) in these subjects.

The retrospective PK/PD and PTA analysis of ceftobiprole using data from NP clinical study subjects is considered to be adequate because the PK parameters were derived from individual subject's covariate data using the population PK model. These PTAs exceeded 95% for a target of 60% $fT > MIC$ with a ceftobiprole MIC 4 mg/L, and demonstrate the adequacy of a 500 mg t.i.d. dose of ceftobiprole.

There are two drivers which explain the apparent large inter-subject variability observed in BAP248/307, the first of which is due to the allocation of concentrations to scheduled sampling times. This is accounted for in the ceftobiprole population PK model by using actual sampling times, which substantially (by up to 5-fold) reduced the variability.

The second factor with an impact on inter-subject variability is related to the heterogeneity of the NP patient population, which is accounted for in the population PK model by using covariates.

Scheduled versus actual sampling times

The protocol for study BAP248/307 did not include precise rules regarding scheduled PK sampling times, particularly in regard to sparse sampling.

Under the protocol, for sparse sampling (N=80), three samples were to be collected:

- Two samples on study day 1, one each at about 2 hours and 6 hours after the start of the first study drug infusion
- One sample on study day 4, at least 30 minutes after the start of the study drug infusion, and before the end of the infusion (2 hours)

For rich PK sampling (N=3), seven samples were to be collected:

- On study day 4±1, one sample immediately before the start of the first study drug infusion, three samples during the infusion (at 15 min, 1 hour, and 2 hours, immediately before end of infusion), and three samples at 4, 6 and 8 hours after the start of infusion

The use of scheduled, rather than actual, sampling times in the PK analysis described in the **BAP248/307 CSR** introduced additional variability in the PK analysis; a re-analysis of the PK data using actual sampling times resulted in a significant reduction of intersubject variability, of up to 5-fold depending on the time-point assessed.

This is shown in Table 14 for the sparse-PK analysis set, which constituted more than 95% of all PK samples obtained in study BAP248/307.

Table 14 Study BAP248/307: Comparison of inter-subject variability of ceftobiprole plasma drug concentrations using scheduled versus actual sampling time (sparse PK data set)

Time-point of PK sampling	Ceftobiprole plasma concentrations (µg/mL)	
Day 1: 2-hour (End of infusion)	Scheduled sampling time	Actual sampling time
	N=68	N=52
	Mean (SD)	18.8 (±9.77)
	CV (%)	52%
Day 1: 6-hour	Scheduled sampling time	Actual sampling time
	N=68	N=55
	Mean (SD)	6.1 (±4.5)
	CV (%)	74%
Day 4: 0.5-hour to 2-hour	Scheduled sampling time	Actual sampling time
	N=58	N=34
	Mean (SD)	13.3 (±7.95)
	CV (%)	60%

CV=coefficient of variation (SD/mean); SD=standard deviation.

Only subjects with a documented actual sampling time are included in the analysis of actual sampling time.

For the 2-hour time point on study day 1 (end of infusion), the actual sampling times ranged from 1.5 to 2 hours, which makes this time-point assessment relatively robust (Coefficient of variation [CV]: 48% in the scheduled sampling analysis versus 52% in the actual sampling analysis). In study CSI-1004 (pivotal phase 1 study for therapeutic dose regimen the level of C_{\max} was shown to be approximately 90% at 1.5 hours after the start of the study infusion. For the 0.5- to 2-hour time point on study day 4, the variability was higher than for the 2-hour time point at day 1 (CV: 120% in the scheduled sampling analysis versus 60% in the actual sampling analysis), which is expected considering that the level of C_{\max} is approximately 60% at 1 hour after the start of the study infusion (based on study CSI-1004). For the 6-hour time point on study day 1, the largest variability was observed (CV: 377% in the scheduled sampling analysis versus 74% in the actual sampling analysis), as the actual sampling times ranged from 3 hours up to 11.5 hours. This refers to a period characterised by a relatively steep decline in the ceftobiprole plasma concentration.

Heterogeneity of patient characteristics

As shown in studies BAP00018 (Phase 1 study in renally-impaired subjects) and CEFTO-NOS-1001 (Phase 1 study in ICU subjects), exposure to ceftobiprole is driven predominantly by creatinine clearance, as ceftobiprole is primarily eliminated by glomerular filtration. The broad range of renal function status in subjects included in study BAP248/307 resulted in variation in ceftobiprole plasma levels which reflects the true biological between-subject variation. For example, the creatinine clearance ranged from 11.8 up to 612.1 mL/min in the PK subset, and from 17.0 to 475.3 mL/min in the NP population.

With such a range of creatinine clearance, inter-subject variability in exposure was anticipated.

To adjust for the biological variation due to renal function status, a population PK model was constructed from pooled plasma concentration data obtained in six studies involving 171 subjects, ranging from healthy subjects to severely ill patients, with rich and sparse sampling. This model was constructed using actual PK sampling times. The six studies were BAP00010 (Phase 1 multiple dose study), BAP00034 (cSSSI Phase 2 study), CS-1004 (pivotal Phase 1 study for therapeutic dose), BAP00018 (Phase 1 study in renally impaired subjects), BAP248/307 (nosocomial pneumonia study), and CEFTO NOS-1001 (Phase 1 study in ICU subjects). The pooled data were best described by a 3-compartment model. The covariates

identified in the population PK model for ceftobiprole were creatinine clearance for systemic clearance, and age for the volume of the central compartment.

In summary, the variability observed in the PK analysis described in the **BAP248/307 CSR** was found to be primarily caused by the use of scheduled versus actual sampling times, with the remaining variability due to inherent heterogeneity in the NP patient population. A population PK model with two covariates of age and creatinine clearance using actual sampling times accounted for the remaining variability.

This model was found to be highly predictive of the actual sparse sampling plasma concentrations observed in study BAP248/307, with an R² value of 0.96 [Muller 2013].

The biological variability of ceftobiprole concentrations observed in the sparse PK sampling set could be appropriately adjusted when using age and creatinine clearance as covariates in the population PK model.

Drug exposure for the individual ceftobiprole-treated subjects in study BAP248/307 was then calculated from regression analysis of their covariate data (N=364) against the population PK model. The resulting exposure data were incorporated into Monte Carlo Simulations to describe the PTA of ceftobiprole in NP subjects. presented in Table 12. This retrospective PTA analysis is considered to be adequate because the PK parameter inputs were derived from a robust population PK model which accounted for inter-subject variability seen in initial PK analyses described in the **BAP248/307 CSR**, yet incorporated the biological variability observed in NP patients.

The PTAs from this retrospective analysis were > 95% for a target of 60% fT>MIC with a ceftobiprole MIC 4 mg/L.

To demonstrate the exposure require to achieve a bactericidal 1 log kill, the company states that PK/PD targets based on 1 log kill were retrospectively calculated using pre-clinical animal models of infection. This suggest that the %T>MIC necessary to achieve bactericidal 1 log kill is 30% of the dosing interval for Gram-positive organisms and 60% of the dosing interval for Gram negative organisms. These targets also correlates with the clinical PK/PD analysis of HAP subjects in BAP 248/307 in which an exposure of 60% fT>MIC appeared to be predictive of outcome.

The company's justification regarding the adequacy of the pre-clinical animal models is also acceptable. It is agreed that ELF measurements in healthy human subjects probably don't correlate with ELF measurements in patients with infections as inflammation and tissue damage could alter tissue permeability.

A retrospective PK/PD analysis of PTA in study BAP248/307 has been provided this suggest that %fT>MIC corresponded to microbiological outcome at end of treatment and clinical response at test of cure. In the NP subjects excluding the VAP subjects. The PTA for Gram positive organisms was found to be 100% and for Gram negative organisms 96.7% (MIC 4µg/ml). The %fT>MIC were comparable in both arms (ceftobiprole and ceftazidime).

Ceftobiprole has demonstrated *in vitro* antibacterial activity against several isolates of MRSA (as defined by oxacillin resistance) which do not have a detectable *mecA* gene.

Oxacillin-resistant *Staphylococcus aureus* isolates from the British Society of Antimicrobial Chemotherapy (BSAC) surveillance programme were tested by polymerase chain reaction (PCR) to detect the presence of the *mecA* gene (encoding PBP-2a) in order to be designated as MRSA. However, several isolates exhibited resistance to oxacillin (according to the BSAC MIC breakpoint for oxacillin resistance of > 2 mg/L) were negative for the presence of *mecA* by PCR. These are described below:

- BSAC surveillance year 2009: 3/107 oxacillin-resistant *S. aureus* were negative by PCR for the *mecA* gene. All had oxacillin MICs of 4 mg/L and ceftobiprole MICs of 2 mg/L [BSAC 2010].
- BSAC surveillance year 2008: 5/119 oxacillin-resistant *S. aureus* were negative by PCR for *mecA*. All had oxacillin MICs of 4 mg/L except one which had an oxacillin MIC of 64 mg/L. Ceftobiprole was active (MICs in the range 0.5–2mg/L) against these five isolates [BSAC 2009].
- BSAC surveillance year 2007: 5/89 oxacillin-resistant *S. aureus* were negative by PCR for *mecA*. Three had oxacillin MICs of 4 mg/L, one of 8 mg/L, and one of 128 mg/L. Ceftobiprole was active (MICs in the range 0.5–1mg/L) against these five isolates [BSAC 2008].

These data from the BSAC surveillance programme demonstrated that ceftobiprole was consistently active against clinical *mecA*-negative-oxacillin-resistant *S. aureus* and activity was equivalent to its activity against the majority of MRSA isolates which tested positive for *mecA* by PCR.

There are two possible explanations for the *mecA*-negative-oxacillin-resistant *S. aureus* phenotype. First, it is possible that the oxacillin resistance phenotype was mediated by hyper-production of beta-lactamase PC1. Such isolates have been termed borderline oxacillin-resistant *S. aureus* (BORSA), and exhibit low level resistance (MICs 2–4) to oxacillin [McDougal 1986, Maalej 2012]. Ceftobiprole is very stable to hydrolysis by the *S. aureus* PC1 enzyme, exhibiting hydrolysis rates 20-fold lower than ceftriaxone, and 10,000-fold lower than penicillin G [Hebeisen 2001]. Furthermore, ceftobiprole was active (MICs of 0.25 mg/L) against *mecA*-negative-oxacillin-resistant isolates of *S. haemolyticus* and *S. hominis* in which beta-lactamase expression was detected [Shang 2007]. These observations would suggest that ceftobiprole would be active against *S. aureus* which overproduce PC1.

In support of this, *S. aureus* suspected to be BORSA were identified from the SENTRY surveillance programme (2002–2004). Isolates were considered BORSA when all of the following characteristics were demonstrated:

- Oxacillin MIC \geq 4 mg/L by consensus of broth microdilution and Etests.
- Disk zones $>$ 20 mm for cefoxitin (30- μ g) and \geq 11 mm for ceftizoxime (30- μ g), and \leq 13 mm for oxacillin (1- μ g) or possible synergy by clavulanate and an amoxicillin/clavulanic zone diameter in the susceptible range.
- *mecA* agglutination test result = negative.

Of 35,545 isolates screened, 13 (0.037%) exhibited the suspected BORSA phenotype, and were tested for susceptibility to ceftobiprole. Ceftobiprole exhibited an MIC₅₀ of 0.5 mg/L, an MIC₉₀ of 1 mg/L, and an MIC range of 0.06–1 mg/L. These data confirm the observations from the BSAC surveillance data in that ceftobiprole is active against *mecA*-negative-oxacillin-resistant *S. aureus*, including suspected BORSA isolates, and MICs are no different to those for *mecA*-positive MRSA.

A second explanation for the *mecA*-negative, oxacillin-resistant phenotype is that these isolates could contain a homolog of *mecA* which is not detectable by conventional molecular tests. Such isolates have been described recently and contain a homolog, *mecALGA251*, that is not detectable by the usual *mecA*-specific PCR tests or PBP2a agglutination tests. This novel *mecA* homolog exhibited 70% identity at DNA level to the *mecA* gene [Kriegeskorte 2012]

Ceftobiprole has been tested against 16 (clinically derived, n = 14; ovine origin, n = 2) oxacillin/cefoxitin-resistant *S. aureus* isolates possessing *mecALGA251*, but lacking the classical *mecA* gene. The ceftobiprole MIC₉₀ (determined by Etest) of strains tested was 1 μ g/mL [Kriegeskorte 2012] and the MIC range 0.38 – 1 μ g/mL [Basilea data on file]. The reference MRSA strain ATCC 44330 had a ceftobiprole MIC of 0.75 μ g/mL. These data show ceftobiprole is active against *mecALGA251* MRSA and MICs were the same as those

against their *mecA* counterparts.

In summary, ceftobiprole is active against *mecA* negative *S. aureus*, both suspected BORSA isolates, and *mecALGA251* MRSA.

Overall conclusions of Pharmacodynamics

Ceftobiprole also appears to be active against *streptococcus pneumoniae*, in particular penicillin susceptible strains. However, it does not appear to be as active against penicillin resistant strains.

In terms of gram-negative bacteria known to cause HAP, ceftobiprole appears to have variable activity; it appeared to be active against *E.coli* generally but not active against ESBL strains. It also appears to be active against *H.influenza*.

It is important to note that ceftobiprole does not appear to have any activity against *K.pneumoniae*. This is significant as *K.pneumoniae* is an important cause of HAP.

There were a number of issues regarding the dose selection. In particular;

- It appeared that the targets selected in the murine thigh infection model and the acute pneumonia models were based on stasis instead of log kill. The %T>MIC necessary to achieve 1 log kill for both gram positive and gram negative bacteria have not been provided i.e. it would appear that the 80% PTA is based on stasis and not log kill.
- It appeared that the dosing rationale is based on a neutropenic mouse thigh infection model which indicated that the effects of ceftobiprole against gram-positive bacteria occurred when drug concentrations exceeded the MIC for 30% of the dosing interval and for gram-negative bacteria for 50% of the dosing interval, therefore PTAs were based on these. However, staphylococcus aureus murine models of acute pneumonia suggest that ceftobiprole penetration into ELF was about 69%, but ELF penetration in human volunteers was only about 25.5% (Rodvold 2009) therefore it is not certain that the plasma targets attained in mice will be adequate in humans. Overall, there remains a lack of certainty regarding the adequacy of the dose and dosing interval selected for the pivotal studies.
- It would normally be expected that the PTA would ideally be 90% and above

In response to the above concerns, the following were provided:

- a retrospective analysis which suggest that that the %T>MIC necessary to achieve bactericidal 1 log kill is 30% of the dosing interval for Gram-positive organisms and 60% of the dosing interval for Gram negative organisms. These targets also correlates with the clinical PK/PD analysis of HAP subjects in BAP 248/307 in which an exposure of 60% fT>MIC appeared to be predictive of outcome
- a retrospective PK/PD analysis of PTA in study BAP248/307 which suggest that %fT>MIC corresponded to microbiological outcome at end of treatment and clinical response at test of cure. In the NP subjects excluding the VAP subjects. The PTA for Gram positive organisms was found to be 100% and for Gram negative organisms 96.7% (MIC 4µg/ml). The %fT>MIC were comparable in both arms (ceftobiprole and ceftazidime).

Overall therefore, the right dose regimen appears to have been selected for the pivotal phase III studies.

Clinical Efficacy

The efficacy of ceftobiprole in the treatment of nosocomial pneumonia and the treatment of community-acquired pneumonia have been investigated in 2 separate randomised, double-

blind, multicentre, Phase 3 non-inferiority studies:

- **Study BAP248/307** investigated the efficacy and safety of ceftobiprole compared to a combination of ceftazidime/linezolid in patients with nosocomial pneumonia.
- **Study CAP-3001** investigated the efficacy and safety of ceftobiprole compared to ceftriaxone with/without linezolid in patients with community-acquired pneumonia.

Table 1 Completed Phase 3 studies supporting the efficacy of ceftobiprole medocaril in nosocomial pneumonia (BAP248/307) and community-acquired pneumonia (CAP-3001)

Study	Design and dosing regimen	Number of subjects Randomization
BAP248/307	Randomized (1:1), double-blind, multicenter, Phase 3 non-inferiority study of ceftobiprole medocaril versus ceftazidime/linezolid in the treatment of nosocomial pneumonia. Treatment: ceftobiprole medocaril (500 mg ceftobiprole equivalent every 8 hours as a 120-min i.v. infusion), or linezolid (600 mg every 12 hours as a 60-min i.v. infusion) plus ceftazidime (2 g every 8 hours as a 120-min i.v. infusion) for 7 to 14 days.	N=781 Ceftobiprole (n=391) Ceftazidime/linezolid (n=390)
CAP-3001	Randomized (1:1), double-blind, multicenter Phase 3 non-inferiority study of ceftobiprole medocaril versus ceftriaxone with/without linezolid in the treatment of community-acquired pneumonia. Treatment: ceftobiprole medocaril (500 mg ceftobiprole equivalent every 8 hours as a 120-min i.v. infusion), or ceftriaxone (2g once daily as a 30-min i.v. infusion), with or without 600 mg linezolid every 12 hours as a 60-min i.v. infusion, for 5 to 14 days.	N=638 Ceftobiprole (n=314) Ceftriaxone (n=324)

Dose-response studies and main clinical studies

Dose response studies

There were no clinical dose-finding studies. The dosing rationale for the efficacy studies was based on animal infection models.

Main study (ies)

Study BAP248/307 in nosocomial pneumonia

A randomised, double-blind, multicentre study comparing the efficacy and safety of ceftobiprole and linezolid plus ceftazidime in subjects with nosocomial pneumonia [includes a subset of patients with ventilator-associated pneumonia (VAP)]

Methods

Study Participants

Subjects who were hospitalised with a clinical diagnosis of nosocomial pneumonia NP (including VAP) were eligible for the study.

Main inclusion criteria

- Men or women ≥ 18 years who provided written informed consent.
- Women postmenopausal for at least 1 year, surgically sterile, or practising an effective method of birth control, with a negative pregnancy test at screening

Were included in the study if suffering from nosocomial pneumonia (including VAP) defined as follows:

Nosocomial pneumonia (subjects must have had all of the following):

- Clinical diagnosis of pneumonia after a minimum of 72 hours of hospitalisation or stay in a chronic care facility.

Exceptions included:

- o ≥ 48 to < 72 hours of hospitalisation or stay in a chronic care facility if at admission no acute inflammatory pulmonary infiltrate was present, white blood cell (WBC) and differential blood counts were normal, and the reason for admission was not an infection, OR
- o discharge from the hospital or stay at a chronic care facility ≤ 48 hours after a hospital stay of ≥ 72 hours.
- Clinical signs or symptoms of pneumonia with at least two of the following:
 - o New onset of purulent sputum production or respiratory secretions or a worsening in character of sputum
 - o Tachypnea (respiratory rate ≥ 20 per minute), particularly if progressive in nature
 - o Hypoxemia with a $PO_2 \leq 60$ mmHg while breathing room air, as determined by pulse oximetry or arterial blood gas, or alveolar arterial O_2 gradient, or respiratory failure requiring mechanical ventilation.
- New or persistent (defined as the infiltrate being radiographically visible for at least 72 hours) radiographic infiltrates not related to another disease process.
- Fever or leucocytosis/leukopenia consistent with a diagnosis of pneumonia with at least one of the following:
 - o Fever ([in the absence of antipyretics], increase in core temperature of $> 1^\circ\text{C}$ or an oral temperature $> 38^\circ\text{C}$, a tympanic temperature $> 38.5^\circ\text{C}$, a rectal/core temperature $> 39^\circ\text{C}$, or hypothermia, defined as a rectal/core body temperature of $< 35^\circ\text{C}$), OR
 - o Leucocytosis (a total WBC count $\geq 10 \times 10^9/\text{L}$ or $\geq 15\%$ immature neutrophils [bands], regardless of total peripheral white count; or leukopenia with total WBC $\leq 4.5 \times 10^9/\text{L}$).

Ventilator-associated pneumonia (VAP)

- Subjects with nosocomial pneumonia (as defined above) who developed pneumonia more than 48 hours after onset of mechanical ventilation.
- With microbiological samples (respiratory secretions) suitable for culture and microscopy.
- APACHE II score ≥ 8 and ≤ 25 .

Main exclusion criteria*General exclusions*

- Women who were pregnant or lactating.
- Any known or suspected hypersensitivity to any related anti-infective (including beta-lactam antibiotics, such as penicillins, cephalosporins, oxazolidinones, or monobactams).
- Any known or suspected condition or concurrent treatment that was contraindicated by the prescribing information for linezolid (any medicinal product that inhibits monoamine oxidases A or B [e.g., phenelzine, isocarboxazid, selegiline, moclobemide] or within 2 weeks of taking any such medicinal product), or ceftazidime.

Exception: Subjects with underlying conditions and/or on concomitant medications that might have put them at risk from monoamine oxidase inhibition were enrolled only if they were hospitalized.

- Known or suspected severe renal impairment (calculated CLCr < 30 mL/min or oliguria < 20 mL/h unresponsive to fluid challenge), or any form of dialysis.
- Known or suspected hepatic dysfunction (total bilirubin, alanine aminotransferase [ALT], or aspartate aminotransferase [AST] $\geq 3 \times$ upper limit of the normal range [ULN]).
- Known to be HIV-positive with CD4 counts of $0.2 \times 10^9/L$ (≤ 200 cells/mm³) (subjects with HIV and counts $> 0.2 \times 10^9/L$ [> 200 cells/mm³] could be included).
- Any other known or suspected condition that may have jeopardized adherence to protocol requirements (e.g., severe COPD, New York Heart Association Class 4 cardiac disease, burn subjects with $> 15\%$ total body burn or any significant third degree burn).
- Myelosuppression or neutropenia (absolute neutrophil count [ANC] $\leq 0.5 \times 10^9/L$ [< 500 PMNs/mm³]), severe anaemia (hemoglobin < 6.5 g/dL), or severe thrombocytopenia ($< 49.9 \times 10^9/mm^3$). Subjects receiving immunosuppressive therapy who were expected to reach a nadir of < 500 PMNs/mm³ during administration of study drug should not have been enrolled.

Exclusions related to clinical conditions that might have interfered with assessments of efficacy

- Sustained shock (e.g., systolic blood pressure < 90 mmHg for > 2 hours despite adequate fluid resuscitation, with evidence of hypoperfusion or need for sympathomimetic agents).
- Any of the following:
 - known bronchial obstruction or a history of post-obstructive pneumonia (subjects with mild and moderate chronic obstructive pulmonary disease [COPD] were allowed), including primary lung cancer or another malignancy metastatic to the lungs
 - cystic fibrosis
 - lung abscess
 - pleural effusion as a primary source of infection
 - active tuberculosis
 - required antibiotic coverage for aspiration pneumonia, atypical pneumonia (including *Legionella pneumophila*), or *P. jirovecii* (*carinii*) pneumonia

Exclusions related to microbiological conditions that might have interfered with assessments of efficacy

- Use of systemic antimicrobial therapy for more than 24 hours in the 48 hours before enrolment.

Exceptions: Systemic antimicrobial therapy for more than 24 hours in the 48 hours prior to enrolment was permitted in the event of any of the following:

- the infection was caused by microbiologically confirmed pathogens that were resistant to the previous antimicrobial agents.
- the subject was clinically failing treatment despite at least 48 hours of antibiotic therapy and was infected with a pathogen that was considered to be susceptible to both ceftobiprole and the comparator regimen.

Note: Subjects with antibiotic use for more than 24 hours in the last 48 hours before enrolment required a confirmed microbiological diagnosis of nosocomial pneumonia.

- Evidence from available surveillance cultures of (co-)infection with pathogen(s) including:
 - Extended spectrum beta-lactamases producers,

- *Proteus vulgaris*, OR
- ceftazidime- or ceftobiprole-resistant non-fermenters.

The inclusion and exclusion criteria are acceptable.

Treatments

Ceftobiprole medocaril (500 mg every 8 hours by 250 mL intravenous infusion over 120-minutes)

Linezolid (600 mg every 12 hours as a 60-minute intravenous infusion) plus ceftazidime (2 g every 8 hours as a 120-minute intravenous infusion)

The scheduled treatment duration was 7 to 14 days. If more than 14 days of treatment were necessary, the subject was to have been discontinued.

Combination therapy with protocol-defined agents (levofloxacin, amikacin, or gentamicin) was permitted in subjects identified to be at risk for pseudomonal infections.

HAP and VAP are commonly poly-microbial and usually caused by aerobic gram-negative bacilli and gram-positive bacteria especially MRSA, hence the choice of Linezolid and ceftazidime as comparators. These are acceptable and in line with IDSA guidelines.

Objectives

Primary objective

The primary objective of this study was to demonstrate the non-inferiority of ceftobiprole compared with linezolid plus ceftazidime with respect to the clinical cure rate at the Test of Cure (TOC) visit in subjects with nosocomial pneumonia.

Secondary objectives

The secondary objectives, to be tested in a hierarchical order, were to compare:

1. Microbiological eradication rate at the TOC visit.
2. Clinical cure rate in subjects with *S. aureus* (including MRSA) at the TOC visit.
3. Clinical cure rate in subjects with VAP at the TOC visit.
4. Clinical relapse rate at the late follow-up (LFU) visit.
5. 30-day pneumonia-specific mortality.

Outcomes/endpoints

The primary efficacy endpoint was the clinical cure rate at the TOC visit, defined as the ratio of the number of subjects who had a clinical outcome of Cure at the TOC visit to the total number of subjects in the analysis set under consideration.

The primary efficacy analysis was performed on the co-primary Clinically Evaluable and ITT analysis sets.

Secondary efficacy endpoints were to demonstrate the non-inferiority of ceftobiprole versus linezolid plus ceftazidime with respect to the following outcomes using a step-down procedure (to protect against a Type I error) in the following order:

- (1) Microbiological eradication rate at the TOC visit,
- (2) Clinical cure rate at the TOC visit in subjects with nosocomial pneumonia caused by *S. aureus* (including MRSA),
- (3) Clinical cure rate at the TOC visit in subjects with VAP, (4) clinical relapse rate at the LFU visit in subjects with nosocomial pneumonia, (5) 30-day pneumonia-specific mortality rates in subjects with nosocomial pneumonia.

The endpoints chosen are overall acceptable.

Sample size

Sample size calculation was based on a non-inferiority design using the CI approach for normal approximation to the difference of two binomial probability distributions. The following assumptions were used in the calculations:

- Clinical cure rate 50% in both groups
- Non-inferiority margin 15%
- Level of significance two-sided 5%
- Power 90%
- Clinically evaluable rate 60%

Based on these assumptions, a total of 770 subjects were to be enrolled to ensure 231 clinically evaluable subjects in each treatment group.

Randomisation

As eligible subjects were identified during baseline, they were randomly assigned to treatment via a central Interactive Voice Response System (IVRS) in a 1:1 ratio to 1 of the 2 treatment groups based on a computer-generated randomization schedule prepared by the Sponsor before the study. The randomization was balanced by using randomly permuted blocks for each of the 2 subject strata (non-VAP and VAP). The subjects were further stratified based on their APACHE II score at baseline, 8 to 19 and 20 to 25. The subjects with VAP were further stratified according to number of days on ventilation, ≥ 5 days and < 5 days. The randomization list was prepared by the Sponsor or the designated statistician, and kept in a secured area until after the study was complete and the database was finalised.

Blinding (masking)

It was a double-blind study with respect to the investigators and the study participants. An unblinded pharmacist was responsible for preparing the study medication. Infusion bags and line tubings were covered by coloured sleeves. The pharmacist was monitored by an unblinded site monitor, who operated independently of the blinded site monitor and all blinded activities.

Statistical methods

Intent-to-Treat (ITT): all subjects randomly assigned to treatment.

Microbiological Intent-to-Treat (mITT): all subjects in the ITT analysis set who had a valid pathogen at baseline.

Clinically Evaluable: all ITT subjects who received at least one dose of study medication excluding those subjects with a derived clinical outcome of Not Evaluable at the TOC visit. The derived clinical outcome was based on clinical assessment of a subject, whereas the clinical outcome collected on the CRF was based on the Investigator's assessment.

Microbiologically Evaluable (ME): all subjects in the mITT analysis set who were also clinically evaluable, excluding those with a microbiological outcome of Not Evaluable at the TOC visit. The microbiological outcomes are Eradication, Presumed Eradication, Colonization, Persistence, Presumed Persistence, Superinfection, or Not Evaluable.

Safety: all subjects in the ITT analysis set who were exposed to any study medication.

Non-inferiority margin

A 15% non-inferiority margin was prospectively defined for study BAP248/307, in accordance with the CHMP *Guideline for the choice of non-inferiority margins* (EMA/CPMP/EWP/2158/99), based on two important elements:

The first was the variability around a point estimate of clinical cure observed in clinical studies involving the treatment of patients with nosocomial pneumonia with a treatment regimen involving combinations of linezolid and ceftazidime. As no studies were identified in which these agents were used together for the treatment of patients with nosocomial pneumonia, studies in which either of these agents were used to treat nosocomial pneumonia were included in the analysis of comparator cure rates.

The second was the estimate of the spontaneous cure rate in patients with nosocomial pneumonia who had a disease comparable to the definition in study BAP00248/307. Since no placebo-controlled studies in patients with nosocomial pneumonia who had disease comparable to that studied in study BAP00248/307 have been published, an estimate of the spontaneous cure rate was derived from patients who received inappropriate antibiotic therapy.

The justification of the non-inferiority margin in the BAP248/307 study required a demonstration that it preserved at least 50% of the benefit of the active comparator over placebo in the treatment of nosocomial pneumonia.

Baseline data

The percentage of male subjects in the ceftobiprole group (71%) was greater than in the linezolid plus ceftazidime group (62%). All other demographic and baseline characteristics for subjects in the ITT analysis set were similar between the 2 treatment groups. The majority (81%) of subjects were white. The mean age of the subjects was 61 years, and ages ranged from 18 to 98 years, with 52% of the subjects being under 65 years of age.

Clinical baseline characteristics

The clinical baseline characteristics of subjects in the ITT analysis set were similar between the 2 treatment groups.

The distribution of subjects by the stratification factors of APACHE II scores (8 to 19 versus 20 to 25), infection type (VAP versus non-VAP), and ventilation duration (2 to <5 days versus ≥ 5 days for VAP subjects) was similar between the 2 treatment groups.

12% of subjects in the ceftobiprole group and 13% in the linezolid plus ceftazidime group had an APACHE II score of 20 to 25.

Two hundred forty-six (63%) of 391 ceftobiprole-treated subjects and 240 (62%) of 390 linezolid plus ceftazidime-treated subjects were never ventilated prior to the onset of pneumonia. Forty-one (10%) of 391 ceftobiprole-treated subjects and 44 (11%) of 390 linezolid plus ceftazidime-treated subjects were ventilated <48 hours prior to the onset of pneumonia and were treated as non-VAP subjects for the purposes of analyses.

A total of 69% of all subjects had valid baseline pathogens.

- 136 (35%) of 391 ceftobiprole-treated subjects and 149 (38%) of 390 linezolid plus ceftazidime-treated subjects had a valid gram-positive pathogen at baseline.
- 196 (50%) of 391 ceftobiprole-treated subjects and 177 (45%) of 390 linezolid plus ceftazidime-treated subjects had a valid gram-negative pathogen at baseline.
- Of those subjects with a valid pathogen at baseline, 174 (65%) of 269 ceftobiprole-treated subjects and 175 (66%) of 267 linezolid plus ceftazidime-treated subjects had monomicrobial infections, defined as an infection involving 1 organism.

Table 11: Clinical and Baseline Characteristics for All Subjects (Study BAP00248/307: Intent-to-Treat Analysis Set)				
	--- Ceftobiprole --- (N=391)	Linezolid/Ceftazidime (N=390)	----- Total ----- (N=781)	P-value
Subjects with a valid pathogen at baseline				
N	391	390	781	
Category, n (%)				
Yes	269 (69)	267 (68)	536 (69)	0.919 ^a
No	122 (31)	123 (32)	245 (31)	
Subjects with a valid gram-positive pathogen at baseline				
N	391	390	781	
Category, n (%)				
Yes	136 (35)	149 (38)	285 (36)	0.321 ^a
No	255 (65)	241 (62)	496 (64)	
Subjects with a valid gram-negative pathogen at baseline				
N	391	390	781	
Category, n (%)				
Yes	196 (50)	177 (45)	373 (48)	0.185 ^a
No	195 (50)	213 (55)	408 (52)	
Subjects with a valid pathogen at baseline				
N	269	267	536	
Category, n (%)				
Monomicrobial	174 (65)	175 (66)	349 (65)	0.835 ^a
Polymicrobial	95 (35)	92 (34)	187 (35)	
APACHE II score				
N	391	390	781	
Category, n (%)				
8-19	344 (88)	338 (87)	682 (87)	0.511 ^a
20-25	46 (12)	51 (13)	97 (12)	
Above 25	1 (<1)	0	1 (<1)	
Missing	0	1 (<1)	1 (<1)	
Infection type				
N	391	390	781	
Category, n (%)				
VAP	104 (27)	106 (27)	210 (27)	0.855 ^a
Non-VAP	287 (73)	284 (73)	571 (73)	
Prestudy ventilation duration				
N	391	390	781	
Category, n (%)				
Never ventilated	246 (63)	240 (62)	486 (62)	0.546 ^a
Ventilated 1-<2 days	41 (10)	44 (11)	85 (11)	
Ventilated 2-<5 days	28 (7)	20 (5)	48 (6)	
Ventilated ≥ 5 days	76 (19)	86 (22)	162 (21)	

^a n value was calculated using the Chi-square test

^a p value was calculated using the Chi-square test.

Less than 30% of the subjects enrolled had VAP and most of the subjects had APACHE II scores less than 20, suggesting that most of the subjects enrolled were not too severely ill. However, about 2/3rd of the subjects enrolled had valid pathogens at baseline.

Concomitant Systemic Antimicrobial Therapy

- In the ITT analysis set, a total of 30% of subjects (33% of ceftobiprole treated and 28% of linezolid plus ceftazidime-treated subjects) received non-study systemic antibiotic therapy effective against gram-positive or gram-negative pathogens between baseline and the TOC visit. Overall, the most commonly received therapies (≥5% of subjects) were carbapenems (9%), fluoroquinolones (9%), cephalosporins (6%), aminoglycosides (6%), and penicillin combinations including those with β-lactamase inhibitors (5%). Subjects who received a non-study systemic antimicrobial for any reason after randomization and before the TOC visit were considered clinical failures at the TOC visit in the ITT analysis set.
- A total of 73 (15%) (18% in the ceftobiprole group versus 12% in the linezolid plus ceftazidime group) of 495 clinically evaluable subjects received allowed anti-pseudomonal therapy from baseline to the TOC visit

Table 13: Allowed Anti-Pseudomonal Therapy Taken From Baseline to the TOC Visit for All Subjects (Study BAP00248/307: Clinically Evaluable Analysis Set)			
Therapeutic Class	Ceftobiprole (N=251)	Linezolid/Ceftazidime (N=244)	Total (N=495)
Pharmacological/Chemical Class			
Medication Generic Term	n (%)	n (%)	n (%)
Total no. subjects with anti-pseudomonal therapy	44 (18)	29 (12)	73 (15)
Antibacterials for systemic use	44 (18)	29 (12)	73 (15)
Other aminoglycosides	29 (12)	19 (8)	48 (10)
Amikacin	28 (11)	18 (7)	46 (9)
Gentamicin	1 (<1)	1 (<1)	2 (<1)
Fluoroquinolones	18 (7)	11 (5)	29 (6)
Levofloxacin	10 (4)	9 (4)	19 (4)
Ciprofloxacin	8 (3)	1 (<1)	9 (2)
Ofloxacin	0	1 (<1)	1 (<1)

Note: Percentage calculated with the total number of subjects as the denominator.

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Numbers analysed

Of the 781 subjects who were randomly assigned to receive treatment in this study, 251 (64%) of 391 subjects in the ceftobiprole group and 244 (63%) of 390 subjects in the linezolid plus ceftazidime group were considered clinically evaluable for efficacy (Table below).

Table 6: Number of Subjects Included in Each Analysis Set for All Subjects (Study BAP00248/307: Intent-to-Treat Analysis Set)			
Analysis Set	Ceftobiprole (N=391)	Linezolid/Ceftazidime (N=390)	Total (N=781)
	n (%)	n (%)	n (%)
Intent-to-Treat	391 (100)	390 (100)	781 (100)
Clinically Evaluable	251 (64)	244 (63)	495 (63)
Microbiological Intent-to-Treat ^a	269 (69)	267 (68)	536 (69)
Microbiologically Evaluable	162 (41)	170 (44)	332 (43)
Safety	386 (99)	386 (99)	772 (99)

Note: Percentages were calculated with the number of ITT subjects as the denominator.

^a Microbiological Intent-to-Treat: Subjects in the ITT Analysis Set with valid pathogen at baseline.

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Outcomes and estimation

The primary endpoint of clinical cure at the TOC visit (7–14 days after completing study drug) in the Clinically Evaluable and ITT analysis sets was similar between the two treatment groups. The clinical cure rates were 69.3% vs 71.3% (Clinically Evaluable analysis set) and 49.9% vs 52.8% (ITT analysis set) in the ceftobiprole and linezolid/ceftazidime groups respectively. The 2-sided 95% confidence interval for the difference in the cure rates (ceftobiprole minus linezolid plus ceftazidime) was -10.0% to 6.1 % in the clinically evaluable analysis set. In the ITT analysis set, the 2-sided 95% confidence interval for the difference in the cure rates (ceftobiprole minus linezolid plus ceftazidime) was -10.0% to 4.1%

Table 17: Clinical Cure Rates at the TOC Visit for All Subjects (Study BAP00248/307: Clinically Evaluable and Intent-to-Treat Analysis Set)								
	Ceftobiprole			Linezolid/ Ceftazidime				
	N	n	%	N	n	%	Diff ^a (%)	95% CI ^b
Clinically Evaluable								
All subjects	251	174	69.3	244	174	71.3	-2.0	(-10.0; 6.1)
Intent-to-Treat								
All subjects	391	195	49.9	390	206	52.8	-2.9	(-10.0; 4.1)

Note: n is the number of subjects with a clinical outcome of Cure.

^a Ceftobiprole minus linezolid/ceftazidime.

^b 2-sided 95% C.I. is based on the Normal approximation to the difference of the 2 proportions. A lower limit greater than or equal to -15% indicates that ceftobiprole is not inferior to linezolid/ceftazidime.

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Non-inferiority of ceftobiprole vs linezolid/ceftazidime was also demonstrated in the pre-specified subgroup of subjects with nosocomial pneumonia only (excluding VAP) subjects. However, non-inferiority of ceftobiprole was not demonstrated in the relatively smaller subset of VAP subjects (see Table 1 below).

Table 1 Clinical cure at TOC (primary endpoint) in study BAP248/307						
Clinical cure at TOC	Ceftobiprole		Linezolid/ceftazidime		Diff. (%) ^a	95% CI [#]
Analysis set	N	n (%)	N	n (%)		
Group						
<u>Intent-to-Treat</u>						
All subjects	391	195 (49.9)	390	206 (52.8)	(-2.9)	(-10.0; 4.1)
NP (excluding VAP)	287	171 (59.6)	284	167 (58.8)	(0.8)	(-7.3; 8.8)
VAP	104	24 (23.1)	106	39 (36.8)	(-13.7)	(-26.0; -1.5)
<u>Clinically Evaluable</u>						
All subjects	251	174 (69.3)	244	174 (71.3)	(-2.0)	(-10.0; 6.1)
NP (excluding VAP)	198	154 (77.8)	185	141 (76.2)	(1.6)	(-6.9; 10.0)
VAP	53	20 (37.7)	59	33 (55.9)	(-18.2)	(-36.4; -0.0)

n is the number of subjects with clinical cure at TOC.

^a Difference ceftobiprole minus linezolid/ceftazidime.

[#] Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

For VAP, it is clear that ceftobiprole is inferior to linezolid/ceftazidime

The reasons for failure were similar between the 2 treatment groups in the clinically evaluable analysis set (subjects may have had more than 1 reason) (Table 18).

- The primary reason for failure was the use of non-study systemic antibiotics for pneumonia (21.5% of ceftobiprole-treated and 18.9% of linezolid plus ceftazidime-treated subjects).
- The second most common reason was that subjects were deemed clinical failures at the TOC visit by the investigator (16.7% of ceftobiprole-treated subjects and 16.8% of linezolid plus ceftazidime treated subjects).
- The third most common reason was that a TOC visit assessment was missing and the final clinical assessment before TOC was 'worsened' or 'unchanged' from baseline (9.6% of ceftobiprole-treated subjects and 6.1% of linezolid plus ceftazidime-treated subjects).

Table 18: Reasons for Failure for All Subjects (Study BAP00248/307: Clinically Evaluable Analysis Set)		
	Ceftobiprole n (%)	Linezolid/Ceftazidime n (%)
Cure	174 (69.3)	174 (71.3)
Sponsor failure	77 (30.7)	70 (28.7)
Non-study antibiotics for NP	54 (21.5)	46 (18.9)
TOC outcome as failure	42 (16.7)	41 (16.8)
Worsened/unchanged last observation carried forward	24 (9.6)	15 (6.1)
Discontinued due to treatment-emergent adverse event	11 (4.4)	9 (3.7)
Subject died of NP	10 (4.0)	13 (5.3)
Discontinued due to lack of efficacy	5 (2.0)	6 (2.5)

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Primary Efficacy Sensitivity Analysis

To assess the robustness of the primary efficacy results, a sensitivity analysis was performed excluding all subjects from the following sites: site BAP00248-517, site BAP00248-563, site BAP00248-577, site BAP00307-006, site BAP00307-473, site BAP00307-191, and site BAP00307-545 as they were identified as being at risk for having made errors in clinical study conduct.

In the sensitivity analysis, the treatment difference between ceftobiprole and linezolid plus ceftazidime with respect to the clinical cure rates in the clinically evaluable analysis set was -

4.2% with a 2-sided 95% confidence interval of -12.6% to 4.2% and for the ITT analysis set, the treatment difference was -4.4% with a 2-sided 95% confidence interval of -11.7% to 2.9%. This is similar to the results obtained for the primary efficacy variable.

Primary Efficacy Subgroup Analyses

Subgroup analyses were performed on the primary efficacy parameter to assess the robustness of the primary efficacy results. The clinical cure rate was compared between the 2 treatment groups for various subgroups for the clinically evaluable analysis set.

Due to the significant difference in clinical cure rates between the two treatment groups with respect to the non-VAP and VAP subject stratum using the Breslow-Day test, the treatment by ventilation status interaction p value was 0.069, further analysis of the cure rates were performed separately for non-VAP and VAP subjects.

For non-VAP subjects in the clinically evaluable analysis set, the clinical cure rates were 77.8% (154/198) in the ceftobiprole group and 76.2% (141/185) in the linezolid plus ceftazidime group and in VAP subjects, the clinical cure rates in the clinically evaluable analysis set were 37.7% (20/53) in the ceftobiprole group and 55.9% (33/59) in the linezolid plus ceftazidime group.

Table 20: Clinical Cure Rates at the TOC Visit for All Subjects by Infection Type (Study BAP00248/307: Clinically Evaluable Analysis Set)								
Infection type	Ceftobiprole			Linezolid/ Ceftazidime			Diff ^a (%)	95% CI ^b
	N	n	%	N	n	%		
Non-VAP	198	154	77.8	185	141	76.2	1.6	(-6.9; 10.0)
VAP	53	20	37.7	59	33	55.9	-18.2	(-36.4; 0.0)

Note: n is the number of subjects with a clinical outcome of Cure.
^a Ceftobiprole minus linezolid/ceftazidime.
^b 2-sided 95% C.I. is based on the Normal approximation to the difference of the 2 proportions.
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The microbiological outcome at the TOC visit in study BAP248/307 was derived through a combination of clinical and microbiological assessment at baseline and at the TOC visit. Given its timing (7–14 days after EOT), a microbiological sample for culture was often not available at the TOC visit, particularly if subjects were cured or substantially improved, as respiratory samples for microbiological work-up are generally not obtained from cured patients. In accordance with the protocol definition, for these subjects, in the absence of a valid microbiological sample, microbiological outcome was ‘presumed’ based solely on the clinical outcome at the TOC visit.

For the analyses of microbiological eradication rates, subjects were classified as having microbiological eradication irrespective of whether ‘eradication’ was ‘confirmed’ (no growth in a valid microbiological culture at TOC regardless of the clinical outcome) or ‘presumed’ (for subjects with clinical cure who had no valid microbiological sample at the TOC visit). Similarly, subjects with clinical failure who had no valid microbiological sample at the TOC visit were classified as microbiological failures due to ‘presumed’ persistence. The majority of subjects (> 95%) in both study groups who were classified as microbiological eradications were ‘presumed’ eradications. The efficacy of ceftobiprole and the comparator by baseline pathogen may therefore be best described by clinical cure rates rather than microbiological eradication rates.

Microbiological outcomes

Overall microbiological outcomes are summarised in Table 15, and are further discussed

below.

The overall microbiological eradication rate in the ceftobiprole group was 62.9% (73/116; 70 'presumed' and 3 'confirmed' eradications), compared to 67.5% (81/120; 78 'presumed' and 3 'confirmed' eradications) in the linezolid/ ceftazidime group. However, in subjects with clinical cure, 73/86 (85%) in the ceftobiprole group, and 81/94 (86%) in the linezolid/ceftazidime group, had an outcome of microbiological eradication (presumed plus confirmed). Of these subjects, 'confirmed' microbiological eradications were comparable between the treatment groups (3/73 in the ceftobiprole group, and 3/81 in the linezolid/ceftazidime group).

Differences in microbiological eradication rates between the ceftobiprole and linezolid/ceftazidime treatment groups (60.3% vs 65.0%, respectively) were only observed in the subgroup of subjects with 'presumed' eradication (Table 15). In contrast, microbiological outcome in subjects with a valid microbiological sample at the TOC visit was comparable between the ceftobiprole and linezolid/ceftazidime groups.

Valid microbiological samples were available at TOC for subjects with a clinical outcome of cure for 16/86 ceftobiprole subjects (18.6%) and 16/94 linezolid/ceftazidime subjects (17.0%). For subjects with a clinical outcome of failure, valid microbiological samples were available at TOC for 7/30 ceftobiprole subjects (23.3%) and 9/26 linezolid/ceftazidime subjects (34.6%).

The rate of 'confirmed' microbiological failure (persistence or colonization) in subjects with an outcome of clinical cure was also comparable between the two treatment groups.

Microbiological failure in clinically cured subjects was observed in 13 of the 86 subjects (15.1%) in the ceftobiprole group and in 13 of the 94 subjects (13.8%) in the linezolid/ceftazidime group (Table 15).

In subjects with clinical failure at the TOC visit, no subjects in either treatment group had 'confirmed' microbiological eradication. The rate of 'confirmed' microbiological failure (i.e., persistence or superinfection) in subjects with clinical failure at TOC was numerically higher in the linezolid/ceftazidime group: 7/30 subjects (23.3%) in the ceftobiprole group and 9/26 subjects (34.6%) in the linezolid/ceftazidime group (Table 15).

Table 15 Study BAP248/307: Distribution of confirmed microbiological outcomes (valid microbiological culture at the TOC visit) by treatment group and clinical outcome (Microbiologically Evaluable analysis set)

Pathogen	Ceftobiprole			Linezolid/ceftazidime		
	Clinical cure N=86 n (%)	Clinical failure N=30 n (%)	All subjects N=116 n (%)	Clinical cure N=94 n (%)	Clinical failure N=26 n (%)	All subjects N=120 n (%)
All subjects						
Microbiological eradication (presumed and confirmed)	73 (84.9%)	0 (0.0%)	73 (62.9%)	81 (86.1%)	0 (0.0%)	81 (67.5%)
No valid microbiological sample at TOC¹	70 (81.4%)	23 (76.7%)	93 (80.2%)	78 (83.0%)	17 (65.4%)	95 (79.2%)
Presumed eradication			70 (60.3%)			78 (65.0%)
Presumed persistence			23 (19.8%)			17 (14.2%)
Valid microbiological sample at TOC	16 (18.6%)	7 (23.3%)	23 (19.8%)	16 (17.0%)	9 (34.6%)	25 (20.8%)
Eradication ²	3 (3.5%)	0 (0%)	3 (2.6%)	3 (3.2%)	0 (0%)	3 (2.5%)
Colonization ³	1 (1.2%)	n.a.	1 (0.9%)	6 (6.4%)	n.a.	6 (5.0%)
Persistence ⁴	12 (14.0%)	4 (13.3%)	16 (13.8%)	7 (7.4%)	5 (19.2%)	12 (10.0%)
Superinfection ⁵	n.a.	3 (10.0%)	3 (2.6%)	n.a.	4 (15.4%)	4 (3.3%)
'Confirmed' microbiological eradication	3 (3.5%)	0 (0%)	3 (2.6%)	3 (3.2%)	0 (0%)	3 (2.5%)
'Confirmed' microbiological failure ⁶	13 (15.1%)	7 (23.3%)	20 (17.2%)	13 (13.8%)	9 (34.6%)	22 (18.3%)

¹ Subjects with clinical cure and no valid microbiological sample at the TOC visit were categorized as "presumed" eradication; subjects with clinical failure and no valid microbiological sample at the TOC visit were categorized as "presumed" persistence. The overall microbiological eradication rate was 73 (70 presumed plus 3 confirmed eradications) out of 116 subjects (62.9%) in the ceftobiprole group and was 81 (78 presumed and 3 confirmed eradications) out of 120 subjects (67.5%) in the linezolid/ ceftazidime group.

² No pathogen growth regardless of clinical cure or failure.

³ Growth of new pathogen in a subject with clinical cure.

⁴ Growth of baseline pathogen regardless of clinical cure or failure.

⁵ Growth of new pathogen in a subject with clinical failure.

⁶ Includes microbiological outcomes of colonization, persistence or superinfection.

Microbiological eradication and clinical cure by pathogen – discussion

Differences in clinical cure and eradication rates by pathogen between treatment groups with a tendency towards higher cure and eradication rates in the comparator group were observed in the analyses of "All subjects" (i.e. 'VAP subjects' and 'NP excluding VAP subjects' combined). However, for Gram-positive pathogens and most of the Gram-negative pathogens, these differences were driven by the inferior outcome of ceftobiprole in the subgroup of VAP subjects (see Table 16). For pathogens in the larger group of NP (excluding VAP) subjects, clinical cure and microbiological eradication rates were similar, in particular for Gram-positive pathogens, but also for most of the Gram-negative pathogens. Clinical cure and microbiological eradication for infections with Gram-negative pathogens are summarised in Table 16 and are discussed in more detail below:

- For the relatively small sample of *Haemophilus* and *Acinetobacter* species at baseline, numerically lower clinical cure and microbiological eradication rates were observed, in both the VAP and NP (excluding VAP) subgroups.
- For the most prevalent Enterobacteriaceae, such as *Escherichia coli*, *Klebsiella*

pneumoniae, *Enterobacter spp.* and *Proteus mirabilis*, and for *Pseudomonas aeruginosa*, clinical cure and microbiological eradication rates were similar between treatment groups in the large group of NP (excluding VAP) subjects. For *K. pneumoniae*, *Enterobacter spp.* *P. mirabilis* and *P. aeruginosa*, there was a tendency towards higher clinical cure and microbiological eradication rates in the ceftobiprole group.

- When members of the family of **Enterobacteriaceae** were combined and analysed as a group, a disparity between the clinical cure rate and the microbiological eradication rate was observed in both the VAP and NP (excluding VAP) ceftobiprole groups. In NP (excluding VAP) subjects, the clinical cure rate was similar between ceftobiprole (72%) and comparator (71%), but microbiological eradication was numerically lower for ceftobiprole (63%, [29/46] compared to 71%, [32/45]). This difference in the microbiological eradication rate arises from 4 of the 33 clinically cured subjects in the ceftobiprole group who had confirmed microbiological persistence. Persistence in these 4 subjects was not confined to any specific species, but was observed for single cases of *P. mirabilis*, *S. marcescens*, *Enterobacter cloacae* and *K. pneumoniae*.

Table 16 Study BAP248/307: Clinical cure and microbiological eradication by pathogen (Microbiologically Evaluable analysis set)

Pathogen	NP (excluding VAP)		VAP		All subjects	
	Ceftobiprole	Linezolid/ ceftazidime	Ceftobiprole	Linezolid/ ceftazidime	Ceftobiprole	Linezolid/ ceftazidime
	N=116 n (%)	N=120 n (%)	N=46 n (%)	N=50 n (%)	N=162 n (%)	N=170 n (%)
<i>S. aureus</i>	39	49	25	28	64	77
Clinical cure	28 (72)	36 (73)	9 (36)	16 (57)	37 (58)	52 (68)
M. eradication	23 (59)	31 (63)	10 (40)	18 (64)	33 (52)	49 (64)
MSSA	20	30	17	19	37	49
Clinical cure	15 (75)	24 (80)	5 (29)	10 (53)	20 (54)	34 (69)
M. eradication	15 (75)	21 (70)	5 (29)	12 (63)	20 (54)	33 (67)
MRSA	19	19	8	9	27	28
Clinical cure	13 (68)	12 (63)	4 (50)	6 (67)	17 (63)	18 (64)
M. eradication	8 (42)	10 (53)	5 (63)	6 (67)	13 (48)	16 (57)
<i>S. pneumoniae</i>	7	14	4	1	11	15
Clinical cure	7 (100)	13 (93)	0 (0)	1 (100)	7 (64)	14 (93)
M. eradication	7 (100)	13 (93)	0 (0)	1 (100)	7 (64)	14 (93)
Enterobacteriaceae	46¹	45²	18	16	64	61
Clinical cure	33 (72)	32 (71)	5 (28)	6 (38)	38 (59)	38 (62)
M. eradication	29 (63)	32 (71)	6 (33)	7 (44)	35 (55)	39 (64)
<i>E. coli</i>	14	11	6	3	20	14
Clinical cure	8 (57)	7 (64)	2 (33)	1 (33)	10 (50)	8 (57)
M. eradication	8 (57)	7 (64)	2 (33)	1 (33)	10 (50)	8 (57)
<i>K. pneumoniae</i>	12	19	4	4	16	23
Clinical cure	11 (92)	15 (79)	0 (0)	1 (25)	11 (69)	16 (70)
M. eradication	10 (83)	15 (79)	0 (0)	1 (25)	10 (63)	16 (70)
<i>Enterobacter spp.</i>	9	7	3	2	12	9
Clinical cure	7 (78)	3 (43)	1 (33)	0 (0)	8 (75)	3 (33)
M. eradication	6 (67)	3 (43)	2 (66)	0 (0)	8 (75)	3 (33)
<i>Proteus spp.</i>	5	5	2	5	7	10
Clinical cure	4 (80)	2 (40)	0 (0)	1 (20)	4 (57)	3 (30)
M. eradication	3 (60)	2 (40)	0 (0)	2 (40)	3 (43)	4 (40)
<i>Serratia spp.</i>	5	4	3	3	8	7
Clinical cure	3 (60)	2 (50)	1 (33)	3 (100)	4 (50)	5 (71)
M. eradication	2 (40)	2 (50)	1 (33)	3 (100)	3 (38)	5 (71)
<i>P. aeruginosa</i>	16	20	11	14	27	34
Clinical cure	12 (75)	14 (70)	5 (45)	10 (71)	17 (63)	24 (71)
M. eradication	9 (56)	11 (55)	4 (36)	8 (57)	13 (48)	19 (56)
<i>A. baumannii</i>	8	12	6	5	14	17
Clinical cure	4 (50)	9 (75)	3 (50)	4 (80)	7 (50)	13 (77)
M. eradication	4 (50)	9 (75)	3 (50)	3 (60)	7 (50)	12 (71)
<i>Haemophilus</i>	5	9	4	0	9	9
Clinical cure	2 (40)	9 (100)	1 (25)	na	3 (33)	9 (100)
M. eradication	2 (40)	9 (100)	1 (25)	na	3 (33)	9 (100)

Numbers in bold refer to the number of subjects with a pathogen in the respective group.

n = number of subjects with an outcome of clinical cure or eradication for the respective pathogen at TOC.

¹ 46 subjects with Enterobacteriaceae isolated at baseline, including: *E. coli* (monomicrobial); n=11, *E. coli* plus *Klebsiella spp.*; n=1, *E. coli* plus *Proteus spp.*; n=1, *E. coli* plus *Providencia spp.*; n=1, *K. pneumoniae* (monomicrobial); n=9, *Klebsiella oxytoca*; n=1, *K. pneumoniae* plus *Proteus spp.*; n=1, *K. pneumoniae* plus *Serratia spp.*; n=1, *K. pneumoniae* plus *Enterobacter spp.*; n=1, *Enterobacter spp.*; n=8, *Serratia spp.*; n=5, *Proteus mirabilis*; n=3, *Citrobacter spp.*; n=2, *Providencia spp.*; n=1.

² 45 subjects with Enterobacteriaceae isolated at baseline, including: *E. coli* (monomicrobial); n=8, *E. coli* plus *K. pneumoniae*; n=1, *E. coli* plus *Proteus spp.*; n=1, *E. coli* plus *K. pneumoniae* plus *Serratia spp.*; n=1, *K. pneumoniae* (monomicrobial); n=14, *Klebsiella oxytoca*; n=1, *Klebsiella spp.*; n=2, *K. pneumoniae* plus *Proteus spp.*; n=2, *K. pneumoniae* plus *Proteus spp.* plus *Serratia spp.*; n=1, *Enterobacter spp.*; n=7, *Serratia spp.*; n=2, *Proteus mirabilis*; n=3, *Citrobacter spp.*; n=1, *Hafnia alvei*; n=1.

Microbiological eradication and clinical cure by pathogen – conclusions

- Analyses of clinical and microbiological outcome by baseline pathogen in the Microbiological Evaluable analysis set show consistent results and comparable results between treatment groups for Gram-positive pathogens, *E.coli*, *K. pneumoniae*, *Enterobacter spp.*, *P. mirabilis*, and *P. aeruginosa*.
- Only for *Acinetobacter baumannii* and *Haemophilus* species, numerically lower clinical cure as well as microbiological eradication rates were observed in the ceftobiprole group, but the sample is relatively small.

For the overall group of Enterobacteriaceae, clinical cure rates were similar between ceftobiprole and linezolid/ceftazidime but a disparity was observed for the microbiological eradication rate which was lower in the ceftobiprole group. This disparity in microbiological eradication was due to single cases of 4 clinically cured subjects with microbiological

persistence of *P. mirabilis*, *S. marcescens*, *Enterobacter cloacae* and *K. pneumoniae*. The analysis of microbiological eradication by pathogen demonstrated that this disparity between clinical and microbiological cure is not apparent for the most prevalent baseline Enterobacteriaceae species (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter spp.* and *Proteus mirabilis*). Therefore, the observed 4 cases of microbiological persistence as described above, do not compromise the conclusion that the microbiological efficacy of ceftobiprole against Enterobacteriaceae species is similar to the comparator.

Microbiological outcomes in specific subgroups

For each of these subgroups, Table 17 displays clinical cure rates in the Clinically Evaluable (CE) analysis set, and microbiological eradication rates in the Microbiologically Evaluable (ME) analysis set at the test-of-cure (TOC) visit.

Table 17 Study BAP248/307: Subgroup analyses of clinical cure and microbiological eradication rates

Group (NP excluding VAP) Outcome at TOC (analysis set)	Ceftobiprole		Linezolid/ ceftazidime		Diff. (%)	95% CI [#]
	N	n (%)	N	n (%)		
Overall study population						
Clinical cure (CE)	198	154 (77.8)	185	141 (76.2)	(1.6)	(−6.9; 10.0)
Microbiological eradication (ME)	116	73 (62.9)	120	81 (67.5)	(−4.6)	(−16.7; 7.6)
Antipseudomonal antibiotics						
Clinical cure (CE)	27	15 (55.6)	19	10 (52.6)	(2.9)	(−26.3; 32.2)
Microbiological eradication (ME) (see Section 1.2)	20	7 (35.0)	13	3 (23.1)	(11.9)	(−19.1; 42.9)
APACHE II score 20–25						
Clinical cure (CE)	13	8 (61.5)	14	7 (50.0)	(11.5)	(−25.7; 48.8)
Microbiological eradication (ME) (see Section 1.3)	9	2 (22.2)	6	4 (66.7)	(−44.4)	(−90.9; 2.0)
No pre-study antibiotics						
Clinical cure (CE)	53	44 (83.0)	59	49 (83.1)	(−0.0)	(−14.0; 13.9)
Microbiological eradication (ME) (see Section 1.4)	39	25 (64.1)	39	34 (87.2)	(−23.1)	(−41.4; −4.7)

Diff.: between-group difference (ceftobiprole minus linezolid/ceftazidime)

[#] Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

CE: Clinically evaluable analysis set; ME: Microbiologically Evaluable analysis set.

The applicant conducted further analyses:

- to assess the impact on clinical cure rates of the higher number of subjects receiving anti-pseudomonal therapy in the ceftobiprole group
- to explore the imbalances between clinical cure rates and microbiological eradication rates in subjects with APACHE II scores of 20–25, and in subjects with no prior antibiotic use

Subgroup analyses of subjects receiving anti-pseudomonal antibiotics

According to the study protocol, empirical antipseudomonal therapy (fluoroquinolones or aminoglycosides) was to be added to the study drug regimen for subjects with a suspected or proven (positive surveillance culture within 72 hours) infection due to *P. aeruginosa*. Criteria for suspected infections to be considered by the investigator included mechanical ventilation > 7 days, prior residence in a nursing home or extended-care facility, and current care in a hospital ward or ICU setting where the incidence of nosocomial pneumonia due to *P. aeruginosa* exceeded 20%. The investigator's decision regarding antipseudomonal therapy was therefore mainly based on the subject's characteristics at enrolment rather than disease progression during therapy.

The fact that more subjects in the ceftobiprole group than in the linezolid/ceftazidime group received anti-pseudomonal antibiotics (27 vs 19 in the CE analysis set, and 20 vs 13 in the

ME analysis set, see Table 18) therefore likely occurred at random, and was not related to an effect of study drug.

Table 18 Study BAP248/307: Subgroup analyses of subjects with and without anti-pseudomonal treatment (Clinically Evaluable and Microbiologically Evaluable analysis sets)

Analysis set – outcome at TOC Group (NP excluding VAP)	Ceftobiprole		Linezolid/ ceftazidime		Diff. (%)	95% CI [#]
	N	n (%)	N	n (%)		
CE – Clinical cure						
All subjects	198	154 (77.8)	185	141 (76.2)	(1.6)	(-6.9; 10.0)
Anti-pseudomonal treatment	27	15 (55.6)	19	10 (52.6)	(2.9)	(-26.3; 32.2)
No anti-pseudomonal treatment	171	139 (81.3)	166	131 (78.9)	(2.4)	(-6.2; 10.9)
ME – Microbiological eradication						
All subjects	116	73 (62.9)	120	81 (67.5)	(-4.6)	(-16.7; 7.6)
Anti-pseudomonal treatment	20	7 (35.0)	13	3 (23.1)	(11.9)	(-19.1; 42.9)
No anti-pseudomonal treatment	96	66 (68.8)	107	78 (72.9)	(-4.1)	(-16.7; 8.4)

Diff.: between-group difference (ceftobiprole minus linezolid/ceftazidime)

[#] Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

To assess the impact of this unequal distribution of anti-pseudomonal antibiotics across treatment groups, subgroup analyses were conducted in subjects who did or did not receive anti-pseudomonal antibiotics.

As shown in Table 18, subjects who received anti-pseudomonal treatment had similarly lower clinical cure and microbiological eradication rates than those who did not, in both treatment groups. These data suggest that a need for anti-pseudomonal treatment was associated with poor prognosis regarding clinical cure and microbiological eradication:

- the clinical cure rates for subjects who received anti-pseudomonal treatment were 55.6% in the ceftobiprole group and 52.6% in the linezolid/ceftazidime group, compared to 81.3% and 78.9%, respectively, for subjects who did not receive anti-pseudomonal treatment
- similarly, the microbiological eradication rates for subjects who received anti-pseudomonal treatment were 35.0% in the ceftobiprole group and 23.1% in the linezolid/ceftazidime group, compared to 68.8% and 72.9%, respectively, for subjects who did not receive anti-pseudomonal treatment.

In summary, the number of subjects who received anti-pseudomonal treatment was not balanced and clinical cure rates and microbiological eradication rates were different in the strata of subjects who received anti-pseudomonal treatment (lower success rates) compared to subjects who did not receive anti-pseudomonal treatment (higher success rates). In the overall analysis (all subjects) the imbalance of more subjects being on ceftobiprole in the subgroup with low success rates (i.e., subjects receiving anti-pseudomonal treatment) did not favour ceftobiprole but had a small effect in favour of the comparator group.

The observation of a higher numerical microbiological eradication rate in the ceftobiprole group within the stratum of subjects receiving anti-pseudomonal treatment is likely to have been a chance finding. It should be noted that due to the very small subject numbers (n=7 in the ceftobiprole group and n=3 in the linezolid/ceftazidime group), the addition of 1 to 2 subjects with microbiological eradication to the comparator group would have resulted in a similar eradication rate between the treatment groups.

Subgroup analyses of subjects with high APACHE II scores

Subjects with APACHE II scores 20–25 were predefined as a subgroup for stratification of randomization. The number of subjects in this stratum was relatively small: 27/383 (7%) in the Clinically Evaluable analysis set, equally distributed between ceftobiprole (n=13) and linezolid/ceftazidime (n=14), and 15/236 (6.4%) in the Microbiologically Evaluable analysis set (ceftobiprole n=9, and linezolid/ceftazidime n=6) (see Table 19).

A comparison of clinical cure in the CE analysis set and microbiological eradication in the ME analysis set between treatment arms in this relatively small subpopulation is therefore limited and needs to be interpreted with caution. This is particularly the case for the ME analysis set, which was constituted in a non-randomized manner based on the availability of microbiological samples.

Table 19 Study BAP248/307: Subgroup analyses by APACHE II scores

Group (NP excluding VAP) Outcome (Analysis set)	Ceftobiprole		Linezolid/ceftazidime			
	N	n (%)	N	n (%)	Diff. (%)	95% CI [#]
APACHE II score 8–19						
Clinical cure (CE)	185	146 (78.9)	171	134 (78.4)	(0.6)	(−8.0; 9.1)
Microbiological eradication (ME)	107	71 (66.4)	114	77 (67.5)	(−1.2)	(−13.6; 11.2)
Clinical cure (ME)	107	81 (75.7)	114	89 (78.1)	(−2.4)	(−13.5; 8.8)
APACHE II score 20–25						
Clinical cure (CE)	13	8 (61.5)	14	7 (50.0)	(11.5)	(−25.7; 48.8)
Microbiological eradication (ME)	9	2 (22.2)	6	4 (66.7)	(−44.4)	(−90.9; 2.0)
Clinical cure (ME)	9	5 (55.6)	6	5 (83.3)	(−27.8)	(−71.9; 16.3)

Diff.: between-group difference (ceftobiprole minus linezolid/ceftazidime)

Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

The ME analysis set included all CE subjects who had a valid pathogen at baseline, i.e., CE subjects were not included in the ME analysis set if they did not have a baseline pathogen identified.

In the ceftobiprole CE group (n=13), 4 subjects had no valid microbiological sample and were not included in the ME population, whereas 8 subjects in the ceftazidime CE group (n=14) had no valid microbiological sample and were not included in the ME population (see Table 20). Because 3 of the 4 subjects from the ceftobiprole group not included were clinical cures and 1 was a clinical failure, the overall clinical cure rate for the ceftobiprole group in the ME analysis set decreased from 65% to 56%. In contrast, because 6 of the 8 subjects in the linezolid/ceftazidime group not included were clinical failures and 2 were clinical cures, the clinical cure rate for the linezolid/ceftazidime group in the ME analysis set increased from 56% to 83% (see Table 20).

Table 20 Study BAP248/307: Subject disposition and clinical cure rates in Clinically Evaluable versus Microbiological Evaluable analysis sets in subjects with APACHE II scores 20–25

Group (NP excluding VAP) Outcome	Clinically Evaluable			
	Ceftobiprole N=13		Linezolid/ceftazidime N=14	
	Clinical cure N=8 (61.5%)	Failure N=5 (38.5%)	Clinical cure N=7 (50.0%)	Failure N=7 (50.0%)
No. of subjects in CE analysis set excluded from ME analysis set (no pathogen at baseline)	3	1	2	6
Microbiologically Evaluable	Ceftobiprole N=9		Linezolid/ceftazidime N=6	
Clinical cure	5 (56%)	–	5 (83%)	–
Clinical failure	–	4 (44%)	–	1 (17%)

This change in clinical cure rates in the ME analysis set directly affected the microbiological eradication rates in this population. Under the protocol, if no microbiological culture was available at the TOC visit for a subject assessed as a clinical cure, eradication was to be ‘presumed’. As a consequence, in the absence of microbiological samples at the TOC visit, microbiological eradication was ‘presumed’ and driven solely by the clinical outcome of cure, rather than a proven negative microbiological culture. As shown in Table 21, all four ‘microbiological eradications’ in the linezolid/ceftazidime group were ‘presumed’ eradications.

For subjects with APACHE scores 20–25, 3 of the 9 subjects in the ceftobiprole group and 2 of the 6 subjects in the linezolid/ceftazidime group had a valid microbiological sample at TOC. All 3 subjects with a valid culture at TOC in the ceftobiprole group were clinically cured but showed persistence of baseline pathogens (*P. aeruginosa*, *P. aeruginosa*/*S. marcescens*, *S. aureus*). Of the 2 subjects with a valid culture at TOC in the linezolid/ceftazidime group, 1 was a clinical failure who showed persistence of *S. aureus*, while the other was clinically cured at TOC and had a tracheal aspiration culture positive for *Haemophilus influenzae* at baseline, but *Acinetobacter baumannii* at the TOC visit. The microbiological outcome for this subject was therefore termed ‘colonization’ in accordance with the protocol definition.

Table 21 Study BAP248/307: Microbiological outcomes in the Microbiologically Evaluable analysis set for subjects with APACHE II scores 20–25

Group (NP excluding VAP) Outcome	Ceftobiprole N=9		Linezolid/ceftazidime N=6	
	Clinical Cure	Failure	Clinical Cure	Failure
Microbiologically Evaluable analysis set	N=5	N=4	N=5	N=1
Eradication	–	–	–	–
Presumed eradication	2	–	4	–
Persistence	3	–	–	1
Presumed persistence	–	4	–	–
Colonization	–	–	1	–
Superinfection	–	–	–	–

It should also be noted that subject characteristics of subjects with an APACHE II score 20–25 were more comparable in the CE analysis set between the two treatment groups than in the ME analysis set, in particular related to age, creatinine clearance, geographic distribution,

low albumin levels, use of anti-pseudomonal antibiotics and chronic care (see Table 22).

Table 22 Study BAP248/307: Characteristics in subjects with APACHE II scores 20–25 (Clinically Evaluable and Microbiologically Evaluable analysis sets)

Variable	Microbiologically Evaluable		Clinically Evaluable	
	Ceftobiprole	Lin/Ceftazidime	Ceftobiprole	Lin/Ceftazidime
	N=9 n (%)	N=6 n (%)	N=13 n (%)	N=14 n (%)
Male	5 (56)	3 (50)	9 (69)	8 (57)
Mean age (years)	72.0	62.5	73.2	71.5
Age > 65 years	8 (89)	3 (50)	12 (92)	11 (79)
CRP > 100 mg/L	8 (89)	5 (83)	9 (69)	9 (64)
EU	3 (33)	3 (50)	5 (38)	5 (36)
US	3 (33)	0	3 (23)	3 (21)
Smoking	4 (44)	1 (17)	5 (38)	3 (21)
Congestive heart failure	4 (44)	2 (33)	5 (38)	2 (14)
Diabetes mellitus	1 (11)	2 (33)	2 (15)	4 (29)
Chronic care	3 (33)	0	4 (31)	3 (21)
Mean CL _{CR} (mL/min)	71.8	109.2	77.7	82.2
Pre-study antibiotics within 24 h	5 (56)	4 (67)	7 (54)	9 (64)
Albumin ≤ 25 g/L	7 (78)	3 (50)	8 (62)	9 (64)
Baseline ventilation	3 (33)	3 (50)	3 (23)	5 (36)
Anti-pseudomonal antibiotics	5 (56)	0	5 (38)	2 (14)
Valid baseline pathogen	9 (100)	6 (100)	9 (69)	6 (43)
Any Gram-positive	4 (44)	4 (67)	4 (31)	4 (29)
Any Gram-negative	7 (78)	4 (67)	7 (54)	4 (29)

Lin=linezolid; CRP= C-reactive protein; CL_{CR}=creatinine clearance.

The differences in subject characteristics between the two treatment groups in the ME analysis set were apparently caused by the effect noted above, that subjects receiving linezolid/ceftazidime who were in the CE analysis set but had no valid pathogen at baseline (and were therefore not included in the ME analysis set) were predominantly clinical failures (poor response/prognosis), while the subjects who were not included in the ME analysis set in the ceftobiprole group were predominantly clinical cures (favourable response/prognosis). This imbalance in subject characteristics between treatment groups in the ME analysis set is considered a chance finding, as assignment to the ME analysis set was linked to the availability of a valid baseline microbiological sample and not to any treatment effect. Therefore, in small subpopulations, such as subjects with APACHE II scores 20–25, the clinical cure rate in the CE analysis set may provide a more reliable assessment of ceftobiprole's efficacy than the rate of microbiological eradication in the ME analysis set. In order to have a more robust comparison of clinical cure and microbiological eradication rates based on APACHE II scores, an alternative segmentation using a threshold score of 15 is supported by the results of the original APACHE II validation study, in which APACHE II scores ≥ 15 were characterised by a relatively steep increment in hospital mortality [Knaus 1985]. In that study, APACHE II scores < 15 were associated with a hospital mortality of 4–15%, compared to 25–40% in subjects with APACHE II scores of 15–24.

Table 23 Study BAP248/307: Subgroup analyses by APACHE II scores (8-19/20-25 and 8-14/15-25)

Group (NP excluding VAP) Outcome (Analysis set)	Ceftobiprole		Linezolid/ceftazidime			
	N	n (%)	N	n (%)	Diff. (%)	95% CI [#]
APACHE II score 8–19						
Clinical cure (CE)	185	146 (78.9)	171	134 (78.4)	(0.6)	(−8.0; 9.1)
Microbiological eradication (ME)	107	71 (66.4)	114	77 (67.5)	(−1.2)	(−13.6; 11.2)
Clinical cure (ME)	107	81 (75.7)	114	89 (78.1)	(−2.4)	(−13.5; 8.8)
APACHE II score 20–25						
Clinical cure (CE)	13	8 (61.5)	14	7 (50.0)	(11.5)	(−25.7; 48.8)
Microbiological eradication (ME)	9	2 (22.2)	6	4 (66.7)	(−44.4)	(−90.9; 2.0)
Clinical cure (ME)	9	5 (55.6)	6	5 (83.3)	(−27.8)	(−71.9; 16.3)
APACHE II score 8–14						
Clinical cure (CE)	131	108 (82.4)	126	103 (81.7)	(0.7)	(−8.7; 10.1)
Microbiological eradication (ME)	76	51 (67.1)	83	61 (73.5)	(−6.4)	(−20.6; 7.8)
Clinical cure (ME)	76	58 (76.3)	83	69 (83.1)	(−6.8)	(−19.3; 5.7)
APACHE II score 15–25						
Clinical cure (CE)	67	46 (68.7)	59	38 (64.4)	(4.2)	(−12.3; 20.8)
Microbiological eradication (ME)	40	22 (55.0)	37	20 (54.1)	(0.9)	(−21.3; 23.2)
Clinical cure (ME)	76	28 (70.0)	37	25 (67.6)	(2.4)	(−18.3; 23.1)

Diff.: between-group difference (ceftobiprole minus linezolid/ceftazidime)

[#]Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

Analyses applying an APACHE II score threshold of 15 resulted in larger comparable subgroups which did not show the imbalances seen in the APACHE II 20-25 stratum, but with comparable clinical and microbiological efficacy of ceftobiprole compared to linezolid/ceftazidime in subjects with high APACHE II scores (see Table 23). It therefore seems unlikely that the lower microbiological eradication rates observed in analyses using the original stratification threshold (i.e., APACHE II 20-25) reflect an actual effect on outcomes for more severely ill subjects.

Analyses in subjects with APACHE II scores 8-19 are provided. Patient characteristics were balanced between treatment groups in this much larger group.

Subgroup analyses of subjects by prior antibiotic use

Clinical cure and microbiological eradication rates by prior antibiotic use are shown in Table 24. Neither the use of prior antibiotics, nor the length of their use (≤ 24 hours, > 24 hours), appeared to have an impact on the microbiological eradication rate in the ceftobiprole arm. Microbiological eradication rates within the ceftobiprole group were comparable between subjects without prior antibiotics (25/39, 64.1%), and subjects with any (i.e., combined ≤ 24 hours and > 24 hours) prior antibiotics (48/77, 62.3%). Consistent with microbiological eradication, clinical cure rates were also not affected by the use of prior antibiotics (Table 24).

Clinical cure rates in the CE analysis set were comparable between the two treatment groups, in a range of 70% to 83% across the three strata (subjects with no prior antibiotics, prior antibiotic use ≤ 24 hours, prior antibiotic use > 24 hours within the 72 hours prior to baseline). In contrast, while microbiological eradication rates in the ME analysis set were comparable (in a range of about 60%) between the two treatment groups in both strata with prior antibiotic use, they were higher in the linezolid/ceftazidime group (87.2% [34/39 subjects]) than in the ceftobiprole group (64.1% [25/39 subjects]) for subjects with no prior antibiotic use.

Table 24 Study BAP248/307: Subgroup analyses by prior antibiotic use

Group (NP excluding VAP) Outcome (analysis set)	Ceftobiprole		Linezolid/ ceftazidime		Diff. (%)	95% CI [#]
	N	n (%)	N	n (%)		
No prior antibiotic use						
Clinical cure (CE)	53	44 (83.0)	59	49 (83.1)	(−0.0)	(−14.0; 13.9)
Microbiological eradication (ME)	39	25 (64.1)	39	34 (87.2)	(−23.1)	(−41.4; −4.7)
Clinical cure (ME)	39	33 (84.6)	39	36 (92.3)	(−7.7)	(−21.8; 6.4)
Antibiotic use ≤ 24 hours						
Clinical cure (CE)	65	52 (80.0)	59	45 (76.3)	(3.7)	(−10.8; 18.3)
Microbiological eradication (ME)	31	19 (61.3)	38	22 (57.9)	(3.4)	(−19.9; 26.6)
Clinical cure (ME)	31	21 (67.7)	38	29 (76.3)	(−8.6)	(−29.9; 12.7)
Antibiotic use > 24 hours						
Clinical cure (CE)	80	58 (72.5)	67	47 (70.1)	(2.4)	(−12.3; 17.0)
Microbiological eradication (ME)	46	29 (63.0)	43	25 (58.1)	(4.9)	(−15.4; 25.2)
Clinical cure (ME)	46	32 (69.6)	43	29 (67.4)	(2.1)	(−17.2; 21.4)

Diff.: between-group difference (ceftobiprole minus linezolid/ceftazidime).

[#]Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

The window for prestudy antibiotics was limited to 72 hours prior to baseline.

To further investigate the subgroup of subjects with no prior antibiotic use, and to explore the observed imbalance between clinical cure in the CE analysis set and microbiological eradication in the ME analysis set, the number of subjects with clinical cure and clinical failure excluded from the ME analysis set, and the distribution of confirmed and presumed microbiological outcomes, were analysed (see Table 25).

Table 25 Study BAP248/307: Disposition of subjects with no prior antibiotic use (Clinically Evaluable and Microbiologically Evaluable analysis sets)

	Clinically Evaluable analysis set			
	Ceftobiprole N=53		Linezolid/ceftazidime N=59	
	Clinical Cure N=44 (83.0%)	Failure N=9 (17.0%)	Clinical Cure N=49 (83.1%)	Failure N=10 (16.9%)
Subjects in CE analysis set excluded from ME analysis set (no valid pathogen at baseline)	11	3	13	7
Microbiol. Evaluable analysis set	n	n	n	n
Eradication	1	–	–	–
Presumed eradication	24	–	34	–
Persistence	7	–	1	1
Presumed persistence	0	5	0	2
Colonization	1	–	1	0
Superinfection	–	1	0	0
Number of subjects with valid microbiological samples at TOC (confirmed microbiological outcome)	9	1	2	1
Total number of subjects with confirmed microbiological outcome	10		3	

As shown in Table 25, there was a lower number of subjects with a valid microbiological sample at the TOC visit in the linezolid/ceftazidime group (N=3) than in the ceftobiprole group (N=10).

Such an imbalance in the availability of microbiological samples may introduce a bias of microbiological outcomes favouring the treatment group with the lower number of available

microbiological samples, for the following reasons:

- In the absence of a valid microbiological sample, the microbiological eradication rate is entirely driven by the clinical cure rate ('presumed eradication' in clinically cured subjects, or 'presumed persistence' in clinically failed subjects).
- Non-availability of microbiological samples at the TOC visit in clinically cured subjects will bias the microbiological outcomes towards success ('presumed eradication').
- As a consequence, microbiological eradication rates will differ from clinical cure rates if the clinical cure rate is similar between treatment groups but the number of available microbiological samples at TOC is imbalanced.
- This may result in higher microbiological success rates in the group that has a lower number of available valid microbiological samples at the TOC visit.

Therefore a comparison of microbiological eradication rates between treatment groups with an imbalance in the number of available microbiological cultures needs to be interpreted with caution.

In the overall study population, a valid microbiological culture at the TOC visit was available in 48/236 subjects (20.3%) in the ME analysis set. Of these 48 subjects, 23 were in the ceftobiprole group and 25 in the linezolid/ceftazidime group. The availability of a valid microbiological sample at the TOC visit was associated with an outcome of microbiological failure (persistence, colonisation or super-infection) in 87.5% of these subjects (42/48); only 12.5% (6 subjects, 3 in each treatment group) with a valid microbiological sample at the TOC visit had no growth of a pathogen.

In the subgroup of subjects without prior antibiotic use (ME analysis set), 36 out of 39 subjects (92.3%) in the linezolid/ceftazidime group had a clinical outcome of cure at the TOC visit (see Table 24); of these 36, 34 did not have a valid microbiological sample at the TOC visit and were therefore classified as 'presumed microbiological eradication'. Only 2 clinically cured subjects in the linezolid/ceftazidime group became microbiological failures due to the availability of positive cultures (1 with persistence and 1 with colonisation) (see Table 25).

In contrast, 33 out of 39 subjects (84.6%) in the ceftobiprole group had a clinical outcome of cure at the TOC visit (see Table 24), but of these 33, only 24 were classified as 'presumed microbiological eradication'. There were 8 clinically cured subjects in the ceftobiprole group who became microbiological failures due to the availability of positive cultures (7 with persistence and 1 with colonization) (Table 25).

Additional analyses of subject disposition in the subgroups of subjects with prior antibiotic use ≤ 24 hours and prior antibiotic use > 24 hours, and the subject characteristics in the three subgroups of prior antibiotic use, are provided in supplementary Table IV and Table V.

Summary and conclusions of the subgroup analyses

- The analyses of imbalances between clinical and microbiological eradication rates in small subgroups, such as the subjects who received prior anti-pseudomonal therapy, had APACHE scores 20–25, or had no prior antibiotics, were limited by the size of these subgroups and therefore need to be interpreted with caution.
- In both treatment groups, clinical cure rates were lower for subjects who received anti-pseudomonal treatment than for subjects who did not. The higher number of subjects in the ceftobiprole group who received anti-pseudomonal treatment therefore did not result in a bias in favor of ceftobiprole.
- The imbalance between the clinical cure rate in the CE analysis set and microbiological eradication rate in the ME analysis set for subjects with APACHE II scores of 20–25 is explained by the fact that in the ceftobiprole group, predominantly subjects with clinical cure were not included in the ME analysis set, whereas in the linezolid/ceftazidime group,

predominantly subjects with clinical failure were not included in the ME analysis set. This resulted in structurally different groups in the ME analysis set, with higher clinical cure rates in the linezolid/ceftazidime group, and a related higher rate of 'presumed' eradication based on a small subset of subjects. Microbiological outcomes were balanced in a larger subgroup of subjects with APACHE II scores ≥ 15 .

- The imbalance between the clinical cure rate in the CE analysis set and microbiological eradication rate in the ME analysis set in subjects with no prior antibiotic use can be explained by an imbalance in the availability of valid microbiological samples at the TOC visit. The lower number of subjects with valid microbiological samples at the TOC visit in the linezolid/ceftazidime group (n=3) than in the ceftobiprole group (n=10) was associated with a high microbiological eradication rate ('presumed' eradications) in subjects who were clinical cures in the linezolid/ceftazidime group.

Issues with the study conduct and data integrity

The identification of sites by the previous sponsor was not based on GCP site audits, but on a review of monitoring visit reports for those sites for which protocol deviations were identified by a screening of the clinical database. As a result of this review, the seven sites were flagged as being at risk of not having fully adhered to the study protocol. However, based on the limitations of this analysis, no conclusions could be drawn regarding the reliability of the data generated at those sites. As such, the sensitivity analysis conducted by the previous sponsor should be regarded as an exploratory analysis to determine the impact of these sites on the primary efficacy outcome in the event that the reliability of the data from these "at risk" sites could not be confirmed. The sensitivity analysis did not question the integrity of data generated at these seven sites, or in the study as a whole.

As noted, an analysis based solely on a combination of a database screening and a review of monitoring visiting reports was not in itself considered sufficiently robust to allow definite conclusions to be drawn regarding the reliability of the data generated at these sites. However, the analysis was helpful for the selection of sites for which a GCP audit should be performed in order to be able to reach such conclusions.

These seven sites were therefore included in a comprehensive GCP audit program commissioned by the applicant to be conducted by an independent audit company (Adamas Consulting, UK). These audits included 100% data verification of 100% of subjects at each audited site.

The audit observations indicated that although the sites did not always adhere fully to all aspects of the study protocol, thorough analysis of the audit observations confirmed that subjects participating in the study had pneumonia (as evidenced by X-rays and clinical characteristics), that subjects received ceftobiprole or the comparator in accordance with the protocol, and that there were no suggestions of a potential bias of the study results due to unblinding. Source data verification furthermore revealed that the clinical database can be considered reliable.

In addition, it could be confirmed that subject protection was maintained throughout the study, and that neither the subjects' rights nor their decisions to participate in the study were significantly affected. The existence of study subjects was confirmed in all cases, and none of the audit observations pointed to fraud or misconduct.

The data from study BAP248/307 are therefore considered reliable and adequate for a benefit/risk assessment of ceftobiprole in the treatment of subjects with nosocomial pneumonia.

This conclusion is consistent with the outcome of inspections of clinical study sites conducted by the MHRA as part of the MAA review, which included site BAP00248-517, one of the seven sites identified by the previous sponsor as being at risk. The MHRA inspection revealed that the observations made did not compromise the reliability of the

overall study results.

Adequacy of a single pivotal study in support of the NP (excluding VAP) indication

Important aspects for the adequacy of a single pivotal study

The adequacy of the development programme to support a marketing authorisation in nosocomial pneumonia needs to be considered in the context of the current CHMP *Points to Consider on marketing authorisation applications with one pivotal study* (CPMP/EWP/2330/99), which indicates specific circumstances in which one pivotal study may be appropriate to support an indication.

An important aspect is the strong pharmacological rationale when using ceftobiprole in the treatment of pneumonia. Ceftobiprole is a beta-lactam antibiotic with a well-known mechanism of action. The comprehensive characterisation of ceftobiprole's *in vitro/in vivo* antimicrobial efficacy against clinically relevant pathogens permits robust prediction of pharmacokinetic/pharmacodynamic effects, and provides a strong pharmacological rationale for the efficacy of ceftobiprole in the proposed NP (excluding VAP) indication. This is further supported by extensive historical evidence for the efficacy of beta-lactam antibiotics in treating severe infections including pneumonia.

Specific points indicated in the guidance are the degree of statistical significance of the primary endpoint, and the consistency of results.

Statistical significance

- A 15% non-inferiority margin was pre-specified for the primary outcome of clinical cure at the TOC visit in study BAP248/307, and the actual lower bound of the 95% CI was –10.0% in both the Clinically Evaluable and ITT analysis sets of the entire study population.
- For subjects with nosocomial pneumonia (excluding VAP), the degree of statistical significance was even more pronounced, with lower bounds of the 95% CI of –6.9% (Clinically Evaluable) and –7.3% (ITT), which is well away from the pre-specified –15% non-inferiority margin, and is also well within the non-inferiority margin of –12.5% stipulated in the recent EMA guidance *EMA/CHMP/776609/2011*.
- Additional discussion of non-inferiority at various significance levels < 0.05 is provided in Section 3.2.

Consistency of results

- The absence of consistent results in the subset of VAP subjects (N=210) in study BAP248/307 is not considered to compromise the validity of the observed outcome in the majority of subjects (nosocomial pneumonia subjects [excluding VAP], N=571), as VAP and NP (excluding VAP) are distinct patient groups
- Consistency of results between clinical cure and microbiological eradication in NP subjects (excluding VAP) is addressed above.
- Consistency of results in other important secondary outcomes such as 30-day pneumonia-specific and 30-day all-cause mortality is described in Section 3.3.

Robustness of non-inferiority in study BAP248/307 related to the primary endpoint of clinical cure at the TOC visit

To assess the robustness of the non-inferiority of ceftobiprole in the clinical cure rate at TOC versus the comparator in study BAP248/307, and to address potential issues related to multiplicity of testing, the results for the primary endpoint in this study were analysed at various alpha levels. As shown in Table 26, an adjustment of the alpha level to 0.0167 (equivalent to the provision of a 98.3% CI), for example to adjust for testing of the primary hypothesis in three groups (all subjects, nosocomial pneumonia subjects [excluding VAP], and VAP subjects) supports the robustness of the non-inferiority of ceftobiprole at both the

pre-specified 15% non-inferiority margin, and at the 12.5% non-inferiority margin recommended in EMA guidance *EMA/CHMP/776609/2011*.

Table 26 Study BAP248/307: Clinical cure rates at TOC at various significance levels

Group	Ceftobiprole		Linezolid/ceftazidime		Diff. (%) ^a	Confidence interval [#]	
Analysis set	N	n (%)	N	n (%)			
All NP subjects						95%	(-10.0; 4.1)
Intent-to-Treat	391	195 (49.9)	390	206 (52.8)	(-2.9)	97.5%	(-11.0; 5.1)
						98.3%	(-11.5; 5.6)
Clinically Evaluable	251	174 (69.3)	244	174 (71.3)	(-2.0)	95%	(-10.0; 6.1)
						97.5%	(-11.2; 7.2)
						98.3%	(-11.8; 7.8)
NP (excluding VAP)						95%	(-7.3; 8.8)
Intent-to-Treat	287	171 (59.6)	284	167 (58.8)	(0.8)	97.5%	(-8.4; 10.0)
						98.3%	(-9.0; 10.6)
Clinically Evaluable	198	154 (77.8)	185	141 (76.2)	(1.6)	95%	(-6.9; 10.0)
						97.5%	(-8.1; 11.2)
						98.3%	(-8.7; 11.8)

n is the number of subjects with clinical cure at TOC.

^a Difference ceftobiprole minus linezolid/ceftazidime.

[#] Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

30-day pneumonia-specific and all-cause mortality

Pneumonia-specific mortality at 30 days was a pre-specified secondary endpoint in study BAP248/307; in addition, 30-day all-cause mortality was also analysed, as it represents a broader and more conservative assessment of mortality rates.

30-day pneumonia-specific mortality and 30-day all-cause mortality in the ITT analysis set are summarised in Table 27, both for all subjects and for NP subjects excluding VAP. All-cause mortality rates and pneumonia-specific mortality rates were similar between treatment groups, with upper bounds of the 95% CI for the between-group difference between 3.9 and 6.5 %.

Table 27 Study BAP248/307: 30-day pneumonia-specific and all-cause mortality (ITT analysis set)

Mortality Group	Ceftobiprole		Linezolid/ceftazidime			
	N	n (%)	N	n (%)	Diff (%) ^a	95% CI [#]
30-day pneumonia-specific mortality						
All subjects	391	26 (6.6)	390	24 (6.2)	(0.5)	(-2.9; 3.9)
NP (excluding VAP)	287	17 (5.9)	284	16 (5.6)	(0.3)	(-3.5; 4.1)
30-day all-cause mortality						
All subjects	391	76 (19.4)	390	72 (18.5)	(1.0)	(-4.5; 6.5)
NP (excluding VAP)	287	48 (16.7)	284	51 (18.0)	(-1.2)	(-7.4; 5.0)

n = number of subjects with the respective outcome

^a Difference ceftobiprole minus linezolid/ceftazidime

[#] Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions

Main secondary endpoints

A total of 332 (42.5%) subjects were considered microbiologically evaluable (i.e., subjects who were clinically evaluable at the TOC visit and from whom a valid pathogen was isolated at baseline).

Table 31: Microbiological Outcome at the TOC Visit for All Subjects (Study BAP00248/307: Microbiologically Evaluable and Microbiological Intent-to-Treat Analysis Sets)		
Microbiologic Outcome	Ceftobiprole n (%)	Linezolid/Ceftazidime n (%)
Microbiologically Evaluable	162	170
Eradication	3 (1.9)	11 (6.5)
Presumed eradication	84 (51.9)	95 (55.9)
Presumed persistence	42 (25.9)	27 (15.9)
Persistence	22 (13.6)	22 (12.9)
Colonization	2 (1.2)	6 (3.5)
Superinfection	9 (5.6)	9 (5.3)
Microbiological Intent-to-Treat	269	267
Eradication	4 (1.5)	11 (4.1)
Presumed eradication	101 (37.5)	114 (42.7)
Presumed persistence	114 (42.4)	86 (32.2)
Persistence	34 (12.6)	33 (12.4)
Colonization	2 (0.7)	7 (2.6)
Superinfection	14 (5.2)	16 (6.0)
teff07_rmicout.rtf generated by rmicout.sas.		

In the Microbiologically Evaluable analysis set, microbiological eradication (including presumed eradication) rates at the TOC visit were 53.7% (87/162) for the ceftobiprole group, and 62.4% (106/170) for the comparator group.

Table 2 Microbiological eradication at TOC

	Ceftobiprole		Linezolid/ceftazidi			
Analysis set	N	n (%)	me		Diff	95% CI #
Group						
<u>Microbiological Intent-to-Treat</u>						
All subjects	269	105 (39.0)	267	127 (47.6)	(−8.5)	(−16.9; -
NP (excluding VAP)	179	87 (48.6)	181	97 (53.6)	(−5.0)	(−15.3; 5.3)
VAP	90	18 (20.0)	86	30 (34.9)	(−14.9)	(−27.9;
<u>Microbiological Evaluable</u>						
All subjects	162	87 (53.7)	170	106 (62.4)	(−8.6)	(−19.2; 1.9)
NP (excluding VAP)	116	73 (62.9)	120	81 (67.5)	(−4.6)	(−16.7; 7.6)
VAP	46	14 (30.4)	50	25 (50.0)	(−19.6)	(−38.8;

n = number of subjects with microbiological eradication at TOC.

^a Difference ceftobiprole minus linezolid/ceftazidime.

[#] Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions

In the microbiologically evaluable and mITT analysis sets, the number of subjects with presumed persistence was higher in the ceftobiprole treatment group; however, the number of subjects with persistence (documented by culture) was similar in both treatment groups

Subgroup Analyses of Microbiological Eradication

The treatment effect differed based on duration of pre-study antibiotic therapy. In subjects who did not receive pre-study antibiotic therapy, lower microbiological eradication rates were observed in the ceftobiprole treatment group compared with the linezolid plus ceftazidime treatment group (50.9% compared with 77.8%, respectively

Non-VAP Subjects

Among non-VAP subjects who were microbiologically evaluable, microbiological eradication (including presumed eradication) rates at the TOC visit were 62.9% (73/116) for the ceftobiprole group and 67.5% (81/120) for the linezolid plus ceftazidime group.

Table 33: Microbiological Outcome at the TOC Visit for Non-VAP Subjects (Study BAP00248/307: Microbiologically Evaluable and Microbiological Intent-to-Treat Analysis Sets)		
Microbiologic Outcome	Ceftobiprole n (%)	Linezolid/Ceftazidime n (%)
Microbiologically Evaluable	116	120
Eradication	3 (2.6)	3 (2.5)
Presumed eradication	70 (60.3)	78 (65.0)
Presumed persistence	23 (19.8)	17 (14.2)
Persistence	16 (13.8)	12 (10.0)
Colonization	1 (0.9)	6 (5.0)
Superinfection	3 (2.6)	4 (3.3)
Microbiological Intent-to-Treat	179	181
Eradication	3 (1.7)	4 (2.2)
Presumed eradication	84 (46.9)	92 (50.8)
Presumed persistence	63 (35.2)	52 (28.7)
Persistence	24 (13.4)	17 (9.4)
Colonization	1 (0.6)	7 (3.9)
Superinfection	4 (2.2)	9 (5.0)

In non-VAP subjects who did not receive pre-study antibiotic therapy, lower microbiological eradication rates were observed in the ceftobiprole treatment group compared with the linezolid plus ceftazidime treatment group (64.1% compared with 87.2%, respectively).

Pneumonia-specific mortality at 30 days

For all subjects in the clinically evaluable analysis set, the 30-day pneumonia-specific mortality rates were 5.6% (14/251) in the ceftobiprole group and 7.0% (17/244) in the linezolid plus ceftazidime group

Non-VAP Subjects

For non-VAP subjects in the clinically evaluable analysis set, the 30-day pneumonia-specific mortality rates were 4.5% (9/198) in the ceftobiprole group and 5.9% (11/185) in the linezolid plus ceftazidime group. For non-VAP subjects in the ITT analysis set, the 30-day pneumonia-specific mortality rates were 5.9% (17/287) in the ceftobiprole group and 5.6% (16/284) in the linezolid plus ceftazidime group.

Table 41: Analysis of 30-Day Pneumonia Specific Mortality Rates for Non-VAP Subjects (Study BAP00248/307: Clinically Evaluable and Intent-to-Treat Analysis Set)								
	Ceftobiprole			Linezolid/ Ceftazidime			Diff ^a (%)	95% CI ^b
	N	n	%	N	n	%		
Clinically Evaluable								
All subjects	198	9	4.5	185	11	5.9	-1.4	(-5.9; 3.1)
Intent-to-Treat								
All subjects	287	17	5.9	284	16	5.6	0.3	(-3.5; 4.1)

Note: n is the number of subjects who died of nosocomial pneumonia.
^a Ceftobiprole minus linezolid/ceftazidime.
^b 2-sided 95% C.I. is based on the Normal approximation to the difference of the 2 proportions.
teff15_np_rmortr.rtf generated by rmortr.sas.

The pneumonia specific mortality appears to be comparable in both treatment groups.

Thirty-day all cause and overall mortality rates in non-VAP subjects

This was similar between treatment groups for all subjects in the ITT analysis set (19.4% vs 18.5% [95% CI -4.5 to 6.5] in the ceftobiprole and comparator treatment groups, respectively). However, it was numerically higher with the comparator in nosocomial pneumonia (excluding VAP) subjects (16.7% in the ceftobiprole group and 18.0% in the comparator group; 95% CI -7.4 to 5.0).

The 30-day all cause mortality appears to be comparable in both treatment groups.

Clinical Cure Rates by Baseline Pathogens

- The clinical cure rates in microbiologically evaluable subjects with MSSA were 54% (20/37) in the ceftobiprole group and 69% (34/49) in the linezolid plus ceftazidime group.
- The clinical cure rates for microbiologically evaluable subjects with MRSA were 63% (17/27) in the ceftobiprole group and 64% (18/28) in the linezolid plus ceftazidime group.
- The clinical cure rates in microbiologically evaluable subjects with *P. aeruginosa* were 63% (17/27) in the ceftobiprole group and 71% (24/34) in the linezolid plus ceftazidime group.
- The clinical cure rates in microbiologically evaluable subjects with *S. pneumoniae* were 69% (11/16) in the ceftobiprole group and 70% (16/23) in the linezolid plus ceftazidime group.

Non-VAP Subjects

- The clinical cure rates in subjects with MSSA were 75% (15/20) in the ceftobiprole group and 80% (24/30) in the linezolid plus ceftazidime group.
- The clinical cure rates in subjects with MRSA were 68% (13/19) in the ceftobiprole group and 63% (12/19) in the linezolid plus ceftazidime group.
- The clinical cure rates in subjects with *P. aeruginosa* were 75% (12/16) in the ceftobiprole group and 70% (14/20) in the linezolid plus ceftazidime group.
- Clinical cure rates by the MIC of the baseline pathogen (grouped together as gram-positive or gram-negative) did not reveal a trend for cure with low MICs, but failure with high MICs

Table 45: Clinical Cure Rates at the TOC Visit for All Non-VAP Subjects by Pathogens Detected at Baseline in at Least 10 Subjects in the Ceftobiprole Group (Study BAP00248/307: Microbiologically Evaluable Analysis Set)

Main Heading Infection Specified Term	Ceftobiprole (N=116)		Linezolid/Ceftazidime - (N=120)	
	Total n	Category, n (%) Cure	Total n	Category, n (%) Cure
Gram-positive				
Staphylococcus, coagulase-positive	39	28 (72)	49	36 (73)
Staphylococcus aureus/MSSA	20	15 (75)	30	24 (80)
Staphylococcus aureus/MRSA	19	13 (68)	19	12 (63)
Gram-negative				
Enterobacteriaceae	46	33 (72)	45	32 (71)
Escherichia coli	14	8 (57)	11	7 (64)
Klebsiella pneumoniae	12	11 (92)	19	15 (79)
Pseudomonas	16	12 (75)	21	14 (67)
Pseudomonas aeruginosa	16	12 (75)	20	14 (70)

Note: n is the number of subjects at baseline with the given pathogen. Subjects with more than 1 pathogen from the same main heading grouping are only counted once at the main heading level.
teff05_np_rclinc_r_blp.rtf generated by rclinc_r_blp.sas.

The clinical cure rates in subjects with confirmed pathogens appear to be comparable in both treatment arms in the HAP population excluding VAP.

Clinical Cure Rates for Subjects with *S. aureus*

- For subjects in the microbiologically evaluable analysis set with *S. aureus* at baseline, the clinical cure rates were 57.8% (37/64) in the ceftobiprole group and 67.5% (52/77) in the linezolid plus ceftazidime group
- For subjects in the microbiologically evaluable analysis set with MRSA at baseline, the clinical cure rates were 63.0% (17/27) in the ceftobiprole group and 64.3% (18/28) in the linezolid plus ceftazidime group
- For subjects whose only baseline pathogen was MRSA, the clinical cure rates were 56% (9/16) in the ceftobiprole group and 62% (8/13) in the linezolid plus ceftazidime group
- For subjects in the microbiologically evaluable analysis set with MSSA at baseline, the clinical cure rates were 54.1% (20/37) in the ceftobiprole group and 69.4% (34/49) in the linezolid plus ceftazidime group.

- For subjects whose only baseline pathogen was MSSA, the clinical cure rates were 67% (12/18) in the ceftobiprole group and 82% (23/28) in the linezolid plus ceftazidime group.

Non-VAP Subjects

- For non-VAP subjects in the microbiologically evaluable analysis set with *S. aureus* at baseline, the clinical cure rates were 71.8% (28/39) in the ceftobiprole group and 73.5% (36/49) in the linezolid plus ceftazidime group.
- In the microbiologically evaluable analysis set, the clinical cure rates in non-VAP subjects with MRSA were 68.4% (13/19) in the ceftobiprole group and 63.2% (12/19) in the linezolid plus ceftazidime group
- For non-VAP subjects whose only baseline pathogen was MRSA, the clinical cure rates were 57% (8/14) in the ceftobiprole group and 63% (5/8) in the linezolid plus ceftazidime group
- In the microbiologically evaluable analysis set, the clinical cure rates in non-VAP subjects with MSSA were 75.0% (15/20) in the ceftobiprole group and 80.0% (24/30) in the linezolid plus ceftazidime group
- For non-VAP subjects whose only baseline pathogen was MSSA, the clinical cure rates were 85% (11/13) in the ceftobiprole group and 88% (15/17) in the linezolid plus ceftazidime group

Table 47: Clinical Cure Rates at the TOC Visit for Non-VAP Subjects with *S. aureus* at Baseline (Study BAP00248/307: Microbiologically Evaluable and Microbiological Intent-to-Treat Analysis Sets)

	Ceftobiprole			Linezolid/ Ceftazidime			Diff ^a (%)	95% CI ^b
	N	n	%	N	n	%		
Microbiologically Evaluable								
All <i>S. aureus</i>	39	28	71.8	49	36	73.5	-1.7	(-20.4; 17.1)
All MRSA ^c	19	13	68.4	19	12	63.2	5.3	(-24.9; 35.4)
All MSSA ^c	20	15	75.0	30	24	80.0	-5.0	(-28.8; 18.8)
PVL-positive MSSA ^c	2	2	100.0	3	2	66.7	33.3	(-20.0; 86.7)
<i>S. aureus</i> , monomicrobial	27	19	70.4	25	20	80.0	-9.6	(-32.9; 13.7)
<i>S. aureus</i> , polymicrobial	12	9	75.0	24	16	66.7	8.3	(-22.6; 39.3)
- gram positive only	2	2	100.0	4	3	75.0	25.0	(-17.4; 67.4)
- mixed	10	7	70.0	20	13	65.0	5.0	(-30.3; 40.3)
Microbiological Intent-to-Treat								
All <i>S. aureus</i>	55	29	52.7	76	43	56.6	-3.9	(-21.1; 13.4)
All MRSA ^c	28	13	46.4	32	15	46.9	-0.4	(-25.7; 24.9)
All MSSA ^c	27	16	59.3	44	28	63.6	-4.4	(-27.7; 19.0)
PVL-positive MSSA ^c	2	2	100.0	5	2	40.0	60.0	(17.1; 100.0)
<i>S. aureus</i> , monomicrobial	35	19	54.3	45	25	55.6	-1.3	(-23.3; 20.7)
<i>S. aureus</i> , polymicrobial	20	10	50.0	31	18	58.1	-8.1	(-36.0; 19.9)
- gram positive only	3	2	66.7	6	4	66.7	0.0	(-65.3; 65.3)
- mixed	17	8	47.1	25	14	56.0	-8.9	(-39.6; 21.7)

Note: n is the number of subjects with a clinical outcome of Cure.

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin susceptible *Staphylococcus aureus*.

^a Ceftobiprole minus linezolid/ceftazidime.

^b 2-sided 95% C.I. is based on the Normal approximation to the difference of the 2 proportions.

^c By either oxacillin test or mecA genotype.

teff03c_np_rcinrc_mi_sg.rtf generated by rcinrc_mi_sg.sas.

For subjects with *S. aureus*, the clinical cure rates for HAP were comparable between both treatment groups.

Clinical Cure Rates for Subjects with *S. pneumoniae*

All Subjects

- Clinical cure rates for all subjects in the microbiologically evaluable analysis set with *S. pneumoniae* were lower in ceftobiprole-treated subjects (63.6%, 7/11) compared with linezolid plus ceftazidime-treated subjects (93.3%, 14/15).
- All subjects in both treatment groups whose only baseline pathogen was *S. pneumoniae* were clinical cures in the microbiologically evaluable analysis set, including 1 (100%) subject with MDRSP and 4 (100%) of 4 subjects with non-MDRSP in each treatment group
- In the microbiologically evaluable analysis set, the 1 subject in the ceftobiprole treatment

group who had *S. pneumoniae* isolated from blood at baseline was a clinical failure. No microbiologically evaluable subjects in the linezolid plus ceftazidime treatment group had *S. pneumoniae* isolated from blood at baseline.

Non-VAP Subjects

For non-VAP subjects in the microbiologically evaluable analysis set with *S. pneumoniae* at baseline, the clinical cure rates were 100% (7/7) in the ceftobiprole group and 92.9% (13/14) in the linezolid plus ceftazidime group.

Table 49: Clinical Cure Rates at the TOC Visit for Non-VAP Subjects with <i>Streptococcus pneumoniae</i> Infection at Baseline (Study BAP00248/307: Microbiologically Evaluable and Microbiological Intent-to-Treat Analysis Sets)						
	Ceftobiprole			Linezolid/ Ceftazidime		
	N	n	%	N	n	%
Microbiologically Evaluable						
All <i>S. pneumoniae</i>	7	7	100.0	14	13	92.9
All MDRSP	1	1	100.0	3	2	66.7
All penicillin resistant <i>S. pneumoniae</i>	1	1	100.0	3	2	66.7
Only in respiratory culture	7	7	100.0	14	13	92.9
Microbiological Intent-to-Treat						
All <i>S. pneumoniae</i>	12	8	66.7	15	13	86.7
All MDRSP	1	1	100.0	3	2	66.7
All penicillin resistant <i>S. pneumoniae</i>	1	1	100.0	3	2	66.7
Only in blood	1	0	0.0	0	0	
Only in respiratory culture	11	8	72.7	15	13	86.7

Note: n is the number of subjects with a clinical outcome of Cure.
MDRSP: multi-drug resistant *S. pneumoniae*.
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For subjects with *S. pneumoniae*, the clinical cure rates for HAP were higher in the ceftobiprole group.

Clinical Cure Rates for Subjects with *P. aeruginosa*

All Subjects

- The clinical cure rates in the microbiologically evaluable analysis set in subjects with *P. aeruginosa* at baseline were 63% (17/27) in the ceftobiprole group and 71% (24/34) in the linezolid plus ceftazidime group.
- For subjects whose only baseline pathogen was *P. aeruginosa*, the clinical cure rates in the microbiologically evaluable analysis set were 83% (10/12) in the ceftobiprole group and 77% (10/13) in the linezolid plus ceftazidime group.
- For subjects with *P. aeruginosa* at baseline who received anti-pseudomonal therapy, the clinical cure rates in the microbiologically evaluable analysis set were 61.5% (8/13) in the ceftobiprole group and 71.4% (10/14) in the linezolid plus ceftazidime group
- For subjects with *P. aeruginosa* at baseline who did not receive anti-pseudomonal therapy, the clinical cure rates in the microbiologically evaluable analysis set were 64.3% (9/14) in the ceftobiprole group and 70.0% (14/20) in the linezolid plus ceftazidime group
- In the microbiologically evaluable analysis set, none of the 2 subjects in the ceftobiprole treatment group who had *P. aeruginosa* isolated from blood at baseline were clinical cures
- No microbiologically evaluable subjects in the linezolid plus ceftazidime treatment group had *P. aeruginosa* isolated from blood at baseline.

Non-VAP Subjects

- The clinical cure rates in the microbiologically evaluable analysis set in non-VAP subjects with *P. aeruginosa* at baseline were 75% (12/16) in the ceftobiprole group and 70% (14/20) in the linezolid plus ceftazidime group.
- For non-VAP subjects with *P. aeruginosa* at baseline who received anti-pseudomonal therapy, the clinical cure rates were 83.3% (5/6) in both treatment groups.

Table 51: Clinical Cure Rates at the TOC Visit for Non-VAP Subjects with <i>Pseudomonas aeruginosa</i> Infection at Baseline (Study BAP00248/307: Microbiologically Evaluable and Microbiological Intent-to-Treat Analysis Sets)						
	Ceftobiprole			Linezolid/ Ceftazidime		
	N	n	%	N	n	%
Microbiologically Evaluable						
With anti-pseudomonal therapy	6	5	83.3	6	5	83.3
Without anti-pseudomonal therapy	10	7	70.0	14	9	64.3
Microbiological Intent-to-Treat						
With anti-pseudomonal therapy	9	6	66.7	9	5	55.6
Without anti-pseudomonal therapy	20	9	45.0	19	10	52.6

Note: n is the number of subjects with a clinical outcome of Cure.
teff03j_np_rclincr_pa.rtf generated by rclincr_pa.sas.

For subjects with pseudomonas the results appear to be comparable in the 2 treatment groups.

Overall comment on BAP248/307

The study design including the endpoints and comparators are acceptable. Using the non-inferiority margin set, ceftobiprole was demonstrated to be non-inferior to the combination of linezolid and ceftazidime for the clinically evaluable set and ITT set as a whole i.e. nosocomial pneumonia plus VAP; the nosocomial pneumonia group excluding the VAP group but the VAP group was demonstrated not to be non-inferior.

With regards to the important secondary endpoints, there were a number of issues considered significant as this is a single pivotal study. In particular

- The numerical imbalance in microbiological outcome with a higher percentage of subjects in the comparator group achieving eradication not in keeping with the clinical outcome where the numbers were comparable. However, It is noted that a limited number microbiological samples were available at the test of cure (TOC) visit; therefore microbiological outcome was presumed based on clinical outcome in most cases.

Overall, there were 3 confirmed eradications in both treatment groups and confirmed microbiological failures in both treatment groups were comparable (15.1% in the ceftobiprole groups versus 13.8% in the ceftazidime group).

When VAP is excluded, there is no difference in microbiological eradication rates observed in the ceftobiprole and ceftazidime group for Gram positive bacteria. However, for Gram negative bacteria, the microbiological eradication rate was lower in the ceftobiprole group which according to the applicant is driven by 4 subjects with confirmed persistence even though clinically cured

Interms of Microbiological outcome in specific sub-groups It would appear that a need for anti-pseudomonas treatment was generally associated with poor prognosis in both groups

- For subjects with APACHE scores 20-25, 3 of the subjects in the ceftobiprole were clinically cured but showed persistence of baseline pathogens.
- Prior antibiotic use appeared to have an impact on microbiological eradication rates but it would appear that there were a lower number of subjects with valid samples at TOC in the ceftazidime group.

In conclusion, the justification provided to explain the imbalance is acceptable.

Study CAP-3001**Study participants**

Subjects with a diagnosis of community acquired pneumonia (CAP).

Main inclusion criteria

- Men or women 18 years of age or older who provided written informed consent.
- Women postmenopausal for at least 1 year, surgically sterile, or practising an effective method of birth control, with a negative pregnancy test at screening.

With a diagnosis of CAP severe enough to require hospitalisation and treatment with intravenous antibiotics for at least 3 days (72 hours), defined as meeting all of the following criteria

- a) Clinical diagnosis of acute bacterial pneumonia acquired in the community. Subjects must have resided in the community (i.e., not in a chronic care facility such as a nursing home or rehabilitation facility) and must not have been hospitalized during the 14 days before the onset of symptoms of pneumonia. Residence in an assisted-living facility where the subject had regular access to the community was allowed.
- b) Clinical signs or symptoms of acute bacterial pneumonia with at least 2 of the following criteria:
 - Cough (new or increased over usual state)
 - Production of purulent sputum or a worsening in the character of sputum
 - Auscultatory findings on pulmonary examination of rales or evidence of pulmonary consolidation (dullness on percussion, bronchial breath sounds, or egophony)
 - Dyspnoea or tachypnoea (respiratory rate ≥ 20 breaths per minute), that was new or worse than usual state
 - New onset hypoxemia on room air ($PO_2 \geq 60$ mmHg on arterial blood gas or O_2 saturation $< 90\%$ by pulse oximetry), or respiratory failure that required mechanical ventilation.
- c) New radiographic infiltrates (not related to another disease process) consistent with the diagnosis of bacterial pneumonia
- d) Fever or leucocytosis/leucopenia (temporally associated with the onset of pneumonia symptoms) with at least 1 of the following:
 - Fever (in the absence of antipyretics) defined as an axillary temperature $\geq 37.5^\circ\text{C}$, an oral temperature $\geq 38^\circ\text{C}$, a tympanic temperature $\geq 38.5^\circ\text{C}$, a rectal temperature $\geq 39^\circ\text{C}$, or hypothermia, defined as a rectal body temperature of $\leq 35^\circ\text{C}$
 - Leukocytosis defined as an elevated total peripheral white blood cell (WBC) count $\geq 10 \times 10^9/\text{L}$ or $\geq 15\%$ immature neutrophils (bands), regardless of total peripheral white count; or leukopenia with total WBC $\leq 4.5 \times 10^9/\text{L}$
- e) Severity of pneumonia required intravenous antibiotic therapy

Main exclusion criteria*General exclusions*

- Any known or suspected condition that may have jeopardized adherence to protocol requirements
(e.g., severe cardiac disease such that even minimal physical activity caused discomfort, New York Heart Association [NYHA] Class 4)
- Any known or suspected hypersensitivity to any related anti-infective (including beta-lactam antibiotics such as penicillins and cephalosporins, oxazolidinones). Subjects in

whom cephalosporins would be used as part of normal clinical practice were not excluded.

- Any known or suspected condition or concurrent treatment that was contraindicated by the prescribing information for ceftriaxone or linezolid.
- Any known or suspected severe renal impairment, ($\text{CLCr} \leq 30 \text{ mL/min}$ or oliguria $\leq 20 \text{ mL/h}$ unresponsive to fluid challenge) or any form of dialysis.
- Had any known or suspected hepatic dysfunction (total bilirubin, or alanine aminotransferase [ALT], or aspartate aminotransferase [AST] $\geq 3 \times$ the upper limit of the normal range [ULN]).
- Had any known or suspected extra-pulmonary infection, including concomitant meningitis, endocarditis, septic arthritis, or osteomyelitis.
- Known or suspected to be human immunodeficiency virus (HIV) positive with CD4 counts of $\leq 0.2 \times 10^9/\text{L}$ ($\leq 200 \text{ cells/mm}^3$). (Subjects with HIV and $>0.2 \times 10^9/\text{L}$ [$>200 \text{ cells/mm}^3$] may have been included).
- Presence of neutropenia, (absolute neutrophil count [ANC] $\leq 0.5 \times 10^9/\text{L}$ [< 500 neutrophils (polymorphonuclear leukocytes) [PMNs/mm³]), severe anemia (hemoglobin $< 6.5 \text{ g/dL}$), or severe thrombocytopenia ($< 49.9 \times 10^9/\text{cm}^3$). In addition, subjects who were receiving immunosuppressive therapy and who were expected to reach a nadir of $\square 500$ PMNs/mm³ during administration of study drug should not have been enrolled.
- Women who were pregnant or lactating.

Exclusions related to clinical conditions that might have interfered with assessments of efficacy

- Sustained shock.
- Any of the following pulmonary conditions:
 - known bronchial obstruction or a history of post-obstructive pneumonia
 - primary lung cancer or another malignancy to the lungs unless surgically resected
 - cystic fibrosis
 - lung abscess
 - pleural effusion as a primary source of infection
 - active tuberculosis
 - suspected or known pneumonia due to aspiration, atypical bacteria (*Legionella* spp., *M. pneumoniae*, and *C. pneumoniae*), viruses, or *Pneumocystis jiroveci* (*carinii*)

Subjects with asthma or chronic obstructive pulmonary disease were not excluded provided they met the criteria of acute new-onset pneumonia.

Exclusions related to microbiological conditions that might have interfered with assessments of efficacy

- Use of systemic antimicrobial therapy for more than 24 hours in the 3 days before enrollment; systemic antimicrobial therapy for more than 24 hours was permitted in the case of a subject with an infection caused by microbiologically-confirmed pathogen(s) that were resistant to the previous antimicrobial agents (e.g., pneumonia due to a macrolide-resistant staphylococci or pneumococci being treated with a macrolide).

Treatments

Ceftobiprole medocaril (500 mg every 8 hours as a 120-minute intravenous infusion)

Ceftriaxone (2 g once daily as a 30-minute intravenous infusion) with or without linezolid (600 mg every 12 hours as a 60-minute intravenous infusion)

A switch from i.v. study drugs to oral cefuroxime axetil (500 mg every 12 hours) was allowed after a minimum of 3 days of i.v. therapy for subjects who met all protocol-specified criteria for improvement and were candidates for hospital discharge.

The total duration of study drug therapy (intravenous plus oral) for all subjects was a minimum of 5 days and a target of 7 days. If in the investigator's opinion a subject required additional days of study therapy, the duration of the therapy could have been extended up to a maximum of 10 days.

Therapy could have been further extended to a maximum of 14 days after approval by the Sponsor's Medical Monitor for subjects with a history of persistent bacteraemia or necrotising pneumonia.

Linezolid was to be added to ceftriaxone treatment for subjects with confirmed ceftriaxone-resistant *Streptococcus pneumoniae* provided the susceptibility of the isolate to linezolid had been confirmed. Linezolid was also to be added to ceftriaxone treatment when the incidence of MRSA in CAP isolates was prevalent (greater than 15%) in the region or institution, or when the subject's initial signs and symptoms were suggestive of infection due to *S. aureus*.

The choice of ceftriaxone as a comparator and dose used is acceptable

Objectives

Primary objective

To demonstrate the non-inferiority of ceftobiprole compared with ceftriaxone with or without linezolid with respect to the clinical cure rate at the test-of-cure (TOC) visit in subjects hospitalized with community-acquired pneumonia (CAP).

Secondary objectives

The secondary objectives, tested in a hierarchical order, were:

- To compare the microbiological eradication rate at the TOC visit following treatment with ceftobiprole versus ceftriaxone with or without linezolid in subjects with CAP requiring hospitalization;
- To compare the clinical cure rate at the TOC visit following treatment with ceftobiprole versus ceftriaxone with or without linezolid in subjects with CAP requiring hospitalization who had a PSI score ≥ 91 ;
- To compare the 30-day pneumonia-specific mortality rates following treatment with ceftobiprole versus ceftriaxone with or without linezolid in subjects with CAP requiring hospitalization.

Outcome/endpoints

Primary efficacy analysis

The primary efficacy endpoint was the clinical cure rate at the TOC visit defined as the ratio of the number of subjects who had a derived clinical outcome of Cure at the TOC visit to the total number of the subjects in the analysis set under consideration.

Secondary efficacy analyses

The secondary efficacy endpoints were tested using a step down hierarchical procedure in the following order:

- 1) microbiological eradication rate at the TOC visit,
- 2) clinical cure rate at the TOC visit for subjects who had a PSI score ≥ 91 , (3) 30-day pneumonia-specific mortality rates.

Sample size

Sample size calculation was based on a non-inferiority design using the CI approach for normal approximation to the difference of two binomial proportions. The sample size calculation was based on the following assumptions for the ITT population:

Clinical cure rate 70% in both treatment groups

- Non-inferiority margin 10%
- Power 80%
- Level of significance two-sided 5%
- Clinically evaluable rate 80%

Based on these assumptions, 670 subjects needed to be randomised (335 in each group).

For the Clinically Evaluable population:

- Clinical cure rate 90% in both treatment groups
- Clinically evaluable rate 80%

A total of 670 subjects would provide 532 clinically evaluable subjects, with 97% power for testing the primary hypothesis in the Clinically Evaluable population.

Non-inferiority margin

The justification of the non-inferiority margin required a demonstration that it preserved at least 50% of the benefit of the active comparator over placebo in the treatment of patients hospitalized with CAP. It was therefore calculated on the basis of two elements from the published literature: the variability around a point estimate of clinical cure observed in clinical trials in patients with CAP treated with ceftriaxone-containing regimens, and an estimate of the spontaneous/placebo cure rate in patients with CAP.

The estimated cure rate from clinical studies with ceftriaxone with/without linezolid from pooled historical data was 90.8% (95% CI 88.8–92.8). In the absence of placebo-controlled studies of CAP, the (maximum) estimated placebo cure rate calculated from the clinical experience with specific causative pathogens of CAP was 53%; a conservative figure of 55% was used in the calculations of the non-inferiority margin.

Based on these figures, the most conservative estimate for a non-inferiority margin which preserved at least 50% of the benefit of the active comparator over placebo, calculated under the formula set out in detail in CSR Appendix 2.2.1.2, was 16.9%. The margin of 10% selected for the study therefore met this requirement.

Randomisation

As eligible subjects were identified during baseline, they were randomly assigned to treatment via a central Interactive Voice Response System (IVRS) in a 1:1 ratio to receive ceftobiprole or the comparator. The randomization was balanced between the 2 treatment groups by using randomly permuted blocks. Subjects were stratified at entry by PSI score: ≤ 90 or ≥ 91 . Subjects were also stratified by the need for anti-staphylococcal therapy (placebo or linezolid) based on signs, symptoms, and medical history at enrolment

Blinding (masking)

It was a double-blind study with respect to the investigators and the study participants. An unblinded pharmacist was responsible for preparing the study medication. Infusion bags and line tubings were covered by coloured sleeves. The pharmacist was monitored by an unblinded site monitor, who operated independently of the blinded site monitor and all blinded activities.

Statistical methods

The primary efficacy endpoint was the clinical cure rate at the TOC visit, defined as the ratio

of the number of subjects who had a derived clinical outcome of cure at the TOC visit to the total number of the subjects in the analysis set under consideration. The hypotheses tested were:

H0: The clinical cure rate of the ceftobiprole group is more than 10% inferior to that of the ceftriaxone with or without linezolid group.

H1: The clinical cure rate of the ceftobiprole group is no more than 10% inferior to that of the ceftriaxone with or without linezolid group.

A two-sided 95% confidence interval was calculated for the between-treatment difference (ceftobiprole minus ceftriaxone with or without linezolid) at the TOC visit. Non-inferiority of ceftobiprole compared with ceftriaxone with or without linezolid was concluded if the lower limit of this confidence interval was greater than or equal to -10%.

The primary efficacy analysis was performed on the clinically evaluable and ITT co-primary analysis sets.

The secondary efficacy endpoints were tested using a step-down hierarchical procedure in the following order:

- 1) microbiological eradication rate at the TOC visit
- 2) clinical cure rate at the TOC visit for subjects who had a PSI score ≥ 91
- 3) the 30-day pneumonia-specific mortality rates

The microbiological eradication rate at the TOC visit was defined as the ratio of the number of subjects with a microbiological outcome of Eradication or Presumed Eradication at the TOC visit to the total number of subjects in the analysis set under consideration at the TOC visit. This analysis was performed on the microbiologically evaluable analysis set.

The clinical cure rate for subjects who had a PSI score ≥ 91 was defined as the ratio of the number of subjects with PSI score ≥ 91 who had a clinical outcome of Cure at the TOC visit to the total number of the subjects with a PSI score ≥ 91 in the analysis set under consideration. The analysis of the clinical cure rate for subjects who had a PSI score ≥ 91 was performed on the clinically evaluable and ITT analysis sets.

Non-inferiority hypotheses similar to the primary hypothesis were tested for the microbiological eradication rate and the clinical response rate in subjects with a PSI score ≥ 91 .

The 30-day pneumonia-specific mortality rate was defined as the ratio of the number of deaths due to pneumonia to the total number of subjects in the analysis set under consideration. This analysis was performed on the clinically evaluable and ITT analysis sets.

A 15% non-inferiority margin was used to test all secondary hypotheses. This was documented prior to database lock, but was not pre-specified in the protocol or the analysis plan. The two-sided 95% confidence interval was computed in the same way as for the primary efficacy analysis.

Analysis sets

Intent-to-Treat (ITT): all subjects randomly assigned to treatment.

Microbiological Intent-to-Treat (mITT): a subset of the ITT analysis set that included all ITT subjects who had a valid pathogen at baseline.

Clinically Evaluable: a subset of the ITT analysis set that included all ITT subjects who

received at least 48 hours of study medication, but excluded those subjects with a derived clinical outcome of Not Evaluable at the TOC visit. The derived clinical outcome was based on clinical assessment and evaluability of a subject, whereas the clinical outcome collected in the eCRF was based on the investigator's assessment.

Microbiologically Evaluable: a subset of the mITT analysis set that included all mITT subjects who were also clinically evaluable subjects, excluding those with a microbiological outcome of Not Evaluable at the TOC visit.

Safety: a subset of the ITT analysis set including all subjects in the ITT analysis set who were exposed to any study medication.

A TOC evaluation was performed 7 to 14 days after the EOT. Subjects were evaluated for relapse 28 to 35 days after the EOT at the LFU visit.

Recruitment

This study was conducted between 2006 and 2007 and included subjects from 103 sites worldwide.

Conduct of study

There were 3 protocol amendments which are not considered to be relevant to the outcome of the study.

Baseline data

- Overall, the mean age of subjects was 54.5 years with a range of 18 to 94 years, and 37% of subjects were 65 years or older.
- Forty-six percent of the subjects were from regions other than the U.S. and Europe; 13% were from the U.S. and 41% were from Europe.
- Overall, the mean BMI was 25.1 kg/m² and the mean weight was 70.8 kg
- In the ITT analysis set, the demographic and baseline characteristics were similar for subjects who switched to oral medication compared with subjects who did not switch
- The demographic and baseline characteristics in the clinically evaluable analysis set were consistent with those of the ITT analysis set

Baseline Clinical Characteristics

- A valid baseline pathogen was isolated from 184 (29%) of 638 subjects in the ITT analysis set: 101 (16%) subjects had at least 1 gram-positive pathogen and 100 (16%) subjects had at least 1 gram-negative pathogen
- A significantly higher percentage of subjects had poly-microbial infections (i.e., 2 or more valid pathogens isolated at baseline) in the ceftobiprole group (16 [18%] of 87 subjects) compared with the ceftriaxone with or without linezolid group (8 [8%] of 97 subjects)
- Twenty-two percent of subjects had a PSI score ≥ 91 . The majority (130 [92%] of 141) of these subjects were in PORT category IV
- Fifty-four percent of the subjects had SIRS
- The baseline clinical characteristics in the clinically evaluable analysis set were consistent with those of the ITT analysis set

Baseline Microbiology Characteristics

- Ceftobiprole susceptibility data from the central laboratory were obtained for 91 gram-positive and 89 gram-negative isolates detected in respiratory or blood cultures from

subjects in the mITT analysis set in both treatment groups.

- All 91 (100%) of the gram-positive isolates and 81 (91%) of the gram-negative isolates had a ceftobiprole MIC ≤ 2 $\mu\text{g/mL}$.
 - *S. pneumoniae* (including 6 isolates of multidrug-resistant *S. pneumoniae* [MDRSP]) and *S. aureus* (including 2 isolates of MRSA) comprised all but 2 of the gram-positive isolates.
 - The 89 gram-negative isolates included 17 different pathogens, with *H. influenzae* (24 isolates), *K. pneumoniae* (14 isolates), *H. parainfluenzae* (13 isolates), *Escherichia coli* (9 isolates), and *M. catarrhalis* (7 isolates) being the most prevalent.
- Of the 57 tested isolates of *S. pneumoniae* from respiratory cultures, 2 isolates were resistant and 2 isolates were intermediate to ceftriaxone. All *S. pneumoniae* isolates were susceptible to linezolid.
 - All of the 20 tested isolates of *S. pneumoniae* from blood cultures were susceptible to ceftriaxone and linezolid
 - All 19 isolates of MSSA from respiratory cultures were susceptible to both ceftriaxone and linezolid. The 2 MRSA isolates were resistant to ceftriaxone and susceptible to linezolid.

Prior Therapies

The frequency and types of antimicrobial agents used by subjects were similar between the 2 treatment groups. Sixty percent of the subjects in the ITT analysis set were treated with antimicrobial agents prior to their participation in the study (Table 13). Overall, the most common prior antimicrobial therapies were cephalosporins (26%), macrolides (13%), fluoroquinolones (12%), penicillin combinations including those with β -lactamase inhibitors (11%), and extended spectrum penicillins (9%).

Prior antibiotic usage among clinically evaluable subjects was similar to that observed in the ITT analysis set.

Extent of Exposure

- In the clinically evaluable analysis set, 103 subjects in the ceftobiprole group and 101 subjects in the ceftriaxone with or without linezolid group received only intravenous therapy and were not switched to oral therapy with cefuroxime axetil.
 - Among these subjects, the 2 most frequent durations of therapy were 5 to <7 days and 7 to <11 days: 24 (23%) subjects received ceftobiprole and 13 (13%) subjects received ceftriaxone for 5 to <7 days; 59 (57%) and 74 (73%) subjects, respectively, received 7 to <11 days of treatment. The mean extents of exposure for ceftobiprole and ceftriaxone were 7.2 and 7.8 days, respectively.
 - Of the 18 subjects who had linezolid added to the ceftriaxone regimen, 7 (39%) subjects received linezolid for 5 to <7 days and 5 (28%) subjects received linezolid for 7 to <11 days. The mean extent of exposure was 5.8 days for linezolid.
- In the clinically evaluable analysis set, 128 subjects in the ceftobiprole group and 137 subjects in the ceftriaxone with or without linezolid group were switched to oral therapy with cefuroxime axetil during the study
 - Among these subjects, 99 (77%) subjects received 7 to <11 days of combined intravenous ceftobiprole and oral cefuroxime axetil treatment; 103 (75%) subjects received 7 to <11 days of combined intravenous ceftriaxone and oral cefuroxime axetil treatment. The mean extents of combined intravenous and oral exposure for the ceftobiprole and ceftriaxone groups were 9.4 and 9.3 days, respectively.
 - Before being switched to oral medication, 69 (54%) and 51 (37%) subjects

received ceftobiprole and ceftriaxone, respectively, for 3 to <5 days; 41 (32%) and 60 (44%) subjects received ceftobiprole and ceftriaxone, respectively, for 5 to <7 days. The mean extents of exposure for ceftobiprole and ceftriaxone before subjects were switched to oral medication were 4.8 and 5.1 days, respectively.

- Before being switched to oral medication, 6 (38%) and 5 (31%) subjects received linezolid for less than 3 days and for 3 to <5 days, respectively. The mean extent of exposure for linezolid before subjects were switched to oral medication was 4.0 days.

Numbers analysed

Table 6: Number of Subjects Included in Each Analysis Set (Study CAP3001: Intent-to-Treat Analysis Set)			
Analysis Set	Ceftobiprole (N=314) n (%)	Ceftriaxone (N=324) n (%)	Total (N=638) n (%)
Intent-to-Treat	314 (100)	324 (100)	638 (100)
Clinically Evaluable	231 (74)	238 (73)	469 (74)
Microbiological Intent-to-Treat ^a	87 (28)	97 (30)	184 (29)
Microbiologically Evaluable	68 (22)	76 (23)	144 (23)
Safety	310 (99)	322 (99)	632 (99)

Note: Percentages were calculated with the number of ITT subjects as the denominator.
^a Microbiological Intent-to-Treat: Subjects in the ITT Analysis Set with valid pathogen at baseline.
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Outcome and estimation

In subjects with CAP requiring hospitalization, non-inferiority of ceftobiprole compared with ceftriaxone with or without linezolid was demonstrated within the 10% non-inferiority margin for the primary efficacy endpoint of clinical cure rate at the TOC visit (7 to 14 days after the EOT visit), for both the clinically evaluable and ITT co-primary analysis sets. Clinical cure rates at the TOC visit were 86.6% and 87.4% in the ceftobiprole and ceftriaxone with or without linezolid groups, respectively, in the Clinically Evaluable analysis set, and 76.4% and 79.3%, respectively, in the ITT analysis set.

Table 6 Results of study CAP-3001: primary and secondary endpoints						
	Ceftobiprole		Ceftriaxone ± linezolid		Diff (%)	95% CI[*]
	N	n (%)	N	n (%)		
Enrolled/completed	314	258 (82)	324	274 (85)		
<i>Primary endpoint</i>						
Clinical cure at TOC						
ITT	314	240 (76.4)	324	257 (79.3)	(-2.9)	(-9.3; 3.6)
Clinically Evaluable	231	200 (86.6)	238	208 (87.4)	(-0.8)	(-6.9; 5.3)
<i>Secondary endpoints</i>						
Microbiological eradication at TOC						
Microbiologically Evaluable	68	60 (88.2)	76	69 (90.8)	(-2.6)	(-12.6; 7.5)
Microbiological ITT	87	70 (80.5)	97	79 (81.4)	(-1.0)	(-12.4; 10.4)
Clinical cure at TOC for subjects with PSI ≥ 91						
ITT	69	56 (81.2)	72	56 (77.8)	3.4	(-9.9; 16.7)
Clinically Evaluable	51	46 (90.2)	58	49 (84.5)	5.7	(-6.7; 18.1)
30-day pneumonia-specific mortality						
ITT	314	1 (0.3)	324	3 (0.9)	(-0.6)	(-1.8; 0.6)
Clinically Evaluable	231	0	238	2 (0.8)	(-0.8)	(-2.0; 0.3)

^{*} Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

Non-inferiority within a 10% non-inferiority margin was also shown for the subgroup of subjects in PORT Risk Classes ≥ III (PSI score ≥ 71) and PORT Risk Classes ≥ IV (PSI score ≥ 91)).

Non-inferiority of ceftobiprole compared with ceftriaxone with or without linezolid was demonstrated for all the pre-specified secondary efficacy endpoints (microbiological eradication at the TOC visit, clinical cure rate at the TOC visit in subjects with a PSI score ≥ 91 [PORT Risk Class $\geq IV$, see Table 1 above], and 30-day pneumonia-specific mortality rate).

Microbiological eradication rates were 88.2% (60/68 subjects) in the ceftobiprole treatment group and 90.8% (69/76) in the ceftriaxone with or without linezolid group in the Microbiologically Evaluable analysis set (two-sided 95% CI for the between group difference of ceftobiprole minus ceftriaxone with or without linezolid: -12.6% to 7.5%).

30-day all-cause mortality (ITT analysis set) was 1.6% in the ceftobiprole group and 2.5% in the ceftriaxone with or without linezolid group. 30-day pneumonia-specific mortality was 0.3% in the ceftobiprole group and 0.9% in the ceftriaxone with or without linezolid group.

Clinical cures were observed for 26 (93%) of 28 microbiologically evaluable subjects in the ceftobiprole group who had *S. pneumoniae* detected at baseline, including cures for both subjects with MDRSP. Clinical cures were observed for all 7 subjects in the ceftobiprole group who had *S. aureus* at baseline, including 1 subject with MRSA.

Clinical cures were observed for all subjects who had *H. influenzae*, *E. coli*, *M. catarrhalis*, *K. oxytoca*, or *Acinetobacter* species isolated at baseline. Clinical cures were observed for 6 (67%) of 9 subjects with *H. parainfluenzae* and 4 (80%) of 5 subjects with *K. pneumoniae*. The microbiological eradication rates for the pathogens listed above were similar to the clinical cure rates for subjects with those pathogens.

In order to support the robustness and clinical relevance of the results of the primary and secondary efficacy endpoints, additional analyses of clinical cure and microbiological eradication rates were performed for subjects in PORT Risk Classes III, IV or V, i.e., those subjects at highest risk for mortality and generally considered for in-patient admission. (The protocol-specified stratum $PSI \geq 91$ corresponds to PORT Risk Classes IV and V). The results are presented in the table below.

Table 7 Results of study CAP-3001: analyses for PORT Risk Classes III–V

	Ceftobiprole		Ceftriaxone ± linezolid		Diff (%)	95% CI [#]
	N	n (%)	N	n (%)		
<i>Primary endpoint subgroup analyses</i>						
Clinical cure at TOC for subjects in PORT Risk Classes III–V						
Modified (PORT Risk Class III, IV or V) ITT	157	125 (79.6)	149	117 (78.5)	(1.1)	(−8.0; 10.2)
Modified (PORT Risk Class III, IV or V) Clinically Evaluable	126	109 (86.5)	117	101 (86.3)	(0.2)	(−8.4; 8.8)
Modified (PORT Risk Class III, IV or V) ITT – no prior antibiotics	62	54 (87.1)	46	36 (78.3)	(8.8)	(−5.7; 23.4)
<i>Secondary endpoint subgroup analyses</i>						
Microbiological eradication at TOC for subjects in PORT Risk Classes III–V						
Modified (PORT Risk Class III, IV or V) Microbiological ITT	54	43 (79.6)	39	29 (74.4)	(5.3)	(−12.1%; 22.7%)
Modified (PORT Risk Class III, IV or V) Microbiologically Evaluable	45	39 (86.7)	30	26 (86.7)	0	(−15.7%; 15.7%)
Modified (PORT Risk Class III, IV or V) Microbiological ITT – no prior antibiotics	24	21 (87.5)	15	12 (80.0)	(7.5)	(−16.7%; 31.7%)

Two-sided 95% CI is based on the Normal approximation to the difference of the two proportions.

Cure rates were analysed for all randomized subjects in Port Risk Classes III, IV, or V who received at least one dose of study drug (Modified Intent To Treat Efficacy [mITT^{III–V}] analysis set). Cure rates were also analysed for clinically evaluable (mCE) and microbiologically evaluable (mME) subsets of the mITT^{III–V} population). The mITT^{III–V} population comprised 306 (48%) of the 638 subjects in the ITT analysis set: 157 (50%) of the 314 subjects in the ceftobiprole group, and 149 (46%) of the 324 subjects in the comparator group.

In the mITT^{III–V} population, the clinical cure rate at the TOC visit was 79.6% for ceftobiprole, and 78.5% for the comparator (treatment difference 1.1 %, 95% CI: –8.0% to 10.2%. In the mCE subset of the mITT^{III–V} population, the clinical cure rate at the TOC visit was 86.5 % for the ceftobiprole treatment group, and 86.3% for the comparator group (treatment difference 0.2%, 95% CI: –8.4% to 8.8%).

Non-inferiority of ceftobiprole was therefore demonstrated within the 10% margin for this subgroup analysis of the primary endpoint in PORT Risk Class III–V subjects in both the mCE and mITT^{III–V} populations.

Clinical Cure Rate at the TOC Visit in Subjects with a PSI Score of at Least 91

- In the clinically evaluable analysis set, the clinical cure rate at the TOC visit for subjects with a PSI score ≥ 91 was 90.2% for the ceftobiprole treatment group and 84.5% for the ceftriaxone with or without linezolid treatment group. The corresponding treatment difference (ceftobiprole minus ceftriaxone with or without linezolid) was 5.7% with a 2-sided 95% confidence interval of -6.7% to 18.1%. Therefore, non-inferiority was demonstrated between the 2 treatment groups within the 15% non-inferiority margin. In the ITT analysis set, the clinical cure rate at the TOC visit for subjects with a PSI score ≥ 91 was 81.2% for the ceftobiprole treatment group and 77.8% for the ceftriaxone with or without linezolid treatment group. The corresponding treatment difference (ceftobiprole minus ceftriaxone with or without linezolid) was 3.4% with a 2-sided 95% confidence interval of -9.9% to 16.7%.

Table 27: Clinical Cure Rates at the TOC Visit for Subjects With a PSI Score of at Least 91 (Study CAP3001: Clinically Evaluable and Intent-to-Treat Analysis Set)								
	-- Ceftobiprole --			--- Ceftriaxone ---				
	N	n	%	N	n	%	Diff ^a (%)	95% CI ^b
Clinically Evaluable								
All subjects	51	46	90.2	58	49	84.5	5.7	(-6.7; 18.1)
Intent-to-Treat								
All subjects	69	56	81.2	72	56	77.8	3.4	(-9.9; 16.7)

Note: PSI score of at least 91 corresponds to PORT categories IV and V.

^a Ceftobiprole minus ceftriaxone with/without linezolid.

^b 2-sided 95% C.I. is based on the Normal approximation to the difference of the 2 proportions.

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Secondary Efficacy Analyses

Microbiological Eradication Rate at the TOC Visit

Table 23: Microbiological Outcome at the TOC Visit (Study CAP3001: Microbiologically Evaluable and Microbiological Intent-to-Treat Analysis Set)		
Microbiologic Outcome	Ceftobiprole n (%)	Ceftriaxone n (%)
Microbiologically Evaluable	68	76
Eradication	2 (2.9)	3 (3.9)
Presumed Eradication	58 (85.3)	66 (86.8)
Presumed persistence	7 (10.3)	6 (7.9)
Persistence	1 (1.5)	0
Superinfection	0	1 (1.3)
Microbiological Intent-to-Treat	87	97
Eradication	2 (2.3)	3 (3.1)
Presumed Eradication	68 (78.2)	76 (78.4)
Presumed persistence	14 (16.1)	17 (17.5)
Persistence	1 (1.1)	0
Colonization	1 (1.1)	0
Superinfection	1 (1.1)	1 (1.0)

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In the microbiologically evaluable analysis set, the microbiological eradication rate at the TOC visit was 88.2% for the ceftobiprole treatment group and 90.8% for the ceftriaxone with or without linezolid treatment group.

Microbiological Eradication Rate Subgroup Analyses

For subjects in the ceftobiprole and the ceftriaxone with or without linezolid groups who switched to oral medication, microbiological eradication was observed in 33 (89.2%) of 37 subjects and 41 (100%) of 41 subjects, respectively; for subjects who did not switch to oral medication, microbiological eradication was observed in 27 (87.1%) of 31 subjects and 28 (80.0%) of 35 subjects, respectively.

Clinical Cure Rates by Pathogens**Table 29: Clinical Cure Rates at the TOC Visit by Pathogens Detected at Baseline
(Study CAP3001: Microbiologically Evaluable Analysis Set)**

Main Heading Infection Specified Term	----- Ceftobiprole ----- (N=68)		----- Ceftriaxone ----- (N=76)	
	Total n	Category, n (%) Cure	Total n	Category, n (%) Cure
Gram-positive				
Enterococcus	0	0	1	1 (100)
<i>Enterococcus faecalis</i>	0	0	1	1 (100)
Gram positive bacillus	1	1 (100)	0	0
<i>Arcanobacterium haemolyticum</i>	1	1 (100)	0	0
Staphylococcus, coagulase-positive	7	7 (100)	6	5 (83)
<i>Staphylococcus aureus</i> /MSSA	6	6 (100)	6	5 (83)
<i>Staphylococcus aureus</i> /MRSA	1	1 (100)	0	0
Streptococcus pneumoniae	28	26 (93)	36	32 (89)
<i>Streptococcus pneumoniae</i> /non-MDRSP	23	21 (91)	29	25 (86)
<i>Streptococcus pneumoniae</i> /unk ^a	6	6 (100)	8	5 (63)
<i>Streptococcus pneumoniae</i> /MDRSP	2	2 (100)	3	3 (100)
Streptococcus, beta-hemolytic	1	0	0	0
<i>Streptococcus pyogenes</i>	1	0	0	0
Gram-negative				
Acinetobacter	3	3 (100)	1	1 (100)
<i>Acinetobacter baumannii</i>	2	2 (100)	1	1 (100)
<i>Acinetobacter junii</i>	1	1 (100)	0	0
Enterobacteriaceae	14	12 (86)	10	9 (90)
<i>Escherichia coli</i>	6	6 (100)	1	0
<i>Klebsiella pneumoniae</i>	5	4 (80)	7	7 (100)
<i>Citrobacter koseri</i>	1	0	0	0
<i>Enterobacter cloacae</i>	1	1 (100)	1	1 (100)
<i>Klebsiella oxytoca</i>	1	1 (100)	0	0
<i>Proteus mirabilis</i>	1	1 (100)	0	0
<i>Morganella morganii</i>	0	0	1	1 (100)
Gram negative coccus	5	5 (100)	5	5 (100)
<i>Moraxella (branhamella) catarrhalis</i>	4	4 (100)	4	4 (100)
<i>Neisseria</i> species	1	1 (100)	1	1 (100)
Haemophilus	16	13 (81)	21	19 (90)
<i>Haemophilus parainfluenzae</i>	9	6 (67)	7	6 (86)
<i>Haemophilus influenzae</i>	7	7 (100)	14	13 (93)
<i>Haemophilus parahaemolyticus</i>	1	1 (100)	0	0
Pseudomonas	1	0	2	2 (100)
<i>Pseudomonas aeruginosa</i>	1	0	2	2 (100)

Note: n is the number of subjects at baseline with the given pathogen. Subjects with more than 1 pathogen from the same main heading grouping are only counted once at the main heading level.

^a Streptococcus pneumoniae with unknown multi-drug resistance status.

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Overall conclusions on CAP-3001

For the important secondary endpoint microbiological eradication, clinical cure rates by pathogens and microbiological eradication by pathogens; ceftobiprole was demonstrated to be comparable to ceftriaxone. Results for subjects within each PORT score category III, IV and V have also been provided which show comparable outcome in both the ceftobiprole and ceftriaxone groups.

CLINICAL SAFETY

The Summary of Clinical Safety includes safety data obtained from a total of 25 completed clinical studies with ceftobiprole, including data from 539 subjects in 20 Phase 1 clinical studies, 40 subjects with complicated skin and soft tissue infections (cSSTIs) in Phase 2 study BAP00034, 632 subjects with community-acquired pneumonia (CAP) requiring hospitalisation in Phase 3 study CAP-3001, 772 subjects with nosocomial pneumonia (NP) in Phase 3 study BAP248/307 and 1,593 subjects with cSSTIs in two Phase 3 studies BAP00154 and BAP00414.

A total of 3,037 subjects, who took ceftobiprole or comparator, are included in this summary of clinical safety from the Phase 2 and Phase 3 ceftobiprole studies. From these studies 1668 subjects took ceftobiprole and 1369 subjects took comparator. Of the 1,404 subjects in the pooled pneumonia studies (CAP-3001 and BAP248/307), 696 subjects received ceftobiprole and 708 received comparator. There were 1633 subjects in the pooled cSSTI studies (BAP00034, BAP00154 and BAP00414), 972 received ceftobiprole and 661 received comparator.

The mean duration of ceftobiprole therapy was 7.6 days in study BAP248/307, with 83% treated for 10 days or less. In study CAP-3001, 98% of the ceftobiprole-treated subjects received 10 or less days medication, with the mean duration being 5.5 days. The mean duration of ceftobiprole therapy was 8.1 days in the cSSTI studies and 83% were treated for up to 10 days. No ceftobiprole-treated subject received more than 21 days therapy. The mean durations of comparator therapy were 8.2, 6.1 and 8.1 days in the nosocomial pneumonia, CAP and pooled skin studies, respectively.

An overview of the AEs in the pooled Phase 3 pneumonia and pooled cSSTI studies is presented in the table below.

Table 18 Summary of treatment-emergent adverse events (Phase 3 pooled pneumonia studies and pooled Phase 2+3 cSSTI studies (Safety analysis sets))				
	Ceftobiprole		Comparator	
	Pooled Pneumonia Studies (N=696)	Pooled cSSTI Studies[#] (N=972)	Pooled Pneumonia Studies (N=708)	Pooled cSSTI Studies[#] (N=661)
Without AE	180 (25.9%)	433 (44.5%)	200 (28.2%)	309 (46.7%)
At least one AE	516 (74.1%)	539 (55.5%)	508 (71.8%)	352 (53.3%)
Treatment-related AEs	207 (29.7%)	306 (31.5%)	181 (25.6%)	179 (27.1%)
Death	97* (13.9%)	3 (0.3%)	93 (13.1%)	4 (0.6%)
Serious AEs	175 (25.1%)	67 (6.9%)	160 (22.6%)	47 (7.1%)
Treatment-related serious AEs	18 (2.6%)	15 (1.5%)	16 (2.3%)	13 (2.0%)
AE leading to discontinuation	72 (10.3%)	47 (4.8%)	52 (7.3%)	38 (5.7%)
Treatment-related AEs leading to discontinuation	24 (3.4%)	35 (3.6%)	17 (2.4%)	24 (3.6%)
* includes the subject 'BAP00307-191-005134' who died on Day 1 after receiving 2 doses of ceftobiprole with no adverse event reported.				
[#] Studies BAP00034, BAP00154, and BAP00414				
Note: Any subjects with missing relationship, possibly, probably or very likely related were counted as related.				
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The table shows an overview of the TEAEs in study CAP-3001 and BAP248/307,

respectively.

Table 19 Summary of treatment-emergent adverse events (Phase 3 pneumonia studies)

	Ceftobiprole		Comparator	
	CAP-3001 (N=310)	BAP248/307 (N=386)	CAP-3001 (N=322)	BAP248/307 (N=386)
Without AE	93 (30.0%)	87 (22.5%)	114 (35.4%)	86 (22.3%)
At least one AE	217 (70.0%)	299 (77.5%)	208 (64.6%)	300 (77.7%)
Treatment-related AEs	111 (35.8%)	96 (24.9%)	83 (25.8%)	98 (25.4%)
Death	9 (2.9%)	88*(22.8%)	9 (2.8%)	84 (21.8%)
Serious AEs	35 (11.3%)	140 (36.3%)	37 (11.5%)	123 (31.9%)
Treatment-related serious AEs	3 (1.0%)	15 (3.9%)	4 (1.2%)	12 (3.1%)
AE leading to discontinuation	18 (5.8%)	54 (14.0%)	12 (3.7%)	40 (10.4%)
Treatment-related AEs leading to discontinuation	7 (2.3%)	17 (4.4%)	3 (0.9%)	14 (3.6%)

* includes the subject 'BAP00307-191-005134' who died on Day 1 after receiving 2 doses of ceftobiprole with no adverse event reported.

Note: Any subjects with missing relationship, possibly, probably or very likely related were counted as related.

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Overall in study CAP-3001 there were more AEs in subjects receiving ceftobiprole than there were in the subjects receiving comparator (70% for ceftobiprole and 64.6% for comparator). The incidence of AEs for study BAP248/307 was the same for subjects receiving ceftobiprole and comparator (77.5% and 77.7% respectively).

The SOC with the most frequently reported AEs in ceftobiprole-treated subjects across all pneumonia studies in decreasing order were gastrointestinal disorders (27.9%), infections and infestations (22%), respiratory, thoracic and mediastinal disorders (21%), general disorder and administration site conditions (18.4%), metabolism and nutrition disorders (18.4%), investigations (17%), nervous system disorders (13.8%), vascular disorders (13.4%) and skin and subcutaneous tissue disorders (10.6%). The picture in the pooled pneumonia studies is generally consistent with the pooled cSSTI studies despite small differences in the SOC ranking. Across all studies the gastrointestinal disorders SOC contained the most frequently reported AEs with a higher incidence of 28.7% in ceftobiprole-treated subjects versus 18.9% in the comparator group for study CAP-3001 and 27.2% of ceftobiprole-treated subjects compared to 31.1% in the comparator group in study BAP248/307.

Subjects who received ceftobiprole in the Phase 3 pneumonia studies and the pooled cSSTI studies had a higher incidence of nausea and vomiting than subjects who received the comparators. Nausea occurred in 9.7% of subjects who received ceftobiprole in study CAP-3001 compared to 4% of those who received comparator. Vomiting occurred in 8.7% of subjects who received ceftobiprole in study CAP-3001 compared to 2.8% of those who received comparator. Nausea occurred in 3.9% of subjects who received ceftobiprole in study BAP248/307 compared to 3.4% of those who received comparator. Vomiting occurred in 7.3% of subjects who received ceftobiprole in study BAP248/307 compared to 3.1% of those who received comparator.

Nausea occurred in 12.8% of subjects who received ceftobiprole in the pooled cSSTI studies compared to 7.4% of those who received comparator. Vomiting occurred in 7.5% of subjects who received ceftobiprole in the pooled cSSTI studies compared to 4.1% of those who received comparator. In these studies, nausea occurred in a higher percentage of subjects who received 500 mg ceftobiprole every 12 hours over a 60-minute infusion (55/389 14%) compared with subjects who received 500 mg ceftobiprole every 8 hours over a 120-minute infusion (58/543 11%). This suggests that the incidence of nausea is related to infusion duration and not daily dose.

Subjects who received ceftobiprole in study CAP-3001 had a higher incidence of hypersensitivity than those who received comparator (5.8% and 3.1%, respectively). Subjects who received ceftobiprole in study BAP248/307 and the pooled cSSTI studies had a lower incidence of hypersensitivity which was particularly apparent in the pooled cSSTI studies (4.6% for ceftobiprole and 10.6% for comparator).

Rash and pruritus were the most commonly reported terms for both ceftobiprole and comparators in all studies.

One ceftobiprole-treated subject and 1 comparator-treated subject had anaphylaxis in the pooled Phase 2 and 3 cSSTI studies; the anaphylaxis was reported as moderate, not serious, and probably related to treatment in the ceftobiprole-treated subject, and as severe, serious, and probably related to treatment in the comparator-treated subject; it resolved in both subjects.

Renal-related adverse events incidences in the ceftobiprole treatment groups were higher in BAP248/307 (8.5%) vs CAP-3001 (1%), similar to the corresponding values in the comparator groups with 6.0% and 3.4%, respectively. Incidences in the pooled cSSTI studies were similar to those observed in CAP-3001, with 2.1% in the ceftobiprole-treated groups vs 3.5% in the comparator groups. Elevation of serum creatinine, defined as an increase in serum creatinine >0.5 mg/dL from baseline and a concentration >1.2 mg/dL, was reported at comparable low frequencies ranging from 0 – 1.8% in ceftobiprole-treated subjects vs 0.3 – 1.0% in the comparator in the Phase 3 pneumonia studies and the pooled cSSTI studies. The incidence of renal and urinary AEs was lower in subjects who received ceftobiprole than those who received comparator for study CAP-3001 (0.3% ceftobiprole and 0.9% comparator) and the pooled cSSTI studies (0.4% ceftobiprole and 1.7% comparator). The incidence of these events in study BAP248/307 was higher in subjects receiving ceftobiprole (6%) compared to subjects receiving comparator (3.1%) although the same numbers of acute renal failure and oliguria along with several low incidences of other events in those given ceftobiprole.

Injection / infusion-site-related AEs occurred more frequently in subjects who received ceftobiprole than in those who received comparators for all studies. The incidence of injection / infusion-site-related AEs in ceftobiprole-treated subjects was 7.1% for study CAP-3001, 6% for study BAP248/307 and 7.5% for the pooled cSSTI studies. The corresponding incidence figures for the comparator groups were: 5% for study CAP-3001, 4.7% for study BAP248/307 and 6.4% for the pooled cSSTI studies.

Table 20 Treatment-emergent adverse events by System Organ Class in Phase 3 pneumonia studies, pooled pneumonia studies and pooled Phase 2 and Phase 3 cSSTI studies (Safety analysis sets)

System Organ Class	Ceftobiprole				Comparator			
	CAP	NP	Pooled Pneumonia Studies	Pooled cSSTI Studies [#]	CAP	NP	Pooled Pneumonia Studies	Pooled cSSTI Studies [#]
	CAP-3001 (N=310)	BAP248/307 (N=386)	(N=696)	(N=972)	CAP-3001 (N=322)	BAP248/307 (N=386)	(N=708)	(N=661)
Total number reporting	217 (70.0%)	299 (77.5%)	516 (74.1%)	539 (55.5%)	208 (64.6%)	300 (77.7%)	508 (71.8%)	352 (53.3%)
Blood and lymphatic system disorders	6 (1.9%)	41 (10.6%)	47 (6.8%)	26 (2.7%)	18 (5.6%)	40 (10.4%)	58 (8.2%)	26 (3.9%)
Cardiac disorders	16 (5.2%)	61 (15.8%)	77 (11.1%)	18 (1.9%)	16 (5.0%)	57 (14.8%)	73 (10.3%)	20 (3.0%)
Congenital, familial and genetic disorders	1 (0.3%)	4 (1.0%)	5 (0.7%)	0	0	3 (0.8%)	3 (0.4%)	0
Ear and labyrinth disorders	4 (1.3%)	2 (0.5%)	6 (0.9%)	4 (0.4%)	2 (0.6%)	4 (1.0%)	6 (0.8%)	5 (0.8%)
Endocrine disorders	1 (0.3%)	7 (1.8%)	8 (1.1%)	0	1 (0.3%)	0	1 (0.1%)	1 (0.2%)
Eye disorders	4 (1.3%)	11 (2.8%)	15 (2.2%)	11 (1.1%)	2 (0.6%)	11 (2.8%)	13 (1.8%)	10 (1.5%)
Gastrointestinal disorders	89 (28.7%)	105 (27.2%)	194 (27.9%)	241 (24.8%)	61 (18.9%)	120 (31.1%)	181 (25.6%)	122 (18.5%)
General disorders and administration site conditions	37 (11.9%)	91 (23.6%)	128 (18.4%)	173 (17.8%)	41 (12.7%)	87 (22.5%)	128 (18.1%)	90 (13.6%)
Hepatobiliary disorders	8 (2.6%)	10 (2.6%)	18 (2.6%)	4 (0.4%)	8 (2.5%)	19 (4.9%)	27 (3.8%)	2 (0.3%)
Immune system disorders	2 (0.6%)	2 (0.5%)	4 (0.6%)	10 (1.0%)	0	2 (0.5%)	2 (0.3%)	11 (1.7%)
Infections and infestations	48 (15.5%)	105 (27.2%)	153 (22.0%)	114 (11.7%)	38 (11.8%)	100 (25.9%)	138 (19.5%)	81 (12.3%)
Injury, poisoning and procedural complications	5 (1.6%)	29 (7.5%)	34 (4.9%)	19 (2.0%)	8 (2.5%)	20 (5.2%)	28 (4.0%)	9 (1.4%)
Investigations	45 (14.5%)	73 (18.9%)	118 (17.0%)	119 (12.2%)	55 (17.1%)	64 (16.6%)	119 (16.8%)	59 (8.9%)
Metabolism and nutrition disorders	34 (11.0%)	94 (24.4%)	128 (18.4%)	68 (7.0%)	42 (13.0%)	78 (20.2%)	120 (16.9%)	28 (4.2%)
Musculoskeletal and connective tissue disorders	15 (4.8%)	21 (5.4%)	36 (5.2%)	60 (6.2%)	13 (4.0%)	16 (4.1%)	29 (4.1%)	36 (5.4%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	5 (1.6%)	2 (0.5%)	7 (1.0%)	1 (0.1%)	3 (0.9%)	5 (1.3%)	8 (1.1%)	1 (0.2%)
Nervous system disorders	37 (11.9%)	59 (15.3%)	96 (13.8%)	169 (17.4%)	40 (12.4%)	51 (13.2%)	91 (12.9%)	76 (11.5%)
Psychiatric disorders	25 (8.1%)	38 (9.8%)	63 (9.1%)	72 (7.4%)	22 (6.8%)	44 (11.4%)	66 (9.3%)	32 (4.8%)
Renal and urinary disorders	7 (2.3%)	50 (13.0%)	57 (8.2%)	28 (2.9%)	9 (2.8%)	25 (6.5%)	34 (4.8%)	30 (4.5%)
Reproductive system and breast disorders	3 (1.0%)	3 (0.8%)	6 (0.9%)	13 (1.3%)	2 (0.6%)	2 (0.5%)	4 (0.6%)	4 (0.6%)
Respiratory, thoracic and mediastinal disorders	34 (11.0%)	112 (29.0%)	146 (21.0%)	48 (4.9%)	41 (12.7%)	91 (23.6%)	132 (18.6%)	45 (6.8%)
Skin and subcutaneous tissue disorders	22 (7.1%)	52 (13.5%)	74 (10.6%)	114 (11.7%)	16 (5.0%)	59 (15.3%)	75 (10.6%)	97 (14.7%)
Surgical and medical procedures	3 (1.0%)	11 (2.8%)	14 (2.0%)	11 (1.1%)	1 (0.3%)	12 (3.1%)	13 (1.8%)	5 (0.8%)
Vascular disorders	27 (8.7%)	66 (17.1%)	93 (13.4%)	79 (8.1%)	25 (7.8%)	54 (14.0%)	79 (11.2%)	42 (6.4%)

[#] Studies BAP00034, BAP00154, and BAP00414

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Serious adverse events and deaths

Serious AEs in study CAP-3001 occurred in 35 (11.3%) ceftobiprole-treated subjects and 37 (11.5%) of the comparator group. In study BAP248/307 the incidence in the ceftobiprole-treated subjects was 140 (36.3%) and 123 (31.9%) for the comparator-treated subjects. Within the pooled cSSTI studies, at least one SAE was reported in 67 (6.9%) of ceftobiprole-treated subjects 47 (7.1%) of comparator subjects. The SOC with the most frequently reported SAEs were Infections and infestations, Respiratory, thoracic and mediastinal disorders, Cardiac disorders, Nervous system disorders and Vascular disorders. A relatively higher number of SAEs was reported in study BAP248/307 compared to the number reported in study CAP-3001 and the pooled cSSTI studies. A SOC level summary of SAEs is provided in the table below.

Table 36 Serious adverse events reported in all subjects by System Organ Class in Phase 3 pneumonia studies CAP-3001 and BAP248/307, pooled pneumonia studies, and pooled Phase 2 and Phase 3 cSSTI studies (Safety analysis set)

System Organ Class	Ceftobiprole				Comparator			
	CAP-3001 (N=310)	BAP248/307 (N=386)	Pooled pneumonia Studies (N=696)	Pooled cSSTI Studies [#] (N=972)	CAP-3001 (N=322)	BAP248/307 (N=386)	Pooled pneumonia Studies (N=708)	Pooled cSSTI Studies [#] (N=661)
Total number reporting	35 (11.3%)	140 (36.3%)	175 (25.1%)	67 (6.9%)	37 (11.5%)	123 (31.9%)	160 (22.6%)	47 (7.1%)
Blood and lymphatic system disorders	1 (0.3%)	3 (0.8%)	4 (0.6%)	3 (0.3%)	1 (0.3%)	5 (1.3%)	6 (0.8%)	0
Cardiac disorders	7 (2.3%)	36 (9.3%)	43 (6.2%)	6 (0.6%)	4 (1.2%)	33 (8.5%)	37 (5.2%)	5 (0.8%)
Congenital, familial and genetic disorders	0	1 (0.3%)	1 (0.1%)	0	0	0	0	0
Gastrointestinal disorders	3 (1.0%)	10 (2.6%)	13 (1.9%)	3 (0.3%)	2 (0.6%)	11 (2.8%)	13 (1.8%)	2 (0.3%)
General disorders and administration site conditions	1 (0.3%)	12 (3.1%)	13 (1.9%)	7 (0.7%)	4 (1.2%)	13 (3.4%)	17 (2.4%)	2 (0.3%)
Hepatobiliary disorders	1 (0.3%)	4 (1.0%)	5 (0.7%)	0	0	5 (1.3%)	5 (0.7%)	1 (0.2%)
Immune system disorders	1 (0.3%)	1 (0.3%)	2 (0.3%)	3 (0.3%)	0	1 (0.3%)	1 (0.1%)	3 (0.5%)
Infections and infestations	12 (3.9%)	50 (13.0%)	62 (8.9%)	29 (3.0%)	16 (5.0%)	36 (9.3%)	52 (7.3%)	21 (3.2%)
Injury, poisoning and procedural complications	0	7 (1.8%)	7 (1.0%)	4 (0.4%)	1 (0.3%)	3 (0.8%)	4 (0.6%)	1 (0.2%)
Investigations	0	9 (2.3%)	9 (1.3%)	1 (0.1%)	1 (0.3%)	7 (1.8%)	8 (1.1%)	0
Metabolism and nutrition disorders	3 (1.0%)	9 (2.3%)	12 (1.7%)	4 (0.4%)	0	5 (1.3%)	5 (0.7%)	0
Musculoskeletal and connective tissue disorders	0	0	0	2 (0.2%)	2 (0.6%)	0	2 (0.3%)	1 (0.2%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	4 (1.3%)	0	4 (0.6%)	1 (0.1%)	3 (0.9%)	3 (0.8%)	6 (0.8%)	0
Nervous system disorders	0	22 (5.7%)	22 (3.2%)	3 (0.3%)	1 (0.3%)	16 (4.1%)	17 (2.4%)	0
Psychiatric disorders	2 (0.6%)	1 (0.3%)	3 (0.4%)	2 (0.2%)	0	0	0	1 (0.2%)
Renal and urinary disorders	0	6 (1.6%)	6 (0.9%)	1 (0.1%)	1 (0.3%)	7 (1.8%)	8 (1.1%)	4 (0.6%)
Reproductive system and breast disorders	0	0	0	0	0	0	0	1 (0.2%)
Respiratory, thoracic and mediastinal disorders	9 (2.9%)	40 (10.4%)	49 (7.0%)	8 (0.8%)	11 (3.4%)	42 (10.9%)	53 (7.5%)	9 (1.4%)
Skin and subcutaneous tissue disorders	0	1 (0.3%)	1 (0.1%)	3 (0.3%)	0	1 (0.3%)	1 (0.1%)	4 (0.6%)
Surgical and medical procedures	0	1 (0.3%)	1 (0.1%)	7 (0.7%)	0	3 (0.8%)	3 (0.4%)	5 (0.8%)
Vascular disorders	1 (0.3%)	15 (3.9%)	16 (2.3%)	4 (0.4%)	2 (0.6%)	9 (2.3%)	11 (1.6%)	3 (0.5%)

#Studies BAP00034, BAP00154, and BAP00414

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In study BAP248/307, 140 (36.3%) subjects in the ceftobiprole group and 123 (31.9%) subjects in the linezolid plus ceftazidime group reported SAEs during the study. The most common SAEs in both treatment groups were associated with the SOCs infections and infestations, respiratory thoracic, and mediastinal disorders, and Cardiac disorders. Within these SOCs, there were more SAEs reported in subjects in the ceftobiprole group versus the linezolid plus ceftazidime group, i.e. for septic shock 12 (3.1%) subjects versus 6 (1.6%), respectively, pneumonia 10 (2.6%) subjects versus 14 (3.6%), respectively, sepsis 11 (2.8%) subjects versus 8 (2.1%), respectively, respiratory distress 6 (1.6%) versus 1 (0.3%), respectively, cardiac failure 7 (1.8%) versus 6 (1.6%), respectively, and cardiac arrest 7 (1.8%) versus 8 (2.1%) respectively. The underlying disease comorbidities are possible causes of the reported events.

In study CAP-3001, 35 (11.3%) subjects in the ceftobiprole group and 37 (11.5%) subjects in the ceftriaxone with or without linezolid group suffered at least one SAE. The most frequently reported SAE in both treatment groups was pneumonia, occurring in 5 (1.6%) subjects in the ceftobiprole group and 10 (3.1%) subjects in the comparator group.

A review of pneumonia reports shows that 12 of the 15 subjects had clinical outcomes of failure at the TOC visit. The remaining 3 subjects (1 ceftobiprole and 2 ceftriaxone with or without linezolid treated subjects) had clinical outcomes of cure at the TOC visit. In all 3

cases the onset of the SAE of pneumonia was at least 14 days after the TOC visit date (CAP-3001 CSR).

Four subjects (1.3%) in the ceftobiprole group and 1 (0.3%) in the comparator group experienced empyema, and 3 (1.0%) in the ceftobiprole group versus 4 (1.2%) in comparator group experienced respiratory failure. Lung neoplasms were reported in 4 (1.2%) subjects in the ceftobiprole treatment group and one (0.3%) subject in the comparator group. All other SAEs were reported in only one subject (i.e. they occurred with an incidence of 0.3%).

In terms of nosocomial pneumonia excluding VAP and VAP status, the SOC with the most common SAEs were associated with Infections and infestations, Respiratory, thoracic, and mediastinal disorders and Cardiac disorders. The higher incidence rates in the VAP subjects treated with ceftobiprole compared to the comparator group could primarily be attributed to higher incidences of infections and infestations (16.5% versus 11.8%, respectively), Cardiac disorders (12.6% versus 8.8%, respectively), and Nervous system disorders (9.7% versus 4.9%, respectively).

In the nosocomial pneumonia excluding VAP ceftobiprole group, SAEs occurring in subjects at a frequency of $\geq 2\%$ were respiratory failure (4.2%), pneumonia (2.8%), sepsis (2.5%), septic shock (2.5%), and cardiac arrest (2.1%). In the nosocomial pneumonia excluding VAP comparator group, SAEs occurring at a frequency of $\geq 2\%$ were respiratory failure (3.9%), pneumonia (3.2%), sepsis (2.5%), and cardiac arrest (2.5%).

In the VAP ceftobiprole treatment group, SAEs occurring at $\geq 2\%$ frequency were septic shock (4.9%), sepsis (3.9%), respiratory failure (3.9%), cardiac failure (2.9%) and bradycardia (2.9%), while in the VAP comparator subjects, SAEs occurring at a frequency of $\geq 2\%$ included pneumonia (4.9%), respiratory failure (2.9%), cardiac failure (2.9%), multi-organ failure (2%) and hyponatraemia (2%).

Treatment-related serious adverse events

In study CAP-3001 treatment-related SAEs were reported for 3 subjects (1%) in the ceftobiprole treatment group and 4 subjects (1.2%) in the ceftriaxone with or without linezolid treatment group. In the ceftobiprole group there were single reports of anaemia, anaphylactic shock and viral infection. In the comparator group there were single reports of *Clostridium difficile* colitis, pneumonia, respiratory failure and deep vein thrombosis. In study BAP248/307 treatment-related SAEs were reported for 15 subjects (3.9%) in the ceftobiprole treatment group and 12 subjects (3.1%) in the ceftazidime plus linezolid group. In the ceftobiprole treatment group the only events reported for more than one subject were hyponatraemia and coma. Hyponatraemia was reported in 4 subjects (1%). Coma was reported in 2 subjects (0.5%). There were single reports of the following events: cardiac arrest, nausea, vomiting, no therapeutic response, pyrexia, hypersensitivity, bronchopneumonia, *Clostridium difficile* colitis, lung abscess, QT prolongation, hepatic enzyme increased, laboratory test abnormal, hypocalcaemia, convulsion, pulmonary oedema, respiratory distress, respiratory failure and shock. In the comparator group there were 3 reports of QT prolongation and 2 reports of hyponatraemia. There were single reports of the following events: myocardial ischaemia, multi-organ failure, peripheral oedema, liver disorder, hypersensitivity, *Clostridium difficile* colitis, increased hepatic enzyme, ALT increased, AST increased, positive blood culture, blood urea increased, hypocalcaemia, hypomagnesaemia, hypokalaemia, oliguria and rash. In the pooled cSSTI studies there were 15 (1.5%) treatment-related SAEs in the ceftobiprole groups and 13 (2%) in the comparator groups. In the ceftobiprole treatment groups the only events for which there was more than one report were hypersensitivity and hyponatraemia. There were 3 (0.3%) reports of hypersensitivity and 2 (0.2%) reports of hyponatraemia. There were single reports of the following events: bradycardia, congestive cardiac failure, nausea, vomiting, colitis, necrosis,

clostridial infection, wound complication, abnormal liver function test, hyperglycaemia, hypochloraemia, hypomagnesaemia, polyarthritis, convulsion, agitation, oliguria, renal failure, respiratory depression, maculopapular rash and skin ulcer. In the comparator groups there were 2 (0.3%) reports of hypersensitivity and pruritus. There were single reports of the following events: congestive cardiac failure, colitis, peripheral oedema, hepatic failure, anaphylactic reaction, *Clostridium difficile* colitis, skin infection, wound infection, arthralgia, renal failure, acute renal failure, asthma, wheezing, rash, flushing and hypotension.

Deaths

There have been a total of 199 deaths to date in the ceftobiprole development programme.

Of the total deaths, the only ones which were considered by investigators to be related to study medication were 4 in each treatment group in the nosocomial pneumonia study. In summary, the deaths which the investigator assessed as treatment-related were complicated cases with significant co-morbidities and in which only one of the ceftobiprole and 2 of the comparator therapy subjects died while on therapy. There have been no deaths in the programme which unequivocally resulted directly from the administration of study drug. In the Phase 3 nosocomial pneumonia study, the death rate was 22.8% for ceftobiprole and 21.8% for ceftazidime plus linezolid. In the Phase 3 CAP-3001 study the death rate was 2.9% for ceftobiprole and 2.8% for ceftriaxone with or without linezolid.

Serum sodium

In study CAP-3001, treatment-emergent and treatment-related hyponatraemia occurred less frequently in subjects receiving ceftobiprole than in those receiving comparators. The incidence of treatment-emergent hyponatraemia was 1.3% in the ceftobiprole group and 2.8% in the comparator group. The incidence of treatment-related hyponatraemia was 0.6% in the ceftobiprole group and 1.2% in the comparator group. In study BAP248/307 hyponatraemia was reported as an AE in 38 (9.8%) subjects in the ceftobiprole treatment group and 24 (6.2%) subjects in the linezolid plus ceftazidime treatment group. Hyponatraemia was considered treatment-related by the investigator for 17 (4.4%) subjects in the ceftobiprole treatment group and 10 (2.6%) subjects in the linezolid plus ceftazidime treatment group. Five subjects (1.3%) in the ceftobiprole treatment group and 2 (0.5%) subjects in the linezolid plus ceftazidime treatment group had hyponatraemia that was considered serious. Four subjects (1%) in the ceftobiprole treatment group and 1 (0.3%) subject in the linezolid plus ceftazidime treatment group discontinued treatment because of hyponatraemia. The majority (84% [32/38]) of reports of hyponatraemia in the ceftobiprole treatment group were considered mild or moderate in severity by the investigator; 5 were considered severe and 1 was considered life-threatening by the investigator. For the linezolid plus ceftazidime group, the majority (92% [22/24]) of reports of hyponatraemia were considered mild or moderate in severity by the investigator; 1 was considered severe and 1 was considered life-threatening by the investigator.

Hepatic-related events

In study CAP-3001, the incidence of study report defined ADR increases in hepatic enzymes was 7% in both treatment groups. One ceftobiprole subject discontinued therapy due to raised liver enzymes, the event was considered severe and an adverse drug reaction was not serious. In study BAP248/307, the incidence of study report defined ADR increases in hepatic enzymes was 6% in the ceftobiprole treatment group and 5% in the linezolid plus ceftazidime treatment group. The events were considered serious in 1 subject in the ceftobiprole group and 2 subjects in the linezolid plus ceftazidime group but led to treatment discontinuation in 2 subjects in the ceftobiprole and 1 subject in the linezolid plus ceftazidime treatment group. In addition, between baseline and EOT for ceftobiprole treated subjects, mean ALT and AST

increased by approximately 25% and GGT by approximately 50% compared to minimal drops in AST and ALT with comparator and an approximate 10% increase in GGT. In the pooled cSSTI studies, treatment-related hepatic adverse events occurring at 1% in the ceftobiprole treated subjects were: ALT increased 1.2% (12 subjects), AST increased 1% (10 subjects). The incidences in the comparator groups were ALT increased 2% (14 subjects) and AST increased 1.6% (11 subjects). A single subject in the ceftobiprole group had a serious adverse event of liver function test abnormal. A single subject in the comparator treatment group had a serious adverse event and discontinued study treatment due to hepatic failure. No subject discontinued ceftobiprole treatment due to an increase in hepatic enzymes.

In study CAP-3001 at baseline, 55 (23.1%) ceftobiprole-treated subjects and 34 (13.9%) ceftriaxone with or without linezolid-treated subjects had AST levels categorised as grade 1 through grade 4; 42 (16.8%) ceftobiprole-treated subjects and 32 (12.7%) of the comparator group had ALT levels categorised as grade 1 through grade 4.

A 1-grade shift of AST from Grade 0 to Grade 1 was observed for 16 (6.7%) subjects in the ceftobiprole group and 28 (11.5%) subjects in the ceftriaxone with or without linezolid group; a shift from Grade 1 to Grade 2 was observed for 6 (2.5%) and 1 (0.4%) subjects, respectively; and there were no shifts from Grade 2 to Grade 3 in either group. A 1-grade shift in ALT from Grade 0 to Grade 1 was observed 42 (16.9%) subjects in the ceftobiprole group and 43 (17.1%) subjects in the comparator group; a shift from Grade 1 to Grade 2 was observed for 7 (2.8%) subjects and 5 (2.0%) subjects respectively and a shift from Grade 2 to Grade 3 occurred in 1(0.4%) subject in both treatment groups.

One (0.4%) subject in the comparator group developed a severe AST elevation (grades 3 and 4) during treatment while severe elevations of ALT occurred in 1.7% (4 subjects) of the ceftobiprole group and in 0.8% (2 subjects) of the comparator group.

In study BAP248/307 at baseline, 60 (25%) ceftobiprole-treated subjects and 62 (26.1%) linezolid plus ceftazidime-treated subjects had AST levels categorised as grade 1 through grade 4 and 59 (22.8%) ceftobiprole-treated subjects and 63 (26.3%) of the comparator group had ALT levels categorised as grade 1 through grade 4.

A 1-grade shift of AST from Grade 0 to Grade 1 was observed for 28 (11.7%) subjects in the ceftobiprole group and 22 (9.3%) subjects in the ceftriaxone with or without linezolid group; a shift from Grade 1 to Grade 2 was observed for 5 (2.1%) and 7 (3.0%) subjects, respectively and there were no shifts from Grade 2 to Grade 3 in either treatment group. A 1-grade shift in ALT from Grade 0 to Grade 1 was observed in 32 (12.4%) subjects in the ceftobiprole group and 24 (9.5%) subjects in the comparator group; a shift from Grade 1 to Grade 2 was observed for 5 (1.9%) subjects and 5 (2.0%) subjects respectively (BAP248/307 CSR).

The percentage of ceftobiprole-treated subjects who developed severe AST elevations (grades 3 and 4) at any time during treatment in study BAP248/307 was 1.3% (3 subjects) for ceftobiprole-treated subjects and 0.8% (2 subjects) of the comparator group, severe elevations of ALT occurred in 1.9% (5 subjects) of the ceftobiprole group and 2.8% (4 subjects) of the comparator group (BAP248/307 CSR).

In the pooled cSSTI studies at baseline, 138 (16.5%) ceftobiprole-treated subjects and 106 (19.4%) subjects in the comparator group had ALT levels categorised as grade 1 through grade 4, 99 (12.4%) ceftobiprole-treated subjects and 68 (12.7%) of the comparator group had AST levels categorised as grade 1 through grade 4.

The percentage of ceftobiprole-treated subjects who developed severe AST elevations (grades 3 and 4) at any time during treatment in the pooled cSSTI studies was 0.2% (2 subjects) in both treatment groups and severe elevations of ALT occurred in 0.4% (3

subjects) of the ceftobiprole group and 0.2% (1 subject) of the comparator group.

Overall, the incidence of treatment-emergent AEs in subjects in the pooled pneumonia studies was slightly higher for ceftobiprole-treated females than ceftobiprole-treated males (75.7% versus 73.3%). In the cSSTI pooled studies the incidence of subjects with at least 1 adverse event was higher for ceftobiprole-treated females than ceftobiprole-treated males (58.1% versus 53.7%).

In the pooled pneumonia studies for the ceftobiprole-treated subjects, a higher percentage of females experienced, nausea, diarrhoea and vomiting (12.3%, 11.1%, and 11.1%, respectively) compared with males (3.3%, 8.4%, and 6.2%, respectively). In the pooled cSSTI studies, similar trends were seen with more females than males experiencing nausea, vomiting and diarrhoea. A similar but less marked pattern was seen for these three AEs in the comparator groups for both the pooled pneumonia and pooled cSSTI studies.

In the pooled pneumonia studies the incidence of hypokalaemia, hyponatraemia and pyrexia was higher in the male subjects (7.7%, 7.3% and 6.0%, respectively) compared to females (7.5%, 3.7% and 4.9% respectively).

Adverse events by age

When comparing the incidence of AEs according to age frequency differences of 2% or more between the age subgroups were considered. In the pooled pneumonia studies, treatment-emergent AEs were reported at a slightly higher frequency of 41% in the ceftobiprole-treated subjects who were under 65 years of age compared to a frequency of 36.4% in the comparator subjects. In subjects over 65 years of age, the incidence was 32.6% in the ceftobiprole-treated subjects versus 35.3% in the comparator subjects. In the pooled cSSTI studies 45% versus 43% of ceftobiprole and comparator treated subjects respectively experienced treatment emergent adverse events in subjects less than 65 years while in subjects greater than 65 years, the incidence was 11% of ceftobiprole-treated subjects versus 9.8% of the comparator-treated subjects.

Discontinuation due to AES

A few more subjects who received ceftobiprole for nosocomial pneumonia discontinued for AEs than in those receiving comparator. The SOC in which the majority of events leading to discontinuation occurred were infections and infestations; respiratory thoracic and mediastinal disorders; gastrointestinal disorders and nervous system disorders in the nosocomial pneumonia and the cSSTI studies.

Just less than 700 patients were exposed to ceftobiprole in the CAP and HAP with most of them being exposed for 7 days or less. Approximately 10% of subjects discontinued because of adverse events.

The most common drug-related treatment emergent adverse reactions were nausea, infusion site reactions, vomiting, diarrhoea, and dysgeusia. It also appears that there were a number of hyponatraemia cases necessitating a change in protocol. There were also a few cases of anaphylactic reaction after ceftobiprole use.

Pharmacovigilance system

The RMS considers that the Pharmacovigilance system as described by the applicant fulfils the requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Risk Management Plan (RMP)

Satisfactory full risk management plan has been provided.

Summary of Product Characteristics (SmPC)

The approved SmPC is satisfactory for this product.

Patient Information Leaflet (PIL)

The final PIL is in line with approved SmPC and is satisfactory.

Labelling

The labelling is satisfactory.

Clinical Expert Report

The clinical expert report is written by an appropriately qualified physician and is a suitable summary of the clinical aspects of the dossier.

Marketing Authorisation Application (MAA) Form

The MAA form is satisfactory from a clinical perspective.

CONCLUSION

There are no objections to the approval of this product from a clinical point of view.

IV OVERALL CONCLUSION AND BENEFIT-RISK ASSESSMENT QUALITY

The important quality characteristics of Zevtera 500 mg powder for concentrate for solution for infusion are well-defined and controlled. The specifications and batch analytical results indicate consistency from batch to batch. There are no outstanding quality issues that would have a negative impact on the benefit/risk balance.

NON-CLINICAL

Satisfactory non-clinical data were submitted.

CLINICAL

Pharmacokinetics

The pharmacokinetics of ceftobiprole appears to be straight forward. Ceftobiprole medocaril (pro-drug) is converted very rapidly to ceftobiprole. There is a dose proportional increase in systemic exposure after single and multiple dose administration. There is low protein binding and the volume of distribution is approximately 18l. One open ring metabolite has been identified which accounts for about 4% of systemic exposure.

Ceftobiprole is eliminated mainly by renal excretion with a half –life of approximately 3 hours which is independent of dose. The total systemic clearance ranged from approximately 4-7L/h and the mean renal clearance was about 3 to 5 L/h covering > 80% of total clearance. Urine recovery including pro-drug, ceftobiprole and the open ring metabolite is about 89%.

Systemic exposure and half-life were increased in patients with renal impairment; in patients with mild renal impairment systemic exposure was about 29% higher, and more than 2.5-fold higher in moderate impairment and in severe impairment 3-fold higher with a corresponding decrease in systemic and renal clearance. In patients with end stage renal disease (ESRD), systemic exposure to ceftobiprole and the open-ring metabolite was much higher in ESRD subjects dosed pre-dialysis or post-dialysis. Based on simulations, the extraction ratio during haemodialysis for ceftobiprole was conservatively set to 0.6, haemodialysis time to 4 hours and the CLCR to 10 mL/min and this suggests that a ceftobiprole dose of 250 mg (2-h) infusion post-dialysis once-daily is an appropriate regimen.

Ceftobiprole does not appear to undergo significant metabolism via the liver and protein binding is also quite low, therefore no study was conducted in patients with hepatic impairment. Overall, this is considered acceptable.

No drug interaction study has been conducted with ceftobiprole due to the non-significant hepatic metabolism, low protein binding and it not being a substrate or inhibitor for p-glycoprotein *in vitro* and cytochrome P450 isozymes, which is considered acceptable.

Pharmacodynamic

Ceftobiprole also appears to be active against *streptococcus pneumoniae*, in particular penicillin susceptible strains. However, it does not appear to be as active against penicillin resistant strains.

In terms of gram-negative bacteria known to cause HAP, ceftobiprole appears to have variable activity; it appeared to be active against *E.coli* generally but not active against ESBL strains. It also appears to be active against *H.influenza*.

It is important to note that ceftobiprole does not appear to have any activity against

K.pneumoniae.

Efficacy

BAP248/307

The study design including the endpoints and comparators are acceptable. Using the non-inferiority margin set, ceftobiprole was demonstrated to be non-inferior to the combination of linezolid and ceftazidime for the clinically evaluable set and ITT set as a whole i.e. nosocomial pneumonia plus VAP; the nosocomial pneumonia group excluding the VAP group but the VAP group was demonstrated not to be non-inferior.

CAP-3001

The study design including endpoints, non-inferiority margin and comparators chosen are acceptable. However, 50% of the study population chosen were not appropriate for treatment with an intra-venous agent as they were subjects with PORT scores I and II.

For the primary endpoint, the overall results for both the clinically evaluable and ITT population; ceftobiprole was demonstrated to be non-inferior to ceftriaxone, the lower limit of the confidence interval for the ITT population was -9.3 and for the clinically evaluable population it was -6.9.

For patients with PORT scores III – V and subjects with PSI \geq 91 (considered the relevant population for the proposed indication) ceftobiprole was also demonstrated to be non-inferior to ceftriaxone. Results for subjects within each PORT score category III, IV and V have also been provided which show comparable outcome in both the ceftobiprole and ceftriaxone groups.

SAFETY

Just less than 700 patients were exposed to ceftobiprole in the CAP and HAP with most of them being exposed for 7 days or less. Approximately 10% of subjects discontinued because of adverse events.

The most common drug-related treatment emergent adverse reactions were nausea, infusion site reactions, vomiting, diarrhoea, and dysgeusia. It also appears that there were a number of hyponatraemia cases necessitating a change in protocol. There were also a few cases of anaphylactic reaction after ceftobiprole use.

PRODUCT LITERATURE

The SmPC, PIL and labelling are satisfactory.

BENEFIT-RISK ASSESSMENT

The quality of the product is acceptable and any non-clinical or clinical concerns have been fully resolved. The risk benefit is, therefore, considered to be positive.

Module 6

STEPS TAKEN AFTER INITIAL PROCEDURE - SUMMARY

Date submitted	Application type	Scope	Outcome