INVESTIGATOR'S BROCHURE

SPONSOR'S NAME: Entasis Therapeutics

Product Name: Sulbactam/ETX2514

Research Number: ETX2514SUL

Trade Name: N/A

Indication: Treatment of infections caused by *Acinetobacter baumannii*

Formulation: Sterile Powder for Injection

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SUMMARY OF INVESTIGATOR'S BROCHURE CHANGES

This version of the Investigator's Brochure has been updated from the previous version, Version 2.1, dated 14 July 2017. Some editorial changes (e.g., capitalizations, hyphenations, typographical errors, and use of abbreviations) have been made for consistency of style. Updates included in this version (Version 3.0, dated 03 December 2018) and in Version 2.1 are listed in the table below. Summary sections have been updated for consistency to changes within the respective section. Editorial changes and changes to summary sections are not captured in this table.

Section No.	Section Title	Revision
1	SUMMARY	Updated to reflect changes to remainder of document.
1.2.4	Projections of human efficacious dose	Created new section to better organize existing data.
2.1.2	Mechanisms of resistance in *A. baumannii*	Updated to include whole genome sequencing results.
2.2	References	Updated references.
3.1	Drug substance	Updated ETX2514SUL drug product description.
3.1.1	ETX2514 sodium	Added additional company codes and Chemical Abstracts Service Registry (CAS) Number. Updated solubility.
3.1.2	Sulbactam sodium	Added Unites States Adopted Name (USAN). Updated solubility.
3.2	Drug product	Updated ETX2514SUL drug product description.
3.2.1	ETX2514 sodium	Added additional information on composition and excipients.
3.2.2	Sulbactam sodium	Updated language about diluent being specified in the clinical pharmacy manual.
4.1.1	Brief summary	Updated to reflect changes to section. Added summary table of nonclinical pharmacology studies.
4.1.2	Primary pharmacology	Removed subsections describing *in vitro* hollow-fiber studies, *in vitro* chemostat model studies, and *in vivo* pharmacokinetic/pharmacodynamic determination and efficacy of sulbactam administered in the presence of ETX2514.

Section No.	Section Title	Revision
4.1.2.1	*In vitro* studies	Removed subsection describing β-lactam partner selection for ETX2514 for *A. baumannii*. Removed subsection describing ETX2514 effects on sulbactam activity in sulbactam-sensitive *A. baumannii* isolates.
4.1.2.1.2	*In vitro* antibacterial activity	Updated section on ETX2514 inhibition of β-lactamases in bacterial cells.
4.1.2.1.3	Development of testing paradigm for the combination of sulbactam and ETX2514	Added new data. Updated title of subsection to reflect new data.
4.1.2.1.5	Spectrum of *in vitro* activity against *A. baumannii*	Updated and revised surveillance data.
4.1.2.1.6.2	Spontaneous resistance to sulbactam/ETX2514 in *A. baumannii*	Updated discussion.
4.1.2.2.1	Static time kill studies	Added new data.
4.1.2.2.2	Post-antibiotic effect	Added new data.
4.1.2.2.3	Activity of sulbactam/ETX2514 in combination with other antibiotics	Added new subsection.
4.1.2.2.4	Evaluation of experimental conditions that may influence sulbactam/ETX2514 susceptibility test results	Added new subsection.
4.2.1	Brief summary	Added new section.
4.3.1	Brief summary	Updated to reflect changes to section. Added summary tables of nonclinical pharmacokinetic and pharmacodynamic studies.
4.3.2.1	Analysis of nonlabeled compound	Added description of additional analytical methods.
4.3.3.1	ETX2514 single-dose PK in nonclinical species	Reorganized section by species.
4.3.3.3	Sulbactam and ETX2514 combination PK	Updated subsection title.
4.3.4.3	Penetration into lung ELF	Added new subsection.
4.3.5.2	*In vivo* mass balance, excretion, tissue distribution, and metabolite characterization	Added mass balance, distribution, and excretion studies. Updated metabolite profiling data.
4.3.7	Projection of a human efficacious dose	Updated data on pharmacokinetic/pharmacodynamic drivers for sulbactam and ETX2514.

Section No.	Section Title	Revision
4.3.7.1	Brief summary of PK/PD drivers of efficacy against *A. baumannii*	Created new subsection.
4.3.7.3	pTA of ETX2514SUL	Updated human dose projections, including exposure assessments in epithelial lining fluid.
4.3.8	Renal dose adjustment	Added new section.
4.4	Toxicology	Updated data and rearranged existing sections.
4.4.1	Brief summary	Updated to reflect changes to section. Added summary table of nonclinical toxicology studies.
4.4.3.5	1-week toxicity study of sulbactam/ETX2514 in rats	Added new subsection.
4.4.3.6	28-day toxicity study of sulbactam/ETX2514 in rats	Added new subsection.
4.4.4	Safety pharmacology	Moved cardiovascular safety pharmacology data into this new section. Added pulmonary and neurologic safety pharmacology data.
4.4.6	Reproductive toxicology	Added new section.
4.5	References	Updated reference list.
5	EFFECTS IN HUMANS	Entire section updated with corrected, final, and quality assurance-reviewed pharmacokinetics data from the first-in-human study and rewritten to reorganize and consolidate data from the first-in-human study, lung penetration study, renal insufficiency study, and Phase 2 study. Added summary table of clinical studies of ETX2514 conducted to date. Updated recommended ETX2514SUL dose adjustments in patients with impaired renal function.
5.2	Efficacy	Added new section.
5.3	Safety	Updated section with new data and reorganized by study.
6	SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR	Entire section rewritten to reflect data from the first-in-human study, lung penetration study, renal insufficiency study, and Phase 2 study.
6.1.2	Clinical data for ETX2514	Added new section.
6.1.3	Possible risks and adverse reactions	Updated section..
6.4	Reference safety information	Added reference safety information section.
6.5	References	Added references section.

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List of Abbreviations and Definition of Terms

The following abbreviations and special terms are used in this investigator's brochure.

Abbreviation or Special Term	Explanation
% T>MIC	Percentage of time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration of the organism
% T>pMIC	Percentage of time in a dosing interval that the drug concentration exceeds the 'potentiated' minimum inhibitory concentration of the organism
ALT	Alanine aminotransferase
AP	Acute pyelonephritis
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC_{0-6}	Area under the plasma concentration-time curve from 0 to 6 hours postdose
AUC_{0-24}	Area under the plasma concentration-time curve from 0 to 24 hours postdose
AUC_{0-t}	Area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed
BAL	Bronchoalveolar lavage
BID	Twice daily
BL/BLI	β-lactam/β-lactamase inhibitor
BLI	β-lactamase inhibitor
bpm	Beats per minute
CDAD	*Clostridium difficile* associated diarrhea
CFU	Colony-forming unit
CLcr	Creatinine clearance
CLSI	Clinical and Laboratory Standards Institute
C_{max}	Maximum plasma concentration
CNS	Central nervous system
cUTI	Complicated urinary tract infection
CYP	Cytochrome P450
DDI	Drug-drug interaction
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram

Abbreviation or Special Term	Explanation
EFD	Embryofetal development
ELF	Epithelial lining fluid
ETX2514SUL	Sulbactam/ETX2514
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
FIH	First-in-human
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practice
hERG	Human Ether à-go-go-related gene
hpd	Hours postdose
IC_{50}	Concentration showing 50% inhibitory effect
ICH	International Council for Harmonisation
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
LC/MS/MS	Liquid chromatography-tandem mass spectrometry
MAD	Multiple ascending dose
MBL	Metallo-β-lactamase
MDR	Multi-drug resistant
MIC	Minimum inhibitory concentration
MIC_{50}	Minimum concentration that inhibits 50% of isolates
MIC_{90}	Minimum concentration that inhibits 90% of isolates
m-MITT	Microbiologically Modified Intent-to-Treat
NaCl	Sodium chloride
NDA	New Drug Application
NDM-1	New Delhi metallo-β-lactamase 1
NOAEL	No-observed-adverse-effect-level
PAE	Post-antibiotic effect
PBP	Penicillin-binding protein
PCE	Polychromatic erythrocytes
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
pMIC	'Potentiated' minimum inhibitory concentration

Abbreviation or Special Term	Explanation
pTA	Probability of target attainment
q3h	Every 3 hours
q6h	Every 6 hours
q12h	Every 12 hours
q24h	Every 24 hours
QTc	Corrected QT interval
QWBA	Quantitative whole body-autoradiography
R^2	Correlation coefficient
Rcs	Regulator of capsule synthesis
SAD	Single ascending dose
SAE	Serious adverse event
SAR	Serious adverse reaction
SmPC	Summary of Product Characteristics
SOC	System organ class
$T>C_T$	Time in a dosing interval that the drug concentration exceeds a critical threshold
T>MIC	Time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration
TEAE	Treatment-emergent adverse events
TK	Toxicokinetic(s)
TOC	Test-of-Cure
V_{dss}	Steady state volume of distribution
WBC	White blood cell

1 SUMMARY

1.1 Background

There is a significant unmet medical need to identify new agents to treat *Acinetobacter baumannii* infections. Sulbactam/ETX2514 (ETX2514SUL) is a novel bactericidal β-lactam/β-lactamase inhibitor (BL/BLI) combination that is being developed for the treatment of infections caused by *A. baumannii*, including multi-drug resistant (MDR) and carbapenem-resistant isolates.

ETX2514 is a novel, rationally designed diazabicyclooctenone β-lactamase inhibitor (BLI). It is a potent inhibitor of Classes A and C β-lactamases and a broad spectrum inhibitor of Class D β-lactamases. ETX2514 is not a β-lactam. It displays a covalent, reversible mechanism of inhibition through β-lactamase active-site serine carbamoylation. ETX2514 exhibits intrinsic activity against some *Enterobacteriaceae* but has no significant clinical activity against *A. baumannii*.

Sulbactam is a penicillin derivative and is used widely as an inhibitor of β-lactamases. Although sulbactam is available as a standalone product in a small number of countries (e.g., Combactam™, Germany), the vast majority of human use is in combination with β-lactams (e.g., Unasyn™, ampicillin/sulbactam). Unasyn is approved by regulatory authorities in the United States, Europe, and the Asia-Pacific region. Sulbactam possesses intrinsic antimicrobial activity against *A. baumannii*. Increasing resistance of *A. baumannii* to sulbactam, however, has reduced its usefulness in treating infections caused by this bacterium.

Entasis Therapeutics is developing ETX2514SUL for the treatment of serious infections caused by *A. baumannii.* The dose of sulbactam, 1 g once every 6 hours (q6h) (maximum daily dose of 4 g), that will be used in combination with ETX2514 is the top dose approved for human use.

1.2 Nonclinical summary

Nonclinical studies of ETX2514 alone or as ETX2514SUL are described in Section 4 of this Investigator's Brochure. Nonclinical data for sulbactam are available from published literature and product information for Unasyn (ampicillin/sulbactam).

1.2.1 Nonclinical pharmacology

- ETX2514 is a novel, diazabicyclooctenone BLI with a spectrum of activity that encompasses clinically-important β-lactamases of Ambler Classes A and C and broad-spectrum inhibition of Class D. ETX2514 displays a covalent, reversible mechanism of inhibition through β-lactamase active-site serine carbamoylation.

- ETX2514 has greater potency than comparator BLIs, including clavulanic acid, tazobactam, and avibactam against all Class A, C, and D enzymes tested. ETX2514 does not inhibit the Class B metallo-β-lactamase (MBL) New Delhi metallo-β-lactamase 1 (NDM-1).

- Sulbactam is classified as a BLI but also has intrinsic antibacterial activity against *A. baumannii.* This intrinsic activity has been shown by target-binding studies, cytological profiling, and resistance mapping to be primarily due to its inhibition of penicillin-binding protein (PBP)3, a transpeptidase that is essential for the final step of bacterial peptidoglycan synthesis.

- Although no breakpoint for *A. baumannii* has been established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical and Laboratory Standards Institute (CLSI) for sulbactam alone, sulbactam is used in combination with ampicillin (in a 2:1 ampicillin:sulbactam ratio) in therapy for *A. baumannii*. CLSI has established breakpoints of 8/4 (ampicillin/sulbactam) mg/L for this agent for *Acinetobacter* species. Because the activity of ampicillin/sulbactam versus *A. baumannii* can be attributed to the sulbactam component alone, the CLSI-defined susceptible breakpoint of 8/4 (ampicillin/sulbactam) mg/L that applies to the combination correlates to a susceptible breakpoint of 4 mg/L for sulbactam alone in *A. baumannii*, and this provisional minimum inhibitory concentration (MIC) breakpoint has been used throughout this document.

- ETX2514 alone exhibits intrinsic antibacterial activity against some *Enterobacteriaceae* but has no clinically important activity against *A. baumannii* (MIC ≥32 mg/L for most strains tested).

- The addition of ETX2514 to sulbactam *in vitro* restores the activity of sulbactam such that the minimum concentration that inhibits 90% of isolates (MIC_{90}) versus a collection of recent *A. baumannii* clinical isolates shifts from >32 to 2 mg/L in the presence of ETX2514 (held constant at 4 mg/L). This activity of sulbactam/ETX2514 was consistent across *A. baumannii* isolates from different geographic regions and infection types and did not change in subsets of meropenem-resistant, colistin-resistant, or MDR isolates, nor did it change over time (a total of 3611 isolates have been tested to date).

- Sulbactam/ETX2514 and sulbactam are bactericidal against *A. baumannii*.

- The frequency of spontaneous resistance to sulbactam/ETX2514 was low (ranging from 2×10^{-8} to 8×10^{-10} at 4X MIC and undetectable at 8X MIC).

- The sulbactam/ETX2514 combination showed no synergy nor antagonism when tested in combination with other antibiotics (and vice versa). In addition, the MICs were not affected by variations in starting inoculum, temperature, atmospheric conditions, divalent cation concentration, surfactant, or the presence of human urine or serum. Sulbactam/ETX2514 MICs were elevated against some isolates at pH 5.0 and pH 6.0, which was exacerbated in the presence of urine.

- *In vitro* experiments supported a susceptibility testing paradigm of holding the concentration of ETX2514 constant at 4 mg/L while varying the sulbactam concentration in 2-fold increments. In addition, a 10 µg sulbactam/10 µg ETX2514 disk mass was determined to have the best correlation with broth MICs for use in disk diffusion tests.

- Evaluation of sulbactam/ETX2514 in *in vivo* animal efficacy models and in *in vitro* hollow-fiber studies indicate that sulbactam/ETX2514 was efficacious against *A. baumannii*, including those strains that are MDR.

1.2.2 Secondary pharmacology

- ETX2514 has minimal binding affinity for the vast majority of cellular receptors at projected efficacious concentrations.

- No major interactions were identified against selected human serine proteases.

1.2.3 PK and drug metabolism in animals

- Intravenous (IV) pharmacokinetics (PK) of ETX2514 in rats were generally dose proportional with increasing dose. Exposure in males and females was comparable. Short half-lives of 0.23 and 0.80 hours in rats and dogs, respectively, resulted in no accumulation. Steady state volume of distribution (V_{dss}) ranged from 0.44 to 0.59 L/kg in rats and from 0.26 to 0.32 L/kg in individual dogs.

- Protein binding of ETX2514 was very low with the percentage of unbound ETX2514 ranging from 78.3% to 100% across human, mouse, dog, rat, and guinea pig, with no evidence of saturation or concentration dependence.

- *In vitro*, ETX2514 was metabolically stable in rat, dog, and human hepatocytes as well as rat and dog microsomes.

- ETX2514 showed no significant inhibition of cytochrome P450 (CYP) enzymes, no *in vitro* evidence for induction potential, and no inhibitory potency against major transporters within a clinically relevant exposure range.

- Clearance of ETX2514 in rats and dogs was predominantly via urinary excretion (59% and 71%, respectively). *In vitro* studies confirmed ETX2514 is a substrate for the kidney transporter OAT1 at concentrations in excess of 500 μM. This transport was inhibited competitively with the addition of probenecid.

1.2.4 Projections of human efficacious dose

- Investigations using the *in vitro* hollow-fiber system and the chemostat model characterized PK/pharmacodynamic (PD) drivers of time in a dosing interval that the drug concentration exceeds the MIC (T>MIC) and area under the plasma concentration-time curve (AUC)/MIC for sulbactam and ETX2514, respectively. The 'potentiated' MIC (pMIC) of sulbactam (MIC in the presence of 4 mg/L ETX2514) was used for both T>MIC and AUC/MIC targets for sulbactam and ETX2514, respectively. E_{max} modeling of *in vivo* animal efficacy conducted in thigh and lung infections suggested T>MIC of 50% of the dosing interval for sulbactam in order to reach a 1-$\log_{10}$ kill. For ETX2514, AUC/MIC ratios of 10 and 30 are associated with stasis and 1-$\log_{10}$ kill endpoints when sulbactam is at 50% of time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration of the organism (% T>MIC). Experimental results obtained in murine infection models have

historically been predictive of human efficacy. Achieving these PK/PD exposures of sulbactam and ETX2514 are anticipated to translate to a favorable clinical outcome.

- A detailed assessment of human dose projection of sulbactam/ETX2514 was performed utilizing PK parameters from Phase 1 data in humans, PK/PD targets, and current MIC distribution versus *A. baumannii*. A preliminary probability of target attainment (pTA) was completed. The result of these analyses suggested a 3-hour infusion of 1 g of sulbactam and 0.5 to 1.0 g of ETX2514 administered q6h is predicted to be effective to treat patients infected with *A. baumannii* with MICs ≤4 mg/L.

1.2.5 Toxicology

- Maximum tolerated, single IV dose escalation studies of ETX2514 performed in rats and dogs did not result in serious test article–related events. Exposure across the dose ranges evaluated were generally dose proportional, and there were no significant differences between males and females.

- 7-day IV repeat-dose range-finding studies in rats and dogs at doses up to the limit dose of 2000 mg/kg/day did not identify serious test article–related findings.

- In pivotal 14-day IV repeat-dose toxicity studies conducted in rats and dogs, ETX2514 administered up to the limit dose of 2000 mg/kg/day was not associated with mortality or significant changes in clinical signs, ophthalmology, urinalysis or hematology parameters, organ weight changes, or changes in gross pathology or histopathology, and there were no toxicologically significant changes in clinical chemistry parameters. Based on these findings, the no-observed-adverse-effect-level (NOAEL) was 2000 mg/kg/day in both species. The associated combined-sex mean steady state maximum plasma concentration (C_{max}) and AUC from 0 to 24 hours postdose (AUC_{0-24}) values on Day 14 in the most sensitive species, rat, at the NOAEL were 1340 µg/mL and 3140 µg·h/mL, respectively. The exposure margin based on AUC_{0-24} relative to human exposures was 6.3-fold.

- In a 7-day IV repeat-dose study of ETX2514 in combination with sulbactam, minimal decreases in body weight gain were noted, and there were no adverse effects on clinical pathology parameters, organ weights, or macroscopic tissue findings. Based on the mild severity of the findings, the NOAEL was the highest combination dose tested, 400 mg/kg/day of each compound.

- The toxicity of ETX2514 alone or in combination with sulbactam was evaluated in a pivotal (Good Laboratory Practice [GLP]) study in rats administered the treatments IV daily for 28 days. ETX2514 administered alone (600 mg/kg/day) was devoid of toxicity. Notable effects in rats treated with the combination of sulbactam/ETX2514 (300 or 600 mg/kg/day of each compound) were consistent with known, monitorable effects of sulbactam. Dose-related inflammatory changes noted microscopically at the infusion site and in liver and lung with both combination doses were considered adverse effects of treatment; however, inflammatory changes in liver and lung were not associated with necrosis and were not present at the end of the recovery period. Thus, while there was no NOAEL identified in the study due to inflammatory changes noted with both combination doses, the adverse changes were monitorable and reversible and occurred after an infusion period of approximately 2-fold the maximum clinical treatment period.

- *In vitro* investigations exhibited no significant interactions with cardiac ion channels expressed in HEK293 and CHO cells at any concentrations ranging from 100 to 1000 µM including human Ether à-go-go-related gene (hERG) (concentration showing 50% inhibitory effect [IC_{50}] >333 µM).

- ETX2514 had no quantitative effects on any electrocardiogram (ECG) parameters including PR interval, QRS interval, QT interval, corrected QT interval (QTc), heart rate, or arterial pressure after single doses up to 2000 mg/kg in telemetered conscious dogs.

- ETX2514 was not toxic to male rats in respiratory and neurologic safety pharmacology studies at doses up to 2000 mg/kg/day.

- ETX2514 was not mutagenic *in vitro* in a bacterial reverse mutation assay, and it was not clastogenic *in vitro* in human lymphocytes or *in vivo* in rats. Thus, ETX2514 is not genotoxic.

- Reproductive toxicology studies with ETX2514 using the IV infusion route assessing embryofetal development (EFD) and male and female fertility have been completed in the rat. In these studies, rats were administered ETX2514 up to the limit dose of 1000 mg/kg/day. No ETX2514-associated adverse effects on male or female fertility or EFD were observed. Thus, the NOAEL for reproductive and embryofetal toxicity in rats is 1000 mg/kg/day.

- Carcinogenicity studies of ETX2514 are not planned because there is no evidence of genotoxicity and the intended duration of therapy is no more than 2 weeks.

- Based on the results of the 28-day sulbactam/ETX2514 combination study in rats, minimal but reversible inflammatory changes in liver and lung have been the only adverse toxicities of note, occurring only after the combination was infused daily for approximately 2 times the maximum duration of clinical use. The C_{max} values of ETX2514 in the combination arms of the 28-day rat study were 3.6- to 6.5-times higher than human exposure, and the C_{max} values of sulbactam ranged from 3.5- to 6.8-fold higher than human exposure. Exposure margins based on AUC_{0-24} compared to human exposures ranged from 0.4- to 1.0-fold for ETX2514 and 0.8- to 1.8-fold for sulbactam.

1.3 Effects in humans

1.3.1 ETX2514 and ETX2514SUL

With respect to PK:

- ETX2514 demonstrated linear PK and had no evidence of accumulation.

- ETX2514 did not interact with sulbactam and/or imipenem/cilastatin.

- ETX2514 was predominantly excreted renally and dose adjustments are necessary for patients with moderate or severe renal insufficiency including dialysis. Similarly, higher doses are recommended for patients with augmented renal clearance or receiving continuous renal replacement therapy.

- ETX2514SUL demonstrated good penetration of the lung epithelium.

With respect to efficacy:

- ETX2514SUL when dosed with imipenem/cilastatin showed comparable efficacy to placebo + imipenem/cilastatin in treatment of hospitalized patients with complicated urinary tract infection (cUTI) or acute pyelonephritis (AP). Among patients with imipenem nonsusceptible infections, imipenem when combined with ETX2514SUL was able to successfully treat 3 out of 3 infections.

With respect to safety and tolerability:

- ETX2514 was generally safe and well tolerated when given alone or with sulbactam and/or imipenem/cilastatin.
- To date, there have been no serious adverse drug reactions or deaths in clinical studies.
- The most common adverse events seen have been headache and infusion site reactions, including phlebitis and vascular pain at the infusion site.
- Mild reductions in total white blood cell (WBC) and absolute neutrophil counts have been observed with multiple doses of ETX2514SUL, although no events of leukopenia or neutropenia have been observed.
- Asymptomatic, transient increases in hepatic enzymes, unaccompanied by increases in bilirubin, have been observed with ETX2514SUL, and these are thought to be related to known effects of sulbactam.
- Allergic reactions including urticaria have been observed at rates <1%, with ETX2514SUL, and all have been mild or moderate.
- No clinically significant changes in vital signs or ECG parameters have been observed with ETX2514SUL in patients. Transient increases in systolic blood pressure within normal ranges were observed in healthy elderly subjects dosed with ETX2514SUL.

1.3.2 Sulbactam

- Sulbactam is a penicillin derivative and is used widely as an inhibitor of β-lactamases. Although sulbactam is available as a standalone product in a small number of countries (e.g., Combactam, Germany), the vast majority of human use is in combination with β-lactams (e.g., Unasyn, ampicillin/sulbactam). Unasyn is approved by regulatory authorities in the United States, Europe, and the Asia-Pacific region.
- The maximum recommended daily dose of sulbactam is 1 g q6h (maximum daily dose of 4 g).
- Sulbactam is a penicillin derivative and, as such, should not be used in subjects with a history of β-lactam hypersensitivity.

- Up-to-date information on sulbactam can be found in United States and European product labels (Unasyn Product Label, Ampicillin/sulbactam Summary of Product Characteristics).

- The PK of ampicillin and sulbactam are linear up to at least 1000 mg. The PK profile of IV ampicillin is unaffected by co-administration of sulbactam. Similarly, the PK profile of sulbactam is not impacted by co-administration of ampicillin.

- The clinical safety of sulbactam has been established by nearly 30 years of experience with Unasyn. Based on the product label, Unasyn is generally safe and well tolerated.

1.4 References

Unasyn® ampicillin sodium and sulbactam sodium injection, powder, for solution (date of revision 12/2014). Accessed at www.Pfizer.com on 10 July 2016.

2 INTRODUCTION

2.1 The prevalence of *A. baumannii*

A. baumannii, a Gram-negative bacterium, is a significant public health concern and is classified as a "serious threat" pathogen in the recent US Centers for Disease Control report "Antibiotic Resistance Threats" (CDC 2013) and is ranked as "critical" on the World Health Organization Priority Pathogens List for R&D of New Antibiotics (WHO 2017).
A. baumannii causes severe infections that are associated with high mortality. Approximately 2% of healthcare-associated infections are caused by *A. baumannii* (Sievert 2013). Patients on mechanical ventilators and those with central line-catheters have the highest proportion of infections caused by *A. baumannii*. Serious infections caused by *A. baumannii* including hospital-acquired bacterial pneumonia/ventilator-associated bacterial pneumonia, cUTI, bloodstream infection, and wound infection are estimated to occur in 70,000 to 90,000 patients in the United States per year, of which ~63% are caused by MDR isolates (Decision Resources Group 2015). Mortality associated with bacteremia and pneumonia caused by *A. baumannii* range from 30% to 50% (Spellberg 2015, Cheng 2015). The risk of death associated with infections caused by *A. baumannii* isolates resistant to carbapenems is even higher (Cheng 2015, CDC 2013).

2.1.1 Antibiotic resistance in *A. baumannii*

In the early 1960s to 1970s, infections due to *Acinetobacter* were managed effectively with β-lactam antibiotics, as the rates of resistance to available antimicrobial classes was low (Doi 2015, Gonzalez-Villoria 2016). But by the end of the 1970s, resistance to aminoglycosides and β-lactam antibiotics had been reported. In the 1980s, carbapenems such as imipenem became the drug of choice for treatment of *A. baumannii* infections; however, widespread carbapenem resistance has now been observed in many countries (CDC 2013, Gonzalez-Villoria 2016).

In the late 1990s to 2000s, the *in vitro* activity and clinical effectiveness of sulbactam against *A. baumannii* was demonstrated (Doi 2015). There has been a steady decline, however, in the *in vitro* susceptibility of *A. baumannii* to sulbactam. Presently, the majority of *A. baumannii* isolates are MDR, defined as nonsusceptible to at least 3 or more antimicrobial categories, and many are extremely drug resistant, defined as nonsusceptibility to at least 1 agent in all but 2 or fewer antimicrobial categories (Magiorakos 2012). For example, data from the Healthcare Safety Network summarizing healthcare-associated infections reported that approximately 60% to 70% of *A. baumannii* isolates causing pneumonia or bacteremia were resistant to carbapenems and were identified as MDR (Sievert 2013).

Due to this extensive resistance, there is an urgent unmet medical need to identify new agents to treat *A. baumannii* infections.

2.1.2 Mechanisms of resistance in *A. baumannii*

A. baumannii has a number of resistance mechanisms that can be utilized and/or acquired. Among the most important of these are the production of β-lactamases, which degrade β-lactam antimicrobials. β-lactamases are classified into 4 groups under the Ambler classification: Classes A, B, C, and D. Within each Ambler Class there is diversity, with multiple different β-lactamases identified.

Whole genome sequencing of 84 recent, diverse, MDR *A. baumannii* clinical isolates from the Entasis bacterial strain collection revealed that all 84 isolates encoded Class D β-lactamase genes, with 84% of these encoding 2 or more Class D types (with OXA-23 and OXA-66 predominating). In addition, 53.5% of the Class D-positive strains also carried Class A genes (predominantly TEM-1), 85% carried both Class D and extended spectrum Class C (ADC-type) genes, and 45% encoded genes for Classes A, C, and D enzymes (Durand-Réville 2017). These results reinforce the notion that only an inhibitor of A, C, and D classes of serine β-lactamases can effectively restore β-lactam activity in *A. baumannii*.

Other resistance mechanisms in *A. baumannii* include: (1) presence of efflux pumps responsible for resistance to aminoglycosides, tetracyclines, and fluoroquinolones; (2) production of aminoglycoside-modifying enzymes responsible for resistance to aminoglycosides; (3) amino acid substitutions in the quinolone resistance-determining region responsible for resistance to fluoroquinolones; and (4) alterations in the target site for colistin on lipid A of lipopolysaccharide resulting in colistin-resistance (Doi 2015).

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3 PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 Drug substance

The drug consists of 2 components, ETX2514 and sulbactam. Both components exist as sodium salts. The co-dosed drug is abbreviated ETX2514SUL. Information for both components is provided below.

3.1.1 ETX2514 sodium

3.1.1.1 Nomenclature

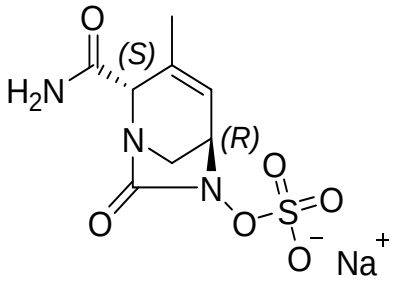
Company/Laboratory Codes: ETX2514, ETX2514 sodium, NaETX2514

Chemical Name (International Union of Pure and Applied Chemistry [IUPAC]): sodium [(2*S*,5*R*)-2-carbamoyl-3-methyl-7-oxo-1,6-diaza-bicyclo[3.2.1]oct-3-en-6-yl] sulfate

Chemical Abstracts Service Registry Number: 1467157-21-6

3.1.1.2 Structural

Structural Formula:



Molecular Formula (sodium salt): $C_8H_{10}N_3NaO_6S$

Molecular Weight (sodium salt): 299.23 g/mole

Molecular Weight (parent, free acid): 277.25 g/mole

Chirality/Stereochemistry: ETX2514 sodium is the pure (2*S*,5*R*)-enantiomer of the trans diastereoisomer, as shown in the structure above.

3.1.1.3 General properties

ETX2514 is a white to yellow amorphous powder, freely soluble in water (>200 mg/mL at 25°C) and dimethyl sulfoxide, and has limited (e.g., <5 mg/mL) solubility in ethanol, isopropanol, acetonitrile, chloroform, ethyl acetate, heptane, and hexane.

3.1.2 Sulbactam sodium

3.1.2.1 Nomenclature

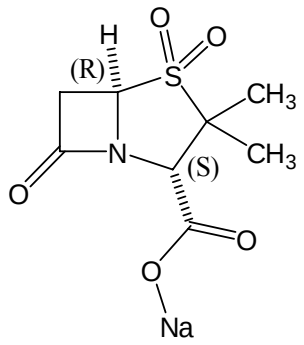
Chemical Name (IUPAC): sodium (2*S*,5*R*)-3,3-Dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide

Chemical Abstracts Service Registry Number: 69388-84-7

United States Adopted Name (USAN): sulbactam sodium

3.1.2.2 Structural

Structural Formula:



Chemical Formula (sodium salt): $C_8H_{10}NNaO_5S$

Molecular Weight (sodium salt): 255.22 g/mole

Molecular Weight (parent, free acid): 233.24 g/mole

Chirality/Stereochemistry: Sulbactam sodium is the pure (2*S*,5*R*)-enantiomer of the cis diastereoisomer, as shown in the structure above.

3.1.2.3 General properties

Sulbactam sodium is a white to off-white crystalline powder which is fully soluble in normal saline (0.9 N sodium chloride [NaCl]) solution at 10 mg/mL.

3.2 Drug product

The drug product consists of 2 components, ETX2514 and sulbactam. Descriptions of each component are provided.

3.2.1 ETX2514 sodium

3.2.1.1 Presentation

ETX2514 sodium drug product is presented as a lyophilized cake in a sterile 10 mL amber glass vial, with a stopper, blue overseal, and clinical label.

3.2.1.2 Composition

The ETX2514 sodium sterile vials are filled with 604 to 614 mg of ETX2514 sodium salt, which is equivalent to 560 to 569 mg of ETX2514 as free acid. The vials contain an 11% to 12% overfill to enable accurate withdrawal of a 500 mg dose following reconstitution with Water for Injection.

3.2.1.3 Excipients

Sodium hydroxide (EP, BP, JP, NF grade), hydrochloric acid (EP, BP, JP, NF grade), Water for Injection (United States Pharmacopeia, EP grade), and nitrogen (NF) are used in the formulation of ETX2514.

3.2.1.4 Diluents

ETX2514 sodium is reconstituted by addition of sterile Water for Injection. Further information about infusion can be found in the handling instructions.

3.2.1.5 Storage conditions and shelf-life

ETX2514 sodium is stored at 2 to 8°C, protected from light. The current retest date is 1 year from the date of manufacture. As additional stability information is obtained, the retest date will be reevaluated, and, if appropriate, revised.

3.2.2 Sulbactam sodium

3.2.2.1 Presentation

Sulbactam sodium drug product is presented as a crystalline powder in a sterile 30 mL clear glass vial, with a stopper, overseal, and clinical label.

3.2.2.2 Composition

The sulbactam sodium sterile vials are filled with 1.2 g of sulbactam sodium salt, which is equivalent to 1.1 g of sulbactam as free acid. The vials contain a 10% overfill to enable accurate withdrawal of a 1.0 g dose following reconstitution with Water for Injection.

3.2.2.3 Excipients

There are no excipients used in the formulation of sulbactam sodium.

3.2.2.4 Diluents

Sulbactam sodium is reconstituted as specified in the pharmacy manual for the respective studies.

3.2.2.5 Storage conditions and shelf-life

Sulbactam sodium is stored at 20 to 25°C and has a retest period of 3 years from the date of manufacture.

4 NONCLINICAL STUDIES

4.1 Nonclinical pharmacology

4.1.1 Brief summary

- ETX2514 is a novel, diazabicyclooctenone BLI with a spectrum of activity that encompasses clinically-important β-lactamases of Ambler Classes A and C and broad-spectrum inhibition of Class D. ETX2514 displays a covalent, reversible mechanism of inhibition through β-lactamase active-site serine carbamoylation.

- ETX2514 has greater potency than comparator BLIs, including clavulanic acid, tazobactam, and avibactam against all Class A, C, and D enzymes tested. ETX2514 does not inhibit the Class B MBL NDM-1.

- Sulbactam is classified as a BLI but also has intrinsic antibacterial activity against *A. baumannii.* This intrinsic activity has been shown by target-binding studies, cytological profiling, and resistance mapping to be primarily due to its inhibition of PBP3, a transpeptidase that is essential for the final step of bacterial peptidoglycan synthesis.

- Although no breakpoint for *A. baumannii* has been established by the EUCAST or CLSI for sulbactam alone, sulbactam is used in combination with ampicillin (in a 2:1 ampicillin:sulbactam ratio) in therapy for *A. baumannii*. CLSI has established breakpoints of 8/4 (ampicillin/sulbactam) mg/L for this agent for *Acinetobacter* species. Because the activity of ampicillin/sulbactam versus *A. baumannii* can be attributed to the sulbactam component alone, the CLSI-defined susceptible breakpoint of 8/4 (ampicillin/sulbactam) mg/L that applies to the combination correlates to a susceptible breakpoint of 4 mg/L for sulbactam alone in *A. baumannii*, and this provisional MIC breakpoint has been used throughout this document.

- ETX2514 alone exhibits intrinsic antibacterial activity against some *Enterobacteriaceae* but has no clinically important activity against *A. baumannii* (MIC ≥32 mg/L for most strains tested).

- The addition of ETX2514 to sulbactam *in vitro* restores the activity of sulbactam such that the MIC_{90} versus a collection of recent *A. baumannii* clinical isolates shifts from >32 to 2 mg/L in the presence of ETX2514 (held constant at 4 mg/L). This activity of sulbactam/ETX2514 was consistent across *A. baumannii* isolates from different geographic regions and infection types and did not change in subsets of meropenem-resistant, colistin-resistant, or MDR isolates, nor did it change over time (a total of 3611 isolates have been tested to date).

- Sulbactam/ETX2514 and sulbactam are bactericidal against *A. baumannii*.

- The frequency of spontaneous resistance to sulbactam/ETX2514 was low (ranging from 2×10^{-8} to 8×10^{-10} at 4X MIC and undetectable at 8X MIC).

- The sulbactam/ETX2514 combination showed no synergy nor antagonism when tested in combination with other antibiotics (and vice versa). In addition, the MICs were not affected by variations in starting inoculum, temperature, atmospheric conditions, divalent cation concentration, surfactant, or the presence of human urine or serum. Sulbactam/ETX2514 MICs were elevated against some isolates at pH 5.0 and pH 6.0, which was exacerbated in the presence of urine.

- *In vitro* experiments supported a susceptibility testing paradigm of holding the concentration of ETX2514 constant at 4 mg/L while varying the sulbactam concentration in 2-fold increments. In addition, a 10 µg sulbactam/10 µg ETX2514 disk mass was determined to have the best correlation with broth MICs for use in disk diffusion tests.

- Evaluation of sulbactam/ETX2514 in *in vivo* animal efficacy models and in *in vitro* hollow-fiber studies indicate that sulbactam/ETX2514 was efficacious against *A. baumannii*, including those strains that are MDR.

An overview of the comprehensive package of nonclinical studies that detail the primary pharmacology of ETX2514 with and without sulbactam is presented in Table 4–1. Details of specific studies follow the table.

Table 4–1. Summary of ETX2514 nonclinical pharmacology studies

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2016-0002	Complete	*In vitro* antimicrobial activity of ETX2514	non-GLP	ETX2514 demonstrates intrinsic antibacterial activity against members of the *Enterobacteriaceae* family. The antimicrobial activity of ETX2514 does not change in the presence of any of the major classes of β-lactamases. Biochemical and morphological effects suggest that ETX2514 primarily targets PBP2.
PC2514-2016-0003	Complete	Sulbactam-ETX2514: Frequencies of spontaneous resistance against sulbactam	non-GLP	The frequency of spontaneous resistance to sulbactam-ETX2514 in 5 clinical isolates of *A. baumannii* ranged from 2.1×10^{-8} to 7.6×10^{-10} at 4X MIC. Mutations in resistant isolates mapped to PBP3, the target of sulbactam, and stringent response genes which are associated with resistance to PBP2 inhibitors.
PC2514-2016-0004	Complete	Frequency and mechanism of spontaneous resistance to ETX2514 alone in *Enterobacteriaceae*	non-GLP	The frequency of spontaneous resistance to 4X MIC ETX2514 was approximately 10^{-6} in both *E. coli* and *K. pneumoniae*. The MIC of ETX2514 for these mutants were increased by 32- to 64-fold compared to their respective parental strains. Resistance mapped to genes encoding for IgaA, a regulator of the Rcs phosphorelay system and the AspS.
PC2514-2016-0005	Complete	β-lactam partner selection for ETX2514 versus *A. baumannii*	non-GLP	The activity of 8 β-lactam partners in the absence or presence of ETX2514 (4 mg/L) was determined against 200 diverse clinical isolates of *A. baumannii* from 2012. The addition of ETX2514 improved the antibacterial activity partners tested with the exception of aztreonam. The greatest effect was seen for sulbactam, whose MIC_{90} was reduced from 64 to 4 mg/L in the presence of 4 mg/L ETX2514.
PC2514-2016-0007	Complete	Degradation of sulbactam by β-lactamases	non-GLP	Sulbactam can be hydrolyzed by β-lactamases from all 4 Ambler Classes, with the greatest susceptibility observed being to TEM-1, KPC-2, and the metallo-β-lactamases.
PC2514-2016-0008	Complete	Sulbactam-ETX2514 activity tested against global isolates of *A. baumannii* from 2014	non-GLP	The *in vitro* activity of sulbactam-ETX2514 and comparator antibiotics was determined against 1131 clinical *A. baumannii* isolates in 2014 from 151 medical centers in 38 countries. Addition of ETX2514 (4 mg/L) improved the MIC_{90} of sulbactam from 64 mg/L to 4 mg/L. This activity was maintained among meropenem-resistant, colistin-resistant, and MDR isolates. Activity was consistent across infection types and geographic locations.

CLSI = Clinical and Laboratory Standards Institute; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IgaA= intracellular growth attenuator protein; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; NA = not applicable; NDM-1 = New Delhi metallo-β-lactamase 1; PBP = penicillin-binding protein; QC = quality control; Rcs = regulator of capsule synthesis.

Table 4–1. Summary of ETX2514 nonclinical pharmacology studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2016-0009	Complete	Mechanism of action of sulbactam combined with ETX2514 in *A. baumannii*	non-GLP	Sulbactam-ETX2514 showed *in vitro* antibacterial activity against *A. baumannii* and species belonging to the *Enterobacteriaceae*. The potent antibacterial activity of sulbactam in the presence of 4 mg/L ETX2514 versus the *Enterobacteriaceae* was primarily due to the intrinsic activity of ETX2514. ETX2514 appears to weakly potentiate the antibacterial activity of sulbactam in sulbactam-sensitive *A. baumannii* strains.
PC2514-2016-0010	Complete	ETX2514: Inhibition of selected class A, B, C, and D β-lactamases	non-GLP	ETX2514 displays potent biochemical inhibition of Class A, C, and D β-lactamases. It does not inhibit the metallo-β-lactamase NDM-1. Acylation efficiency was highest against Class A enzymes, then Class C, then Class D. The potency of ETX2514, as measured by IC_{50}, exceeded that of avibactam by as much as 100-fold for some enzymes.
PC2514-2016-0011	Complete	X-ray crystallography of ETX2514 covalent adducts with β-lactamases and penicillin-binding protein 3	non-GLP	The X-ray crystallographic structures of ETX2514 in complex with the Class C AmpC β-lactamase from *P. aeruginosa*, the Class D OXA-24 β-lactamase from *A. baumannii*, and PBP3 from *P. aeruginosa* were determined. These studies revealed that ETX2514 was covalently bound to the catalytic serine residue of these enzymes.
PC2514-2016-0035	Complete	ETX2514: Inhibition of β-lactamases in *Pseudomonas aeruginosa* and *A. baumannii* isogenic panels	non-GLP	ETX2514 was capable of inhibiting selected Ambler Class A, C, and D β-lactamases expressed in an isogenic panel of *P. aeruginosa* or *A. baumannii* strains, as measured by the restoration of β-lactam susceptibility. ETX2514 was unable to inhibit Class B metallo-β-lactamases.
PC2514-2017-0001	Complete	Sulbactam-ETX2514 Tier 2 broth microdilution MIC QC	non-GLP	Sulbactam-ETX2514 M23 studies for broth microdilution susceptibility tests resulted in QC ranges of 0.5-2 mg/L against *A. baumannii* QC strain NCTC 13304. These were approved by CLSI in January 2017.
PC2514-2017-0002	Complete	Six-month stability testing of sulbactam-ETX2514	non-GLP	This study demonstrated that microbroth dilution panels containing sulbactam-ETX2514 may be stored frozen up to 6 months with no loss of activity.

CLSI = Clinical and Laboratory Standards Institute; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IgaA= intracellular growth attenuator protein; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; NA = not applicable; NDM-1 = New Delhi metallo-β-lactamase 1; PBP = penicillin-binding protein; QC = quality control; Rcs = regulator of capsule synthesis.

Table 4–1. Summary of ETX2514 nonclinical pharmacology studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2017-0003	Complete	Preliminary assessment of the optimal disk content for sulbactam-ETX2514	non-GLP	Sulbactam-ETX2514 disk masses of 10/10 µg and 10/5 µg were recommended to proceed to M23 Phase 3 disk diffusion susceptibility testing.
PC2514-2017-0004	Complete	Assessment of the optimal disk content for sulbactam-ETX2514	non-GLP	Sulbactam-ETX2514 M23 Tier 2 studies showed the optimal disk content for disk diffusion susceptibility testing was 10/10 µg.
PC2514-2017-0005	Complete	*In vitro* antibacterial activity of sulbactam-ETX2514 against diverse, global isolates of *A. baumannii-calcoaceticus* complex (2013- 2015)	non-GLP	The goal of this study was to perform *in vitro* antibacterial susceptibility testing of sulbactam in the presence or absence of ETX2514 as a dual agent or in a triple combination with either imipenem or meropenem against recent (2013, 2014, and 2015) geographically diverse *A. baumannii-calcoaceticus* complex isolates. The MIC_{90} of sulbactam-ETX2514 was 2 mg/L. The addition of imipenem or meropenem (fixed at 2 mg/L) reduced the MIC_{50} from 1 mg/L to 0.12 and 0.25 mg/L, respectively, but did not lower the MIC_{90}. The potent activity of these combinations was consistent over time and across geographic locations and type of infection.
PC2514-2017-0006	Complete	Evaluation of experimental conditions that may influence sulbactam-ETX2514 susceptibility test results	non-GLP	A variety of experimental conditions were tested for their effect on the activity of sulbactam-ETX2514 versus 10 clinical isolates of *A. baumannii*, including inoculum, incubation temperature, atmosphere, pH, divalent cations, serum, albumin, surfactant, and urine. The only condition that decreased the activity of the combination was the presence of 50% urine at pH 5.0. Sulbactam-ETX2514 was also found to be bactericidal against most strains tested.
PC2514-2017-0007	Complete	Activity of sulbactam-ETX2514 in combination with other antibiotics	non-GLP	Combinations of sulbactam-ETX2514 and 11 antibacterial agents or 1 antifungal agent were tested against 10 *A. baumannii* and CLSI QC reference strains in checkerboard assays. Interactions between sulbactam-ETX2514 and imipenem against 12 *Enterobacteriaceae* were also examined. Few instances of synergy and no antagonism was observed.

CLSI = Clinical and Laboratory Standards Institute; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IgaA= intracellular growth attenuator protein; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; NA = not applicable; NDM-1 = New Delhi metallo-β-lactamase 1; PBP = penicillin-binding protein; QC = quality control; Rcs = regulator of capsule synthesis.

Table 4–1. Summary of ETX2514 nonclinical pharmacology studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2017-0008	Complete	Effect of relative concentrations of sulbactam and ETX2514 on antibacterial activity	non-GLP	The optimal paradigm for microbroth susceptibility testing of the combination of sulbactam and ETX2514 is 2-fold titrations of sulbactam in the presence of ETX2514 fixed at 4 mg/L.
PC2514-2017-0009	Complete	Sulbactam-ETX2514 *in vitro* static time kill studies	non-GLP	In static time kill studies against 10 clinical isolates, 4X MIC sulbactam plus 4 mg/L ETX2514 resulted in 4 isolates with >3-$\log_{10}$ reduction and 3 isolates showed a 2.4-2.8 $\log_{10}$ reduction in viable cell counts after 24 hours. The remaining 3 isolates exposed to 4X MIC sulbactam-ETX2514 showed some rebound of bacterial growth at the 24 hours. Sulbactam-ETX2514 was bactericidal against a majority of the 10 clinical isolates examined, each with a significant reduction in bacterial cell counts within 24 hours at 8X MIC.
PC2514-2017-0010	Complete	Post antibiotic effect of sulbactam-ETX2514	non-GLP	Sulbactam-ETX2514 demonstrated little to no post-antibiotic effect when tested against 5 isolates of *A. baumannii*.
Not assigned	In progress	Activity of ETX2514SUL and comparators versus globally diverse isolates of *A. baumannii-calcoaceticus* complex (2016-2018)	non-GLP	NA
Not assigned	In progress	Activity of ETX2514SUL and comparators versus defined panels of *A. baumannii* from regions with high rates of multidrug resistance	non-GLP	NA

CLSI = Clinical and Laboratory Standards Institute; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IgaA= intracellular growth attenuator protein; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; NA = not applicable; NDM-1 = New Delhi metallo-β-lactamase 1; PBP = penicillin-binding protein; QC = quality control; Rcs = regulator of capsule synthesis.

Table 4–1. Summary of ETX2514 nonclinical pharmacology studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
Not assigned	In progress	Activity of sulbactam-ETX2514 in *Acinetobacter*-infected macrophages	non-GLP	NA
Not assigned	In progress	Potential for induction of AmpC by sulbactam-ETX2514 in *Acinetobacter* spp.	non-GLP	NA

CLSI = Clinical and Laboratory Standards Institute; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IgaA= intracellular growth attenuator protein; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; NA = not applicable; NDM-1 = New Delhi metallo-β-lactamase 1; PBP = penicillin-binding protein; QC = quality control; Rcs = regulator of capsule synthesis.

4.1.2 Primary pharmacology

4.1.2.1 *In vitro* studies

4.1.2.1.1 Mechanism of action

4.1.2.1.1.1 Sulbactam

Although sulbactam is classified as a BLI of several Class A enzymes, it also has intrinsic antibacterial activity against *A. baumannii*. This intrinsic activity has been shown by target-binding studies, cytological profiling, and resistance mapping to be primarily due to its inhibition of PBP3, a transpeptidase that is essential for the final step of bacterial peptidoglycan synthesis (Penwell 2015).

4.1.2.1.1.2 ETX2514

ETX2514 is a BLI. The β-lactamase inhibitory activity of ETX2514 was measured by determining IC_{50} values and inhibition kinetics against a panel of purified β-lactamases representative of all 4 Ambler molecular classes (A through D) (Study Report PC2514-2016-0010; Durand-Réville 2017). ETX2514 was more potent than comparator compounds (avibactam, clavulanic acid, and tazobactam) against all Class A, C, and D enzymes tested (Table 4–2). ETX2514 did not inhibit the MBL NDM-1. The acylation efficiency of ETX2514 was highest against Class A enzymes, followed by Class C, then Class D. The potency of ETX2514 (as measured by IC_{50} values) exceeded that of avibactam, a similar compound in clinical use as a BLI, by as much as 100-fold for some enzymes. This differential potency in favor of ETX2514 was even greater, in most cases, when compared with 2 other clinically used BLIs, tazobactam and clavulanic acid. The potency of inhibition of a broad range of Class D β-lactamases by ETX2514 is especially noteworthy because no other BLI in clinical use has a meaningful level of potency against these enzymes, with the exception of inhibition of OXA-48 (a Class D β-lactamase) by avibactam.

Like avibactam (Ehmann 2013), ETX2514 displayed time-dependent inactivation and reversible inhibition of β-lactamases (Study Report PC2514-2016-0010). Acylation efficiency was observed to be highest against Class A enzymes, followed by Class C, then Class D. Half-times for deacylation of ETX2514 from β-lactamases were in the range of 1 minute to 1 hour with Classes A and C enzymes, and many hours with Class D enzymes.

X-ray crystallographic structures of ETX2514 in complex with the Class C AmpC β-lactamase from *Pseudomonas aeruginosa* or the Class D OXA-24 β-lactamase from

A. baumannii were solved at high resolutions (1.3 and 1.9 Å, respectively). These studies revealed ETX2514 covalently binds to the catalytic serine residues of these enzymes, namely S90 of AmpC and S91 of OXA-24 (Study Report PC2514-2016-0011).

ETX2514 was also tested for its ability to acylate PBPs, the enzymes responsible for the final step of peptidoglycan biosynthesis in bacteria. The ability of ETX2514 and comparator compounds to target *Escherichia coli*, *P. aeruginosa*, and *A. baumannii* PBP1a, PBP2, and PBP3 proteins was evaluated in BOCILLIN FL competition assays with both fluorescence anisotropy and gel-based assays (Study Report PC2514-2016-0002). ETX2514 was found to acylate purified *E. coli* and *A. baumannii* PBP2 and to a lesser degree PBP1a. ETX2514 acylation of the *P. aeruginosa* PBPs tested was comparatively weaker. Similar results were observed in gel-based assays using membrane extracts from *E. coli* and *A. baumannii* (Study Report PC2514-2016-0002).

ETX2514 was co-crystallized with PBP3 of *P. aeruginosa* at a resolution of 2.4 Å. As was seen for the β-lactamases, ETX2514 was found to form a covalent bond with the active site serine, S294, of PBP3 (Study Report PC2514-2016-0011).

Table 4–2. IC_{50} values (µM) for ETX2514 inhibition of clinically important β-lactamases

	Ambler Class									
	A			C		D				B
Compound	**TEM-1**	**CTX-M15**	**KPC-2**	**P99**	**AmpC**	**OXA-10**	**OXA-23**	**OXA-24/40**	**OXA-48**	**NDM-1**
ETX2514	0.0012	0.00083	0.0043	0.0013	0.014	0.23	0.18	0.19	0.0063	>200
Avibactam	0.010	0.0045	0.18	0.18	0.54	23	9.2	18	0.70	ND
Clavulanic acid	0.13	0.034	>200	>200	>200	0.59	17	37	15	ND
Tazobactam	0.041	0.0051	39	6.1	3.4	4.6	3.2	77	3.6	ND

All results are averages of n ≥2 measurements except for ETX2514 versus OXA-23 and NDM-1, where n = 1.
IC_{50} = concentration showing 50% inhibitory effect; ND = not determined; NDM-1 = New Delhi metallo-β-lactamase 1.

4.1.2.1.2 *In vitro* antibacterial activity

The ability of ETX2514 to restore β-lactam activity in bacterial cells was evaluated by characterization in an isogenic panel of 6 β-lactamases expressed in the β-lactam susceptible laboratory strain ATCC 17978. This panel was used to test the activity of ETX2514 in combination with piperacillin, ceftazidime, or meropenem (Study Report PC2514-2016-0035). As shown in Table 4–3, β-lactamase overexpression in *A. baumannii* correlated to a reduction of β-lactam susceptibility, which was restored by avibactam against Classes A and C enzymes but not Class D enzymes. In contrast, ETX2514 fully restored β-lactam activity against Classes A, C, and D expressing strains. In addition, ETX2514 was also several fold-more potent against Classes A and C expressing strains than avibactam, similar to what was observed in *P. aeruginosa* (Study Report PC2514-2016-0035).

Table 4–3. MIC values (mg/L) of β-lactams alone or in the presence of β-lactamase inhibitors ETX2514 or avibactam against an isogenic panel of β-lactamases expressed in *A. baumannii*

Strain ID	Overexpressed β-lactamase	Piperacillin			Ceftazidime			Meropenem			Sulbactam		
		Alone	ETX2514	AVI	Alone	ETX2514	AVI	Alone	ETX2514	AVI	Alone	ETX2514	AVI
ARC6542	none	8	4	8	4	2	4	0.25	0.125	0.25	1	0.5	2
ARC6544	TEM-1	>64	2	8	4	1	4	0.25	0.125	0.125	16	0.5	2
ARC6545	KPC-2	>64	2	8	32	2	4	32	0.125	0.5	32	0.5	2
ARC6543	ADC-30	32	2	8	16	2	8	0.25	0.125	0.25	1	0.5	1
ARC6546	OXA-23	>64	2	>64	4	2	4	16	0.25	16	4	0.5	2
ARC6547	OXA-24/40	>64	4	>64	4	2	4	64	0.5	64	4	0.5	4
ARC6548	OXA-66	32	4	32	2	2	2	2	0.125	2	1	0.5	2

ETX2514 and AVI were added at a fixed concentration of 4 mg/L. Values shown represent the average of N ≥ 2 replicates.
AVI = avibactam; ID = identification; MIC = minimum inhibitory concentration.

4.1.2.1.3 Development of testing paradigm for the combination of sulbactam and ETX2514

The *in vitro* antibacterial activity of the combination of sulbactam and varying concentrations of ETX2514 was evaluated by broth dilution against 91 strains of recent, distinct *A. baumannii* clinical isolates with defined β-lactamase content. The broth MICs were evaluated in weight to weight ratios of sulbactam/ETX2514 of 1:1, 2:1, 4:1, and 8:1. In addition, sulbactam MICs were evaluated in the presence of fixed concentrations of ETX2514 at 1, 2, 4, or 8 mg/L (Study Report PC2514-2017-0008). Determination of the most effective combinations and ratios was performed by consideration of individual β-lactamase content and PBP3 mutations, comparison of histograms and scattergram plots of the different testing conditions, and comparison of minimum concentration that inhibits 50% of isolates (MIC_{50}) and MIC_{90} values of all of the combinations. Determination of sulbactam MICs at a constant concentration of 4 or 8 mg/L of ETX2514 most accurately separated susceptible isolates from resistant isolates. Therefore, the optimal susceptibility testing paradigm is to hold the concentration of ETX2514 constant at 4 mg/L while varying the sulbactam concentration in 2-fold increments. In addition, a 10 µg sulbactam/10 µg ETX2514 disk mass was determined to have the best correlation with broth MICs for use in disk diffusion tests (Study Reports C2514-2017-0003 and PC2514-2017-0004).

4.1.2.1.4 General *in vitro* spectrum of activity

The broth dilution MIC values of sulbactam, ETX2514, and sulbactam plus ETX2514 at a fixed concentration of 4 mg/L were determined against a collection of well characterized Gram-positive and Gram-negative organisms using standard CLSI methods (CLSI 2015). For sulbactam alone, no activity was detected against *Candida albicans*, *Staphylococcus aureus*, or wild-type *P. aeruginosa* (Study Report PC2514-2016-0009) (Table 4–4). Weak antibacterial activity for sulbactam alone (MICs of 16-64 mg/L) was observed against 2 strains of *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and efflux-disabled *P. aeruginosa*. Sulbactam alone had an MIC of 16 mg/L against ARC3495, a strain of *A. baumannii* bearing genes encoding for Class D β-lactamases OXA-40 and OXA-119.

ETX2514 alone was found to have intrinsic antibacterial activity against *Enterobacteriaceae* with MICs ranging from 0.125 mg/L for *E. coli* to 4 mg/L for *E. cloacae.* ETX2514 demonstrated weak (32 mg/L) activity against wild-type *P. aeruginosa* and *A. baumannii* and no activity against *C. albicans* or *S. aureus* (Study Report PC2514-2016-0009) (Table 4–4). ETX2514 alone had an MIC against the efflux mutant of *P. aeruginosa* of 4 mg/L, suggesting that it is a substrate of the efflux pumps of this organism. Notably, ETX2514 alone was also

found to have intrinsic antibacterial activity against 10 colistin-resistant *mcr-1*$^{+}$ *E. coli* environmental isolates, with MICs ranging from 0.125 to 2 mg/L (Study Report PC2514-2016-0002), suggesting that the presence of *mcr-1* does not confer resistance to ETX2514.

When sulbactam was combined with 4 mg/L ETX2514, the MIC against *A. baumannii* ARC3495 dropped 32-fold from 16 to 0.5 mg/L (Study Report PC2514-2016-0009). For *C. albicans*, *S. aureus*, and wild-type *P. aeruginosa*, there was still no detectable activity with this combination of sulbactam/ETX2514; however, its MIC against the efflux mutant of *P. aeruginosa* dropped to ≤0.02 mg/L, suggesting that both components are substrates of the efflux pump in this organism. The MIC of sulbactam/ETX2514 ranged from ≤0.02-0.25 mg/L against *Enterobacteriaceae*, which was expected considering the intrinsic antibacterial activity of ETX2514 alone against these organisms.

Table 4–4. *In vitro* antibacterial activity of sulbactam, ETX2514, or a combination of both against Gram-negative and Gram-positive bacteria

Strain ID	Species	Description	MIC (mg/L)				
			ETX2514	SUL	ETX2514SUL	CAZ	LZD
ARC3495	*A. baumannii*	OXA-40; OXA-119	32	16	0.5	>64	64
ARC3528	*E. cloacae*	Chromosomal AmpC	4	64	0.25	>64	>64
ARC4	*E. coli*	ATCC25922	0.125	32	≤0.02	0.5	64
ARC4053	*E. coli*	ATCC25922 ΔtolC (efflux mutant)	0.125	32	≤0.02	0.25	2
ARC16	*E. coli*	ATCC35218 TEM-1	0.125	16	≤0.02	0.125	>64
ARC545	*P. aeruginosa*	PAO1 lab strain	32	>64	>16	2	>64
ARC546	*P. aeruginosa*	PAO1ΔmexABDXY (efflux mutant)	4	64	≤0.02	1	8
ARC1865	*K. pneumoniae*	Clinical isolate	0.25	32	≤0.02	0.5	>64
ARC561	*K. pneumoniae*	ATCC700603 SHV-18, OXA-2, OKP-6	1	64	≤0.02	64	>64
ARC3190	*S. aureus*	USA100 MRSA	>32	>64	>16	>64	1
ARC1192	*C. albicans*	ATCC90028	>32	>64	>16	>64	>64

CAZ = ceftazidime; ETX2514SUL = sulbactam/ETX2514; ID = identification; LZD = linezolid; MIC = minimum inhibitory concentration; SUL = sulbactam.

4.1.2.1.5 Spectrum of *in vitro* activity against *A. baumannii*

Table 4–5 shows the activity over time of sulbactam versus sulbactam/ETX2514 against more recent *A. baumannii* isolates (Study Report C2514-2017-0005). Two hundred isolates collected in 2013, 2014, and 2015 (608 total) from geographically diverse medical centers in the United States, Europe, Latin America, and the Asia-Pacific region were chosen for testing. Sulbactam/ETX2514 was found to be highly active against *A. baumannii* isolates with 98.4% of isolates inhibited at a sulbactam/ETX2514 concentration of 4 mg/L compared to 27.0% inhibited by 4 mg/L sulbactam alone (Table 4–5). The MIC_{90} of the combination against this collection of 608 strains was 2 mg/L.

The activity of sulbactam/ETX2514 in a triple combination with either imipenem or meropenem against recent *A. baumannii* isolates was also evaluated in this study (Study Report PC2514-2017-0005). The carbapenems were included because of their potential use as background therapy during clinical testing. The addition of imipenem or meropenem (at a fixed concentration of 2 mg/L) reduced the MIC_{50} from 1 mg/L to 0.12 and 0.25 mg/L, respectively, but did not lower the MIC_{90}. The potent activity of these combinations was consistent over time, across geographic locations and type of infection (Table 4–6).

Similar surveillance studies are ongoing to generate susceptibility data on global, contemporary collections of clinically relevant strains to allow an assessment of the potential clinical efficacy of sulbactam-ETX2514, as summarized in Table 4–7. Notably, the imipenem resistance rates observed in these studies ranged from 54% to 73%.

Table 4–5. Frequency of distribution of 608 recent *A. baumannii* isolates inhibited by various combinations of sulbactam, imipenem, or meropenem and ETX2514

Drug	Number (Cumulative %) of Isolates Inhibited at MIC (mg/L)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**>32**
Sulbactam	0	0	0	0	11	67	49	37	58	115	138	133
	0.0%	0.0%	0.0%	0.0%	1.8%	12.8%	20.9%	27.0%	36.5%	55.4%	78.1%	100.0% [2]
Sulbactam-ETX2514 [1]	0	1	4	55	178	200	129	31	4	4	1	1
	0.0%	0.2%	0.8%	9.9%	39.1%	72.0%	93.3% [2]	98.4%	99.0%	99.7%	99.8%	100.0%
Sulbactam-ETX2514-Imipenem [1]	291	10	19	50	91	85	49	6	4	2	0	1
	47.9%	49.5%	52.6%	60.9%	75.8%	89.8%	97.9% [2]	98.8%	99.5%	99.8%	99.8%	100.0%
Sulbactam-ETX2514-Meropenem [1]	237	14	20	60	79	120	55	14	6	2	0	1
	39.0%	41.3%	44.6%	54.4%	67.4%	87.2%	96.2% [2]	98.5%	99.5%	99.8%	99.8%	100.0%
Imipenem	0	0	13	126	6	19	12	8	11	9	44	360
	0.0%	0.0%	2.1%	22.9%	23.8%	27.0%	28.9%	30.3%	32.1%	33.6%	40.8%	100.0% [2]
Meropenem	0	0	4	62	53	23	21	13	3	18	47	364
	0.0%	0.0%	0.7%	10.9%	19.6%	23.4%	26.8%	28.9%	29.4%	32.4%	40.1%	100.0% [2]

1. Sulbactam-ETX2514 concentration fixed at 4 mg/L; meropenem and imipenem concentrations fixed at 4 mg/L.
2. MIC_{90}.

MIC = minimum inhibitory concentration; MIC_{90} = minimum concentration that inhibits 90% of isolates.

Table 4–6. Antibacterial activity of various combinations of sulbactam, ETX2514, imipenem, or meropenem as a function of geographic location or source of infection

	N	Sulbactam (mg/L)		Sulbactam-ETX2514 [1] (mg/L)		Sulbactam-ETX2514-IPM [1] (mg/L)		Sulbactam-ETX2514-MEM [1] (mg/L)	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Year									
2013	201	32	>32	1	4	0.25	2	0.25	2
2014	205	32	>32	1	2	0.25	2	0.25	2
2015	202	32	>32	1	2	<0.03	1	0.12	1
Region									
Asia-Pacific	107	32	>32	1	2	0.5	2	0.5	2
Europe	196	32	>32	1	2	0.25	2	0.25	2
North America	194	4	>32	1	2	<0.03	1	0.25	1
Latin America	111	16	>32	1	2	<0.03	1	0.5	1
Total	608	16	>32	1	2	0.12	2	0.25	2
Infection Source									
Blood	106	16	>32	1	2	0.25	2	0.25	2
Respiratory	301	16	>32	1	2	0.25	1	0.25	2
Skin/soft tissue	122	16	>32	1	2	<0.03	1	0.25	2
Urinary tract	33	8	>32	0.5	2	<0.03	1	<0.03	1
Other	46	16	>32	1	2	<0.03	2	0.06	2
Total	608	16	>32	1	2	0.12	2	0.25	2

1. Sulbactam-ETX2514 concentration fixed at 4 mg/L; MEM and IPM concentrations fixed at 2 mg/L.

IPM = imipenem; MEM = meropenem; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; N = number of isolates.

Table 4–7. Summary of surveillance studies of imipenem, sulbactam, or ETX2514SUL activity against contemporary clinical isolates of *Acinetobacter* spp. collected globally between 2011 and 2017

Vendor/Study Number	Year(s)	N	Imipenem (mg/L)			Sulbactam (mg/L)		ETX2514SUL (mg/L)	
			MIC_{50}	MIC_{90}	%R [1]	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
IHMA/2474	2011	199	32	>32	68%	16	64	1	2
IHMA/PC2514-2016-0008	2014	1131	>8	>8	65%	16	64	1	4
JMI/16-ETS-02	2013	201	>32	>32	73%	16	>32	1	4
JMI/16-ETS-02	2014	205	>32	>32	68%	16	>32	1	2
JMI/16-ETS-02	2015	202	>32	>32	63%	16	>32	1	2
IHMA/2896	2016	848	16	64	56%	8	32	1	2
IHMA/2896	2017	825	32	>64	54%	8	64	1	4
Cumulative to date	2011-2017	3611	>8	64	58%	16	64	1	2

MIC testing for ETX2514SUL was performed by serially diluting sulbactam in the presence of ETX2514 at a fixed concentration of 4 mg/L.

1. According to 2018 Clinical and Laboratory Standards Institute criteria.

ETX2514SUL = sulbactam/ETX2514; MIC = minimum inhibitory concentration; MIC_{50} = minimum concentration that inhibits 50% of isolates; MIC_{90} = minimum concentration that inhibits 90% of isolates; N = number of isolates; R = resistance rate.

4.1.2.1.6 *In vitro* selection of resistant mutants

4.1.2.1.6.1 Spontaneous resistance to sulbactam in *A. baumannii*

A recent study showed that the frequency of spontaneous resistance to sulbactam in 5 distinct, susceptible strains of *A. baumannii* was quite low. Of strains tested, the frequency of resistance was ~4 $\times 10^{-9}$ at 2X and 4X MIC for 2 of the strains (ARC2058 and ATCC 17978) and ~2 $\times$ 10^{-9} at 8X MIC for ARC2058 (Penwell 2015). The resistance frequency was below the limit of detection at higher concentrations of sulbactam for these 2 strains and at all concentrations tested for the remaining strains (ARC593, ARC5468, and ARC2461). In this study, sulbactam resistance at 4X MIC mapped to *pbp3* in both strain backgrounds, resulting in a serine-to-threonine substitution at position 390 in the resistant ATCC 17978 mutant and a serine-to-phenylalanine substitution at position 395 in one of the resistant ARC2058 mutants. Both of these mutations are at or near the reported sulbactam binding site of PBP3 (Papp-Wallace 2012). Both strains with PBP3 mutations showed high levels of resistance to sulbactam, with MIC increases of 32- to 64-fold. Neither of these sulbactam-resistant mutants was cross-resistant to other classes of drugs tested (i.e., aztreonam, ceftazidime, meropenem, ciprofloxacin, and colistin). These PBP3 mutations may confer a fitness penalty, as their proliferation rates in both rich media and murine blood were notably reduced as compared to their wild-type parent strains (Penwell 2015).

4.1.2.1.6.2 Spontaneous resistance to sulbactam/ETX2514 in *A. baumannii*

A similar study was conducted to determine the frequency of spontaneous resistance for the sulbactam/ETX2514 combination in 4 *A. baumannii* strains (Study Report C2514-2016-0003; McLeod 2018). The frequencies of spontaneous resistance were low at 2X to 4X MIC, ranging from 2.1 $\times$ 10^{-8} to 7.6 $\times$ 10^{-10}. Colonies could not be isolated at 8X MIC, corresponding to frequencies of spontaneous resistance ranging from <7.6 $\times$ 10^{-10} to <9.0 $\times$ 10^{-10} at that concentration.

The susceptibility to sulbactam/ETX2514 of the few mutants that could be isolated decreased 8- to 64-fold (Table 4–8). Mutations in those resistant isolates mapped to genes encoding for PBP3, AspS, and GltX. As described above, PBP3 has been implicated as the target for sulbactam in *A. baumannii* and the mutants raised in this study were cross-resistant to sulbactam but not to other control compounds tested. The tRNA synthases AspS and GltX are elements of the stringent response that, in other organisms, have been implicated in resistance against inhibitors of PBP2 (Vinella 1992). The sulbactam/ETX2514-resistant tRNA synthase

mutants were not cross-resistant to sulbactam but showed some decreased susceptibility to meropenem or ceftazidime (Table 4–8). These findings support previous data suggesting that, in addition to the activity of ETX2514 as a BLI, PBP2 inhibition by ETX2514 contributes to the antibacterial activity observed for the combination of sulbactam/ETX2514.

In a recent surveillance study, 6 out of 608 diverse *A. baumannii* isolates from 2013 to 2015 were found to have elevated (≥16 mg/L) MICs for sulbactam/ETX2514 (Study Report PC2514-2017-0005). Whole genome sequencing of these isolates revealed that all 6 strains had a variety of *bla*$_{ADC}$ genes (Class C) and 1 or more *bla*$_{OXA}$ Class D genes, which are unlikely to contribute to resistance. The most resistant isolate had the *bla*$_{NDM-1}$ gene, encoding the Class B MBL NDM-1, which is not inhibited by ETX2514. Four of the 6 resistant isolates had a T526S variant of PBP3 and 1 of these isolates also encoded an N256K mutation in PBP1A. One of the isolates had an A579T mutation in PBP3. Unlike the mutations found previously (Table 4–8), neither T526S nor A579T mutations map near the active site (Han 2011), although preliminary results from a separate study suggest the T526S variant may be associated with elevated sulbactam MICs (Study Report PC2514-2017-0008). Therefore, it is likely that the resistance in the non-MBL-containing strains may be associated with these target PBP mutations. It is also possible that 1 or more of the β-lactamases that are usually inhibited by ETX2514 is expressed to sufficiently high levels to render sulbactam/ETX2514 less active. Other possible mechanisms of resistance include efflux pump up-regulation, porin down-regulation, or other as-yet-undefined molecular drivers of resistance.

Table 4–8. MICs of sulbactam/ETX2514-resistant *A. baumannii* mutants

Strain ID	Gene Product	Amino Acid Change	MIC (mg/L)				
			Sulbactam/ ETX2514	SUL	MEM	CAZ	CIP
ARC2058 parent	NA	NA	1	4	0.5	4	0.5
Aba2058 2x-1	AspS (aspartyl-tRNA synthetase)	Q47P	16	4	16	16	1
Aba2058 2x-2	GltX (glutamyl-tRNA synthetase)	M240I	16	4	8	4	0.5
Aba2058 2x-3	GltX (glutamyl-tRNA synthetase)	R117S	64	4	32	8	0.5
Aba2058 2xL-1	FtsI (penicillin-binding protein 3)	V505L	16	16	0.25	4	0.25
ARC2681 parent	NA	NA	2	8	32	256	>64
Aba2681 4x-1	FtsI (penicillin-binding protein 3)	S390T	>64	>64	32	128	>64
ARC2782 parent	NA	NA	0.5	32	16	>512	8
Aba2782 2x-1	FtsI (penicillin-binding protein 3)	T511A	4	64	16	>512	16

CAZ = ceftazidime; CIP = ciprofloxacin; ID = identification; MEM = meropenem; MIC = minimum inhibitory concentration; NA = not applicable; SUL = sulbactam.

4.1.2.1.7 Spontaneous resistance to ETX2514 in *Enterobacteriaceae*

Because ETX2514 has intrinsic antibacterial activity in *Enterobacteriaceae*, the frequency of resistance to this compound in *E. coli* and *K. pneumoniae* was also assessed and found to be fairly high (Study Report PC2514-2016-0004). Resistance frequencies ranged from 1.3×10^{-6} to 9.8×10^{-6} at both 4X and 8X MIC for both species. The MIC values for ETX2514 of both the *E. coli* and *K. pneumoniae* resistant mutants increased by 32- to 64-fold compared to their isogenic parental strain (Table 4–9). For the *E. coli* variants, the MIC of mecillinam, a PBP2-specific inhibitor, increased by more than 16-fold, and a 4-fold increase in imipenem MIC was observed as well. For the *K. pneumoniae* variants, the MIC of ciprofloxacin increased by 4-fold and the imipenem MIC increased by 4- to 8-fold. One of the *K. pneumoniae* isolates also showed a 4-fold increase in aztreonam MIC. The MIC values for colistin and gentamicin were not affected.

Table 4–9. MICs of ETX2514-resistant *Enterobacteriaceae* isolates

Strain	Variant	MIC (mg/L)						
		ETX2514	MEC	ATM	IPM	COL	GEN	CIP
E. coli ARC16	Parent	0.25	4	0.125	0.25	0.5	2	0.015
	8x-1	16	>64	0.06	1	0.5	1	0.015
	8x-2	8	>64	0.06	1	0.25	1	0.015
K. pneumoniae ARC4427	Parent	0.25	>64	0.5	0.125	0.5	64	2
	8x-1	16	>64	2	0.5	0.5	64	8
	8x-2	16	>64	0.125	1	0.25	64	8

ATM = aztreonam; CIP = ciprofloxacin; COL = colistin; GEN = gentamicin; IPM = imipenem; MEC = mecillinam; MIC = minimum inhibitory concentration.

To assess the mechanism of resistance, ETX2514-resistant isolates in *Enterobacteriaceae* were subjected to whole genome sequencing (Study Report PC2514-2016-0004). Both *E. coli* resistant isolates carried mutations in intracellular growth attenuator protein, which negatively regulates the regulator of capsule synthesis (Rcs) phosphorelay system that is primarily known for its role in regulating the synthesis of the colonic acid capsular exopolysaccharide (Majdalani 2005). Activation of the Rcs phosphorelay is seen upon inhibition of PBPs and leads to increased survival in the presence of antibiotics that inhibit PBPs (Laubacher 2008).

In *K. pneumoniae*, ETX2514 resistance mapped to AspS. Mutations in tRNA synthetase genes have been previously documented to lead to resistance to inhibitors of PBP2 by induction of the stringent response (Vinella 1992).

4.1.2.1.7.1 ETX2514 β-lactamase inhibitory activity in ETX2514-resistant isolates

The ability of ETX2514 to act as a BLI in the ETX2514-resistant mutants of *E. coli* and *K. pneumoniae* was also evaluated (Study Report PC2514-2016-0004). The MICs of several different β-lactam antibiotics alone or in combination with ETX2514 at 4 mg/L were measured against 2 *E. coli*- and 2 *K. pneumoniae*-resistant isolates. This concentration of ETX2514 is 2- to 4-fold below its MIC in these resistant mutants, yet was found to restore activity of the β-lactam partners. This suggests that, even though these strains are not very susceptible to the antibacterial activity of ETX2514 alone, ETX2514 still acts as a BLI in these strains. This is particularly evident with piperacillin, where the MIC in combination with ETX2514 drops from 16- to >1066-fold compared to the MIC of piperacillin alone.

4.1.2.2 Miscellaneous microbiological properties

4.1.2.2.1 Static time kill studies

In order to determine the rate of bacterial cell death of *A. baumannii* in the presence of sulbactam/ETX2514, 10 clinical isolates were selected for static time kill testing based on their range of susceptibilities to sulbactam and their varied β-lactamase gene content, which had been previously determined by whole genome sequencing (Study Report PC2514-2017-0009). Sulbactam in combination with 4 mg/L ETX2514 was found to be bactericidal against a majority of these isolates, with each showing a significant reduction in bacterial cell counts within 24 hours at 8-fold the MIC. At this concentration, 6 of the isolates demonstrated a >3-$\log_{10}$ reduction in viable cells, and 3 additional isolates showed a 2.5- to 2.9-$\log_{10}$ reduction. One isolate showed only a 1.2-$\log_{10}$ reduction (ARC 3488 encoding ADC-76; OXA-235-like; OXA-68). After 24 hours in the presence of 4X MIC sulbactam plus 4 mg/L ETX2514, 4 isolates resulted in a >3-$\log_{10}$ reduction, and 3 additional isolates showed a 2.4- to 2.8-$\log_{10}$ reduction in viable cell counts. Three of the isolates exposed to 4X MIC sulbactam plus 4 mg/L ETX2514 showed some rebound of bacterial growth at the 24-hour time point. In total, these results showed that sulbactam/ETX2514 is bactericidal against most isolates of *A. baumannii*. Similar results were seen for sulbactam alone at 8X MIC.

4.1.2.2.2 Post-antibiotic effect

Post-antibiotic effect (PAE) is the suppression of bacterial growth after short exposure to antibiotic compounds. The PAE of sulbactam or sulbactam/ETX2514 was measured in 5 different clinical isolates of *A. baumannii*, which were selected based on their range of susceptibilities to sulbactam and the β-lactamase gene content (Study Report PC2514-2017-0010). Neither sulbactam nor sulbactam/ETX2514 demonstrated any PAE, consistent with what has been seen for other related BL/BLI combinations such as ceftazidime, ceftaroline, or aztreonam combined with avibactam against Gram-negative organisms (Pillar 2016).

4.1.2.2.3 Activity of sulbactam/ETX2514 in combination with other antibiotics

The antibacterial activity of sulbactam/ETX2514 in combination with other antimicrobial agents was measured to determine if there was antagonism, synergy, or indifference between them (Study Report PC2514-2017-0007). Using the checkerboard assay technique, the *in vitro* antimicrobial activity of sulbactam/ETX2514 was tested in combination with 12 other

antimicrobial agents typically used to treat infections caused by Gram-negative and Gram-positive bacteria or fungi, including amikacin, colistin, meropenem, imipenem, ciprofloxacin, cefepime, and ceftazidime, in the presence of a fixed concentration (4 mg/L) of avibactam (ceftazidime-avibactam), rifampicin, linezolid, vancomycin, or fluconazole. Ten *A. baumannii* clinical isolates were tested against all 12 antimicrobial agents in combination with sulbactam plus 4 mg/L ETX2514. Reference strains *P. aeruginosa* ATCC 27853, *S. aureus* USA100, and *C. albicans* ATCC 90028 were used as controls for Gram-negative, Gram-positive, or antifungal agents, respectively. Interactions between sulbactam/ETX2514 and imipenem against 12 *Enterobacteriaceae* isolates were also examined.

Few instances of synergy and no antagonism was observed between sulbactam/ETX2514 and the other antimicrobial agents tested. The vast majority of these combinations demonstrated indifferent interactions. These results demonstrated that sulbactam/ETX2514 could be co-dosed with other antimicrobial agents with no expected interactions.

4.1.2.2.4 Evaluation of experimental conditions that may influence sulbactam/ETX2514 susceptibility test results

Variation in the experimental conditions has been shown to affect the *in vitro* susceptibility test results for some antibiotics. To evaluate the effects of experimental conditions on the sulbactam/ETX2514 *in vitro* susceptibility test, MICs were determined as a function of the starting inoculum concentration, incubation temperature and atmosphere, pH of the growth medium, and concentration of divalent cations in the growth medium (Study Report PC2514-2017-0006). The minimal bactericidal concentration was also determined using both the standard and higher inocula. The effect of human urine over a range of pH values on bacterial susceptibility to sulbactam/ETX2514 was also tested. In addition, the potential of sulbactam/ETX2514 protein binding to alter antibacterial activity was measured by performing *in vitro* susceptibility testing in the presence of surfactant, human albumin, and human serum. Testing was performed against 10 *A. baumannii* clinical isolates, which were selected based on their range of susceptibilities to sulbactam and their β-lactamase gene content.

Altering the size of the starting inoculum; temperature; atmospheric conditions; divalent cation concentration; or the presence of urine, surfactant, or human serum did not affect the activity of sulbactam/ETX2514 against a majority of the isolates tested (Study Report PC2514-2017-0006). A 4-fold decrease in susceptibility to sulbactam/ETX2514 for 5 of the 10 isolates at pH 5.0 and 4 of the isolates at pH 6.0 was

observed (Table 4–10). This phenotype at pH 5.0 was exacerbated in the presence of urine, with 8 of 10 isolates showing a 4X to 8X shift with urine present. However, a decrease in susceptibility to sulbactam/ETX2514 at pH 6.0 was only seen for 1 strain in the presence of urine (Table 4–10). This pH effect is similar to what was reported for ceftazidime-avibactam (Keepers 2015). The MIC values under all of the other conditions tested were within 1 to 2 dilutions of the values seen in the presence of the CLSI standard testing conditions. Minimum bacterial concentration values at the standard inoculum indicated that sulbactam/ETX2514 was bactericidal against 9 out of 10 of the *A. baumannii* isolates examined; while at higher inocula, the combination was bactericidal against only 4 of these isolates (Study Report PC2514-2017-0006).

Table 4–10. MIC (mg/L) values for sulbactam plus 4 mg/L ETX2514 in the presence of urine and varying pH for 10 clinical isolates of *A. baumannii*

Media Condition	ATCC 19606 (ARC593)	ARC2058	ARC3486	ARC3488	ARC3492	ARC3495	ARC5079	ARC5081	ARC5092	NCTC 13304 (ARC6476)
MHBII	0.5	1	0.5	0.5	0.5	0.5	1	4	0.5	1
MHBII pH 5.0	2	2	2	2	1	2	4	4	2	2
MHBII pH 6.0	2	2	2	1	2	1	4	8	1	2
MHBII pH 7.0	2	1	1	1	0.5	1	1	4	1	1
MHBII pH 8.0	0.5	1	0.5	1	0.25	0.5	1	2	0.5	1
MHBII + 50% urine pH 5.0	4	4	2	2	2	2	4	4	2	2
MHBII + 50% urine pH 6.0	1	2	1	1	1	1	8	4	1	2
MHBII + 50% urine pH 7.0	0.5	1	0.5	0.5	0.5	0.5	1	4	1	1
MHBII + 50% urine pH 8.0	0.25	0.5	0.5	0.25	0.25	0.5	0.5	2	0.5	0.5

MIC = minimum inhibitory concentration.

4.2 Secondary pharmacology

4.2.1 Brief summary

- ETX2514 has minimal binding affinity for the vast majority of cellular receptors at projected efficacious concentrations.
- No major interactions were identified against selected human serine proteases.

In vitro studies to assess effects on cardiac ion channels, binding to enzymes, and effect on human serine proteases are described below. Pivotal cardiovascular, pulmonary, and neurologic safety pharmacology studies are detailed in the toxicology section (Section 4.4.4).

4.2.2 General *in vitro* binding profile

ETX2514 was evaluated *in vitro* at 100 µM (approximately 28 µg/mL ETX2514) to assess its affinity for various enzymes and receptors in a series of radioligand binding assays. Overall, ETX2514 showed no affinity for the majority of enzymes and receptors. At higher concentrations of 300 µM, ETX2514 mediated inhibition of 42%, 31%, and 40% was determined for ERb, GAL1, and 15-LO, respectively. At 1 mM, ETX2514 demonstrated 30% inhibition of cathepsin S, 37% inhibition of COX-2, and 35% inhibition of TxA2 synthetase (Study Report PC2514-2017-14).

4.2.3 Activity on human serine proteases

The specificity of ETX2514 to β-lactamases has been assessed by testing for activity against a panel of 12 human serine proteases (chymotrypsin, trypsin, cathepsin G, cathepsin B, neutrophil elastase 2, factor VIIa, factor Xa, plasma kallikrein, plasmin, urokinase, tissue plasminogen activator, and tryptase). Significant inhibition/stimulation (>50%) was not observed at 1 mM (276 µg/mL) for any of the proteases with the exception of chymotrypsin. Follow-up IC_{50} studies against chymotrypsin suggested minimal inhibitory potency of ETX2514 with an IC_{50} >200 µM (Study Report PC2514-2017-14).

4.3 PK and product metabolism in animals

4.3.1 Brief summary

- IV PK of ETX2514 in rats were generally dose proportional with increasing dose. Exposure in males and females was comparable. Short half-lives of 0.23 and 0.80 hours in rats and dogs, respectively, resulted in no accumulation. V_{dss} ranged from 0.44 to 0.59 L/kg in rats and from 0.26 to 0.32 L/kg in individual dogs.

- Protein binding of ETX2514 was very low with the percentage of unbound ETX2514 ranging from 78.3% to 100% across human, mouse, dog, rat, and guinea pig, with no evidence of saturation or concentration dependence.

- *In vitro*, ETX2514 was metabolically stable in rat, dog, and human hepatocytes as well as rat and dog microsomes.

- ETX2514 showed no significant inhibition of CYP enzymes, no *in vitro* evidence for induction potential, and no inhibitory potency against major transporters within a clinically relevant exposure range.

- Clearance of ETX2514 in rats and dogs was predominantly via urinary excretion (59% and 71%, respectively). *In vitro* studies confirmed ETX2514 is a substrate for the kidney transporter OAT1 at concentrations in excess of 500 µM. This transport was inhibited competitively with the addition of probenecid.

- An overview of nonclinical PK and PD studies conducted to date is presented in Table 4–11 and Table 4–12, respectively.

Table 4–11. Summary of ETX2514 nonclinical PK studies

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2015-09	Complete	Rat plasma LC/MS/MS assay validation of ETX2514	GLP	A LC/MS/MS assay was successfully validated in rat plasma. The assay has a dynamic range of 5-5000 µM and utilizes a 1:1 dilution of K_2EDTA plasma with a protease cocktail (SIGMAFAST™) for long-term storage stability.
PC2514-2015-10	Complete	Dog plasma LC/MS/MS assay validation of ETX2514	GLP	A LC/MS/MS assay was successfully validated in dog plasma. The assay has a dynamic range of 5-5000 µM and utilizes a 1:1 dilution of K_2EDTA plasma with a protease cocktail (SIGMAFAST) for long-term storage stability.
PC2514-2016-0030	Complete	Rat plasma LC/MS/MS non-GLP assay of ETX2514	non-GLP	A non-GLP LC/MS/MS assay was qualified in rat plasma using K_2EDTA-treated samples. The assay has a dynamic range of 5-10000 µM and uses 2 µL of sample.
PC2514-2016-0031	Complete	Dog plasma LC/MS/MS non-GLP assay of ETX2514	non-GLP	A non-GLP LC/MS/MS assay was qualified in dog plasma using K_2EDTA-treated samples. The assay has a dynamic range of 10-5000 µM and uses 10 µL of sample.
PC2514-2016-0026	Complete	IV PK of ETX2514 in beagle dogs	non-GLP	ETX2514 PK was characterized in beagle dogs following IV administration of 0.4 mg/kg. V_{dss} = 0.28 ± 0.03 L/kg; CL = 5.06 ± 1.52 mL/min/kg; $t_{½}$ = 0.80 ± 0.13 hours; CL_r = 3.58 mL/min/kg.
PC2514-2016-0027	Complete	IV PK of ETX2514 in Han Wistar rats	non-GLP	ETX2514 PK was characterized in Han Wistar rats following IV administration of 3 mg/kg. V_{dss} = 0.51 L/kg; CL = 44.6 mL/min/kg; $t_{½}$ = 0.23 hours; CL_r = 23.3 mL/min/kg.
PC2514-2016-0032	Complete	*In vivo* ADME of ETX2514 in bile-duct cannulated rats	non-GLP	ETX2514 PK/excretion was characterized in Han Wistar rats following IV administration of 25 mg/kg. V_{dss} = 0.40 ± 0.10 L/kg; CL = 21.6 ± 0.6 mL/min/kg; $t_{½}$ = 0.6 ± 0.0 hours; CL_r = 13.9 ± 6.2 mL/min/kg; Biliary CL = 0.1 mL/min/kg.
PC2514-2016-0024	Complete	*In vitro* protein binding of ETX2514	non-GLP	Protein binding was determined in human, rat, mouse, dog, and guinea pig plasma. Percent unbound ranged from 78%-100% across a concentration range of 1-100 µM with no evidence of saturation.

ADME = absorption, distribution, metabolism, and excretion; BCRP = breast cancer resistance protein; CL = clearance; CL_{int} = intrinsic clearance; CL_r = renal clearance; CYP = cytochrome P450; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IV = intravenous; K_2EDTA = potassium ethylenediaminetetraacetic acid; K b/p = blood to plasma ratio; LC/MS/MS = liquid chromatography-tandem mass spectrometry; MIC = minimum inhibitory concentration; PD = pharmacodynamic; PK = pharmacokinetic; q6h = every 6 hours; QWBA = quantitative whole body-autoradiography; SLC = solute carrier; $t_{½}$ = terminal half-life; V_{dss} = steady state volume of distribution.

Table 4–11. Summary of ETX2514 nonclinical PK studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2016-0025	Complete	Induction properties of ETX2514 against CYP	non-GLP	ETX2514 demonstrated no induction potential of CYP 1A2, 2B6, or 3A4 at concentrations up to and including 300 µM.
PC2514-2016-0014	Complete	Inhibition potential of ETX2514 versus CYP 2A6, 2B6, 2C8, 2E1, and 3A4/5	non-GLP	Minor inhibition of CYP 2A6 and 3A4/5 (2.99% and 7.22%, respectively) was observed at 500 µM of ETX2514. No other interactions were observed at 500 µM for the other isoforms.
PC2514-2016-0015	Complete	Inhibition potential of ETX2514 versus CYP 1A2, 2C9, 2C19, 2D6, and 3A4/5	non-GLP	No inhibition of CYP 1A2, 2C9, 2C19, 2D6, or 3A4/5 was determined for ETX2514 at concentrations up to and including 300 µM.
PC2514-2016-0016	Complete	Inhibition potential of ETX2514 versus human P-glycoprotein in MDR1-MDCK cells	non-GLP	No inhibition of P-glycoprotein mediated transport was determined for ETX2514 at concentrations up to and including 300 µM in MDR1-MDCK cells.
PC2514-2016-0017	Complete	Inhibition potential of ETX2514 versus human BCRP in Caco-2 cells	non-GLP	ETX2514 demonstrated a concentration dependent inhibition of BCRP-mediated transport of rosuvastatin with an IC_{50} >300 µM (34% inhibition observed at 300 µM).
PC2514-2016-0018	Complete	*In vitro* and *in vivo* metabolism of ETX2514	non-GLP	Incubations of ETX2514 at 1 µM resulted in minimal turnover (CL_{int}<1 µL/min/10^6cells). Metabolism of ETX2514 was observed in rat and human cytosol fortified microsomes with ring opening of the diazabicyclooctenone and the formation of several bio-conjugated metabolites.
PC2514-2016-0019	Complete	Human dose prediction of ETX2514	non-GLP	Initial population PK and Monte Carlo simulation used in conjunction with recent surveillance (MIC) data suggests a dose of 1 g ETX2514 + 1 g sulbactam q6h (3-hour infusion) would meet PK/PD requirements for both compounds in a projected patient population.
PC2514-2016-0033	Complete	Inhibition potential of ETX2514 versus OATP1B1 in HEK293 cells	non-GLP	ETX2514 demonstrated minimal inhibition of OATP1B1-mediated transport of estradiol 17β-glucuronide (23% at 300 µM).

ADME = absorption, distribution, metabolism, and excretion; BCRP = breast cancer resistance protein; CL = clearance; CL_{int} = intrinsic clearance; CL_r = renal clearance; CYP = cytochrome P450; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IV = intravenous; K_2EDTA = potassium ethylenediaminetetraacetic acid; K b/p = blood to plasma ratio; LC/MS/MS = liquid chromatography-tandem mass spectrometry; MIC = minimum inhibitory concentration; PD = pharmacodynamic; PK = pharmacokinetic; q6h = every 6 hours; QWBA = quantitative whole body-autoradiography; SLC = solute carrier; $t_{½}$ = terminal half-life; V_{dss} = steady state volume of distribution.

Table 4–11. Summary of ETX2514 nonclinical PK studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2016-0034	Complete	Inhibition potential of ETX2514 versus OAT1 and OAT3 in HEK293 cells	non-GLP	The uptake ratio relative to control of ETX2514 was >2 at the top 2 concentrations of 500 and 900 µM. In the presence of the known inhibitor probenecid, the uptake ratio was reduced by 67%. These results suggest that ETX2514 is a substrate of OAT1 at the higher concentrations.
PC2514-2016-0036	Complete	Time dependent inhibition potential of ETX2514 versus CYPs in human liver microsomes	non-GLP	There was no time-dependent inhibition observed for ETX2514 at 10 µM and 50 µM for CYP 1A2, 2C9, 2C19, 2D6, or 3A4/5.
PC2514-2016-0037	Complete	*In vitro* partitioning of ETX2514 to human red blood cells	non-GLP	Following a 30-minute incubation of ETX2514 at a concentration of 10 µM, the red blood cell partitioning (K b/p) was determined to be 0.58 suggesting ETX2514 does not bind extensively to red blood cells.
PC2514-2018-0001	Ongoing	[^{14}C]-ETX2514, Mass balance, QWBA, and metabolite identification in rats	non-GLP	Rat [^{14}C]-ETX2514 QWBA, mass balance, and PK following IV administration of 250 mg/kg has been completed and metabolite profiling and characterization has been initiated. Urinary excretion of total radioactivity (87.3%) predominated as the major form of elimination.
PC2514-2018-0004	Complete	*In vitro* assessment of ETX2514 SLC drug interactions	non-GLP	ETX2514 was not an inhibitor of OAT1, OAT3, and OCT2 in TransportoCells™, with an IC_{50}>900 µM. ETX2514 was a substrate of OAT1 in TransportoCells at 900 µM and likely at 500 µM. ETX2514 was not a substrate of OAT3 and OCT2 in TransportoCells.

ADME = absorption, distribution, metabolism, and excretion; BCRP = breast cancer resistance protein; CL = clearance; CL_{int} = intrinsic clearance; CL_r = renal clearance; CYP = cytochrome P450; GLP = Good Laboratory Practice; IC_{50} = concentration showing 50% inhibitory effect; IV = intravenous; K_2EDTA = potassium ethylenediaminetetraacetic acid; K b/p = blood to plasma ratio; LC/MS/MS = liquid chromatography-tandem mass spectrometry; MIC = minimum inhibitory concentration; PD = pharmacodynamic; PK = pharmacokinetic; q6h = every 6 hours; QWBA = quantitative whole body-autoradiography; SLC = solute carrier; $t_{½}$ = terminal half-life; V_{dss} = steady state volume of distribution.

Table 4–12. Summary of ETX2514 nonclinical PD studies

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2016-0020	Complete	*In vivo* efficacy of ETX2514SUL in a murine neutropenic thigh model (4:1 ratio)	non-GLP	Five MDR *A. baumannii* isolates were tested in the model to assess the % T>f_{MIC} of sulbactam required for efficacy where sulbactam was administered in the presence of ETX2514 at a constant ratio of 4:1. The mean free % T>MIC magnitudes required for a 1-$\log_{10}$ and 2-$\log_{10}$ reduction, and the EC_{80} were 34% ± 4%, 39% ± 4%, and 43% ± 5%, respectively. These magnitudes are consistent with clinical exposures of sulbactam (30%-50% T>MIC) used to successfully treat *A. baumannii* infections.
PC2514-2016-0021	Complete	*In vivo* efficacy of ETX2514SUL in a murine neutropenic lung model (4:1 ratio)	non-GLP	Four recent MDR *A. baumannii* clinical isolates whose β-lactamase genotypes had been determined (ARC3484, ARC3486, ARC5079, and ARC5081) were run in the neutropenic lung model as dose response studies to determine the % T>f_{MIC} required by sulbactam when administered in the presence of ETX2514 at a constant 4:1 ratio. The mean free % T>MIC magnitudes required for a 1-$\log_{10}$ and 2-$\log_{10}$ reduction, and the EC_{80} in the neutropenic lung were 46% ± 5%, 56% ± 6%, and 85% ± 6%, respectively.
PC2514-2016-0022	Complete	*In vivo* efficacy of ETX2514SUL in a murine neutropenic thigh model (constant sulbactam)	non-GLP	With sulbactam held at constant dose of 15 mg/kg q3h the mean ETX2514 % T>C_T of 2.5 µg/mL magnitudes required for a 1-$\log_{10}$ reduction, 2-$\log_{10}$ reduction, and EC_{50} were 23% ± 14%, 40% ± 14%, and 28% ± 8%, respectively. While the intent of the present study was to determine the magnitude of % T>C_T of 2.5 µg/mL for ETX2514, daily AUCs associated with greater than 2-$\log_{10}$ kill (nearly maximal activity) were ~130 µg·h/mL achieved with a 50 mg/kg q3h dose of ETX2514.
PC2514-2016-0023	Complete	PK/PD driver assessment of ETX2514SUL in an *in vitro* hollow-fiber model system	non-GLP	A PK/PD driver of % T>MIC was correlated to efficacy for sulbactam versus a susceptible *A. baumannii* strain confirming recently published data. AUC and % T>C_T correlated to the restoration of sulbactam efficacy for ETX2514 versus MDR *A. baumannii* strains ARC5081 and ARC5079 with the strongest correlation with C_T of 2-2.5 µg/mL. Based on these data AUC and C_T value of 2.5 µg/mL will be utilized initially to predict PK/PD magnitudes required by ETX2514 to restore sulbactam activity against resistant *A. baumannii* strains.

% T>C_T = percentage of time in a dosing interval that the drug concentration exceeds the critical threshold; % T>f_{MIC} = percentage of time in a dosing interval that the unbound (free) drug concentration exceeds the MIC of the organism; % T>MIC = percentage of time in a dosing interval that the drug concentration exceeds the MIC of the organism; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}Tau}$ = area under the plasma concentration-time curve to the end of the dosing period; C_T = critical threshold; EC_{50} = concentration that causes 50% of the maximum effect; EC_{80} = concentration that causes 80% of the maximum effect; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; hERG = human Ether à-go-go-related gene; IC_{50} = concentration showing 50% inhibitory effect; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; PD = pharmacodynamic; PK = pharmacokinetic; q3h = every 3 hours; R^2 = correlation coefficient.

Table 4–12. Summary of ETX2514 nonclinical PD studies (continued)

Study Number	Status	Short Title	GLP Compliance	Main Findings
PC2514-2016-0011	Complete	PK/PD driver assessment of ETX2514SUL in a chemostat model system	non-GLP	Dose fractionation of ETX2514 was performed with an MDR *A. baumannii* strain using a 1-compartment *in vitro* infection model. The PK/PD index associated with efficacy of ETX2514 in combination with sulbactam against a single *A. baumannii* isolate was determined to be % $T>C_T$ than ETX2514 threshold of 0.75 mg/L (R^2 =0.827). The $AUC_{0\text{-}Tau}$:MIC ratio also described the data well, as observed by a high R^2 value of 0.703. Both AUC and % $T>C_T$ of 0.75 mg/L are potential PK/PD targets.
PC2514-2017-0013	Complete	*In vivo* efficacy of ETX2514SUL in a murine neutropenic thigh model against high MIC strains	non-GLP	Bactericidal activity was observed when concentrations of sulbactam met or approached exposures which exceeded the potentiated MIC for 50% of the dosing interval and AUC/MIC ratios of ETX2514 exceeded 8. These data suggest the combination of sulbactam and ETX2514 is highly effective against MDR strains of *A. baumannii*.
PC2514-2017-0012	Complete	ETX2514 in cardiac ion channel electrophysiological assays *in vitro*	non-GLP	The effects of ETX2514 on cardiac ion channels was evaluated using electrophysiologic measurement of ion flux through recombinant voltage-gated channels expressed in mammalian cells. The channels tested included: hCav1.2/β2/α2δ (ICaL), hCav3.2 (ICaT), hHCN4 (IF), hKv1.5 (IKUR), hKv11.1 (hERG; IKR), hKv4.3/hKChIP2.2 (ITO), hKv7.1/hKCNE1 (IKS), and hNav1.5 (INa). ETX2514 did not significantly inhibit ion channel activity for any of the channels evaluated up to the maximum concentrations tested, 100-1000 µM (IC_{50} >333 µM for all channels).

% $T>C_T$ = percentage of time in a dosing interval that the drug concentration exceeds the critical threshold; % $T>f_{MIC}$ = percentage of time in a dosing interval that the unbound (free) drug concentration exceeds the MIC of the organism; % T>MIC = percentage of time in a dosing interval that the drug concentration exceeds the MIC of the organism; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}Tau}$ = area under the plasma concentration-time curve to the end of the dosing period; C_T = critical threshold; EC_{50} = concentration that causes 50% of the maximum effect; EC_{80} = concentration that causes 80% of the maximum effect; ETX2514SUL = sulbactam/ETX2514; GLP = Good Laboratory Practice; hERG = human Ether à-go-go-related gene; IC_{50} = concentration showing 50% inhibitory effect; MDR = multi-drug resistant; MIC = minimum inhibitory concentration; PD = pharmacodynamic; PK = pharmacokinetic; q3h = every 3 hours; R^2 = correlation coefficient.

4.3.2 Analytical methods

4.3.2.1 Analysis of nonlabeled compound

High performance liquid chromatography-tandem mass spectrometry (LC/MS/MS) methods for the determination of ETX2514 have been developed and validated for use with plasma samples from rat, dog, and human studies. The method requires whole blood to be processed for plasma utilizing potassium ethylenediaminetetraacetic acid as anticoagulant, and the plasma then diluted 2-fold in SigmaFAST™ solution, a protease inhibitor used to stabilize the sample. A stable, labeled [$^{13}C_2$, $^{15}N_2$] ETX2514 was used as an internal standard. Additional methods were developed in human urine, bronchoalveolar lavage (BAL), and dialysate in support of Phase 1 studies evaluating ETX2514 and sulbactam excretion, tissue penetration, and partitioning into the dialysate. Supporting LC/MS/MS methods for urea in plasma and BAL to convert concentrations of ETX2514 and sulbactam in BAL to epithelial lining fluid (ELF) have also been utilized. Table 4–13 presents the dynamic range and long-term matrix storage stability data. Nonvalidated assays in plasma and urine utilizing protein precipitation/LC/MS/MS methodology were used in support of non-GLP investigations in rats and dogs (Study Report PC2514-2016-30 and Study Report PC2514-2016-31, respectively).

Table 4–13. Summary of bioanalytical assay characteristics

Species	Analyte	Extraction method	LLOQ-ULOQ	Validation (study)	Storage Stability
Rat plasma	ETX2514	Protein precipitation	5-5000 µM	PC2514-2016-0030	-70°C; 186 days
Dog plasma	ETX2514	Protein precipitation	5-5000 µM	PC2514-2016-0031	
Rat plasma GLP	ETX2514	Protein precipitation	5-5000 µM	SN-002514-2015-09	-70°C; 81 days
Dog plasma GLP	ETX2514	Protein precipitation	5-5000 µM	SN-002514-2015-10	-70°C; 81 days
Rat plasma	ETX2514/sulbactam	Protein precipitation	10-10000 ng/mL	Covance 8374339	
Human plasma	ETX2514	Protein precipitation	10-10000 ng/mL	Covance 8351072	-70°C; 148 days
Human plasma	ETX2514/sulbactam	Protein precipitation	10-10000 ng/mL	Covance 8351072B	-70°C; 468 days (ETX) 106 days (sul)
Human dialysate	ETX2514/sulbactam	Protein precipitation	10-10000 ng/mL	Covance 8372079	
Human urine	ETX2514	Dilution	50-50000 ng/mL	Covance 8351071	
Human urine	ETX2514/sulbactam	Dilution	50-50000 ng/mL	Covance 8351071C	-70°C; 279 days (ETX) 66 days (sul)
Human BAL	ETX2514/sulbactam	Dilution	2-1000 ng/mL	Keystone M170505	
Human BAL	Urea	Dilution	0.2-10 µg/mL	Keystone 110505	
Human plasma	Urea	Protein precipitation	100-3000 µg/mL	Keystone 110506	
Human plasma	Imipenem	Protein precipitation	10-10000 ng/mL	Covance 8356242	-70°C; 77 days
Human urine	Imipenem	Dilution	50-50000 ng/mL	Covance 8356247	-70°C; 122 days
Human plasma	Cilastatin	Protein precipitation	10-10000 ng/mL	Covance 8356245	-70°C; 16 days
Human urine	Cilastatin	Dilution	50-50000 ng/mL	Covance 8367882	-70°C; 11 days

BAL = bronchoalveolar lavage; ETX = ETX2514; GLP = Good Laboratory Practice; LLOQ = lower limit of quantification; sul = sulbactam; ULOQ = upper limit of quantification.

4.3.3 Absorption

4.3.3.1 ETX2514 single-dose PK in nonclinical species

Single-dose IV PK parameters of ETX2514 were determined in rats and dogs at doses of 3 and 0.45 mg/kg, respectively (Study Report PC2514-2016-0027 and Study Report PC2514-2016-0026). ETX2514 clearance was low to moderate, and V_{dss} was nearly equivalent to body water in both species. Elimination half-life was less than 1 hour in both rats and dogs. The data suggest that exposure generally increases dose proportionally, and the terminal half-life was consistent across all dose levels (Study Report PC2514-2016-0029 and Study Report PC2514-2016-0028).

Table 4–14 presents the mean single-dose PK parameters obtained from maximum tolerated dose and dose range-finding toxicology studies in rats and dogs.

Table 4–14. Mean PK parameters calculated during MTD studies (male only) after single IV dosing (1-hour infusion)

Study/Species	Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (µg·h/mL)	$t_{1/2}$ (h)
	250	262 ± 9	0.92	319 ± 44	0.59
	750	1248 ± 483	0.92	1342 ± 402	0.78
MTD, 7-day DRF/rat	1500	2618 ± 111	0.92	3161 ± 238	0.69
	300	643	0.92	1151	0.72
	1000	2135	0.92	4049	0.80
MTD, 7-day DRF/dog	2000	3993	0.92	7849	0.76

$AUC_{0-\infty}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; C_{max} = maximum plasma concentration; DRF = dose range finding; IV = intravenous; MTD = maximum tolerated dose; PK = pharmacokinetic; $t_{½}$ = terminal half-life; T_{max} = time to reach maximum plasma concentration.

4.3.3.2 ETX2514 multiple-dose PK

PK parameter estimates completed as part of the 14-day GLP toxicology studies in rats and dogs are summarized in Section 4.4. Exposure increased in a dose proportional manner, with little evidence of accumulation. Exposure was consistent between males and females (<2-fold difference) for both species.

4.3.3.3 Sulbactam and ETX2514 combination PK

The PK parameters of ETX2514 and sulbactam in combination have been determined in mice in support of *in vivo* efficacy studies. Table 4–15 presents population PK parameters for sulbactam and ETX2514. The toxicokinetic (TK) parameters of ETX2514 and sulbactam in combination dosing have also been established in rats in 7-day dose range-finding and 28-day toxicology studies (see Section 4.4). No significant differences were observed between the exposure of ETX2514 or sulbactam administered alone or in combination.

Table 4–15. Derived population PK parameters for sulbactam and ETX2514

Parameter [1]	Sulbactam alone	Sulbactam in the presence of ETX2514	ETX2514 in the presence of sulbactam
K_a (1/h)	6 [2]	12 [2]	13.5 (32.6)
V_1 (L/kg)	1.8847	1.0177 (7.0)	1.2508 (8.4)
V_2 (L/kg)	0.57845	0.1332 (12.4)	0.2282 (14.5)
Cl_1 (L/kg/h)	4.4514	2.5617 (5.2)	3.0747 (7.3)
Cl_2 (L/kg/h)	0.09736	0.0299 (11.0)	0.0641 (14.8)
SD [3]		0.639 (4.9)	0.554 (4.4)
Ω_{Cl}		0.019	0.014
Ω_{V2}		0.204	0.052

1. Predicted value (% coefficient of variance).
2. K_a was fixed, as the model was not able to accurately estimate it. Increasing K_a beyond the values used resulted in very little change in the fit quality.
3. SD refers to the SD of Epsilon.

PK = pharmacokinetic; SD = standard deviation.

4.3.4 Distribution

4.3.4.1 *In vitro* interspecies plasma protein binding

In vitro plasma protein binding was determined in rat, mouse, dog, guinea pig, and human using ultracentrifugation methods (Study Report PC2514-2016-0024) and was evaluated at 1, 10, and 100 µM. Table 4–16 presents ETX2514 plasma protein binding across species. Protein binding was minimal across all 5 species and generally unchanged across the 3 concentrations, with the fraction unbound ranging from 78.3% to 100% and no evidence of binding saturation.

Table 4–16. ETX2514 plasma protein binding across species

Species	Fraction Unbound (mean ± SD)		
	1 µM	10 µM	100 µM
Human	99.3 ± 22.3	115 ± 19.1	82.9 ± 8.7
Wistar Hanover rat	97.9 ± 17.2	88.1 ± 4.9	78.3 ± 15.8
CD-1 mouse	84.9 ± 9.6	98.9 ± 26.7	103.1 ± 12.9
Beagle dog	105.4 ± 14.7	96.3 ± 9.1	128.3 ± 12.5
Guinea pig	100.9 ± 18.1	97.3 ± 7.3	105.2 ± 10.9
SD = standard deviation.			

4.3.4.2 Blood/plasma partitioning

In vitro evaluation of blood cell partitioning of ETX2514 was investigated in fresh human whole blood at a concentration of 10 µM (Study Report PC2514-2016-0033). The results of this investigation showed a blood:plasma ratio of 0.58, suggesting ETX2514 does not accumulate into red blood cells.

4.3.4.3 Penetration into lung ELF

The penetration of ETX2514 and sulbactam was investigated in mouse lung in support of the lung efficacy studies. In infected animals, the lung penetration measurements (ELF:plasma total drug AUC ratios) were 0.63 and 0.32 for ETX2514 and sulbactam, respectively (Study Report PC2514-2016-0021). ELF penetration measurements in humans (ELF:plasma total drug AUC ratios) were 0.37 and 0.50 for ETX2514 and sulbactam, respectively.

4.3.5 Drug metabolism

4.3.5.1 *In vitro* metabolism studies

Intrinsic clearance of ETX2514 at a concentration of 1 µM was found to be <1 µL/min/10^6 cells in rat, dog, and human hepatocytes, suggesting minimal metabolism of the compound in the liver (Study Report PC2514-2016-0018). Incubation of ETX2514 microsomes in the presence of cytosol as well as hepatocytes using a substrate concentration of 100 µM showed the formation of ring opened bioconjugated adducts. Initial nucleophilic attack of the diazabicyclooctenone carbonyl with cysteine was common across all species evaluated, resulting in the formation of metabolite M1. Further conjugation with cysteine, glutathione, and glycine resulted in the formation of M2, M3, and M4 metabolites, respectively, in rat, dog, and human hepatocytes. The cysteine conjugate M1 was also

characterized in rat and human microsomes/cytosol, and its formation was independent of the presence of nicotinamide adenine dinucleotide phosphate cofactor suggesting oxidative metabolism was not a prerequisite for its formation. Qualitative profiling of all the metabolites suggested the metabolic disposition was consistent across all species including humans.

4.3.5.2 *In vivo* mass balance, excretion, tissue distribution, and metabolite characterization

Rat absorption, distribution, metabolism, and excretion studies have been completed using ^{14}C-ETX2514 at a dose of 250 mg/kg (ca. 100 µCi/kg) administered as a single 2-hour infusion to male Sprague-Dawley rats (aged 7 to 15 weeks). These studies consisted of: (1) PK of total radioactivity; (2) excretion and mass balance; (3) tissue distribution (quantitative whole body-autoradiography [QWBA]) in nonpigmented and pigmented animals; and (4) metabolite profiling in excreta and plasma.

Following a 2-hour infusion of a 250 mg/kg ^{14}C labeled dose of ETX2514, a C_{max} of 969 µg equiv/g and an AUC of 2155 µg equiv·h/g were achieved in Sprague-Dawley rats. Total radioactivity declined to 0.771 µg equiv by the final time point taken at 72 hours postdose (hpd). Mass balance studies demonstrated a mean total recovery of 96.5%, with 87.3% of total radioactivity excreted in the urine – consistent with previous studies that suggested minimal dose excreted into the bile following IV administration. Nearly 98% of the radioactivity in the urine was recovered in the first 24 hpd. Less than 0.5% of radioactivity remained in the carcass by 168 hpd. Consistent with the mass balance data, QWBA studies in nonpigmented animals demonstrated high concentrations of total radioactivity in the bladder and kidneys (>upper limit of quantification of 1448 µg equiv/g at 2 hours [end of infusion]). With the exception of liver, skin, and kidney (all ~2 µg equiv/g), all other tissue concentrations were not detected or were <lower limit of quantification of 1.05 µg equiv/g.

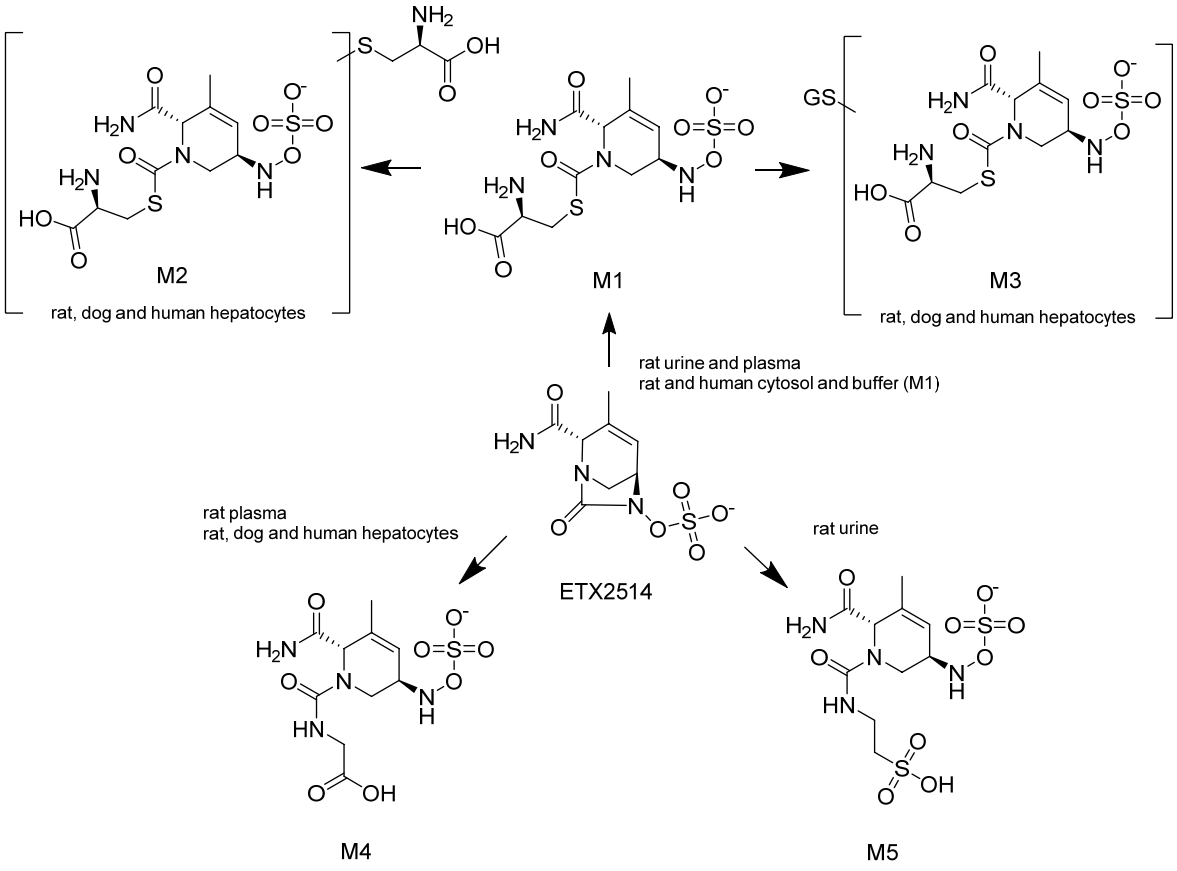
Metabolite profiling of rats receiving ^{14}C-ETX2514 is ongoing; however, nonradiolabeled studies completed to date suggested that in addition to unchanged ETX2514, the glycine adduct M4 was the major circulating metabolite in rat plasma and was also identified in rat urine. Unchanged ETX2514 and a ring opened taurine conjugate (M5) were the major constituents identified in the urine. Hydrolysis and decarboxylation yield the diamine (M6) metabolite. Table 4–17 presents the steady state exposure of ETX2514 and the diamine metabolite (M6) in rats and humans. M6 was found to be circulating at an exposure of ~12% of the ETX2514 AUC in human FIH plasma samples. Although M6 represented only 3% of

circulating dose in rat toxicology studies (2000 mg/kg), the absolute exposure exceeded that of humans, thus qualifying this metabolite in terms of safety in humans. The overall metabolite scheme of ETX2514 in rats, dogs, and humans is shown in Figure 4–1.

Table 4–17. Steady state exposure of ETX2514 and diamine metabolite (M6) in rats and humans

Parameter (Unit)	Rat Toxicology Study 2000 mg/kg		Human Efficacious Dose (1 g ETX2514)	
	ETX2514	M6	ETX2514	M6
AUC (µg·h/mL)	3120	105	423	50.8
C_{max} (µg/mL)	1340	23.6	28.0	4.88
AUC = area under the plasma concentration-time curve; C_{max} = maximum plasma concentration.				

Figure 4–1. Metabolic scheme of ETX2514 in rats, dogs, and humans



4.3.5.3 P450 inhibition

The *in vitro* CYP inhibitory properties of ETX2514 towards human CYP isoforms 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5 (Study Report PC2514-2016-0014 and Study Report PC2514-2016-0015) were investigated in human liver microsomes in the presence of CYP marker substrates. Minimal ETX2514 inhibition of 2.99% and 7.22% were observed versus CYP2A6 and CYP3A4/5 (testosterone substrate), respectively, at a concentration of 500 µM. No inhibition was characterized versus the other isoforms.

The ability of ETX2514 to act as a time-dependent inhibitor was also evaluated at 10 and 50 µM versus CYP isoforms 1A2, 2C9, 2C19, 2D6, and 3A4/5. No time-dependent inhibition was observed for ETX2514 at these concentrations.

4.3.5.4 Interactions with transporters

ETX2514 is not an inhibitor of the human efflux transporter P-glycoprotein (MDR1, ABCB1; Study Report PC2514-2016-0016), and demonstrated minimal inhibition (IC_{50} >300 µM) against the human breast cancer resistance protein (BCRP, ABCG2; Study Report PC2514-2016-0017) and the human hepatic transporter OAT1B1 (Study Report PC2514-2016-0033).

Against the renal transporters OAT1, OAT3, and OCT2, ETX2514 demonstrated no inhibition (IC_{50} >900 µM). ETX2514 was also not a substrate for OAT3 and OCT2, but was characterized as a substrate for OAT1 at 900 µM and likely at 500 µM (Study Report PC2514-2016-0034 and Study Report PC2514-2018-0004). Taken collectively, transporter inhibitory interactions of ETX2514 with gut, hepatic, and kidney transporters are projected to be low at clinically efficacious exposures of ETX2514. As a substrate, ETX2514 demonstrated an uptake ratio of greater than 2-fold (relative to controls) *in vitro* at concentrations greater than 500 µM versus the kidney transporter OAT1. This efflux was inhibited by 67% using the OAT1 inhibitor probenecid, suggesting ETX2514 is a substrate for OAT1 at high concentrations.

4.3.5.5 *In vitro* enzyme induction potential

The potential for ETX2514 to induce CYP isoenzymes 1A2, 2B6, and 3A4 was evaluated *in vitro* using HepaRG® terminally differentiated hepatic cells (Study Report PC2514-2016-0025). No induction was observed for any of the isoforms upon 24-hour exposure of any concentration of ETX2514 to HepaRG cells.

4.3.6 Excretion

Excretion of ETX2514 following IV administration was determined in the urine of rats and dogs (Study Report PC2514-2016-0027 and Study Report PC2514-2016-0026, respectively) and the bile of rats (Study Report PC2514-2016-0032). Urinary excretion of unchanged drug accounted for 59% of a 3 mg/kg dose administered to rats and 71% of a 0.45 mg/kg dose administered to dogs. Recovery of unchanged drug in the bile of rats following administration of a 25 mg/kg dose of ETX2514 to bile duct cannulated rats was very low, ranging from 0.2% to 0.5% of the dose. In this same study, urinary excretion of unchanged drug was quite variable, ranging from 36.0% to 94.1% of the dose (mean = 64.7 ± 29.1%). Analysis of urinary concentrations of unchanged ETX2514 and sulbactam from Phase 1 FIH and renal impairment studies demonstrated that both compounds are primarily cleared via urinary excretion. ETX2514 mean recovery in urine ranged from 50% to 76% and sulbactam recovery ranged from 62% to 66% across FIH cohorts.

4.3.7 Projection of a human efficacious dose

4.3.7.1 Brief summary of PK/PD drivers of efficacy against *A. baumannii*

- Investigations using the *in vitro* hollow-fiber system and the chemostat model characterized PK/PD drivers of T>MIC and AUC/MIC for sulbactam and ETX2514, respectively. The pMIC of sulbactam (MIC in the presence of 4 mg/L ETX2514) was used for both T>MIC and AUC/MIC targets for sulbactam and ETX2514, respectively. E_{max} modeling of *in vivo* animal efficacy conducted in thigh and lung infections suggested T>MIC of 50% of the dosing interval for sulbactam in order to reach a 1-$\log_{10}$ kill. For ETX2514, AUC/MIC ratios of 10 and 30 are associated with stasis and 1-$\log_{10}$ kill endpoints when sulbactam is at 50% T>MIC. Experimental results obtained in murine infection models have historically been predictive of human efficacy. Achieving these PK/PD exposures of sulbactam and ETX2514 are anticipated to translate to a favorable clinical outcome.

- A detailed assessment of human dose projection of sulbactam/ETX2514 was performed utilizing PK parameters from Phase 1 data in humans, PK/PD targets, and current MIC distribution versus *A. baumannii*. A preliminary pTA was completed. The result of these analyses suggested a 3-hour infusion of 1 g of sulbactam and 0.5 to 1.0 g of ETX2514 administered q6h is predicted to be effective to treat patients infected with *A. baumannii* with MICs ≤4 mg/L.

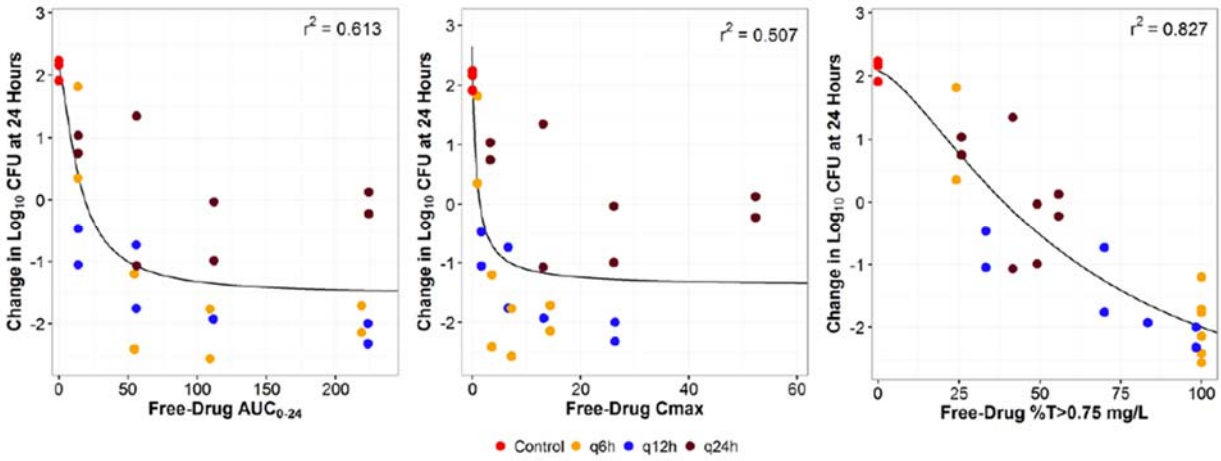
4.3.7.2 PK/PD drivers for sulbactam and ETX2514

In vitro PK/PD studies conducted in a hollow-fiber infection model have demonstrated and confirmed the PK/PD driver of sulbactam to be T>MIC (Study Report PC2514-2016-0023). *In vivo* thigh and lung dose response studies demonstrated that 1-$\log_{10}$ kill is achieved when sulbactam exposure exceeds the MIC for 50% of the dosing interval, and this magnitude has been shown to be efficacious in clinical use of sulbactam against susceptible isolates (Study Report PC2514-2016-0020 and Study Report PC2514-2016-0021). *In vitro* MIC testing and time kill studies have demonstrated restoration of sulbactam antibacterial activity when combined with ETX2514. In the presence of 4 mg/L of ETX2514, the vast majority of isolates with elevated MICs to sulbactam are restored to a pMIC of ≤4 mg/L. The pMIC specifically refers to the sulbactam MIC in the presence of 4 mg/L ETX2514.

In vitro dynamic model studies were pursued in a chemostat model to determine the PK/PD driver for ETX2514 (Study Report PC2514-2017-0011). In these studies, a clinical isolate (ARC5081) with a mean pMIC of 2.9 mg/L was used in initial dose response and dose fractionation experiments. Dose response studies using a q6h regimen of sulbactam that exceeded the pMIC for 100% of the dosing interval, yet was low enough to be ineffective on its own, was used in all dose arms (except control) in order to determine the exposure of ETX2514 required to restore sulbactam activity. Restoration of sulbactam activity was considered at a 24-hour endpoint, where the amount of ETX2514 added to the system resulted in no net growth or kill in bacterial colonies, the PK/PD endpoint equivalent of an MIC. Doses of ETX2514 above and below this net stasis endpoint were further dose fractionated to elucidate the PK/PD driver. Total daily doses of 0.5, 2, 4, and 8 g ETX2514 per day spanning an unbound AUC range of 13.9 to 222 µg·h/mL were administered via doses every 24 hours (q24h), every 12 hours (q12h), and q6h over 24 hours. The 24-hour change of colony-forming unit (CFU) burden was plotted relative to AUC, C_{max}, and time in a dosing interval that the drug concentration exceeds a critical threshold (T>C_T), and the data was fitted to a nonlinear E_{max} model to determine the index most closely associated with the observed activity across the dose range.

Iterative evaluation of several concentrations showed that T>C_T of 0.75 mg/L provided the best fit to the data with a correlation coefficient (R^2) of 0.83 (Figure 4–2). While AUC demonstrated an inferior correlation (R^2 = 0.61), inspection of the data by regimen suggested this correlation was largely driven by the q24h regimen, whereas data obtained with the q12h and q6h regimens revealed a superior fitting of the data. As suggested by the most recent guidance on PK/PD driver assessments of BL/BLI combinations, AUC and T>C_T often are both highly correlated with drugs that demonstrate time dependent activity (CHMP 2016).

Figure 4–2. Relationship between PK/PD parameters of ETX2514 against *A. baumannii* ARC5081 response in the *in vitro* chemostat model

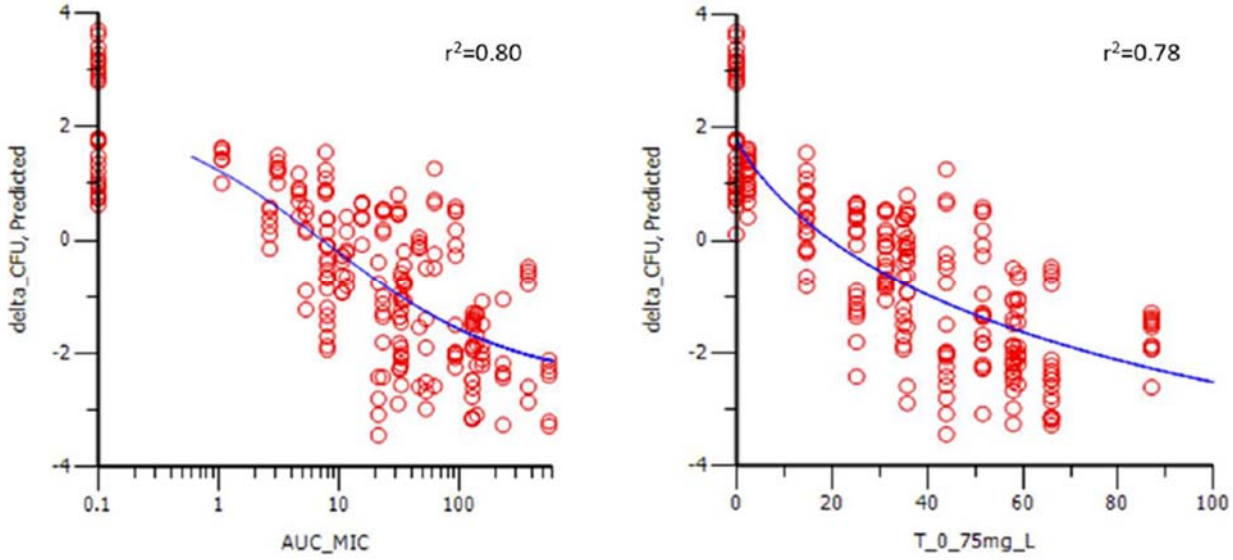


% T>0.75 mg/mL = percentage of time in a dosing interval that the drug concentration exceeds 0.75 mg/L; AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; CFU = colony-forming unit; C_{max} = maximum plasma concentration; PD = pharmacodynamic; PK = pharmacokinetic; q6h = every 6 hours; q12h = every 12 hours; q24h = every 24 hours; r^2 = correlation coefficient.

This observation concerning AUC and $T>C_T$ was particularly true in murine neutropenic thigh infection studies utilizing a once every 3 hours (q3h) dosing regimen. Change in CFU burden data plotted versus AUC and $T>C_T$ revealed similar R^2 correlations of ~0.80 (Figure 4–3). *In vivo* dose response studies utilized sulbactam exposure at 50% of time in a dosing interval that the drug concentration exceeds the pMIC of the organism (% T>pMIC), an exposure target generally associated with a 1-log_{10} kill versus susceptible isolates. Addition of ETX2514 via the same dose frequency (i.e., q3h) using a broad range of concentrations suggested AUC/MIC targets of 10 and 30, for stasis and 1-log_{10} kill, respectively, across the 5 strains.

Figure 4–3. Relationship between change in bacterial density at 24 hours and ETX2514 exposure *in vivo* versus 5 strains

free-drug AUC_{0-24}/MIC free-drug % T>0.75 mg/L

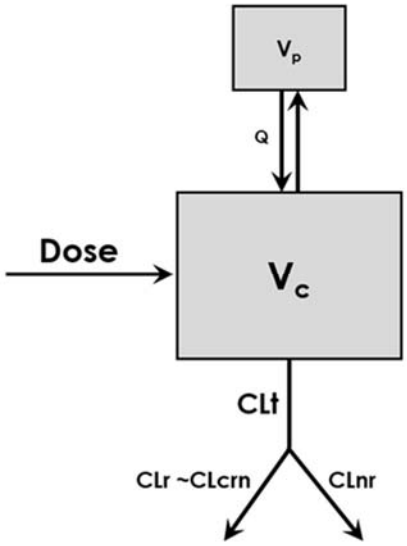


% T>0.75 mg/mL = percentage of time in a dosing interval that the drug concentration exceeds 0.75 mg/L; AUC = area under the plasma concentration-time curve; AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; CFU = colony-forming unit; MIC = minimum inhibitory concentration; r^2 = correlation coefficient.

4.3.7.3 pTA of ETX2514SUL

The human dose projections for ETX2514SUL were developed using a population PK model initiated at the Institute for Clinical Pharmacodynamics. For sulbactam, a published population PK model derived from PK in patients with varying degrees of renal impairment was utilized in support of pTA simulations (Yokoyama 2015). Plasma concentration and demographic data from Phase 1 FIH and renal PK studies were used to construct a population PK model for ETX2514. Predicted performance of the model was assessed using plasma concentration data from the Phase 2 and Phase 1 lung penetration studies. Covariate analysis suggested a strong correlation of creatinine clearance (CLcr) with ETX2514 clearance across clinical subjects, necessitating dose adjustment for individuals with various degrees of renal impairment. Figure 4–4 presents population PK parameter estimates of the population model. Along with excretion of unchanged drug, a nonrenal, metabolic component to ETX2514 clearance is suggested.

Figure 4–4. Parameter estimates of the population PK model for ETX2514

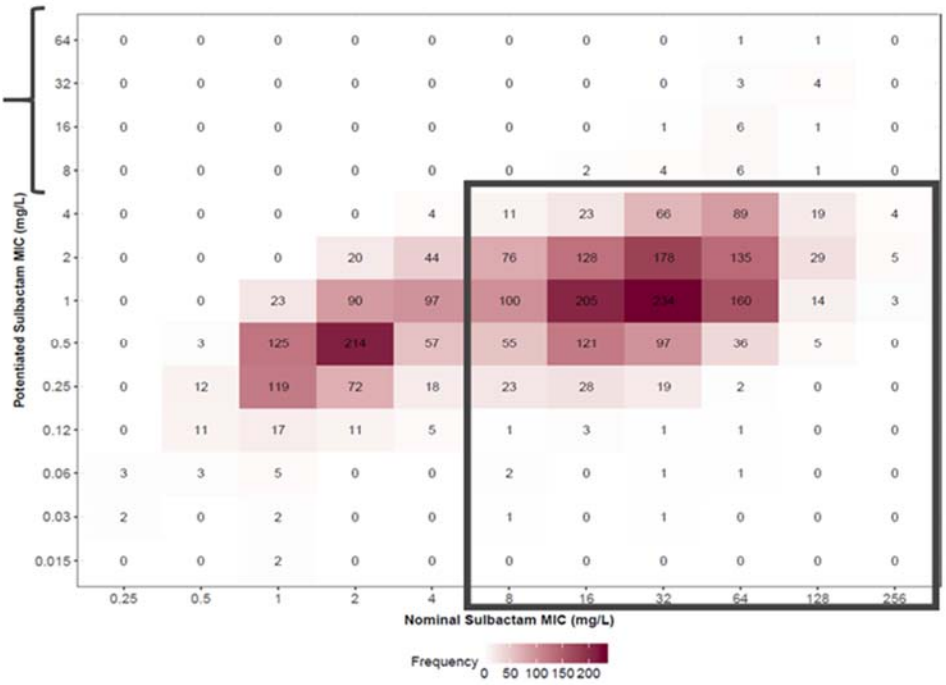


Parameter	Value	Units
CLt	7.62	L/h
V_c	12.1	L
Q	3.20	L/h
V_p	5.84	L
Exponent on normalized CLcr	1.14	---
Fraction excreted renally	0.479	---
IIV on CL	40.5	%CV
IIV on V_c	19.6	%CV
IIV on Q	28.1	%CV
Proportional RV	11.7	%CV
Additive RV	0.000114	mg/L

CL = clearance; CLcr/CLcrn = creatinine clearance; CLnr = nonrenal clearance; CLr = renal clearance; CLt = apparent total body clearance; CV = coefficient of variation; IIV = interindividual variability; PK = pharmacokinetic; Q = intercompartmental clearance; RV = random effects variance V_c = volume of the central compartment; V_p = volume of the peripheral.

Figure 4–5 shows the pTA for clinical dose projections determined using susceptibility data against a contemporary panel of 2866 isolates. The sulbactam MIC_{90} was 64 mg/L and the ETX2514SUL MIC (i.e., sulbactam pMIC) was 2 mg/L. Nearly 98.4% of strains (1877/1907) exhibiting sulbactam MICs >4 mg/L were successfully restored to susceptibility by the addition of ETX2514.

Figure 4–5. Sulbactam alone ('nominal' MIC) and in the presence of ETX2514 at 4 mg/L ('potentiated' MIC) MIC distribution of *A. baumannii* strains (n = 2866)



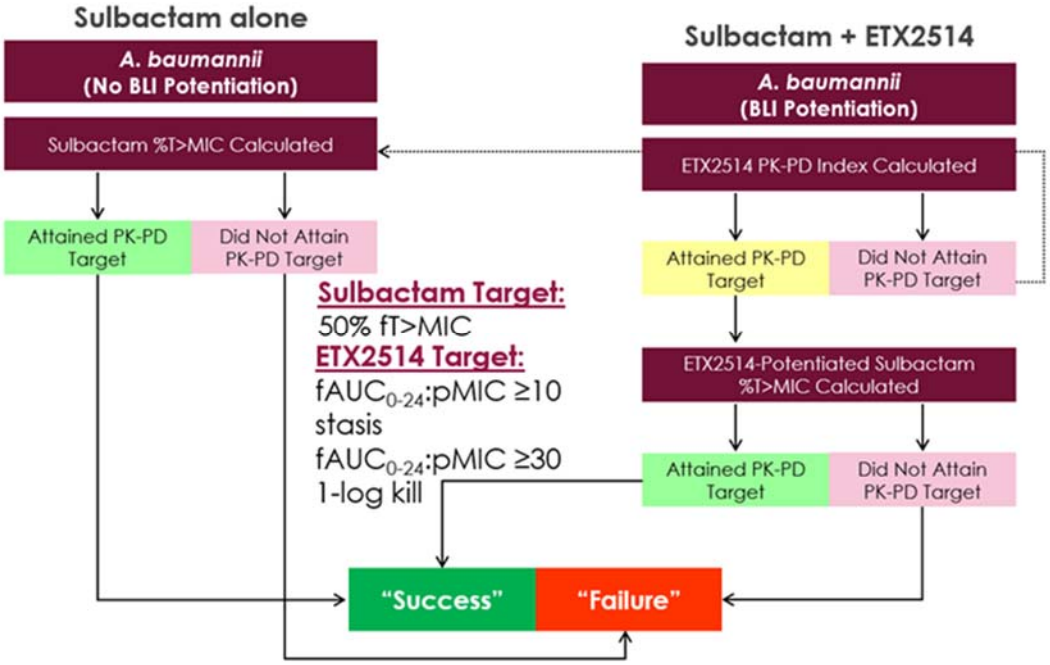
MIC = minimum inhibitory concentration; pMIC = 'potentiated' minimum inhibitory concentration.

Figure 4–6 shows the algorithm for meeting PK/PD exposure targets was utilized as criteria for target attainment at an MIC of 4 mg/L (the proposed ETX2514SUL susceptibility breakpoint). This included strains that were already sulbactam susceptible (MICs of ≤4 mg/L) as well as those isolates that achieved MICs ≤4 mg/L in the presence of ETX2514 (i.e., pMIC ≤4 mg/L). PK/PD targets of 50% T>MIC of 4 mg/L for sulbactam and AUC/MIC ratios of 10 (stasis) and 30 (1-$\log_{10}$ kill) for ETX2514 were considered in Monte Carlo simulations. Joint target attainment for a 1-$\log_{10}$ kill endpoint (sulbactam 50% T>MIC of 4 mg/L, ETX2514 AUC/pMIC = 10 and 30) was performed across 8 renal categories spanning a CLcr range of 0 to 200 mL/min. For each renal category, 1000 patient simulations were performed assuming uniform distribution across the CLcr range.

Based upon the outcome of the chemostat PK/PD driver assessment, stasis (ETX2514 AUC/pMIC =10) was considered as the primary exposure endpoint for ETX2514. This decision was based on the premise that this exposure is required to restore activity of sulbactam relative to its pMIC. An exposure target of 50% T>pMIC was selected for sulbactam as this endpoint is associated with achieving 1-$\log_{10}$ kill in *in vitro* and *in vivo* infection model studies against susceptible strains. *In vivo* studies conducted with sulbactam

targeted at 50% T>pMIC generally resulted in achieving stasis at ETX2514 AUC/pMICs of 10 or less and 1-log_{10} kill at AUC/pMICs of 30 or less. The higher ratio of 30 was considered in a dose sensitivity analysis in the combination.

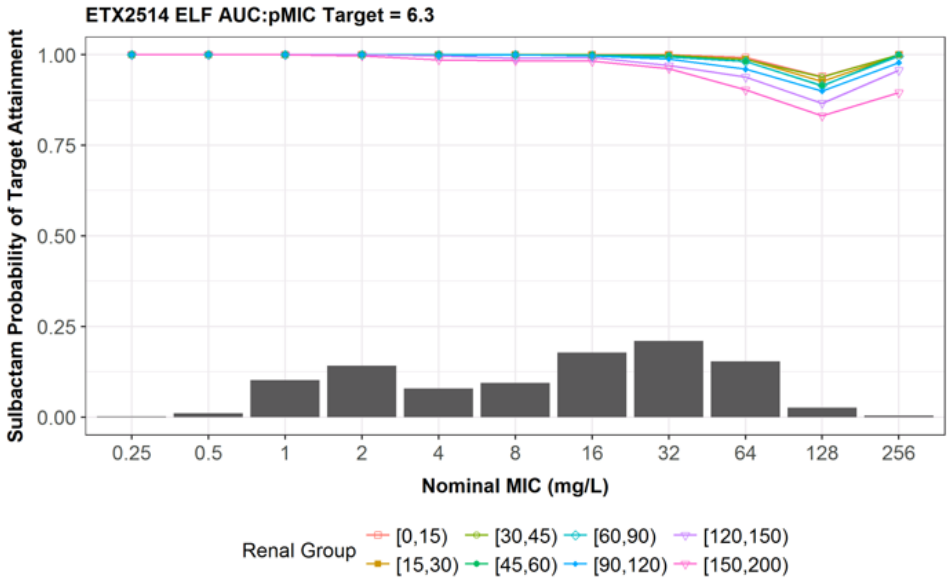
Figure 4–6. Algorithm for sulbactam-ETX2514 PK/PD pTA



% T>MIC = percentage of time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration of the organism; 50% fT>MIC = 50% of time in a dosing interval that the drug concentration exceeds the pMIC of the organism; BLI = β-lactamase inhibitor; $fAUC_{0-24}$ = free area under the plasma concentration-time curve from 0 to 24 hours postdose; PD = pharmacodynamic; PK = pharmacokinetic; pMIC = 'potentiated' minimum inhibitory concentration; pTA = probability of target attainment.

One key clinical target for ETX2514SUL therapy is pneumonia. Therefore, exposure based assessments within the lung are relevant. Using mouse and human ELF penetration data, systemic ETX2514 AUC/pMIC targets of 10 and 30 were normalized to lung levels of 6.3 and 18.9 for stasis and 1-log_{10} kill, respectively. Figure 4–7 and Figure 4–8 show Monte Carlo simulations across all renal categories in consideration of the nominal sulbactam MICs (accounting for both sulbactam-susceptible strains as well as those made susceptible in the presence of ETX2514) based on the ETX2514SUL 1 g/1 g regimen. These pTA assessments were conducted using the stasis and 1-log_{10} kill ETX2514 AUC/pMICs in the ELF. Results of this analysis suggested pTA for ETX2514SUL 1 g/1 g in excess of 90% at a nominal MIC of 64 mg/L, where greater than 90% of the strains would be covered.

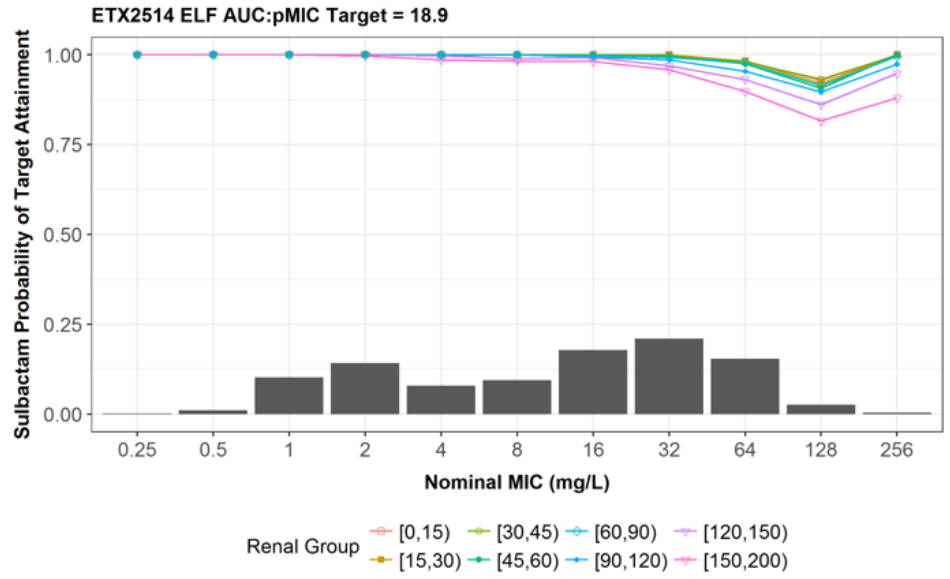
Figure 4–7. Sulbactam pTA in ELF at 50% T>MIC sulbactam and stasis



Based on the ETX2514SUL 1 g/1 g regimen. Stasis = ETX2514 AUC/pMIC ratio of 6.3.

% T>MIC = percentage of time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration of the organism; AUC = area under the plasma concentration-time curve; ELF = epithelial lining fluid; ETX2514SUL = sulbactam/ETX2514; MIC = minimum inhibitory concentration; pMIC = 'potentiated' minimum inhibitory concentration; pTA = probability of target attainment.

Figure 4–8. Sulbactam pTA in ELF fluid at 50% T>MIC sulbactam and 1-$\log_{10}$ kill

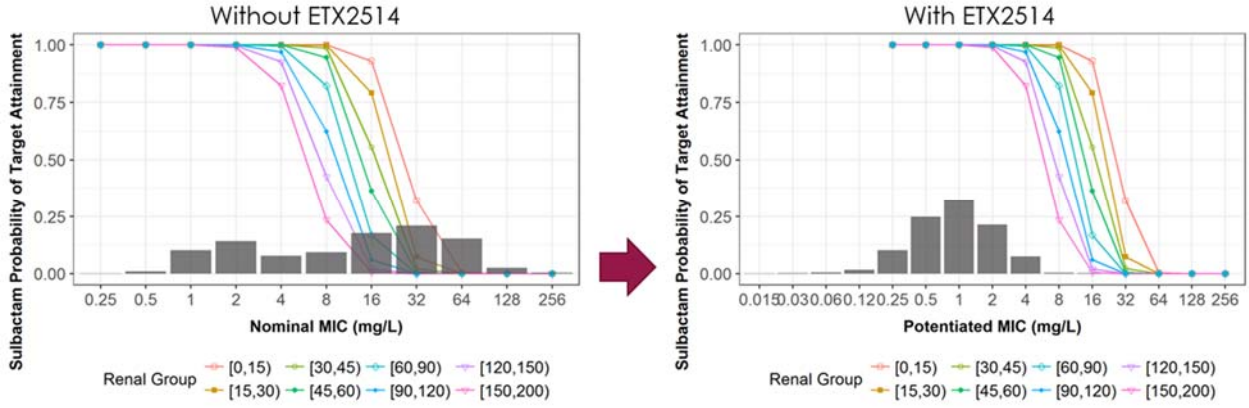


Based on the ETX2514SUL 1 g/1 g regimen. 1-$\log_{10}$ kill = ETX2514 AUC/pMIC ratio of 18.9.

% T>MIC = percentage of time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration of the organism; AUC = area under the plasma concentration-time curve; ELF = epithelial lining fluid; ETX2514SUL = sulbactam/ETX2514; MIC = minimum inhibitory concentration; pMIC = 'potentiated' minimum inhibitory concentration; pTA = probability of target attainment.

Interestingly, the pTA did not change significantly between the stasis and the 1-$\log_{10}$ kill ETX2514 AUC/pMIC ratios, suggesting sulbactam exposure had the predominate influence on achieving pTA across renal categories. As demonstrated in Figure 4–9, the distribution of nominal MIC shifts to a unimodal distribution with a 'potentiated' minimum concentration that inhibits 90% of isolates <4 mg/L; a plasma-based pTA >90% was realized across all renal categories except for those with CLcr in excess of 150 mL/min. By definition, patients with CLcr in excess of 130 mL/min are classified as being in a condition of hyperfiltration or augmented renal clearance. This important segment of the patient population makes up >10% of all ventilator-associated pneumonias (Ambrose 2010). In order to increase the pTA to >90% in patients with CLcr>130 mL/min, a dose adjustment will need to be considered.

Figure 4–9. Sulbactam pTA in plasma at 50% T>MIC sulbactam and 1-$\log_{10}$ kill (n = 2866 strains)



Based on the ETX2514SUL 1 g/1 g regimen. 1-$\log_{10}$ kill = ETX2514 AUC/MIC ratio 30.

% T>MIC = percentage of time in a dosing interval that the drug concentration exceeds the minimum inhibitory concentration of the organism; AUC = area under the plasma concentration-time curve; ETX2514SUL = sulbactam/ETX2514; MIC = minimum inhibitory concentration; pTA = probability of target attainment.

4.3.8 Renal dose adjustment

In consideration of patients across the entire spectrum of renal function (i.e., those with end-stage renal disease on hemodialysis to those with elevated renal clearance such as augmented renal clearance), renal-based dose adjustments will be required.

Table 4–18 details proposed renal-based dose adjustments. The proposed dose adjustment in patients on hemodialysis remains to be determined. Table 4–19 presents simulations and pTA analysis of these regimens. Joint target attainment can be achieved with pTA >90% across all renal categories. These recommendations are based on population PK data that incorporates

plasma concentration collected in Study CS2514-2017-0002 (see Section 5.1.3 for a summary of data).

Table 4–18. Dose adjustment of ETX2514SUL based upon patient renal function

CLcr (mL/min)	Sulbactam/ETX2514 Dose (mg/mg)
0-14	1000/1000 q12h
15-29	1000/1000 q8h
30-130	1000/1000 q6h
130-200	1500/1500 q6h
Hemodialysis/CRRT	To be determined

All regimens use a 3-hour IV infusion.
CLcr = creatinine clearance; CRRT = continuous renal replacement therapy; ETX2514SUL = sulbactam/ETX2514; IV = intravenous; q6h = every 6 hours; q8h = every 8 hours; q12h = every 12 hours.

Table 4–19. pTA of ETX2514SUL at a pMIC of 4 mg/L across all renal categories using proposed renal dose-adjusted regimens (unbound plasma and ELF exposure targets)

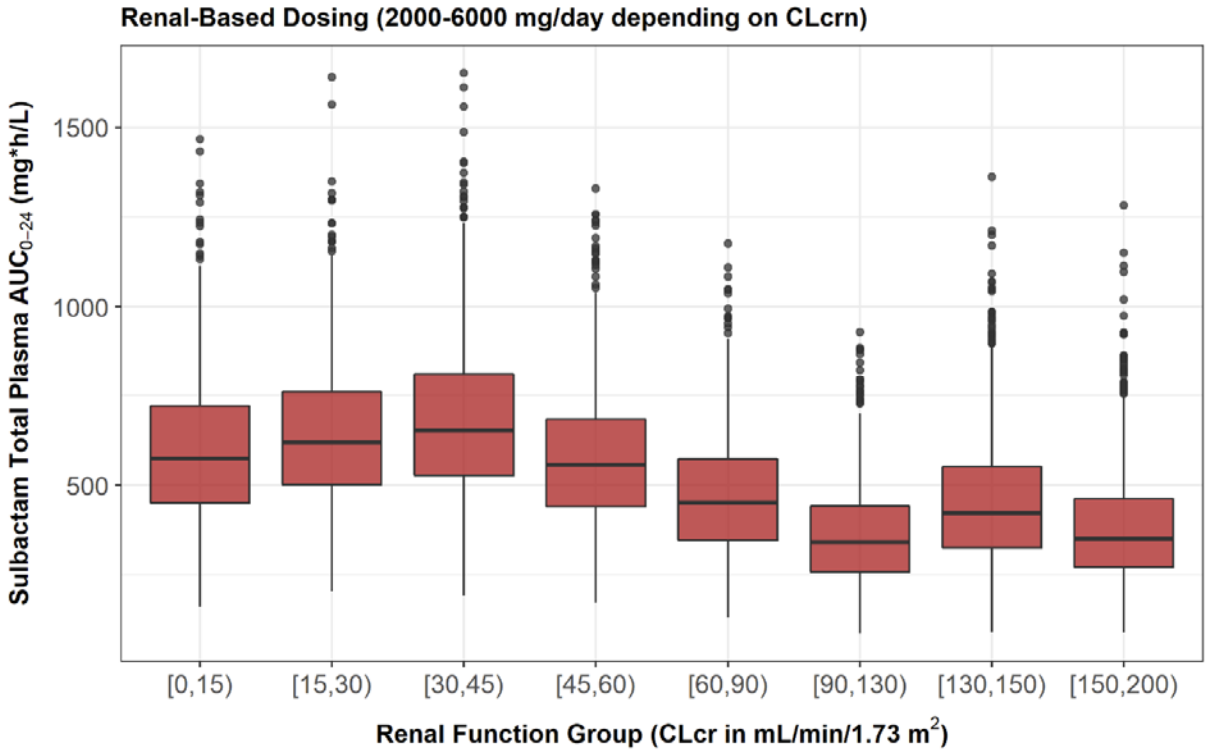
MIC (mg/L)	CLcr (mL/min)	pTA	
		Free-Drug Plasma	ELF
4	0-15	0.98	0.966
4	15-30	0.999	0.998
4	30-45	1	1
4	45-60	1	0.997
4	60-90	0.995	0.988
4	90-130	0.959	0.918
4	130-150	0.989	0.969
4	150-200	0.97	0.933

CLcr = creatinine clearance; ELF = epithelial lining fluid; ETX2514SUL = sulbactam/ETX2514; MIC = minimum inhibitory concentration; pMIC = 'potentiated' minimum inhibitory concentration; pTA = probability of target attainment.

Figure 4–10 and Figure 4–11 present the simulation-based predicted sulbactam plasma AUC values across all renal categories using the proposed renal-based dose-adjusted regimens. The 3 proposed renal-based dose-adjusted regimens result in sulbactam C_{max} values well below current clinical use in Unasyn (New Drug Application [NDA] # 050608). Similarly, the AUC exposures for the 3 proposed renal-based dose-adjusted regimens are not anticipated to exceed the potential sulbactam exposure achieved when 3 g Unasyn q6h (i.e., 1 g sulbactam) is dosed in patients with CLcr >30 mL/min, the maximum dose permitted per the current

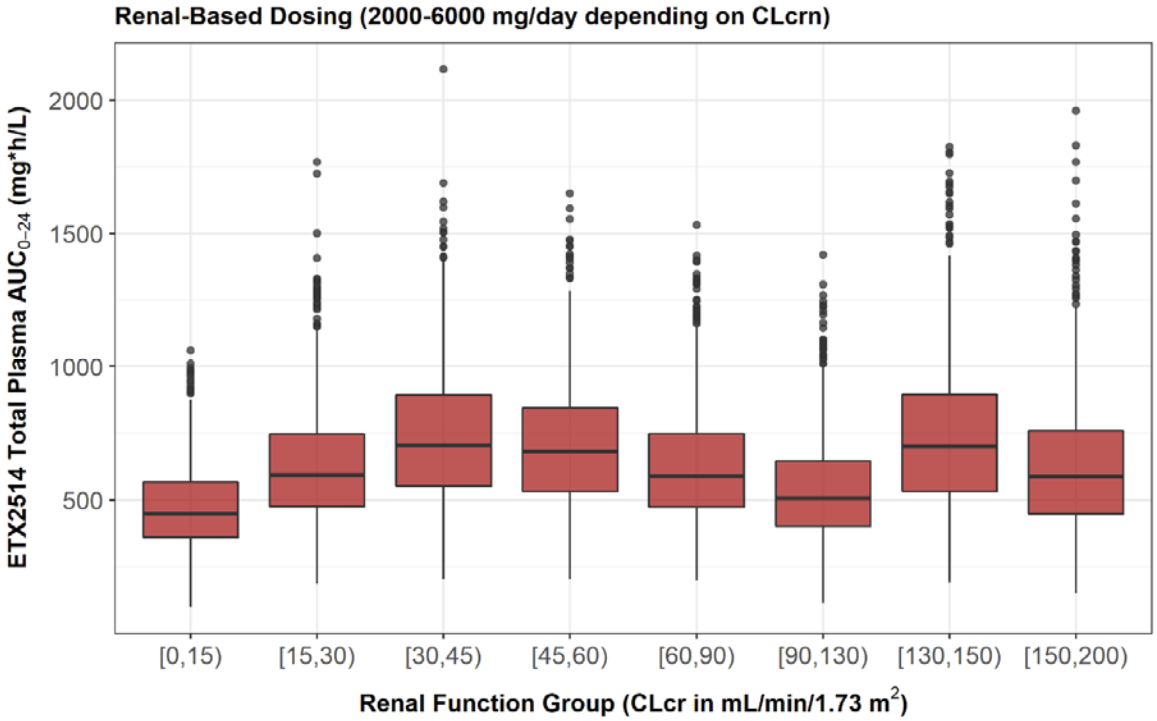
Unasyn product circular (NDA # 050608). With regard to ETX2514, safety margins presented in Section 4.4 are relative to mean Phase 2 C_{max} and AUC_{0-24} values of 37 µg/mL and 501 µg·h/mL, respectively, which are exposures associated with clinical efficacy. While higher exposure is expected in some of the renal-based dose adjustments, ETX2514 exposures are not expected to exceed a C_{max} associated with a single 8 g dose (C_{max} = 175 µg/mL) or an 8 g daily dose (2 g dose of ETX2514 administered q6h; mean AUC_{0-24} = 740 µg·h/mL). Both 8 g dose regimens were tolerated with no adverse events in FIH single ascending dose (SAD) and multiple ascending dose (MAD) cohorts (Study CS2514-2016-0001).

Figure 4–10. Simulation-based predicted sulbactam plasma AUC across all renal categories using proposed renal-based dose-adjusted regimens



AUC = area under the plasma concentration-time curve; AUC_{0-24} = Area under the plasma concentration-time curve from 0 to 24 hours postdose; CLcr/CLcrn = creatinine clearance.

Figure 4–11. Simulation-based predicted ETX2514 plasma AUC across all renal categories using proposed renal-based dose-adjusted regimens



AUC = area under the plasma concentration-time curve; AUC_{0-24} = Area under the plasma concentration-time curve from 0 to 24 hours postdose; CLcr/CLcrn = creatinine clearance.

4.4 Toxicology

4.4.1 Brief summary

- Maximum tolerated, single IV dose escalation studies of ETX2514 performed in rats and dogs did not result in serious test article–related events. Exposure across the dose ranges evaluated were generally dose proportional, and there were no significant differences between males and females.

- 7-day IV repeat-dose range-finding studies in rats and dogs at doses up to the limit dose of 2000 mg/kg/day did not identify serious test article–related findings.

- In pivotal 14-day IV repeat-dose toxicity studies conducted in rats and dogs, ETX2514 administered up to the limit dose of 2000 mg/kg/day was not associated with mortality or significant changes in clinical signs, ophthalmology, urinalysis or hematology parameters, organ weight changes, or changes in gross pathology or histopathology, and there were no toxicologically significant changes in clinical chemistry parameters. Based on these findings, the NOAEL was 2000 mg/kg/day in both species. The associated combined-sex mean steady state C_{max} and AUC_{0-24} values on Day 14 in the most sensitive species, rat, at the NOAEL were 1340 µg/mL and 3140 µg·h/mL, respectively. The exposure margin based on AUC_{0-24} relative to human exposures was 6.3-fold.

- In a 7-day IV repeat-dose study of ETX2514 in combination with sulbactam, minimal decreases in body weight gain were noted, and there were no adverse effects on clinical pathology parameters, organ weights, or macroscopic tissue findings. Based on the mild severity of the findings, the NOAEL was the highest combination dose tested, 400 mg/kg/day of each compound.

- The toxicity of ETX2514 alone or in combination with sulbactam was evaluated in a pivotal (GLP) study in rats administered the treatments IV daily for 28 days. ETX2514 administered alone (600 mg/kg/day) was devoid of toxicity. Notable effects in rats treated with the combination of sulbactam/ETX2514 (300 or 600 mg/kg/day of each compound) were consistent with known, monitorable effects of sulbactam. Dose-related inflammatory changes noted microscopically at the infusion site and in liver and lung with both combination doses were considered adverse effects of treatment; however, inflammatory changes in liver and lung were not associated with necrosis and were not present at the end of the recovery period. Thus, while there was no NOAEL identified in the study due to inflammatory changes noted with both combination doses, the adverse changes were monitorable and reversible and occurred after an infusion period of approximately 2-fold the maximum clinical treatment period.

- *In vitro* investigations exhibited no significant interactions with cardiac ion channels expressed in HEK293 and CHO cells at any concentrations ranging from 100 to 1000 µM including hERG (IC_{50} >333 µM).

- ETX2514 had no quantitative effects on any ECG parameters including PR interval, QRS interval, QT interval, QTc, heart rate, or arterial pressure after single doses up to 2000 mg/kg in telemetered conscious dogs.

- ETX2514 was not toxic to male rats in respiratory and neurologic safety pharmacology studies at doses up to 2000 mg/kg/day.

- ETX2514 was not mutagenic *in vitro* in a bacterial reverse mutation assay, and it was not clastogenic *in vitro* in human lymphocytes or *in vivo* in rats. Thus, ETX2514 is not genotoxic.

- Reproductive toxicology studies with ETX2514 using the IV infusion route assessing EFD and male and female fertility have been completed in the rat. In these studies, rats were administered ETX2514 up to the limit dose of 1000 mg/kg/day. No ETX2514-associated adverse effects on male or female fertility or EFD were observed. Thus, the NOAEL for reproductive and embryofetal toxicity in rats is 1000 mg/kg/day.

- Carcinogenicity studies of ETX2514 are not planned because there is no evidence of genotoxicity and the intended duration of therapy is no more than 2 weeks.

- In summary, based on the results of the 28-day sulbactam/ETX2514 combination study in rats, minimal but reversible inflammatory changes in liver and lung have been the only adverse toxicities of note, occurring only after the combination was infused daily for approximately 2 times the maximum duration of clinical use. The C_{max} values of ETX2514 in the combination arms of the 28-day rat study were 3.6- to 6.5-times higher than human exposure, and the C_{max} values of sulbactam ranged from 3.5- to 6.8-fold higher than human exposure. Exposure margins based on AUC_{0-24} compared to human exposures ranged from 0.4- to 1.0-fold for ETX2514 and 0.8- to 1.8-fold for sulbactam.

ETX2514 by itself or in combination with sulbactam has been assessed in a comprehensive toxicology and secondary pharmacology package, in accordance with International Council for Harmonisation (ICH) guidelines. An overview of nonclinical toxicology and secondary pharmacology studies conducted to date is presented in Table 4–20. Key details of each study are also described in the sections below.

Table 4–20. Summary of ETX2514 toxicology and secondary pharmacology studies

Study No.	Status	Short Title	NOAEL (mg/kg/day)	AUC (µg·h/mL) /Margin	C_{max} (µg/mL) /Margin	Main Findings
SN00251 4-2015-03	Complete	14-day repeat dose IV, rat	2000	3122/6.2X	1340/36X	• Crl:CD(Sprague-Dawley) rats were given sterile saline or ETX2514 at a dose level of 500, 1000, or 2000 mg/kg/day via intermittent IV 2-hour infusion once daily for 14 days. • Clinical pathology changes were mild in severity. ETX2514-related microscopic findings included minimal to slight edema and/or minimal to slight hemorrhage in the lamina propria in the ceca of animals given ≥500 mg/kg/day correlating with the macroscopic observation of cecal enlargement. • Due to the mild severity of the findings and the lack of impact on the health and wellbeing, effects in animals given 2000 mg/kg/day were not considered adverse.
SN00251 4-2015-04	Complete	14-day repeat dose IV, dog	2000	5414/11X	2293/62X	• Male and female beagles were given sterile saline or ETX2514 at a dose level of 500, 1000, or 2000 mg/kg/day via IV 2-hour infusion once daily for 14 days. • No toxicologically important clinical pathology changes were noted. Microscopic findings were limited to minimally to slightly decreased cellularity of the sternum marrow and lymphoid depletion of the GALT/Peyer's patch of males given ≥500 mg/kg/day and minimal lymphoid depletion of the mandibular and mesenteric lymph nodes of males given 2000 mg/kg/day. • Due to the mild severity of the findings and the lack of impact on the health and wellbeing, effects in animals given 2000 mg/kg/day were not considered adverse.

Phase 2 clinical exposure used for margin calculation: AUC_{0-24} = 501 µg·h/mL, C_{max} = 37 µg/mL.

AUC = area under the plasma concentration-time curve; AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; CNS = central nervous system; CV = cardiovascular; DART = development and reproductive toxicology; ECG = electrocardiogram; EFD = embryofetal development; FDA = Food and Drug Administration; GALT = gut-associated lymphoid tissue; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; IV = intravenous; NA = not applicable; NaCl = sodium chloride; No. = number; NOAEL = no-observed-adverse-effect-level; PK = pharmacokinetic; QTc = corrected QT interval; TK = toxicokinetic; USP = United States Pharmacopeia.

Table 4–20. Summary of ETX2514 toxicology and secondary pharmacology studies (continued)

Study No.	Status	Short Title	NOAEL (mg/kg/day)	AUC (µg·h/mL) /Margin	C_{max} (µg/mL) /Margin	Main Findings
PC2514-2017-0018	Ongoing	28-day repeat dose IV combination in rat with 28-day recovery period	Not identified			• ETX2514 alone (600 mg/kg/day) or in combination with sulbactam (300 mg/kg/day or 600 mg/kg/day of each compound in combination) was evaluated in male and female Sprague-Dawley rats administered for 2 hours/day via intermittent IV infusion for 28 days. • ETX2514 administered alone was devoid of toxicity, and notable effects in rats treated with the combination of ETX2514-sulbactam were consistent with known effects of sulbactam. • ETX2514 in combination with sulbactam produced nonadverse reductions in body weight and food consumption, and nonadverse changes in hematology, clinical chemistry, and urinalysis parameters that were generally dose related and completely reversible. • Combination treatment and dose-related reversible increases in liver weights were associated with glycogen accumulation, a known effect of sulbactam. • Dose-related inflammatory changes were noted microscopically at the infusion site and in liver and lung. Inflammation was considered an adverse effect of treatment; however, inflammatory changes in liver and lung were not associated with necrosis and were not present at the end of the recovery period. • Based on adverse inflammatory changes noted at both combination dose levels in liver and lung at the end of the treatment period, a NOAEL was not identified. However, the inflammatory changes were minimal to moderate, not associated with necrosis, completely reversible, clinically monitorable, and occurred only after a treatment duration approximately 2 times the 14-day maximum clinical treatment period.

Phase 2 clinical exposure used for margin calculation: AUC_{0-24} = 501 µg·h/mL, C_{max} = 37 µg/mL.

AUC = area under the plasma concentration-time curve; AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; CNS = central nervous system; CV = cardiovascular; DART = development and reproductive toxicology; ECG = electrocardiogram; EFD = embryofetal development; FDA = Food and Drug Administration; GALT = gut-associated lymphoid tissue; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; IV = intravenous; NA = not applicable; NaCl = sodium chloride; No. = number; NOAEL = no-observed-adverse-effect-level; PK = pharmacokinetic; QTc = corrected QT interval; TK = toxicokinetic; USP = United States Pharmacopeia.

Table 4–20. Summary of ETX2514 toxicology and secondary pharmacology studies (continued)

Study No.	Status	Short Title	NOAEL (mg/kg/day)	AUC (µg·h/mL) /Margin	C_{max} (µg/mL) /Margin	Main Findings
PC2514-2017-0019	Audited draft report	Female Fertility and Embryo-Fetal Development (EFD) in the rat	1000	1600/3.2X	614/17X	• 4 groups of 25 female Crl:CD(Sprague-Dawley) rats were administered 0 (control article), 100, 300, or 1000 mg/kg/day by IV infusion at a volume of 2.5 mL/kg/hour. The control article was 0.9% NaCl injection, USP (sterile saline). • No effect on mating, fertility, or the progress or outcome of pregnancy was noted; and no test article–related malformations or variations were observed.
PC2514-2018-0003	Ongoing	ETX2514 Male Fertility and Early Embryonic Development in the rat	1000			• 4 groups of 20 male Crl:CD(Sprague-Dawley) rats were administered 0 (control article), 100, 300, or 1000 mg/kg/day of ETX2514 by IV infusion at a volume of 2.5 mL/kg/hour. The control article was 0.9% NaCl injection, USP (sterile saline). Males were dosed for up to 21 days (2 weeks prior to pairing and during the pairing phase until confirmation of mating). • 4 groups of 20 female Crl:CD(Sprague-Dawley) rats were used for mating and were not dosed. • Male mating, fertility, and fecundity was unaffected by treatment with ETX2514. No effect of male administration with ETX2514 was noted on pregnancy outcomes or male reproductive organ weights. No macroscopic findings were related to treatment with ETX2514. Administration of up to 1000 mg/kg/day ETX2514 to male Sprague-Dawley rats was well tolerated.

Phase 2 clinical exposure used for margin calculation: $AUC_{0\text{-}24}$ = 501 µg·h/mL, C_{max} = 37 µg/mL.

AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24}$ = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; CNS = central nervous system; CV = cardiovascular; DART = development and reproductive toxicology; ECG = electrocardiogram; EFD = embryofetal development; FDA = Food and Drug Administration; GALT = gut-associated lymphoid tissue; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; IV = intravenous; NA = not applicable; NaCl = sodium chloride; No. = number; NOAEL = no-observed-adverse-effect-level; PK = pharmacokinetic; QTc = corrected QT interval; TK = toxicokinetic; USP = United States Pharmacopeia.

Table 4–20. Summary of ETX2514 toxicology and secondary pharmacology studies (continued)

Study No.	Status	Short Title	NOAEL (mg/kg/day)	AUC (µg·h/mL) /Margin	C_{max} (µg/mL) /Margin	Main Findings
PC2514-2018-0005	Complete	ETX2514 Non-GLP Dose range finding study in non-pregnant rabbits to assess PK and toleration	Not identified	375/0.7X (150 mg/kg/day)	187/5X	• Doses of 150, 500, and 750 mg/kg/day ETX2514 were given by IV infusion to nonpregnant rabbits to assess PK and tolerability. • All doses associated with a marked reduction in food consumption, plus either body weight loss or body weight stasis necessitating cessation of drug administration. There were no findings at macroscopic necropsy. • Based on the TK exposures at these nontolerated doses, the dose setting required for a minimally maternally toxic dose for a rabbit EFD study will not achieve exposures adequate to assess the human therapeutic range. • Ethically, Entasis does not believe it is appropriate to continue in the rabbit (especially an infusion model) when the exposures obtained cannot adequately inform for human risk. • Based on existing precedent exemplified by the nonclinical registration data package for ertapenem and ceftolozane-tazobactam, there have been situations where mouse has been accepted as the second species for EFD assessment of antibacterials. • With FDA approval, Entasis to use mouse as the second species for EFD assessment.
PC2514-2018-0006	Complete	Non-GLP 7-day dose range finding study in mouse to assess PK and toleration	1600	940/13X	485/2X	• Mice were administered ETX2514 subcutaneously for 7 days at doses of 800 and 1600 mg/kg/day. No major findings from gross necropsy or clinical/anatomical pathology. • Mean mouse C_{max} and $AUC_{0\text{-}24}$ were 485 mg/mL and 956 mg·h/mL, which correspond to 13X and 2X margins, respectively, relative to ETX2514 clinical Phase 2 free drug exposures. • Mouse provides adequate tolerability and exposure by the subcutaneous administration route to be utilized as a second DART species as outlined in the ICH Guidance S5(R2) and approved by the FDA.

Phase 2 clinical exposure used for margin calculation: $AUC_{0\text{-}24}$ = 501 µg·h/mL, C_{max} = 37 µg/mL.

AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24}$ = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; CNS = central nervous system; CV = cardiovascular; DART = development and reproductive toxicology; ECG = electrocardiogram; EFD = embryofetal development; FDA = Food and Drug Administration; GALT = gut-associated lymphoid tissue; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; IV = intravenous; NA = not applicable; NaCl = sodium chloride; No. = number; NOAEL = no-observed-adverse-effect-level; PK = pharmacokinetic; QTc = corrected QT interval; TK = toxicokinetic; USP = United States Pharmacopeia.

Table 4–20. Summary of ETX2514 toxicology and secondary pharmacology studies (continued)

Study No.	Status	Short Title	NOAEL (mg/kg/day)	AUC (µg·h/mL) /Margin	C_{max} (µg/mL) /Margin	Main Findings
PC2514-2015-08	Complete	CV safety pharmacology evaluation in male telemetry instrumented conscious dogs	2000	5414/11X	2293/62X	• 4 male beagles were administered control article (0.9% NaCl injection) or 500, 1000, or 2000 mg/kg ETX2514 sodium salt on Days 1, 5, 8, and 12 of the dosing phase in a Latin square dosing design. Dose formulations were administered via 2-hour IV infusion at a dose volume of 10 mL/kg (5 mL/kg/hour). • No qualitative ECG effects or changes in PR interval, QRS duration, QT interval, QTc interval, heart rate, or arterial pressures were attributed to ETX2514 sodium salt administration up to 2000 mg/kg.
PC2514-2017-0015	Complete	Respiratory Safety Pharmacology Evaluation in Male Rats	2000	3122/6.2X	1340/36X	• 32 male Crl:CD(Sprague-Dawley) rats were assigned to 4 groups (8 animals/group). Animals were administered a single IV infusion via a catheter implanted in the femoral vein of control article (0.9% NaCl) or 500, 1000, or 2000 mg/kg ETX2514 in sterile water (vehicle) at a dose volume of 10 mL/kg/day (5 mL/kg/hour). • No ETX2514-related effects on mortality, clinical observations, or respiratory function were noted through 24 hours postdose.
PC2514-2018-0002	Complete	CNS safety pharmacology evaluation in male rats	2000	3122/6.2X	1340/36X	• 32 male Crl:CD(Sprague-Dawley) rats were randomly assigned to 4 groups (8 animals/group). Each group was administered a single dose of control article (0.9% NaCl for injection, USP) or 500, 1000, or 2000 mg/kg ETX2514. Dose formulations were administered via IV infusion at a dose volume of 10 mL/kg (5 mL/kg/hour). • No ETX2514-related effects on any component of the modified Irwin battery of testing (including body temperature) were noted.

Phase 2 clinical exposure used for margin calculation: $AUC_{0\text{-}24}$ = 501 µg·h/mL, C_{max} = 37 µg/mL.

AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24}$ = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; CNS = central nervous system; CV = cardiovascular; DART = development and reproductive toxicology; ECG = electrocardiogram; EFD = embryofetal development; FDA = Food and Drug Administration; GALT = gut-associated lymphoid tissue; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; IV = intravenous; NA = not applicable; NaCl = sodium chloride; No. = number; NOAEL = no-observed-adverse-effect-level; PK = pharmacokinetic; QTc = corrected QT interval; TK = toxicokinetic; USP = United States Pharmacopeia.

Table 4–20. Summary of ETX2514 toxicology and secondary pharmacology studies (continued)

Study No.	Status	Short Title	NOAEL (mg/kg/day)	AUC (µg·h/mL) /Margin	C_{max} (µg/mL) /Margin	Main Findings
SN002514-2015-05	Complete	*In vitro* Bacterial Reverse Mutation Assay	NA	NA	NA	• ETX2514 was negative in the assay under the conditions, and according to the criteria, of the test protocol.
SN002514-2015-06	Complete	*In vitro* Human Lymphocyte Micronucleus Assay	NA	NA	NA	• ETX2514 was considered negative for inducing micronuclei in cultured human peripheral blood lymphocytes, with and without metabolic activation under the conditions utilized in this study.
SN002514-2015-07	Complete	*In vivo* Rat Bone Marrow Micronucleus Assay	2000	3122/6.2X	1340/36X	• ETX2514 was negative in the rat bone marrow micronucleus assay under the conditions of this assay.

Phase 2 clinical exposure used for margin calculation: AUC_{0-24} = 501 µg·h/mL, C_{max} = 37 µg/mL.

AUC = area under the plasma concentration-time curve; AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; CNS = central nervous system; CV = cardiovascular; DART = development and reproductive toxicology; ECG = electrocardiogram; EFD = embryofetal development; FDA = Food and Drug Administration; GALT = gut-associated lymphoid tissue; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; IV = intravenous; NA = not applicable; NaCl = sodium chloride; No. = number; NOAEL = no-observed-adverse-effect-level; PK = pharmacokinetic; QTc = corrected QT interval; TK = toxicokinetic; USP = United States Pharmacopeia.

4.4.2 Single-dose toxicity

Nonpivotal single-dose IV infusion studies to establish a maximum tolerated dose of ETX2514 were conducted in rats and dogs at doses up to the limit dose of 2000 mg/kg. No dose-limiting toxicity was noted in these studies, and in subsequent repeat-dose studies, the NOAEL remained 2000 mg/kg/day.

4.4.3 Repeat-dose toxicity

4.4.3.1 7-Day tolerability study of ETX2514 in rats

Groups of 5 Sprague-Dawley rats per sex were administered ETX2514 daily at 0 (0.9% sterile saline), 1000, or 2000 mg/kg/day (limit dose) for 2 hours via IV infusion for 7 days (Study Report SN002514-2015-01). Toxicity was assessed based on mortality, clinical observations, food consumption, and clinical and anatomic pathology.

Table 4–21 presents TK parameters for rats given daily IV infusions of ETX2514 for 7 days.

Table 4–21. Mean TK parameters in rats given daily IV infusions of ETX2514 for 7 days

Interval	Dose Level (mg/kg/day)	C_{max} (µg/mL)		AUC_{0-t} (µg·h/mL)	
		Males	Females	Males	Females
Day 1	1000	761	703	1830	1590
	2000	1530	1890	3940	4390
Day 7	1000	1000	773	1850	1910
	2000	2100	1440	4840	3840

AUC_{0-t} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; IV= intravenous; TK = toxicokinetic.

There was no mortality during the dosing period, and there were no clinical observations or changes in body weight in any group. Slight to minimal decreases in food consumption were observed for animals given 1000 or 2000 mg/kg/day. ETX2514-related clinical pathology changes were limited to minimally increased alanine aminotransferase (ALT) activities in animals given ≥1000 mg/kg/day that correlated with minimal hepatocellular hypertrophy in animals given 2000 mg/kg/day and were considered nonadverse. Based on the lack of significant clinical observations, lack of body weight changes, and the mild clinical and anatomic pathology findings, the NOAEL is considered to be 2000 mg/kg/day. The associated combined-sex steady state mean C_{max} and AUC from 0 to the time of the last

quantifiable concentration was observed (AUC_{0-t}) at the NOAEL on Day 7 were 1770 µg/mL and 4340 µg·h/mL, respectively.

4.4.3.2 Definitive 14-day study of ETX2514 in rats

Groups of 10 Sprague-Dawley rats per sex were administered ETX2514 daily at 0 (0.9% sterile saline), 500, 1000, or 2000 mg/kg/day (limit dose) for 2 hours via IV infusion for 14 days (Study Report SN002514-2015-03). The study was conducted in compliance with US Food and Drug Administration (FDA) GLP for Nonclinical Laboratory Studies.

TK parameters are presented in Table 4–22. On both Days 1 and 14, C_{max} and AUC_{0-24} increased proportionally with increasing dose in males and females, and there was no difference in exposure or accumulation with repeated dosing between males and females.

Table 4–22. Mean TK parameters in rats given daily IV infusions of ETX2514 for 14 days

Interval	Dose Level (mg/kg/day)	C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
		Males	Females	Males	Females
Day 1	500	296	296	735	738
	1000	710	619	1710	1510
	2000	1460	1110	3540	2820
Day 14	500	326	238	715	NC
	1000	823	621	1770	1290
	2000	1480	1200	3430	2850

AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; IV= intravenous; NC = not calculated due to insufficient data; TK = toxicokinetic.

There was no mortality in the study; no remarkable ophthalmic observations; and no toxicologically significant effects on clinical chemistry, hematology, or urinalysis. Clinical observations included yellow urine discoloration in all groups dosed with ETX2514, and mildly decreased body weight and food consumption in males administered 2000 mg/kg/day. Treatment-related changes in organ weights were limited to high-dose males and were associated with decreased body weight; the changes were not considered adverse.

Microscopic changes related to ETX2514 treatment were minimal to slight edema and/or minimal to slight hemorrhage in the lamina propria in the ceca of animals dosed with ≥500 mg/kg/day, and slight congestion of the cecum noted in 1 female dosed with 1000 mg/kg/day. These findings were generally correlated with the macroscopic observation

of cecal enlargement in animals dosed with ≥500 mg/kg/day. No ETX2514-related microscopic findings were present at the catheter or infusion sites of the animals.

The NOAEL is 2000 mg/kg/day based on the minimal clinical observations; mild decrease in body weight and food consumption in males at the highest dose; mild, nonadverse clinical pathology findings; and the absence of toxicologically significant pathology changes. The associated combined-sex steady state mean C_{max} and AUC_{0-24} values at the NOAEL on Day 14 were 1340 µg/mL and 3140 µg·h/mL, respectively.

4.4.3.3 7-day tolerability study of ETX2514 in dogs

One male and 1 female beagle dog per group were administered ETX2514 at doses of 500, 1000, or 2000 mg/kg daily for 2 hours via IV infusion for 7 days (Study Report SN002514-2015-02). No vehicle-only (0.9% sterile saline control) animals were included in this study.

Table 4–23 presents TK parameters in dogs given daily IV infusions of ETX2514 for 7 days. On both Days 1 and 7, C_{max} and AUC_{0-t} increased approximately dose proportionally in males and females. No accumulation was observed in either males or females, and there were no differences in exposure between males and females.

Table 4–23. Mean TK parameters in dogs given daily IV infusions of ETX2514 for 7 days

Interval	Dose Level (mg/kg/day)	C_{max} (µg/mL)		AUC_{0-t} (µg·h/mL)	
		Males	Females	Males	Females
Day 1	500	768	767	1690	1800
	1000	1300	1590	3010	3690
	2000	3080	3770	9200	9500
Day 7	500	717	727	1760	1850
	1000	1370	1510	3360	3640
	2000	3060	3040	7830	7640

AUC_{0-t} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; IV = intravenous; TK = toxicokinetic.

There was no mortality in the study. Intermittent tremors were noted in all animals. Animals dosed with 1000 mg/kg/day displayed decreased activity and red pinna (female only), and clinical signs noted at 2000 mg/kg/day included emesis, decreased activity, lethargy,

salivation, and red pinna. There were no ETX2514-related effects on body weight, food consumption, clinical pathology parameters, or macroscopic or microscopic findings.

Based on the lack of significant (dose-limiting) clinical observations, lack of body weight changes, and the absence of macroscopic and microscopic pathology findings, the NOAEL is considered to be 2000 mg/kg/day. The associated combined-sex mean steady state C_{max} and AUC_{0-t} values at 2000 mg/kg/day on Day 7 were 3050 µg/mL and 7740 µg·h/mL, respectively.

4.4.3.4 Definitive 14-day study of ETX2514 in dogs

Three beagle dogs per sex per group were administered ETX2514 daily at 0 (0.9% sterile saline), 500, 1000, or 2000 mg/kg/day (limit dose) for 2 hours via IV infusion for 14 days (Study Report SN002514-2015-04). The study was conducted in compliance with FDA GLP for Nonclinical Laboratory Studies.

Table 4–24 presents TK parameters in dogs given daily IV infusions of ETX2514 for 14 days. On both Days 1 and 14, C_{max} and AUC_{0-24} increased dose proportionally in males and females. No accumulation was observed in either males or females, and there were no differences in exposure between males and females.

Table 4–24. Mean TK parameters in dogs given daily IV infusions of ETX2514 for 14 days

Interval	Dose Level (mg/kg/day)	C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
		Males	Females	Males	Females
Day 1	500	627	619	1450	1460
	1000	1300	1160	3180	2760
	2000	2460	2150	6160	5160
Day 14	500	613	627	1380	1480
	1000	1230	1190	2900	2790
	2000	2550	2030	6300	4530

AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; C_{max} = maximum plasma concentration; IV = intravenous; TK = toxicokinetic.

There was no mortality in the study. Clinical observations were limited to sporadic excessive salivation/emesis/vomitus in animals dosed with 2000 mg/kg/day. There were no ETX2514-related ophthalmic changes, and no toxicologically significant effects on body weight, body weight change, or food consumption.

ETX2514 treatment was associated with lower heart rate on Day 13 of dosing in males (-39 beats per minute [bpm]; 31% reduction) and females (-26 bpm; 21% reduction) dosed with 2000 mg/kg/day when compared to controls. Sinus bradycardia was noted in 1 male dosed with 2000 mg/kg/day. No other ETX2514-related ECG changes were noted.

There were no toxicologically significant effects of ETX2514 on clinical pathology parameters, and no clear ETX2514-related effects on urinalysis were identified.

ETX2514-related microscopic findings were limited to minimally to slightly decreased cellularity of the sternum marrow and lymphoid depletion of the gut-associated lymphoid tissue/Peyer's patch of males given ≥500 mg/kg/day, and minimal lymphoid depletion of the mandibular and mesenteric lymph nodes of males given 2000 mg/kg/day. There were no correlates to these changes observed in peripheral hematology parameters. There were no toxicologically significant microscopic changes noted in females at any dose. Additionally, no ETX2514-related microscopic findings were present at the dosing (catheter or infusion) sites in any of the control or treated animals.

Based on the transient nature of clinical observations and minimally decreased heart rates in animals dosed with 2000 mg/kg/day, minor decreases in body weight change and food consumption in males dosed with 2000 mg/kg/day, lack of toxicologically significant clinical pathology changes, and minimal microscopic findings, which were not considered adverse, the NOAEL was considered to be 2000 mg/kg/day. The associated combined-sex mean steady state C_{max} and AUC_{0-24} values on Day 14 at the NOAEL were 2290 µg/mL and 5420 µg·h/mL, respectively.

4.4.3.5 1-week toxicity study of sulbactam/ETX2514 in rats

The toxicity and TK parameters of sulbactam alone or in combination with ETX2514 were evaluated in Sprague-Dawley rats (Study Report PC2514-2017-0016). Animals (N = 5/sex/group for the main study and 3 to 6/sex/group for the TK evaluation) were administered vehicle (0.9% sterile saline), sulbactam alone (100, 200, or 400 mg/kg/day), or ETX2514 plus sulbactam (100, 200, or 400 mg/kg/day of each test article in combination) for 2 hours via IV infusion daily for 7 days.

Table 4–25 and Table 4–26 present sulbactam and ETX2514 TK parameters and show that C_{max} and AUC_{0-t} ($AUC_{0-tlast}$) increased proportionally with increasing dose in males and females, and there was no difference in exposure or accumulation with repeated dosing between males and females.

Table 4–25. Mean TK parameters of sulbactam in rats given daily IV infusions of sulbactam alone or in combination with ETX2514 for 7 days

Interval	Sulbactam Dose Level (mg/kg)	ETX2514 Dose Level (mg/kg)	Sex	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-}tlast}$ (µg·h/mL)
	100	0	MF	42.0	0.5	96.9
	200	0	MF	79.4	0.5	177
	400	0	MF	139	NC	319
	100	100	MF	45.3	NC	98.7
	200	200	MF	84.2	0.5	194
Day 1	400	400	MF	175	0.5	433
	100	0	MF	43.3	0.5	90.4
	200	0	MF	69.0	0.5	165
	400	0	MF	148	NC	302
	100	100	MF	41.6	NC	96.0
	200	200	MF	105	0.5	240
Day 7	400	400	MF	188	0.5	433

$AUC_{0\text{-}tlast}$ = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; IV= intravenous; MF = male and female data combined; NC = not calculated due to insufficient data; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

Table 4–26. Mean TK parameters of ETX2514 in rats given daily IV infusions of ETX2514 in combination with sulbactam for 7 days

Interval	Sulbactam Dose Level (mg/kg)	ETX2514 Dose Level (mg/kg)	Sex	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-}tlast}$ (µg·h/mL)
	100	100	MF	53.4	NC	119
	200	200	MF	98.3	0.5	202
Day 1	400	400	MF	191	0.5	473
	100	100	MF	52.7	NC	120
	200	200	MF	121	0.5	286
Day 7	400	400	MF	219	0.5	496

$AUC_{0\text{-}tlast}$ = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; IV = intravenous; MF = male and female data combined; NC = not calculated due to insufficient data; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

There was no test article–related mortality, clinical or macroscopic observations, or changes in organ weights in the study. The deaths of 3 animals in the TK evaluation group that were administered the 400 mg/kg/day sulbactam/ETX2514 combination were attributed to blood sampling and had no relationship to the test articles.

Minor decreases in body weight gain were noted in animals administered sulbactam alone or in combination with ETX2514, and decreases in food consumption were noted in animals administered 400 mg/kg/day sulbactam/ETX2514. Along with clinical pathology changes including lower absolute neutrophil count, higher absolute lymphocyte and absolute basophil counts, and higher albumin concentration were noted but not considered adverse due to their small magnitude. The NOAEL for the 7-day combination treatment was 400 mg/kg/day sulbactam/ETX2514 due to the mild severity of the findings and the lack of impact on animal health and wellbeing. This dose level corresponded to sex-combined mean C_{max} and $AUC_{0\text{-}t}$ ($AUC_{0\text{-}tlast}$) values of 188 µg/mL and 433 µg·h/mL, respectively, for sulbactam and 219 µg/mL and 496 µg·h/mL, respectively, for ETX2514 on Day 7.

4.4.3.6 28-day toxicity study of sulbactam/ETX2514 in rats

The toxicity of ETX2514 alone (600 mg/kg/day) or in combination with sulbactam (300 mg/kg/day or 600 mg/kg/day of each compound in combination) was evaluated in male and female Sprague-Dawley rats when administered for approximately 2 hours/day via intermittent IV infusion for 28 days (15/sex/group) (Study Report PC2514-2017-0018). Control rats were dosed with 0.9% NaCl. Assessment of toxicity was based on mortality, clinical and ophthalmic observations, body weights, food consumption, and clinical and anatomic pathology. Assessment of delayed onset and/or reversibility of toxicity was made during a 4-week recovery period. The TK profiles of ETX2514 and sulbactam were also assessed in satellite groups of animals. There was no test article–related mortality in the study, and no adverse clinical signs or ophthalmic effects were noted in any treatment group.

Table 4–27 and Table 4–28 present TK parameters for ETX2514 and sulbactam, respectively, in rats given daily IV infusions of ETX2514 alone or in combination with sulbactam for 28 days. On both Days 1 and 28, C_{max} and $AUC_{0\text{-}t}$ ($AUC_{0\text{-}tlast}$) increased proportionally with increasing dose in males and females, and there was no difference in exposure or accumulation with repeated dosing between males and females.

Table 4–27. Mean TK parameters of ETX2514 in rats given daily IV infusions of ETX2514 alone or in combination with sulbactam for 28 days

Interval	Sulbactam Dose Level (mg/kg/day)	ETX2514 Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-t} (µg·h/mL)
	600	0	MF	247	2.25	441
	300	300	MF	146	2.25	247
Day 1	600	600	MF	315	2.25	530
	600	0	MF	245	2.25	439
	300	300	MF	134	2.25	223
Day 28	600	600	MF	242	2.25	470

AUC_{0-t} = Area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; IV = intravenous; MF = male and female data combined; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

Table 4–28. Mean TK parameters of sulbactam in rats given daily IV infusions of sulbactam in combination with ETX2514 for 28 days

Interval	Sulbactam Dose Level (mg/kg/day)	ETX2514 Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-t} (µg·h/mL)
	600	0	MF	NC	NC	NC
	300	300	MF	116	2.25	193
Day 1	600	600	MF	275	2.25	451
	600	0	MF	NC	NC	NC
	300	300	MF	120	2.25	201
Day 28	600	600	MF	237	2.25	450

AUC_{0-t} = Area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; IV = intravenous; MF = male and female data combined; NC = not calculated due to insufficient data; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

ETX2514 administered alone at 600 mg/kg/day was devoid of toxicity, and notable effects in rats treated with the combination of sulbactam/ETX2514 were consistent with known effects of sulbactam. ETX2514 in combination with sulbactam produced nonadverse reductions in body weight and food consumption, and nonadverse changes in hematology, clinical chemistry, and urinalysis parameters that were generally dose related and completely reversible. Combination treatment and dose-related reversible increases in liver weights were associated with glycogen accumulation, a known effect of sulbactam. Nonadverse microscopic changes were noted in spleen, bone marrow (femur and sternum), kidney, and thyroid at the end of the dosing phase; these changes were completely reversed by the end of the recovery period. Dose-related inflammatory changes were noted microscopically at the

infusion site and in liver and lung. Inflammation was considered an adverse effect of treatment; however, inflammatory changes in liver and lung were not associated with necrosis and were not present at the end of the recovery period, demonstrating reversibility.

Based on adverse inflammatory changes noted at both combination dose levels in liver and lung at the end of the treatment period, a NOAEL was not identified. However, the inflammatory changes were minimal to moderate, were not associated with necrosis, were completely reversible, and occurred only after a treatment duration of 28 days (double the intended duration of clinical treatment). Systemic exposures (C_{max} and AUC_{0-t} [$AUC_{0-tlast}$]) to ETX2514 on Day 28 in the combination arms of the study ranged from 134 to 242 µg/mL and 223 to 470 µg·h/mL for C_{max} and AUC_{0-t}, respectively, and corresponding exposures to sulbactam ranged from 120 to 231 µg/mL and 201 to 450 µg·h/mL, for C_{max} and AUC_{0-t}, respectively. Relative to efficacious human clinical exposures, the C_{max} values of ETX2514 in the combination arms were 3.6- to 6.5-times higher than human exposure, and the C_{max} values of sulbactam ranged from 3.5- to 6.8-fold higher than human exposure. Exposure margins based on AUC_{0-24} compared to human exposures ranged from 0.4- to 1.0-fold for ETX2514 and 0.8- to 1.8-fold for sulbactam.

4.4.4 Safety pharmacology

Cardiovascular safety pharmacology of ETX2514 was evaluated *in vitro* (effects on ion channels, including hERG) (Study Report PC2514-2017-0012) and *in vivo* in telemetered dogs (Study Report SN-002514-2015-08). Pulmonary (Study Report PC2514-2017-0015) and central nervous system (CNS) (Study Report PC2514-2018-0002) safety pharmacology assessments of ETX2514 were conducted in Sprague-Dawley rats.

A non-GLP *in vitro* study of the effects of ETX2514 on cardiac ion channels was conducted using electrophysiologic measurement of ion flux through recombinant voltage-gated channels expressed in mammalian cells. The channels tested included: hCav1.2/β2/α2δ (ICaL), hCav3.2 (ICaT), hHCN4 (IF), hKv1.5 (IKUR), hKv11.1 (hERG; IKR), hKv4.3/hKChIP2.2 (ITO), hKv7.1/hKCNE1 (IKS) and hNav1.5 (INa); maximum concentrations tested ranged from 100 to 1000 µM. Potency (IC_{50}) values were calculated if ETX2514 induced a mean inhibitory effect on ion current of >50%. The study was carried out consistent with the Committee for Proprietary Medicinal Products Guidance on Safety Pharmacology Studies for Human Pharmaceuticals (The European Agency for the Evaluation of Medicinal Products, November 16, 2000), and appropriate sections of ICH S9: Note for Guidance on Nonclinical Evaluation for Anticancer Pharmaceuticals (December, 2008).

ETX2514 did not produce >50% inhibition of ion channel activity for any of the 8 human cardiac channels evaluated, including hKv11.1 (hERG), at up to the maximum concentrations tested, 100 to 1000 µM.

Potential *in vivo* cardiovascular effects of ETX2514 were evaluated in male beagle dogs (N = 4/group) given 2-hour IV infusions of vehicle (0.9% NaCl for injection) or ETX2514 at 500, 1000, and 2000 mg/kg using a Latin-Square crossover design. The study was conducted in compliance with US FDA GLP regulations. Animals were surgically implanted telemetry devices and each animal received each treatment with 3 to 4 days between doses. Hemodynamic parameters (body temperature, heart rate, diastolic, systolic, mean arterial blood pressure, and arterial pulse pressure) and ECG parameters (PR, QTc, and QRS intervals) were continuously monitored for 90 minutes before and at least 20 hours after the start of infusion.

IV administration of ETX2514 to male beagle dogs at doses up to the limit dose of 2000 mg/kg did not result in any ETX2514-related deaths or body weight changes, but produced minor, nonadverse increases in body temperature at ≥1000 mg/kg (0.6 to 1.1°C). No treatment-related qualitative ECG effects or quantitative changes in PR interval, QRS duration, QT interval, QTc, heart rate, or arterial pressures were noted. Thus, ETX2514 infusion did not produce any adverse effects on cardiovascular function in male beagle dogs given single doses up to the limit dose of 2000 mg/kg. Systemic exposures were not assessed in this study; however, in the 14-day repeat-dose toxicology study in dogs (Study Report SN002514-2015-04), mean Day 1 C_{max} and AUC_{0-24} in males at 2000 mg/kg were 8900 µM and 22,200 µM·h, respectively.

The effects of ETX2514 on pulmonary function were assessed using whole body plethysmography in conscious male rats (N = 8/group). ETX2514 administered via infusion at doses up to the limit dose of 2000 mg/kg/day produced no mortality or abnormal clinical observations, and there were no ETX2514-related effects on tidal volume, respiration rate, or minute volume at any dose through 24 hours postdose. In the 14-day repeat-dose toxicology study in rats (Study Report SN002514-2015-03), the associated combined-sex steady state mean C_{max} and AUC_{0-24} values at the NOAEL on Day 1 were 1285 µg/mL and 3180 µg·h/mL, respectively.

Neurologic (CNS) safety pharmacology of ETX2514 administered as a single dose via IV infusion was evaluated in male rats (N = 8/group) administered doses up to the limit dose of 2000 mg/kg. CNS effects were measured using a modified Irwin battery of neurological function tests. No mortality, morbidity, or abnormal clinical observations were noted. No

ETX2514-related effects were noted on any component of the modified Irwin battery of testing (including body temperature). In the 14-day repeat-dose toxicology study in rats (Study Report SN002514-2018-03), the associated combined-sex steady state mean C_{max} and AUC_{0-24} values at the NOAEL on Day 1 were 1285 µg/mL and 3180 µg·h/mL, respectively.

The results of the nonclinical safety pharmacology studies are consistent with absence of significant acute CNS, pulmonary, or cardiovascular effects noted in Phase 1 clinical populations.

4.4.4.1 Cardiovascular study of sulbactam as a component of ampicillin/sulbactam in dogs

Ampicillin/sulbactam was administered by 15-minute infusion to 4 anesthetized beagle dogs at a dose of 67 mg/kg of sulbactam and 133 mg/kg of ampicillin. After a 10-minute interval, ampicillin/sulbactam was administered by infusion to the same dogs at a dose of 124 mg/kg sulbactam and 266 mg/kg of ampicillin. No significant changes in ECG, mean blood pressure, or heart rate were reported during this study.

4.4.5 Genotoxicity

The genotoxic potential of ETX2514 was assessed in a series of assays that evaluated mutagenicity in bacteria, deoxyribonucleic acid (DNA) damage in human lymphocytes *in vitro*, and DNA damage in polychromatic erythrocytes (PCE) in rat bone marrow *in vivo*. Study designs and dose selection were consistent with ICH and Organization for Economic Cooperation and Development guidances, and the studies were conducted in compliance with FDA GLP for Nonclinical Laboratory Studies. *In vitro* tests were conducted with and without exogenous metabolic activation at drug concentrations up to those limited by cytotoxicity or solubility.

4.4.5.1 *In vitro* bacterial reverse mutation assay

ETX2514 was not mutagenic in bacteria. ETX2514 was evaluated in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in the presence or absence of an exogenous mammalian metabolic activation system (Aroclor™-induced rat liver S9) (Study Report SN002514-2015-05). The study was conducted in compliance with FDA GLP for Nonclinical Laboratory Studies. At concentrations of ETX2514 ranging from 0.000160 to 5 µg/plate, ETX2514 did not produce positive increases in reverse mutations with any of the tester strains in the presence or absence of S9.

4.4.5.2 *In vitro* micronucleus assay

ETX2514 did not induce micronuclei in cultured human peripheral blood lymphocytes with and without metabolic activation. Cultured human peripheral lymphocytes using whole blood cultures and cytochalasin B block methodology, with and without Aroclor-induced rat liver S9 was used to assess *in vitro* micronuclei induction by ETX2514 (Study Report SN002514-2015-06). The study was conducted in compliance with FDA GLP for Nonclinical Laboratory Studies. The range of ETX2514 concentrations tested was 2.20 to 325 µg/mL. No statistically significant increase in the number of lymphocytes with micronuclei was observed in any of the cultures treated with ETX2514 with or without metabolic activation by S9.

4.4.5.3 *In vivo* micronucleus assay

ETX2514 was negative in the rat bone marrow micronucleus assay.

The *in vivo* clastogenic activity of ETX2514 was evaluated by measuring its ability to induce micronuclei in PCE in rat bone marrow (Study Report SN002514-2015-07). The study was conducted in compliance with FDA GLP for Nonclinical Laboratory Studies, and consistent with the ICH Technical Requirements for Registration of Pharmaceuticals for Human Use Guidance on Genotoxicity Testing and Data Interpretation S2 (R1). Groups of 12 (vehicle control and high-dose) or 6 (low- and mid-dose) rats were administered ETX2514 dissolved in sterile Water for Injection by IV infusion over a period of approximately 2 hours. A separate group of 3 male Sprague-Dawley rats were dosed via oral gavage with 60 mg/kg cyclophosphamide as the positive control. The micronucleus frequency (expressed as the percentage of micronucleated cells) was determined in bone marrow smears prepared 24 or 48 hours after dosing by analyzing the number of micronucleated PCEs from at least 4000 PCEs per animal.

There was no mortality and no clinical signs of severe toxicity at any dose evaluated in the study. Cyclophosphamide induced a statistically significant increase in PCE with micronuclei compared to the vehicle controls, supporting the validity of the study. ETX2514 did not induce statistically significant increases in micronucleated PCEs at any dose given (500, 1000, or 2000 mg/kg). Additionally, ETX2514 was not cytotoxic to bone marrow at any dose (i.e., no effect on PCE:normochromatic erythrocyte ratio).

4.4.6 Reproductive toxicology

Reproductive toxicology studies with ETX2514 using the IV infusion route assessing EFD and fertility have been completed in the rat. A repeat-dose tolerability study in the rabbit has shown the rabbit is an unsuitable species for EFD testing. The second species EFD work has not been completed yet.

4.4.6.1 Female fertility and EFD in the rat

Four groups of 25 female Sprague-Dawley rats were administered 0 (control article), 100, 300, or 1000 mg/kg/day ETX2514 by IV infusion daily for up to 46 days (covering the period from 2 weeks prior to pairing, during the pairing phase, and up to Gestation Day 17) (Study Report PC2514-2017-0019). Four groups of 25 male Sprague-Dawley rats were used for mating and were not dosed.

Assessment of toxicity was based on clinical observations, body weights, food consumption, estrous cycles, mating, fertility, and pregnancy indices. The progress and outcome of pregnancy were evaluated, and fetuses were examined for malformations and variations. Blood for TK evaluation was collected on Pre-Pairing Day 1 and on Gestation Day 17.

Exposure, as assessed by ETX2514 C_{max} and AUC_{0-24}, increased with the increase in dose level from 100 to 1000 mg/kg/day on Day 1 of the pre-pairing phase and on Gestation Day 17. The increases in C_{max} and AUC_{0-24} values were roughly dose proportional. Table 4–29 summarizes TK parameters of ETX2514 in pregnant rat plasma. Day 1 exposures were based upon a suboptimal bleeding schedule and thus appear much lower than Day 17. As steady state exposures on Day 17 are most relevant, safety margins should be considered relative to those TK results. For doses of 100 to 1000 mg/kg/day, C_{max} and daily AUC ranged from 70.1 to 614 µg/mL and 149 to 1600 µg·h/mL, respectively.

No test article–related mortality or clinical observations occurred. Between Pre-Pairing Days 1 and 4, the mean body weight gain showed a statistically significant, dose-related increase for animals administered 1000 mg/kg/day. No effect on mean maternal body weight was noted during gestation. Between Pre-Pairing Days 1 and 4, mean food consumption was statistically significantly reduced for all ETX2514-treated groups compared with controls. In addition, statistically significantly increased food consumption was recorded between Gestation Days 18 and 20 for animals administered 300 or 1000 mg/kg/day.

There were no ETX2514-related effects on estrous cycles, mating, fertility, or fecundity indices, and no test article–related changes to pregnancy parameters. The mean number of corpora lutea, the number of live fetuses/female, the percentage of male fetuses, and mean fetal weights were unaffected by ETX2514.

In controls and groups administered 100, 300, or 1000 mg/kg/day, 17, 25, 24, and 22 litters were examined at necropsy, with a total of 237, 359, 349, or 311 fetuses, respectively. No fetal malformations or variations were attributed to ETX2514.

In conclusion, administration of up to 1000 mg/kg/day ETX2514 was well tolerated by female rats. No effect on mating, fertility, or the progress or outcome of pregnancy was noted, and no test article–related malformations or variations were observed in the study. Therefore, the NOAEL for fertility, maternal toxicity, and EFD was 1000 mg/kg/day. ETX2514 C_{max} and AUC exposures at 1000 mg/kg/day of 614 µg/mL and 1600 µg·h/mL, respectively, were 17-fold and 3.2-fold higher than clinical efficacious exposures.

Table 4–29. Mean TK parameters of ETX2514 in pregnant rat plasma

Interval	Dose Level (mg/kg/day)	C_{max} (µg/mL)	T_{max} (h) [1]	AUC_{0-t} (µg·h/mL) [2]
Day 1	100	14.0	3.00	29.2
	300	23.3	3.00	50.2
	1000	155	3.00	32.4
Day 17	100	70.1	1.92	149
	300	244	1.92	497
	1000	614	1.92	1600

Day 1 was Day 1 of the pre-pairing phase. Day 17 was Gestation Day 17.

1. T_{max} values relative to the start of the infusion on each sampling interval.
2. Concentrations were measurable through 9 hours post the start of the infusion on Day 1 and were measureable through 7 hours post the start of the infusion on Day 17.

AUC_{0-t} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

4.4.6.2 Male fertility in the rat

Four groups of 20 male Sprague-Dawley rats were administered 0 (control article), 100, 300, or 1000 mg/kg/day by IV infusion for up to 21 days (covering the period 2 weeks prior to pairing and during the pairing phase until confirmation of mating). Four groups of 20 female Sprague-Dawley rats were used for mating and were not dosed

(Study Report PC2514-2018-003). Males were retained after the completion of dosing until the outcome of the female pregnancies was confirmed.

Assessment of toxicity was based on clinical observations; body weights; food consumption; and mating, fertility, and pregnancy indices. Complete necropsies were performed on all animals, with a recording of macroscopic abnormalities for all tissues. The progress and outcomes of pregnancies were evaluated. Blood for TK evaluation was collected at Pre-Pairing Day 14 and for exposure confirmation at 3 hours after the end of infusion on Day 21 of dosing.

There were deaths in the study associated with the cannulation process used to administer the daily dose to each of animal. Three decedents were noted in the control group. Four, 2, and 1 decedents were noted in the groups administered 100, 300, or 1000 mg/kg/day, respectively. There were no ETX2514-associated clinical observations. Observations of sores/lesions on the tail (associated with the infusion port tail cuff) were recorded across all groups, including controls.

Dose-related, nonadverse decreases in mean body weight gain and food consumption were noted during the pre-pairing phase and/or early in the dosing phase.

Male mating, fertility, and fecundity were unaffected by treatment with ETX2514. No effect of administration of ETX2514 to male rats was noted on pregnancy outcomes or male reproductive organ weights.

In conclusion, administration of up to 1000 mg/kg/day ETX2514 to male Sprague-Dawley rats was well tolerated. No test article–related mortality occurred, and no clinical observations were noted. A transient reduction in food consumption at all dose levels and lower body weight gain were considered not adverse. There was no effect on mating behavior, fertility, or fecundity. Thus, the NOAEL for male fertility is 1000 mg/kg/day. Table 4–30 presents the mean TK parameters of ETX2514 in male rats. ETX2514 C_{max} and AUC exposures at 1000 mg/kg/day of 617 µg/mL and 1220 µg·h/mL, respectively, were 17- and 2.4-fold higher than clinical efficacious exposures.

Table 4–30. Mean TK parameters of ETX2514 in male rat fertility study

Interval	Dose Level (mg/kg/day)	C_{max} (µg/mL)	T_{max} (h) [1]	AUC_{0-t} (µg·h/mL) [2]
Day 14	100	51.5	1.92	112
	300	137	1.92	282
	1000	617	1.92	1220

Day 14 was Day 14 of the pre-pairing phase.

1. T_{max} values relative to the start of the infusion on each sampling interval.
2. Concentrations were measurable through 7 hours post the start of the infusion on Day 14 of the pre-pairing phase.

AUC_{0-t} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

4.4.6.3 Preliminary study in nonpregnant rabbit

Three groups of 3 nonpregnant female New Zealand White rabbits were administered 150, 500, or 750 mg/kg/day (75, 250, or 375 mg/kg/twice daily [BID]) by IV infusion (administered as two 1-hour infusions 4 hours apart) BID for up to 10 consecutive days (Study Report PC2514-2018-0005).

The 150 and 750 mg/kg/day dosing groups were terminated early due to lack of tolerability. There was a marked reduction of food consumption over the 6 days of dosing at 500 and 750 mg/kg/day (down to only approximately 7% and 2.5% of predose values, respectively). Significant body weight loss was also recorded in both groups. Due to the severity of these effects, the group administered 750 mg/kg/day was terminated on Day 8 of study. The 3 animals administered 150 mg/kg/day were euthanized on Day 8 of the dosing phase due to clinical intolerance. All 3 animals had a large amount of red staining on the cage tray liner on Day 8, which was assumed to be blood. As a result, further evaluation of female fertility and EFD will be conducted in mice.

Table 4–31 summarizes the mean TK parameters of ETX2514 in nonpregnant rabbit.

Table 4–31. Mean TK parameters of ETX2514 in nonpregnant rabbit

Interval	ETX2514 Dose Level (mg/kg)	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-tlast}}$ (µg·h/mL)
	0	0	0	0
	75 + 75 [1]	187 ± 31	3.59 ± 2.31	375 ± 106
	250 + 250	664 ± 52	0.92 ± 0.00	1340 ± 85
Day 1	375 + 375 [2]	778 ± 125	2.25 ± 2.31	1720 ± 347
	0	0	0	0
	75 + 75 [1]	80 ± 57	3.95 ± 1.69	69 ± 21
	250 + 250	582 ± 83	3.59 ± 2.31	1210 ± 168
Day 8/10	375 + 375 [2]	897 ± 50	4.92 ± 0.00	1710 ± 149

1. Group 3 (75 + 75 mg/kg) animals were terminated following the collection of blood samples and completion of the second daily dose on Day 8 of dosing.
2. Group 2 (375 + 375 mg/kg) animals were terminated on Day 8 of dosing.

$AUC_{0\text{-tlast}}$ = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; TK = toxicokinetic; T_{max} = time to reach maximum plasma concentration.

4.4.7 Sulbactam

As described in the product label for Unasyn (ampicillin/sulbactam), conventionally designed nonclinical repeat-dose toxicity studies in animals did not demonstrate special hazard for humans during administration of ampicillin/sulbactam (Unasyn Product Label).

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5 EFFECTS IN HUMANS

As of November 2018, 268 humans have been exposed to placebo or at least 1 dose of ETX2514 (alone or in combination with sulbactam and/or imipenem/cilastatin) in clinical studies, including 132 healthy volunteers and 79 patients with renal insufficiency or with cUTI or pyelonephritis. All studies have been unblinded, and these data represent the actual number of individuals exposed to ETX2514. Table 5–1 summarizes the clinical studies of ETX2514 conducted to date.

Table 5–1. Summary of ETX2514SUL clinical studies

Study Number and Description	Study Design	Status	Route/Dose	Treatment Duration	Study Population	Endpoints
CS2514-2016-0001 FIH including SAD, MAD, DDI	SAD: DB, R, PC	Completed dosing	SAD: IV ETX2514 0.25, 0.5, 1, 2, 4, and 8 g plus elderly cohort 1 g	SAD: 3-hour IV infusion (except a single cohort 1 g 2-hour IV infusion)	SAD: 64 healthy adult subjects (40 M, 24 F) aged 19-74 years	PK (plasma and urine) Safety (includes effect of age and gender)
	MAD: DB, R, PC		MAD: IV ETX2514 0.25, 0.5, 1, 2, and 4 g	MAD: 3-hour IV infusion q6h for 8 days (29 doses)	MAD: 32 healthy adult subjects (21 M, 11 F) aged 19-40 years	
	DDI (single doses): Factorial, DB, R, PC		DDI: IV ETX2514 1 g ± 1 g sulbactam ± 0.5 g/0.5 g imipenem/cilastatin	DDI: ETX2514 3-hour IV infusion; sulbactam 3-hour IV infusion; imipenem/cilastatin 30-minute infusion	DDI: 16 healthy adult subjects (9 M, 7 F) aged 20-40 years	
	Long-term therapy: DB, R, PC		Long-term therapy: IV sulbactam-ETX2514 1 g/1 g plus imipenem/cilastatin 0.5 g/0.5 g	Long-term therapy: Sulbactam-ETX2514 3-hour IV infusion plus imipenem/cilastatin 30-minute infusion for 11 days (41 doses)	Long-term therapy: 12 healthy adult subjects (9 M, 3 F) aged 21-44 years	
CS2514-2017-0001 Lung penetration	OL	Completed dosing	IV sulbactam-ETX2514 1 g/1 g	3-hour IV infusion for 3 doses	30 healthy adult subjects (18 M, 12 F); mean age 41.7 years	PK (plasma ELF, and AM) Safety

AM = alveolar macrophage; cUTI = complicated urinary tract infection; DB = double-blind; DDI = drug-drug interaction; ELF = epithelial lining fluid; ETX2514SUL = sulbactam/ETX2514; F = female; FIH = first-in-human; IV = intravenous; M = male; MAD = multiple ascending dose; OL = open-label; PC = placebo-controlled; PG = parallel group; PK = pharmacokinetic; q6h = every 6 hours; R = randomized; SAD = single ascending dose.

Table 5–1. Summary of ETX2514SUL clinical studies (continued)

Study Number and Description	Study Design	Status	Route/Dose	Treatment Duration	Study Population	Endpoints
CS2514-2017-0002 Renal insufficiency study	OL, PG	Completed dosing	Normal, mild, and moderate renal function: IV sulbactam-ETX2514 1 g single dose Severe renal function: IV sulbactam-ETX2514 0.5 g single dose Hemodialysis: IV sulbactam-ETX2514 0.5 g pre- and post-dialysis	3-hour IV infusion	Healthy adult subjects and patients with renal insufficiency	PK (plasma and urine) Safety
CS2514-2017-0003 Phase 2 cUTI	DB, R, PC	Completed dosing	Active: IV sulbactam-ETX2514 1 g/1 g plus imipenem/cilastatin 0.5 g/0.5 g Comparator: IV placebo plus imipenem/cilastatin 0.5 g/0.5 g	Sulbactam-ETX2514/Placebo: 3-hour IV infusion q6h for 7 days Imipenem/cilastatin: 30-minute infusion q6h for 7 days Option to extend treatment up to 14 days if bacteremia	80 adult patients with cUTI requiring hospitalization (randomized 2:1 sulbactam-ETX2514:placebo)	Safety Efficacy PK (plasma)

AM = alveolar macrophage; cUTI = complicated urinary tract infection; DB = double-blind; DDI = drug-drug interaction; ELF = epithelial lining fluid; ETX2514SUL = sulbactam/ETX2514; F = female; FIH = first-in-human; IV = intravenous; M = male; MAD = multiple ascending dose; OL = open-label; PC = placebo-controlled; PG = parallel group; PK = pharmacokinetic; q6h = every 6 hours; R = randomized; SAD = single ascending dose.

5.1 PK and drug metabolism in humans

5.1.1 Study CS2514-2016-0001

ETX2514, either alone or in combination with sulbactam and/or imipenem/cilastatin, has been evaluated in an FIH study, "A Phase I, Double-blind, Randomized, Placebo-controlled Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Intravenous ETX2514 Administered in Healthy Subjects" (Study CS2514-2016-0001). All 124 healthy subjects (94 ETX2514; 30 placebo) aged 18 to 55 years (and 1 cohort of healthy subjects aged >65 years) were enrolled into the study and received at least 1 dose of ETX2514/placebo. This study evaluated SAD and MAD as well as assessed any clinically significant drug-drug interaction (DDI) between sulbactam and ETX2514 or ETX2514/sulbactam and imipenem/cilastatin.

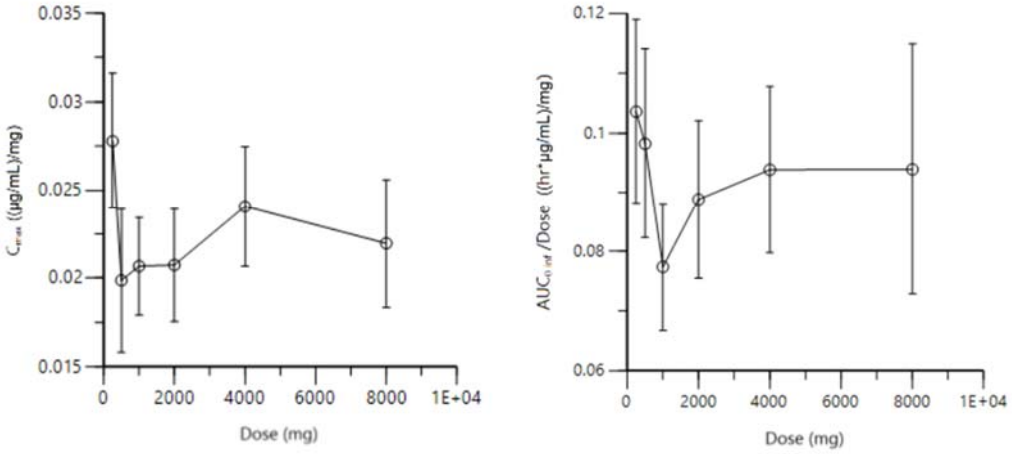
The study comprised 4 parts: Part A, SAD of ETX2514; Part B, MAD of ETX2514; Part C, single-dose DDI; and Part D, multiple-dose safety cohort. The cohorts were generally similar with respect to demographic parameters; the elderly cohort had a mean age of 69.8 years.

5.1.1.1 Results

5.1.1.1.1 PK data

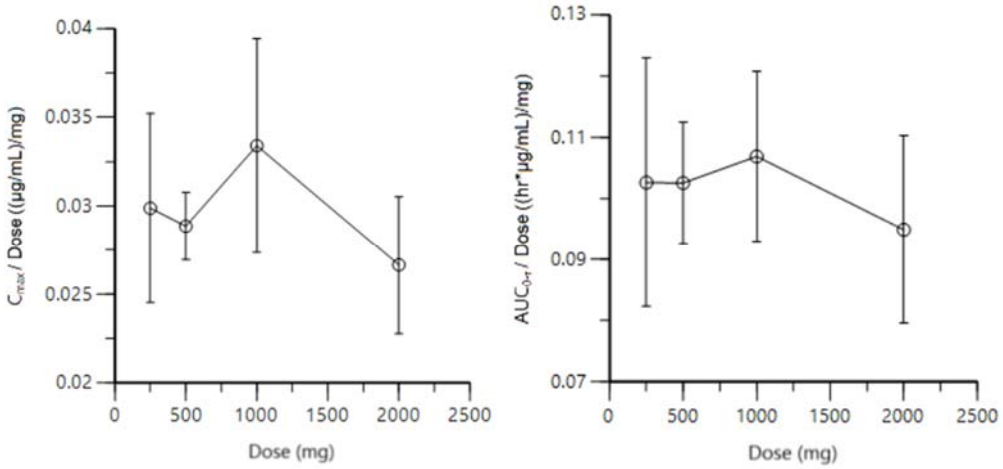
In Part A, SAD, 48 subjects (including 6 elderly subjects) received ETX2514 in single doses ranging from 0.25 to 8.0 g, and 16 subjects received placebo. In Part B, MAD, 24 subjects received ETX2514 in doses ranging from 0.25 to 2.0 g q6h for 7 days, with 1 dose on Day 8, and 8 subjects received placebo. ETX2514, as single and multiple doses, demonstrated linear dose proportional exposure across the dose range studied, with minimal accumulation at Day 8 in MAD cohorts. Table 5–2 and Table 5–3 present the key ETX2514 PK parameters from SAD and MAD dose escalation, respectively. Figure 5–1 and Figure 5–2 show the relationship between ETX2514 dose and dose-normalized C_{max} and AUC for the SAD and MAD periods, respectively.

Figure 5–1. Dose-normalized C_{max} and AUC versus ETX2514 dose in SAD escalation cohorts (Part A)



AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}inf}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; C_{max} = maximum plasma concentration; SAD = single ascending dose.

Figure 5–2. Dose-normalized C_{max} and AUC versus ETX2514 dose in MAD escalation cohorts (Part B)



AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}t}$ = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; C_{max} = maximum plasma concentration; MAD = multiple ascending dose.

Table 5–2. Key ETX2514 PK parameters from SAD escalation ranging from 0.25–8.0 g (Part A), including an elderly cohort (Cohort 8)

Treatment Group	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0-\infty}$ (h·µg/mL)	CL (mL/h)	V_{dss} (mL)
Cohort 1 ETX2514 0.25 g IV (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	1.45	2.83	6.95	12	25.5	9990	16,600
	SD	0.11	0.41	0.95	0.02	3.7	1570	3550
Cohort 2 ETX2514 0.5 g IV (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	2.04	2.92	9.93	18	47.7	10,700	25,800
	SD	0.405	0.49	2.03	6.57	7.56	1860	2850
Cohort 3 ETX2514 1.0 g IV (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	1.99	2.67	20.7	16	76.1	13,400	25,700
	SD	0.26	0.52	2.8	6.2	10.5	1850	4950
Cohort 4 ETX2514 1.0 g IV (2-hour infusion)	N	6	6	6	6	6	6	6
	Mean	2.23	2	31.3	22	101	10,600	22,700
	SD	0.23	0.01	6.9	4.9	28.9	2730	6350
Cohort 5 ETX2514 2.0 g IV (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	2.16	2.5	41.5	24	173	11,800	24,300
	SD	0.18	0.55	6.4	0.02	26	1890	3190
Cohort 6 ETX2514 4.0 g IV (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	2.71	3	96.2	26.028	368	11,100	22,400
	SD	0.58	0	13.6	4.895	55.9	1760	3550

$AUC_{0-\infty}$ = area under the curve from time of dosing extrapolated to infinity; CL = clearance; C_{max} = maximum plasma concentration; IV = intravenous; N = number of subjects; PK = pharmacokinetic; SAD = single ascending dose; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–2. Key ETX2514 PK parameters from SAD escalation ranging from 0.25–8.0 g (Part A), including an elderly cohort (Cohort 8) (continued)

Treatment Group	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0-\infty}$ (h·µg/mL)	CL (mL/h)	V_{dss} (mL)
Cohort 7 ETX2514 8.0 g IV (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	2.77	3.178	176	34.017	732	11,400	28,100
	SD	0.38	0.404	29	9.038	163	2340	9530
Cohort 8 ETX2514 1.0 g IV Elderly Subjects (3-hour infusion)	N	6	6	6	6	6	6	6
	Mean	2.4	2.333	37.8	24.033	151	6660	14,700
	SD	0.26	1.033	2.52	0.051	14	612	2000

$AUC_{0-\infty}$ = area under the curve from time of dosing extrapolated to infinity; CL = clearance; C_{max} = maximum plasma concentration; IV = intravenous; N = number of subjects; PK = pharmacokinetic; SAD = single ascending dose; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–3. Key ETX2514 PK parameters from MAD escalation ranging from 0.25–2.0 g (Part B)

	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{Tau} (h·µg/mL)	T_{last} (h)	AUC_{0-t} (h·µg/mL)	C_{last} (µg/mL)	$AUC_{0-\infty}$ (h·µg/mL)	C_{tau} (µg/mL)	C_{avg} (µg/mL)	CL_{ss} (mL/h)	V_{dss} (mL)	Acc. Index
Cohort 9: ETX2514 0.25 g IV q6h														
Day 1	N		6	6	6									
	Mean		2.833	6.89	23.1									
	SD		0.408	1.38	4.9									
Day 8	N	6	6	6	6	6	6	6	6	6	6	6	6	6
	Mean	1.87	2.5	7.47	26.2	14	29.4	0.084	29.6	1.26	4.36	9820	18,500	1.13
	SD	0.425	0.548	1.34	5.2	4.9	6.4	0.04	6.4	0.44	0.86	1650	2910	0.07
Cohort 10: ETX2514 0.5 g IV q6h														
Day 1	N		6	6	6									
	Mean		3	14.9	50.3									
	SD		0	2.2	9									
Day 8	N	4	4	4	4	4	4	4	4	4	4	4	4	4
	Mean	2.62	3	14.8	53.4	24	60.7	0.013	60.7	2.48	8.9	9420	18,100	1.26
	SD	0.05	0	1.1	4.8	0	5.4	0.002	5.4	0.25	0.8	90	1800	0.01
Cohort 11: ETX2514 1.0 g IV q6h														
Day 1	N		6	6	6									
	Mean		2.337	26.9	79.8									
	SD		0.81	13.1	35.9									
Day 8	N	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	3.51	2.8	33.4	109	31.2	123	0.016	123	4.78	18.1	9310	17,200	1.45
	SD	0.85	0.447	6	14	6.57	17	0.006	17	1	2.3	1143	1980	0.18

Acc. Index = accumulation index; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; AUC_{0-t} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; AUC_{Tau} = partial area under the plasma concentration-time curve from time of dosing to tau; C_{avg} = average concentration; C_{last} = observed concentration corresponding to time to last concentration; CL_{ss} = estimate of total body clearance; C_{max} = maximum plasma concentration; C_{tau} = concentration at time of dosing plus tau; IV = intravenous; MAD = multiple ascending dose; N = number of subjects; PK = pharmacokinetic; q6h = every 6 hours; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–3. Key ETX2514 PK parameters from MAD escalation ranging from 0.25–2.0 g (Part B) (continued)

	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{Tau} (h·µg/mL)	T_{last} (h)	AUC_{0-t} (h·µg/mL)	C_{last} (µg/mL)	$AUC_{0-\infty}$ (h·µg/mL)	C_{tau} (µg/mL)	C_{avg} (µg/mL)	CL_{ss} (mL/h)	V_{dss} (mL)	Acc. Index
Cohort 12: ETX2514 2.0 g IV q6h														
Day 1	N		6	6	6									
	Mean		2.833	51.9	179									
	SD		0.408	8	30									
Day 8	N	6	6	6	6	6	6	6	6	6	6	6	6	6
	Mean	10.1	2.67	53.3	193	46	224	0.015	225	9.79	32.1	10,600	21,800	2.98
	SD	2.92	0.52	7.8	31.2	4.9	42	0.002	43	2.46	5.2	1520	2005	0.69

Acc. Index = accumulation index; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; AUC_{0-t} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; AUC_{Tau} = partial area under the plasma concentration-time curve from time of dosing to tau; C_{avg} = average concentration; C_{last} = observed concentration corresponding to time to last concentration; CL_{ss} = estimate of total body clearance; C_{max} = maximum plasma concentration; C_{tau} = concentration at time of dosing plus tau; IV = intravenous; MAD = multiple ascending dose; N = number of subjects; PK = pharmacokinetic; q6h = every 6 hours; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

In Part C, single-dose DDI, 6 subjects received single doses of 1.0 g ETX2514 alone or in combination with 1.0 g sulbactam; 6 subjects received single doses of 1.0 g ETX2514 alone or in combination with 1.0 g sulbactam and 0.5 g imipenem/cilastatin; and 4 subjects received placebo alone or in combination with 1.0 g sulbactam and/or 0.5 g imipenem/cilastatin. In Part D, multiple-dose safety cohort, 10 subjects received 1.0 g ETX2514 in combination with 1.0 g sulbactam and 0.5 g imipenem/cilastatin, and 2 subjects received placebo in combination with 1.0 g sulbactam and 0.5 g imipenem/cilastatin. There was no significant DDI (either way) between ETX2514 and sulbactam or between ETX2514SUL and imipenem/cilastatin. Table 5–4 and Table 5–5 present key ETX2514 and sulbactam PK parameters from the single-dose, 2-way, DDI study of ETX2514 and sulbactam, and key ETX2514, imipenem, cilastatin, and sulbactam PK parameters from the single-dose, 2-way, DDI study of ETX2514 and imipenem/cilastatin, respectively. Table 5–6 and Table 5–7 present key ETX2514 and sulbactam PK parameters following multiple doses of ETX2514 and sulbactam plus imipenem/cilastatin, respectively.

Renal excretion was the predominant clearance mechanism. ETX2514 demonstrated lower total and renal clearance in the elderly cohort (aged ≥65 years), consistent with renal excretion as a predominant clearance mechanism.

Table 5–4. Key ETX2514 and sulbactam PK parameters from the single dose, 2-way, DDI study of ETX2514 and sulbactam (Part C)

	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0-\infty}$ (h·µg/mL)	CL (mL/h)	V_{dss} (mL)
Cohort 13: ETX2514 PK								
ETX2514 1.0 g IV	N	6	6	6	6	6	6	6
	Mean	2.04	2.83	26.9	20	104	9600	17,600
	SD	0.37	0.41	3.6	6.2	6	608	2080
ETX2514 1.0 g IV + sulbactam 1.0 g IV	N	6	6	6	6	6	6	6
	Mean	2.02	2.83	28.1	20	105	9520	17,400
	SD	0.44	0.41	2.5	6.2	6	563	1690
Cohort 13: Sulbactam PK								
Sulbactam 1.0 g IV	N	6	6	6	6	6	6	6
	Mean	1.3	3	20.7	12	68.5	14,700	18,000
	SD	0.09	0	0.7	0	4.7	1020	1560
ETX2514 1.0 g IV + sulbactam 1.0 g IV	N	6	6	6	6	6	6	6
	Mean	1.29	2.83	22	12	73	13,800	18,100
	SD	0.08	0.41	2.72	0	6.2	1160	1680

$AUC_{0-\infty}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; CL = clearance; C_{max} = maximum plasma concentration; DDI = drug-drug interaction; IV = intravenous; N = number of subjects; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–5. Key ETX2514, imipenem, cilastatin, and sulbactam PK parameters from the single dose, 2-way, DDI study of ETX2514 and imipenem/cilastatin (Part C)

	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0-\infty}$ (h·µg/mL)	CL (mL/h)	V_{dss} (mL)
Cohort 14: ETX2514 PK								
ETX2514 1.0 g IV	N	6	6	6	6	6	6	6
	Mean	1.76	2.83	30	16	106	9900	15,900
	SD	0.43	0.41	9.2	6.2	31	2090	2660
ETX2514 1.0 g IV + imipenem 0.5 g IV/ cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.65	2	26.6	13.3	104	9930	15,400
	SD	0.29	0	51.4	5.5	23	1830	1870
ETX2514 1.0 g IV + sulbactam 1.0 g IV + imipenem 0.5 g IV/cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.91	2.5	30.4	18	116	9000	14,900
	SD	0.42	0.55	7.7	6.5	28	1760	2310
Cohort 14: Imipenem PK								
Imipenem 0.5 g IV/cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.2	0.505	34.4	10.7	43.7	11,800	14,300
	SD	0.14	0.012	5.3	2.1	9.2	2050	2160
ETX2514 1.0 g IV + imipenem 0.5 g IV/ cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.21	0.5	31.3	10.7	42.2	12,000	15,200
	SD	0.16	0	2.3	2.1	5.6	1430	1210
ETX2514 1.0 g IV + sulbactam 1.0 g IV + imipenem 0.5 g IV/ cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.21	0.5	32.7	11.3	45.7	11,000	13,900
	SD	0.07	0	3.97	1.6	5.1	1160	928

$AUC_{0-\infty}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; CL = clearance; C_{max} = maximum plasma concentration; DDI = drug-drug interaction; IV = intravenous; N = number of subjects; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–5. Key ETX2514, imipenem, cilastatin, and sulbactam PK parameters from the single dose, 2-way, DDI study of ETX2514 and imipenem/cilastatin (Part C) (continued)

	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0-\infty}$ (h·µg/mL)	CL (mL/h)	V_{dss} (mL)
Cohort 14: Cilastatin PK								
Imipenem 0.5 g IV/cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.19	0.505	46	10	49.6	10,400	9610
	SD	0.19	0.012	8.3	2	9.8	1800	1400
ETX2514 1.0 g IV + imipenem 0.5 g IV/ cilastatin 0.5 g	N	6	6	6	6	6	6	6
	Mean	1.22	0.5	44	10	47.1	10,800	9870
	SD	0.2	0	6.1	2.19	7.2	1810	1170
ETX2514 1.0 g IV + sulbactam 1.0 g IV+ imipenem 0.5 g IV/cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.23	0.5	40.7	10.7	46.9	10,800	10,400
	SD	0.23	0	4.9	2.1	6.6	1600	899
Cohort 14: Sulbactam PK								
ETX2514 1.0 g IV + sulbactam 1.0 g IV + imipenem 0.5 g IV/cilastatin 0.5 g IV	N	6	6	6	6	6	6	6
	Mean	1.26	2.67	23.7	12	80.6	13,000	15,200
	SD	0.07	0.52	6.1	0	2.1	2570	2720

$AUC_{0-\infty}$ = area under the plasma concentration-time curve from time of dosing extrapolated to infinity; CL = clearance; C_{max} = maximum plasma concentration; DDI = drug-drug interaction; IV = intravenous; N = number of subjects; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–6. Key ETX2514 PK parameters following multiple doses of combined IV ETX2514 1.0 g/sulbactam 1.0 g plus imipenem 0.5 g/cilastatin 0.5 g (Part D)

	Stat.	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{Tau} (h·µg/mL)	T_{last} (h)	$AUC_{0\text{-}last}$ (h·µg/mL)	C_{last} (µg/mL)	$AUC_{0\text{-}\infty}$ (h·µg/mL)	C_{tau} (µg/mL)	C_{avg} (µg/mL)	CL_{ss} (mL/h)	V_{dss} (mL)	Acc. Index
Cohort 15: sulbactam-ETX2514 1.0 g IV + imipenem 0.5 g IV/cilastatin 0.5 g IV for 11 days														
Day 1	N	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	1.28	2.8	27.1	91.8	77.5	NC	6.04	NC	5.53	15.3	10,900	NC	1.04
	SD	0.18	0.4	1.3	5.9	0	NC	1.02	NC	0.65	0.9	726	NC	0.02
Day 11	N	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	4.31	2.4	28.1	96.3	33.6	112	15.4	112	5.33	16.1	10,500	21,400	1.63
	SD	3.07	0.5	8.6	11.6	9.47	13	6.02	13	2.48	19.3	1220	4900	0.71

Acc. Index = accumulation index; $AUC_{0\text{-}\infty}$ = area under the curve from time of dosing extrapolated to infinity;$AUC_{0\text{-}last}$ = area under the curve from time of dosing to time of last measurable concentration; AUC_{Tau} = partial area under the curve from time of dosing to tau; C_{avg} = average concentration; C_{last} = observed concentration corresponding to time to last concentration; CL_{ss} = estimate of total body clearance; C_{max} = maximum plasma concentration; C_{tau} = concentration at time of dosing plus tau; IV = intravenous; N = number of subjects; NC = Not calculated due to insufficient data; PK = pharmacokinetic; SD = standard deviation; Stat. = statistic; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

Table 5–7. Key sulbactam PK parameters following multiple doses of combined IV ETX2514 1.0 g/sulbactam 1.0 g plus imipenem 0.5 g/cilastatin 0.5 g (Part D)

	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{Tau} (h·µg/mL)	T_{last} (h)	$AUC_{0\text{-}last}$ (h·µg/mL)	C_{last} (µg/mL)	C_{tau} (µg/mL)	C_{avg} (µg/mL)	CL_{ss} (mL/h)	V_{dss} (mL)	Acc. Index
Cohort 15: sulbactam-ETX2514 1.0 g IV + imipenem 0.5 g IV/cilastatin 0.5 g IV for 11 days													
Day 1	N	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	1	2.7	23.9	76.7	71	349	3.13	3.18	12.8	13,100	NC	1.02
	SD	0.01	0.5	1.3	5.8	20.7	88	0.81	0.51	1	1040	NC	0.01
Day 11	N	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	2	2.6	22.3	69.7	14.4	75.1	0.138	2.49	11.6	14,500	20,300	1.16
	SD	1.03	0.5	6.2	7.6	7.6	8.3	.053	1.45	1.3	1470	5790	0.21

Acc. Index = accumulation index; $AUC_{0\text{-}last}$ = area under the curve from time of dosing to time of last measurable concentration; AUC_{Tau} = partial area under the plasma concentration-time curve from time of dosing to tau; C_{avg} = average concentration; C_{last} = observed concentration corresponding to time to last concentration; CL_{ss} = estimate of total body clearance; C_{max} = maximum plasma concentration; C_{tau} = concentration at time of dosing plus tau; IV = intravenous; Max = maximum; N = number of subjects; NC = not calculated due to insufficient data; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_{dss} = steady state volume of distribution.

5.1.1.2 PK conclusions

- In SAD escalation, ETX2514 demonstrated linear dose proportional exposure across the dose range studied (0.25 to 8 g).
- In MAD escalation, ETX2514 demonstrated linear dose proportional exposure across the dose range studied (0.25 to 1 g q6h), with minimal accumulation at Day 8.
- Renal excretion was the predominant clearance mechanism.
- ETX2514 demonstrated lower total and renal clearance in elderly subjects (aged ≥65 years).
- There was no DDI (either way) between ETX2514 and sulbactam and/or imipenem/cilastatin.

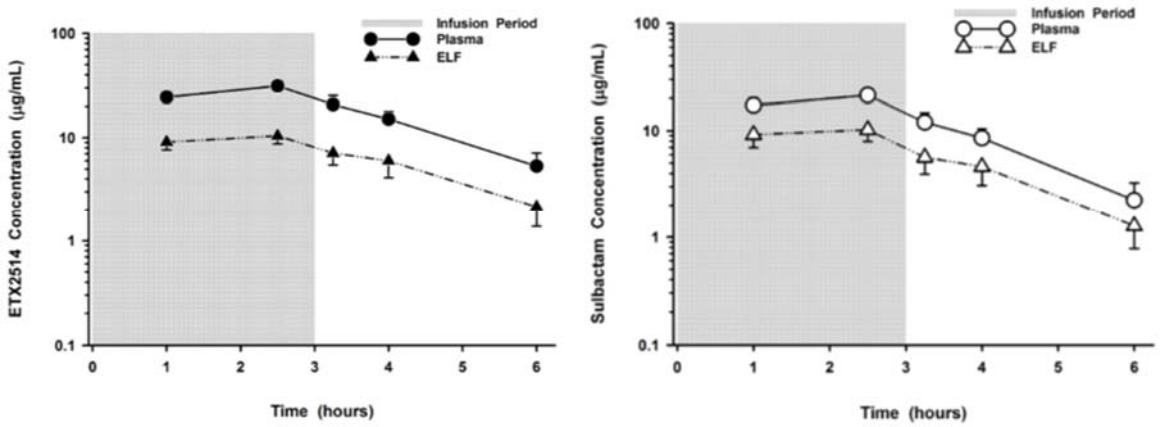
5.1.2 Study CS2514-2017-0001

ETX2514 in combination with sulbactam, has been evaluated in the Phase 1, multiple dose, open-label, PK study, "A Phase 1 Study to Determine and Compare Plasma, Epithelial Lining Fluid (ELF), and Alveolar Macrophage (AM) Concentrations of Intravenous ETX2514 and Sulbactam Administered to Healthy Adult Subjects" (Study CS2514-2017-0001). All 30 healthy subjects aged 18 to 55 years enrolled into the study received 3 doses of ETX2514 1.0 g and sulbactam 1.0 g via IV infusion over 3 hours, administered q6h. Each subject underwent 1 standardized bronchoscopy with BAL at one of several time points following the start of the infusion of the third dose.

5.1.2.1 Results

IV infusion of ETX2514 1.0 g given concurrently with IV sulbactam 1.0 g, administered q6h as a 3-hour infusion, for 3 consecutive doses, achieved a similar time course of concentrations for both agents in plasma, ELF, and alveolar macrophage. The magnitude of total plasma concentrations of ETX2514 at the BAL sampling times was approximately 1.78-fold (2.0 to 12.2 µg/mL) greater than sulbactam. In comparison, ELF concentrations of ETX2514 were approximately 1.27-fold (0.12 to 2.0 µg/mL) greater than sulbactam. Figure 5–3 shows the mean (± standard deviation) concentrations of ETX2514 and sulbactam in plasma (total) and ELF at the bronchopulmonary sampling times.

Figure 5–3. Mean (± standard deviation) concentrations of ETX2514 and sulbactam in plasma (total) and ELF at the bronchopulmonary sampling times



ELF = epithelial lining fluid.

Table 5–8 presents the AUC from 0 to 6 hours postdose (AUC_{0-6}) of ETX2514 based on mean and median concentrations at BAL sampling times. The intrapulmonary penetration of ETX2514 and sulbactam based on AUC_{0-6} values using the mean values of ELF and total plasma concentrations at the BAL sampling times were 37% and 50%, respectively, for ETX2514 and sulbactam. If unbound plasma concentrations of ETX2514 and sulbactam are considered, these values increase to 41% and 81%, respectively. These results lend support to exploring the combination of ETX2514 and sulbactam (i.e., ETX2514SUL) as a potential antimicrobial agent for the treatment of lower respiratory tract bacterial infections caused by susceptible extracellular pathogens.

Table 5–8. AUC_{0-6} values of ETX2514 based on mean and median concentrations at BAL sampling times

	AUC_{0-6} Based on Mean Concentrations (µg·h/mL)		**AUC_{0-6} Based on Median Concentrations (µg·h/mL)**	
Sampling Site	**ETX2514**	**Sulbactam**	**ETX2514**	**Sulbactam**
Plasma (Total)	107.80	68.87	108.70	69.12
Epithelial Lining Fluid	40.07	34.71	39.38	34.48

AUC_{0-6} = Area under the plasma concentration-time curve from 0 to 6 hours postdose;
BAL = bronchoalveolar lavage.

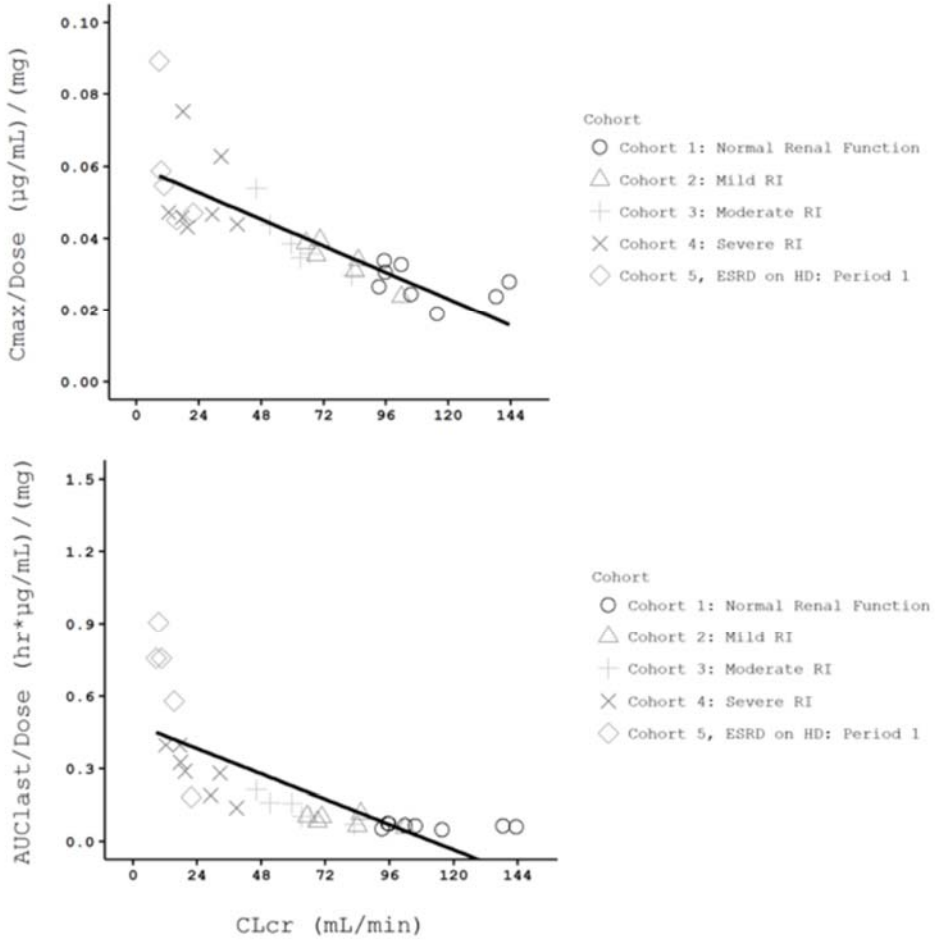
5.1.3 Study CS2514-2017-0002

ETX2514 in combination with sulbactam, has been evaluated in the Phase 1, single dose, PK, safety, and tolerability study, "A Phase 1 Study to determine the plasma and urine pharmacokinetics and safety of ETX2514SUL in healthy adult subjects and adult patients with renal insufficiency" (Study CS2514-2017-0002). This study evaluated the PK, safety, and tolerability of a single dose of ETX2514 and sulbactam (i.e., ETX2514SUL) when concurrently administered by separate IV infusions in patients with various degrees of renal impairment, patients with end-stage renal disease who are on hemodialysis, and in healthy, control-matched subjects with normal renal function. In total, 8 patients with normal renal function and 26 patients with mild and moderate renal insufficiency were administered a single dose of ETX2514SUL 1.0 g via IV infusion over 3 hours. Patients with severe renal impairment received a single dose of ETX2514SUL 0.5 g via IV infusion over 3 hours, and patients on hemodialysis received ETX2514SUL 0.5 g via IV infusion pre- and post-dialysis.

5.1.3.1 Results

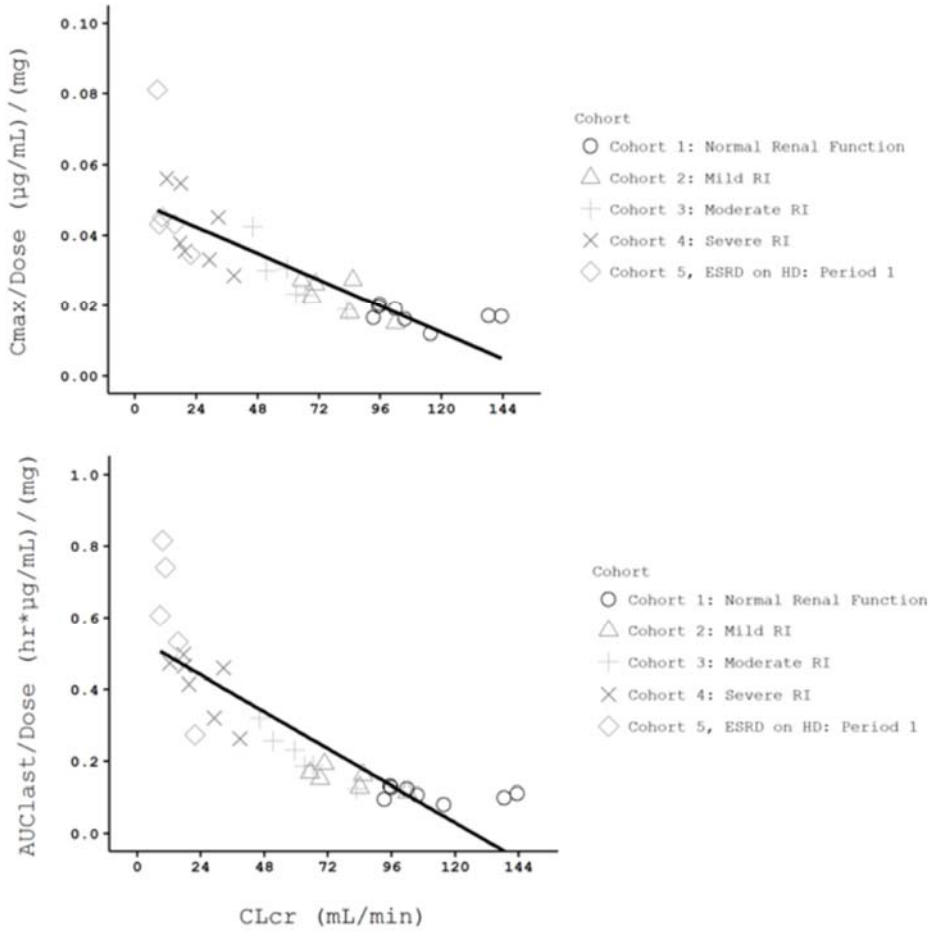
Sulbactam and ETX2514 are predominantly cleared renally, and exposures of both drugs were significantly impacted by reductions in CLcr in this study. Groups that were particularly affected were those with CLcr values below 30 mL/min. Table 5–9 and Table 5–10 present ETX2514 and sulbactam PK parameters, respectively. Figure 5–4 and Figure 5–5 show the relationship between CLcr and dose-normalized C_{max} and AUC for ETX2514 and sulbactam, respectively.

Figure 5–4. Linear regression of ETX2514 dose-normalized C_{max} and AUC versus CLcr



AUC = Area under the plasma concentration-time curve; AUC_{last} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; CLcr = creatinine clearance; C_{max} = maximum plasma concentration; ESRD = end-stage renal disease; HD = hemodialysis; RI = renal impairment.

Figure 5–5. Linear regression of sulbactam dose-normalized C_{max} and AUC versus CLcr



AUC = Area under the plasma concentration-time curve; AUC_{last} = area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration was observed; CLcr = creatinine clearance; C_{max} = maximum plasma concentration; ESRD = end-stage renal disease; HD = hemodialysis; RI = renal impairment.

Table 5–9. Key ETX2514 PK parameters by level of renal impairment

Treatment Group	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0\text{-}last}$ (h·µg/mL)	$AUC_{0\text{-}\infty}$ (h·µg/mL)	CL (mL/h)	V_z (mL)
Cohort 1 Normal renal function ETX2514 1.0 g IV (N = 8)	n	7	8	8	8	8	7	7	7
	Mean	2.34	NC	27.4	NC	110	111	9220	31,000
	SD	0.1	NC	4.98	NC	17.5	18.4	1700	4820
Cohort 2 Mild renal impairment ETX2514 1.0 g IV (N = 6)	n	6	6	6	6	6	6	6	6
	Mean	3	NC	33.8	NC	153	153	6740	29,000
	SD	0.434	NC	5.82	NC	28.2	28.3	1300	6470
Cohort 3 Moderate renal impairment ETX2514 1.0 g IV (N = 6)	n	6	6	6	6	6	6	6	6
	Mean	3.84	NC	39.4	NC	218	218	5010	26,200
	SD	0.84	NC	8.5	NC	67.3	67.3	1700	3450
Cohort 4 Severe renal impairment ETX2514 0.5 g IV (N = 8)	n	7	7	7	7	7	7	7	7
	Mean	5.47	NC	26.1	NC	206	207	2540	19,300
	SD	0.984	NC	6.08	NC	43.6	43.6	667	2400
Cohort 5 ESRD on hemodialysis Pre-dialysis ETX2514 0.5 g IV (N = 6)	n	5	5	5	5	5	5	5	5
	Mean	7.44	NC	29.5	NC	297	299	1940	18,400
	SD	2.18	NC	8.87	NC	105	107	988	2430
Cohort 5 ESRD on hemodialysis Post-dialysis ETX2514 0.5 g IV (N = 6)	n	5	6	6	6	6	5	5	5
	Mean	7.39	NC	22.4	NC	127	127	4240	42,700
	SD	1.92	NC	4.84	NC	32.2	36.2	1410	9770

$AUC_{0\text{-}\infty}$ = area under the curve from time of dosing extrapolated to infinity; $AUC_{0\text{-}last}$ = area under the curve from time of dosing to time of last measurable concentration; CL = clearance; C_{max} = maximum plasma concentration; ESRD = end-stage renal disease; IV = intravenous; n = number of subjects/patients dosed; N = number of subjects/patients in cohort; NC = not calculated due to insufficient data; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_z = volume of distribution based on terminal phase.

Table 5–10. Key sulbactam PK parameters by level of renal impairment

Treatment Group	Statistic	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	T_{last} (h)	$AUC_{0\text{-}last}$ (h·µg/mL)	$AUC_{0\text{-}\infty}$ (h·µg/mL)	CL (mL/h)	V_z (mL)
Cohort 1 Normal renal function Sulbactam 1.0 g IV (N = 8)	n	8	8	8	8	8	8	8	8
	Mean	1.89	NC	17.2	NC	63.3	63.6	16,000	43,100
	SD	0.962	NC	2.69	NC	8.85	8.93	2430	21,200
Cohort 2 Mild renal impairment Sulbactam 1.0 g IV (N = 6)	n	6	6	6	6	6	6	6	6
	Mean	1.97	NC	22.7	NC	88.1	88.4	12,000	33,400
	SD	0.287	NC	5.22	NC	23.4	23.1	3430	7400
Cohort 3 Moderate renal impairment Sulbactam 1.0 g IV (N = 6)	n	6	6	6	6	6	6	6	6
	Mean	3.2	NC	28.1	NC	134	135	8490	34,200
	SD	1.6	NC	8.31	NC	52.5	52.4	3460	6020
Cohort 4 Severe renal impairment Sulbactam 0.5 g IV (N = 8)	n	7	7	7	7	7	7	7	7
	Mean	4.95	NC	20.8	NC	144	145	3920	25,300
	SD	1.41	NC	5.35	NC	48.5	48.6	1710	3780
Cohort 5 ESRD on hemodialysis Pre-dialysis Sulbactam 0.5 g IV (N = 6)	n	5	5	5	5	5	5	5	5
	Mean	10	NC	24.7	NC	318	329	2150	23,500
	SD	4.04	NC	9.1	NC	139	149	1840	4890
Cohort 5 ESRD on hemodialysis Post-dialysis Sulbactam 0.5 g IV (N = 6)	n	5	6	6	6	6	5	5	5
	Mean	10.8	NC	20.5	NC	134	139	4120	55,800
	SD	4.11	NC	4.41	NC	42.6	49.5	1910	10,600

$AUC_{0\text{-}\infty}$ = area under the curve from time of dosing extrapolated to infinity; $AUC_{0\text{-}last}$ = area under the curve from time of dosing to time of last measurable concentration; CL = clearance; C_{max} = maximum plasma concentration; ESRD = end-stage renal disease; IV = intravenous; n = number of subjects/patients dosed; N = number of subjects/patients in cohort; NC = not calculated due to insufficient data; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = terminal half-life; T_{last} = time to last concentration; T_{max} = time to reach maximum plasma concentration; V_z = volume of distribution based on terminal phase.

Table 5–11 presents recommended dose adjustments for ETX2514SUL in patients with impaired renal function based on data from this study and a population PK analysis of overall exposure in all ETX2514 studies.

Table 5–11. Recommended ETX2514SUL dose adjustments in patients with impaired renal function

Creatinine Clearance (mL/min)	Sulbactam/ETX2514 Dose [1] (mg/mg)
0-14	1000/1000 q12h
15-29	1000/1000 q8h
30-130	1000/1000 q6h
130-200	1500/1500 q6h
Hemodialysis	1000/1000 q12h [2]
CRRT	1500/1500 q6h

1. All regimens used a 3-hour IV infusion.
2. The dialysis session should occur toward the end of a dosing interval, and the daily dose should be administered after the dialysis session has ended.

CRRT = continuous renal replacement therapy; ETX2514SUL = sulbactam/ETX2514; IV = intravenous; q6h = every 6 hours; q8h = every 8 hours; q12h = every 12 hours.

5.1.4 Overall conclusions with respect to PK

- ETX2514 demonstrated linear PK and had no evidence of accumulation.
- ETX2514 did not interact with sulbactam and/or imipenem/cilastatin.
- ETX2514 was predominantly excreted renally and dose adjustments are necessary for patients with moderate or severe renal insufficiency including dialysis. Similarly, higher doses are recommended for patients with augmented renal clearance or receiving continuous renal replacement therapy.
- ETX2514SUL demonstrated good penetration of the lung epithelium.

5.2 Efficacy

Efficacy of ETX2514SUL in the background of imipenem/cilastatin was evaluated in a single Phase 2 study in patients with cUTI or AP. Efficacy of ETX2514SUL against *A. baumannii* will be evaluated in Phase 3.

5.2.1 Study CS2514-2017-0003

Efficacy of ETX2514 in combination with sulbactam and background imipenem/cilastatin, has been evaluated in the Phase 2 study, "A Double-blind, Randomized, Placebo-controlled Study to Evaluate the Safety and Efficacy of Intravenous Sulbactam-ETX2514 in the Treatment of Hospitalized Adults With Complicated Urinary Tract Infections, Including Acute Pyelonephritis" (Study CS2514-2017-0003). This study, conducted at 20 sites in 4 countries (Belarus, Bulgaria, Russia, and Ukraine), was a double-blind, randomized, placebo-controlled study to evaluate the safety and efficacy of IV ETX2514SUL in patients with cUTIs who were otherwise relatively healthy. Patients providing informed consent and meeting all study eligibility criteria were enrolled and had a pre-treatment urine and blood sample obtained and submitted to the local laboratory. Eighty patients were randomized 2:1 to receive either 1 g ETX2514/1 g sulbactam IV (infused over 3 hours) or matching placebo q6h. Randomization was stratified by baseline diagnosis (symptomatic cUTI versus AP). At least 30% of patients were to have a diagnosis of AP at study entry. All patients received background therapy with 500 mg IV imipenem/cilastatin q6h. Therapy was continued for 7 days, with the option to increase up to 14 days if bacteremia was present.

5.2.1.1 Patient accounting and demographics

Of the 80 patients enrolled into the study, 53 patients received ETX2514SUL and 27 patients received placebo. All patients received at least 1 dose of study drug. Two (2.5%) patients did not complete the study treatment due to adverse events: 2 patients in the ETX2514SUL group and no patients in the placebo group. The adverse events leading to discontinuation from study therapy were acute urticaria (Patient 103-004 [Severity: moderate; related]) and mild reduction in CLcr to <70mL/min that would require modification to the imipenem/cilastatin dose (Patient 303-002 [Preferred Term: blood creatinine increased; Severity: moderate; related]). See Table 5–12, Table 5–13, and Table 5–14 for patient disposition by treatment, a summary of demographic and baseline characteristics, and a summary of baseline pathogens, respectively.

Table 5–12. Patient disposition by treatment

	ETX2514SUL [1] (N = 53) n (%)	**Placebo [1] (N = 27) n (%)**	**Total (N = 80) n (%)**
Patients who were randomized	53 (100)	27 (100)	80 (100)
Patients who received study drug	53 (100)	27 (100)	80 (100)
Patients who completed the study treatment	51 (96.2)	27 (100)	78 (97.5)
Patients who did not complete the study treatment	2 (3.8)	0 (0)	2 (2.5)
Adverse event	2 (3.8)	0 (0)	2 (2.5)
Patients who completed the study	52 (98.1)	27 (100)	79 (98.8)
Patients who did not complete the study	1 (1.9)	0 (0)	1 (1.3)
Adverse event	1 (1.9)	0 (0)	1 (1.3)

Percentage was calculated using the number of patients in the column heading as the denominator.

1. All patients also received imipenem/cilastatin.

ETX2514SUL = sulbactam/ETX2514; n = number of observations; N = number of patients.

Table 5–13. Summary of demographic and baseline characteristics: m-MITT Population

Demographics/Characteristics Category/Statistics	ETX2514SUL [1] (N = 53) n (%)	Placebo [1] (N = 27) n (%)	Total (N = 80) n (%)
Age (years)			
Mean ± Standard Deviation	51.4 ± 17.55	54.9 ± 15.92	52.6 ± 17.00
Median (Minimum, Maximum)	52.0 (18, 82)	59.0 (22, 78)	54.5 (18, 82)
Age Group (n, %)			
<65 years	36 (67.9)	19 (70.4)	55 (68.8)
65 – 75 years	13 (24.5)	6 (22.2)	19 (23.8)
>75 years	4 (7.5)	2 (7.4)	6 (7.5)
Gender (n, %)			
Male	26 (49.1)	16 (59.3)	42 (52.5)
Female	27 (50.9)	11 (40.7)	38 (47.5)
Race (n, %)			
White	53 (100)	27 (100)	80 (100)
Ethnicity (n, %)			
Hispanic or Latino	1 (1.9)	0 (0)	1 (1.3)
Not Hispanic or Latino	52 (98.1)	27 (100)	79 (98.8)
Weight (kg)			
Mean ± Standard Deviation	83.79 ± 20.644	85.76 ± 17.929	84.45 ± 19.677
Median (Minimum, Maximum)	84.00 (49.5, 135.0)	86.50 (43.6, 125.0)	84.90 (43.6, 135)
Height (cm)			
Mean ± Standard Deviation	172.6 ± 8.81	173.1 ± 8.44	172.8 ± 8.63
Median (Minimum, Maximum)	174.0 (155, 198)	172.0 (157, 187)	173.0 (155, 198)
BMI (kg/m^2)			
Mean ± Standard Deviation	28.09 ± 6.661	28.63 ± 5.856	28.27 ± 6.368
Median (Minimum, Maximum)	27.64 (18.4, 52.1)	29.38 (17.0, 39.5)	28.01 (17.0, 52.1)
Screening Creatinine Clearance (mL/min)			
Mean ± Standard Deviation	94.3 ± 23.76	91.7 ± 18.19	93.4 ± 21.96
Median (Minimum, Maximum)	86.0 (68, 159)	88.7 (70, 143)	88.7 (68, 159)

Percentage was calculated using the number of patients in the column heading as the denominator. Baseline was defined as the last measurement or assessment prior to the first dose of study drug.

1. All patients also received imipenem/cilastatin.

BMI = body mass index; ETX2514SUL = sulbactam/ETX2514; m-MITT = Microbiologically Modified Intent-to-Treat; n = number of observations; N = number of patients in cohort; UTI = urinary tract infection.

Table 5–13. Summary of demographic and baseline characteristics: m-MITT Population (continued)

Demographics/Characteristics Category/Statistics	ETX2514SUL [1] (N = 53) n (%)	Placebo [1] (N = 27) n (%)	Total (N = 80) n (%)
Screening Creatinine Clearance Group (n, %)			
<30 mL/min	0 (0.0)	0 (0.0)	0 (0.0)
30 – <70 mL/min	1 (1.9)	0 (0.0)	1 (1.3)
≥70 mL/min	52 (98.1)	27 (100)	79 (98.8)
Infection Type [2]			
Complicated UTI	36 (67.9)	18 (66.7)	54 (67.5)
Acute pyelonephritis	17 (32.1)	9 (33.3)	26 (32.5)

Percentage was calculated using the number of patients in the column heading as the denominator. Baseline was defined as the last measurement or assessment prior to the first dose of study drug.

1. All patients also received imipenem/cilastatin.

BMI = body mass index; ETX2514SUL = sulbactam/ETX2514; m-MITT = Microbiologically Modified Intent-to-Treat; n = number of observations; N = number of patients in cohort; UTI = urinary tract infection.

Table 5–14. Summary of baseline pathogens: m-MITT Population

Baseline Pathogen	ETX2514SUL + IMI (N = 47) n (%)	Placebo + IMI (N = 21) n (%)	Total (N = 68) n (%)
Escherichia coli	23 (48.9)	7 (33.3)	30 (44.1)
Klebsiella pneumoniae	10 (21.3)	7 (33.3)	17 (25.0)
Enterococcus faecalis	8 (17.0)	3 (14.3)	11 (16.2)
Pseudomonas aeruginosa	3 (6.4)	2 (9.5)	5 (7.4)
Citrobacter freundii	3 (6.4)	0 (0.0)	3 (4.4)
Enterobacter cloacae	0 (0.0)	3 (14.3)	3 (4.4)
Klebsiella oxytoca	2 (4.3)	0 (0.0)	2 (2.9)
Alcaligenes faecalis	1 (2.1)	0 (0.0)	1 (1.5)
Klebsiella aerogenes	1 (2.1)	0 (0.0)	1 (1.5)
Proteus hauseri	1 (2.1)	0 (0.0)	1 (1.5)
Serratia marcescens	1 (2.1)	0 (0.0)	1 (1.5)

Percentage was calculated using the number of patients in the column heading as the denominator.

ETX2514SUL = sulbactam/ETX2514; IMI = imipenem/cilastatin; m-MITT = Microbiologically Modified Intent-to-Treat; n = number of observations; N = number of patients in cohort.

5.2.1.2 Results

The overall efficacy in the ETX2514SUL and the placebo groups was similar using FDA CFU criteria. This was expected since all patients received background therapy with imipenem/cilastatin.

The evaluation in the Microbiologically Evaluable Population and in the Clinically Evaluable Population are most representative of the true efficacy of the regimen since patients with missing data are excluded from these populations. In the Microbiologically Modified Intent-to-Treat (m-MITT) Population analyses, patients with missing data at the Test-of-Cure (TOC) Visit were imputed as failures; the treatment difference in the m-MITT Population analyses was due to 2 patients in the ETX2514SUL group with missing data at the TOC Visit. Table 5–15 presents response at the TOC Visit based on FDA criteria.

Table 5–15. Response at TOC Visit based on FDA criteria

Population	ETX2514SUL + IMI % (n/N)	Placebo + IMI % (n/N)	Treatment Difference (95% CI)
Overall Success (Clinical and Microbiological Success)			
m-MITT Population			
Overall	76.6 (36/47)	81.0 (17/21)	-4.4 (-28.5, 19.8)
Complicated UTI [1]	67.7 (21/31)	87.5 (14/16)	-19.8 (-47.6, 8.1)
Acute Pyelonephritis	93.8 (15/16)	60.0 (3/5)	33.8 (-23.9, 91.4)
Microbiological Success			
ME Population	80.0 (36/45)	81.0 (17/21)	-1.0 (-24.9, 23.0)
Clinical Success			
CE Population	100 (52/52)	100 (27/27)	–

Percentage was calculated using the number of patients in the column heading as the denominator.

Per FDA Criteria, a microbiologic outcome of eradication is defined as the demonstration that the baseline bacterial pathogen(s) is reduced to $<10^4$ CFU/mL on urine culture and negative on repeat blood culture (if positive at baseline).

1. Patients with complicated UTI without acute pyelonephritis.

CE = clinically evaluable; CFU = colony-forming unit; CI = confidence interval; ETX2514SUL = sulbactam/ETX2514; FDA = Food and Drug Administration; IMI = imipenem/cilastatin; ME = microbiologically evaluable; m-MITT = Microbiologically Modified Intent-to-Treat; n = number of observations; N = number of patients in cohort; TOC = Test-of-Cure; UTI = urinary tract infection.

Table 5–16. Analysis of overall response at TOC Visit based on FDA criteria – m-MITT Population – patients with pathogen(s) resistant to imipenem at baseline

Overall Response	ETX2514SUL + IMI (N = 1) n (%)	Placebo + IMI (N = 3) n (%)	Treatment Comparison [1]	
			Difference (%)	95% CI
Success [2]	1 (100.0)	3 (100.0)	NE	NE
Failure	0 (0.0)	0 (0.0)	–	–
Indeterminate	0 (0.0)	0 (0.0)	–	–

Percentage was calculated using the number of patients in the column heading as the denominator.

Per FDA Criteria, a microbiologic outcome of eradication is defined as the demonstration that the baseline bacterial pathogen(s) was reduced to $<10^4$ CFU/mL on urine culture and negative on repeat blood culture (if positive at baseline).

1. Treatment difference was the difference in the overall success rate between the 2 treatment groups (ETX2514SUL + IMI and placebo + IMI). The 95% CI (2-sided) was computed using a continuity-corrected Z-statistic.
2. Overall success was defined as clinical cure and microbiologic eradication.

CFU = colony-forming unit; CI = confidence interval; ETX2514SUL = sulbactam/ETX2514; FDA = Food and Drug Administration; IMI = imipenem/cilastatin; m-MITT = Microbiologically Modified Intent-to-Treat; n = number of observations; N = number of patients in cohort; NE = Not estimable; TOC = Test-of-Cure.

An ad hoc exploratory analysis of isolates that were imipenem nonsusceptible was conducted. In addition to the 4 patients with resistant infections, 3 additional patients were noted to have infections with intermediate-resistance organisms (2 patients in the ETX2514SUL + imipenem/cilastatin group and 1 patient in the placebo + imipenem/cilastatin group). At the TOC Visit, 3 (100.0%) patients in the ETX2514SUL + imipenem/cilastatin group and 3 (75.0%) patients in the placebo + imipenem/cilastatin group with baseline infections due to imipenem-nonsusceptible strains had evidence of overall success based on FDA criteria in the m-MITT Population. Only 1 patient in the study was noted to have bacteremia with an imipenem-resistant organism. Patient 401-002 was successfully treated with ETX2514SUL in combination with imipenem/cilastatin for a dual infection with *K. pneumoniae* (imipenem sensitive) and *Proteus mirabilis* (imipenem resistant) at baseline.

Table 5–17. Overall success at the TOC Visit by baseline pathogen based on FDA criteria – m-MITT Population – patients with pathogen(s) nonsusceptible to imipenem at baseline

Baseline Pathogen	ETX2514SUL + IMI (N = 3) n/N' (%)	Placebo + IMI (N = 4) n/N' (%)
Klebsiella pneumoniae	0/0 (0.0)	1/2 (50.0)
Proteus mirabilis	1/1 (100.0)	0/0 (0.0)
Pseudomonas aeruginosa	2/2 (100.0)	2/2 (100.0)

Percentage was calculated using N', the number of patients with the relevant pathogen, as the denominator.

Per FDA Criteria, a microbiologic outcome of eradication was defined as the demonstration that the baseline bacterial pathogen(s) was reduced to $<10^4$ CFU/mL on urine culture and negative on repeat blood culture (if positive at baseline).

Overall success was defined as clinical cure and microbiologic eradication.

Nonsusceptible was defined as resistant or intermediate.

CFU = colony-forming unit; ETX2514SUL = sulbactam/ETX2514; FDA = Food and Drug Administration; IMI = imipenem/cilastatin; m-MITT = Microbiologically Modified Intent-to-Treat; n = number of observations; N = number of patients in cohort; N' = number of patients with the relevant pathogen; TOC = Test-of-Cure.

5.2.2 Conclusions with respect to efficacy

- ETX2514SUL when dosed with imipenem/cilastatin showed comparable efficacy to placebo + imipenem/cilastatin in treatment of hospitalized patients with cUTI or AP. Among patients with imipenem nonsusceptible infections, imipenem when combined with ETX2514SUL was able to successfully treat 3 out of 3 infections.

5.3 Safety

A brief description of safety parameters/variables observed in each clinical study conducted to date is detailed below followed by a summary of safety of ETX2514 with or without sulbactam.

5.3.1 Study CS2514-2016-0001

The safety of ETX2514 and ETX2514SUL has been evaluated in the FIH study, "A Phase I, Double-blind, Randomized, Placebo-controlled Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Intravenous ETX2514 Administered in Healthy Subjects" (Study CS2514-2016-0001).

5.3.1.1 Part A, SAD

In Part A, SAD, 48 subjects (including 6 elderly subjects) received ETX2514 in doses ranging from 0.25 to 8.0 g, and 16 subjects received placebo. No deaths, serious treatment-emergent adverse events (TEAEs), or TEAEs leading to study drug discontinuation or interruption were reported. Most TEAEs were of mild severity; there were no severe events. Headache was the most frequently reported TEAE (33.3% of subjects who received ETX2514 and 12.5% of subjects who received placebo) and ETX2514-related TEAE.

There were no trends or changes in hematology or urinalysis results following ETX2514 administration at any dose, and there were no notable differences in hematology or urinalysis results between the ETX2514 and placebo groups.

Increases in mean systolic and diastolic blood pressure were observed in the elderly cohort (ETX2514 1.0 g IV). The greatest change in mean systolic blood pressure was an increase from baseline of 23.5 mmHg on Day 7, with the maximum increase from baseline in the placebo group of 6.6 mmHg on Day 5. The greatest change in mean diastolic blood pressure was an increase from baseline of 10.3 mmHg on Days 1 and 3, with a maximum increase from baseline in the placebo group of 8.7 mmHg on Day 7. There were no potentially clinically significant blood pressure results that were reported as TEAEs.

5.3.1.2 Part B, MAD

In Part B, MAD, 24 subjects received ETX2514 in doses ranging from 0.25 to 2 g, q6h for 7 days with 1 dose on Day 8, and 8 subjects received placebo. Three subjects did not receive the full 8 days of dosing: 1 subject in the ETX2514 1.0 g group was withdrawn from the study because of TEAEs that occurred during the administration of the first dose on Day 1 (infusion site reaction and somnolence); 1 subject in the ETX2514 0.5 g group had study drug administration stopped but remained in the study after the first dose on Day 2 because of TEAEs (nausea and somnolence); and 1 subject in the ETX2514 0.5 g group had administration stopped after the second dose on Day 6 because of a serious adverse event (SAE).

The SAE involved a severe anaphylactic reaction in Subject 210003, a 33-year-old Black male subject with an ongoing medical history of allergy to Brazil nuts undisclosed at the time of screening. This event occurred on Day 6, resolved the same day, and was considered unrelated to ETX2514 (subject ate a dessert containing Brazil nuts in the clinic that day). The subject was treated in the Emergency Room, recovered, and remained in the study, but study drug was discontinued.

The greatest number of subjects reported TEAEs in the system organ class (SOC) "General Disorders and Administration Site Conditions," with the most frequently reported TEAE and ETX2514-related TEAE being catheter site phlebitis (50.0% of subjects who received ETX2514; 12.5% of subjects who received placebo). For phlebitis, there appeared to be a trend towards an increasing number of events with increasing dose, with 1 event reported in the 0.25 g ETX2514 cohort (n = 6), 2 events in the 1.0 g cohort (n = 6), and 3 events in the 2.0 g cohort (n = 6). Most TEAEs were of mild severity.

Decreases in mean WBC counts were observed in the ETX2514 group without apparent dose relationship. The greatest observed decreases from baseline in mean WBC counts were 1.08×10^9/L (Day 7), 1.72×10^9/L (Day 22), 1.32×10^9/L (Day 22), and 1.42×10^9/L (Day 22) in subjects who received 0.25 g, 0.5 g, 1.0 g, and 2.0 g ETX2514, respectively, compared with a maximum decrease from baseline of 0.36×10^9/L (Day 22) in the placebo group. However, mean WBC count values remained within the reference range at all time points, and these changes were not considered clinically significant by the Investigator.

Decreases in mean neutrophil counts were observed in the ETX2514 group, without apparent dose relationship. The greatest observed decreases from baseline in mean neutrophil counts were 1.17×10^9/L (Day 7), 1.47×10^9/L (Days 7 and 22), 1.20×10^9/L (Day 22), and 1.32×10^9/L (Day 22) in subjects who received 0.25 g, 0.5 g, 1.0 g, and 2.0 g ETX2514, respectively, compared with a maximum decrease from baseline of 0.28×10^9/L (Day 22) in the placebo group. However, mean neutrophil count values remained within the reference range at all time points without incidence of neutropenia in any subject, and these changes were not considered clinically significant by the Investigator.

There were no trends or changes in clinical chemistry or urinalysis results following ETX2514 administration at any dose, and there were no notable differences in clinical chemistry or urinalysis results between the ETX2514 and placebo groups.

Increases in mean systolic blood pressure and heart rate were observed in the 2.0 g IV ETX2514 group. The greatest change in mean systolic blood pressure was an increase from baseline of 15.0 mmHg at 12 hours after the start of infusion on Day 3, with a maximum increase from baseline in the placebo group of 9.6 mmHg at 12 hours after the start of infusion on Day 4. The greatest change in mean heart rate in the 2.0 g IV ETX2514 group was an increase of 13.3 bpm at 12 hours after the start of infusion on Day 3, with a maximum increase from baseline of 13.4 bpm at 12 hours after the start of infusion on Day 7 in the placebo group.

5.3.1.3 Part C, single-dose DDI

In Part C, single-dose DDI, 6 subjects received single doses of 1.0 g ETX2514 alone or in combination with 1.0 g sulbactam; 6 subjects received single doses of 1.0 g ETX2514 alone or in combination with 1.0 g sulbactam and 0.5 g imipenem/0.5 g cilastatin; and 4 subjects received placebo alone or in combination with 1.0 g sulbactam and/or 0.5 g imipenem/0.5 g cilastatin.

TEAEs were reported in 5 (83.3%) subjects who received ETX2514 with sulbactam, 1 (16.7%) subject who received ETX2514 and imipenem/cilastatin, and in 1 (50.0%) subject in each placebo combination group. ETX2514-related TEAEs were reported in 2 (33.3%) subjects who received ETX2514 with sulbactam; sulbactam-related TEAEs were reported in 2 (33.3%) subjects who received ETX2514 with sulbactam; and an imipenem/cilastatin-related TEAE was reported in 1 (16.7%) subject who received ETX2514 and imipenem/cilastatin. No deaths or serious TEAEs were reported. A TEAE of catheter site extravasation leading to study drug interruption was reported in 1 subject who received placebo and imipenem/cilastatin. The greatest number of subjects reported TEAEs in the SOC "General Disorders and Administration Site Conditions." No individual TEAE in any SOC was reported in more than 1 subject. Only 2 TEAEs were considered related to sulbactam/ETX2514: abdominal discomfort and headache. Most TEAEs were of mild severity, and no TEAEs were considered severe.

Decreases in mean WBC counts were observed in subjects who received 1.0 g ETX2514 with 0.5 g imipenem/0.5 g cilastatin. The greatest observed decreases from baseline in mean WBC counts were 1.95×10^9/L (Day 7) and 1.92×10^9/L (Day 22). In comparison, the decreases from baseline in mean WBC counts were 0.40×10^9/L (Day 7) and 1.95×10^9/L (Day 22) in subjects who received placebo and 0.5 g imipenem/0.5 g cilastatin on the same days. However, mean WBC count values remained within the reference range at all time points, and these changes were not considered clinically significant by the Investigator.

There were no trends or changes in clinical chemistry or urinalysis results following ETX2514 administration with or without sulbactam and imipenem/cilastatin, and no notable differences in clinical chemistry or urinalysis results between ETX2514 and placebo groups.

There were no trends or changes in mean absolute change from baseline in vital signs following administration of ETX2514 with or without sulbactam and imipenem/cilastatin, and no notable differences in mean absolute change from baseline in vital signs between active and placebo groups.

5.3.1.4 Part D, multiple-dose safety cohort

In Part D, multiple-dose safety cohort,10 subjects received 1.0 g ETX2514 in combination with 1.0 g sulbactam and 0.5 g imipenem/cilastatin, and 2 subjects received placebo in combination with 1.0 g sulbactam and 0.5 g imipenem/cilastatin.

No deaths or serious TEAEs were reported, and no TEAEs leading to study drug discontinuation or interruption were reported. The greatest number of subjects reported TEAEs in the SOC "Nervous System Disorders." The TEAEs reported in more than 1 subject in any SOC were dysgeusia and headache (each reported in 2 subjects in the ETX2514 combination group), both often considered related to study drug; and upper respiratory tract infection (reported in 1 subject in each treatment group). Most TEAEs were of mild severity, and no TEAEs were considered severe. One subject was lost to follow-up after receiving all but 1 planned dose of 1.0 g ETX2514 with sulbactam and imipenem/cilastatin.

Increases in mean ALT were observed in both treatment groups. The greatest observed increases from baseline in mean ALT were 0.209 IU/L (Day 13) in the ETX2514 with sulbactam and imipenem/cilastatin group and 0.290 IU/L (Day 11) in the placebo with sulbactam and imipenem/cilastatin group. No individual subject had ALT values greater than 0.97 IU/L (Day 13; normal range: ≤0.73 IU/L). No hyperbilirubinemia was associated with these minor changes. These changes were not considered clinically significant by the Investigator.

There were no trends or changes in hematology or urinalysis results following administration of ETX2514 with sulbactam and imipenem/cilastatin, and no notable differences in hematology or urinalysis results between the active and placebo groups.

There were no trends or changes of mean absolute change from baseline in vital signs following administration of ETX2514 with or without sulbactam and imipenem/cilastatin, and no notable differences in mean absolute change from baseline in vital signs between the active and placebo groups.

In summary, ETX2514 by itself or in combination with sulbactam and/or imipenem/cilastatin was generally well tolerated among healthy volunteers in Study CS2514-2016-0001. There was no evidence of a DDI between ETX2514, sulbactam, and imipenem/cilastatin.

5.3.1.5 Safety tables for Study CS2514-2016-0001

Table 5–18. Summary of TEAEs by system organ class and preferred term (blinded to treatment, occurring in >1 person in any preferred term in any cohort) – Part A: SAD (Safety Population)

System Organ Class Preferred Term	Cohort 1 (N = 8) n (%) m	Cohort 2 (N = 8) n (%) m	Cohort 3 (N = 8) n (%) m	Cohort 4 (N = 8) n (%) m	Cohort 5 (N = 8) n (%) m	Cohort 6 (N = 8) n (%) m	Cohort 7 (N = 8) n (%) m	Cohort 8 (N = 8) n (%) m
Subjects with at least 1 TEAE	6 (75.0%) 12	5 (62.5%) 12	6 (75.0%) 10	2 (25.0%) 2	5 (62.5%) 11	4 (50.0%) 9	4 (50.0%) 6	3 (37.5%) 4
Nervous system disorders	1 (12.5%) 1	3 (37.5%) 6	3 (37.5%) 4	0 (0.0%) 0	3 (37.5%) 4	3 (37.5%) 4	1 (12.5%) 2	2 (25.0%) 2
Headache	1 (12.5%) 1	2 (25.0%) 3	2 (25.0%) 2	0 (0.0%) 0	1 (12.5%) 1	2 (25.0%) 2	1 (12.5%) 1	2 (25.0%) 2
Infections and infestations	2 (25.0%) 2	3 (37.5%) 3	1 (12.5%) 1	1 (12.5%) 1	1 (12.5%) 1	1 (12.5%) 2	1 (12.5%) 1	0 (0.0%) 0
Upper respiratory tract infection	1 (12.5%) 1	2 (25.0%) 2	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	1 (12.5%) 1	1 (12.5%) 1	0 (0.0%) 0
General disorders and administration site conditions	1 (12.5%) 1	1 (12.5%) 2	1 (12.5%) 1	0 (0.0%) 0	2 (25.0%) 2	0 (0.0%) 0	1 (12.5%) 2	0 (0.0%) 0
Musculoskeletal and connective tissue disorders	2 (25.0%) 2	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 2
Respiratory, thoracic, and mediastinal disorders	2 (25.0%) 3	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	2 (25.0%) 2	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0
Skin and subcutaneous tissue disorders	2 (25.0%) 2	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	1 (12.5%) 1	0 (0.0%) 0
Gastrointestinal disorders	1 (12.5%) 1	0 (0.0%) 0	2 (25.0%) 2	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0
Nausea	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 2	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0
Injury, poisoning, and procedural complications	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0
Immune system disorders	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0
Reproductive system and breast disorders	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0

Note: Adverse events are classified into system organ class and preferred term using MedDRA Version 19.1. TEAEs are sorted in descending order of total system organ class frequency across all groups and then total preferred term frequency across all groups.

m = number of events occurring in n subjects; MedDRA = Medical Dictionary for Regulatory Affairs; N = number of subjects per cohort; n = number of subjects with event; SAD = single ascending dose; TEAE = treatment-emergent adverse event.

Table 5–19. Summary of TEAEs by system organ class and preferred term (blinded to treatment, occurring in >1 person in any preferred term in any cohort) – Part B: MAD (Safety Population)

System Organ Class Preferred Term	Cohort 9 (N = 8) n (%) m	Cohort 10 (N = 8) n (%) m	Cohort 11 (N = 8) n (%) m	Cohort 12 (N = 8) n (%) m
Subjects with at least 1 TEAE	6 (75.0%) 19	5 (62.5%) 14	8 (100%) 21	8 (100%) 14
General disorders and administration site conditions	4 (50.0%) 7	2 (25.0%) 3	6 (75.0%) 6	3 (37.5%) 3
Catheter site phlebitis	1 (12.5%) 2	1 (12.5%) 1	3 (37.5%) 3	3 (37.5%) 3
Nervous system disorders	3 (37.5%) 5	3 (37.5%) 3	3 (37.5%) 5	1 (12.5%) 1
Headache	3 (37.5%) 4	1 (12.5%) 1	1 (12.5%) 1	1 (12.5%) 1
Dizziness	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 3	0 (0.0%) 0
Vascular disorders	0 (0.0%) 0	1 (12.5%) 1	2 (25.0%) 2	3 (37.5%) 3
Phlebitis	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 2	3 (37.5%) 3
Gastrointestinal disorders	2 (25.0%) 3	3 (37.5%) 3	0 (0.0%) 0	0 (0.0%) 0
Respiratory, thoracic, and mediastinal disorders	1 (12.5%) 1	2 (25.0%) 2	1 (12.5%) 1	1 (12.5%) 1
Nasal congestion	0 (0.0%) 0	2 (25.0%) 2	0 (0.0%) 0	0 (0.0%) 0
Musculoskeletal and connective tissue disorders	1 (12.5%) 1	1 (12.5%) 1	1 (12.5%) 1	1 (12.5%) 1
Skin and subcutaneous tissue disorders	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 3	1 (12.5%) 1
Macule	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 2	0 (0.0%) 0
Injury, poisoning, and procedural complications	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	2 (25.0%) 2
Psychiatric disorders	1 (12.5%) 1	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0
Blood and lymphatic system disorders	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1
Eye disorders	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0
Immune system disorders	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0	0 (0.0%) 0
Infections and infestations	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0

Note: Adverse events are classified into system organ class and preferred term using MedDRA Version 19.1. TEAEs are sorted in descending order of total system organ class frequency across all groups and then total preferred term frequency across all groups.

m = number of events occurring in n subjects; MAD = multiple ascending dose; MedDRA = Medical Dictionary for Regulatory Affairs; N = number of subjects per cohort; n = number of subjects with event; TEAE = treatment-emergent adverse event.

Table 5–19. Summary of TEAEs by system organ class and preferred term (blinded to treatment, occurring in >1 person in any preferred term in any cohort) – Part B: MAD (Safety Population) (continued)

System Organ Class Preferred Term	Cohort 9 (N = 8) n (%) m	Cohort 10 (N = 8) n (%) m	Cohort 11 (N = 8) n (%) m	Cohort 12 (N = 8) n (%) m
Renal and urinary disorders	0 (0.0%) 0	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1
Reproductive system and breast disorders	0 (0.0%) 0	0 (0.0%) 0	1 (12.5%) 1	0 (0.0%) 0

Note: Adverse events are classified into system organ class and preferred term using MedDRA Version 19.1. TEAEs are sorted in descending order of total system organ class frequency across all groups and then total preferred term frequency across all groups.

m = number of events occurring in n subjects; MAD = multiple ascending dose; MedDRA = Medical Dictionary for Regulatory Affairs; N = number of subjects per cohort; n = number of subjects with event; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.

Table 5–20. Summary of TEAEs by system organ class and preferred term (blinded to treatment, occurring in >1 person in any preferred term in any cohort) – Part C: single-dose DDI (Safety Population)

System Organ Class Preferred Term	Cohort 13 (N = 8) n (%) m	Cohort 14 (N = 8) n (%) m
Subjects with at least 1 TEAE	6 (75.0%) 8	2 (25.0%) 2
General disorders and administration site conditions	3 (37.5%) 3	1 (12.5%) 1
Gastrointestinal disorders	1 (12.5%) 1	1 (12.5%) 1
Musculoskeletal and connective tissue disorders	2 (25.0%) 2	0 (0.0%) 0
Nervous system disorders	1 (12.5%) 1	0 (0.0%) 0
Respiratory, thoracic, and mediastinal disorders	1 (12.5%) 1	0 (0.0%) 0

Note: Adverse events are classified into system organ class and preferred term using MedDRA Version 19.1. TEAEs are sorted in descending order of total system organ class frequency across all groups and then total preferred term frequency across all groups.

DDI = drug-drug interaction; m = number of events occurring in n subjects; MedDRA = Medical Dictionary for Regulatory Affairs; N = number of subjects per cohort; n = number of subjects with event; TEAE = treatment-emergent adverse event.

Table 5–21. Summary of TEAEs by system organ class – Part D: multiple-dose safety cohort (Safety Population)

System Organ Class Preferred Term	Cohort 15	
	1.0 g IV ETX2514 with sulbactam and imipenem/cilastatin (N = 10) n (%) m	Placebo with sulbactam and imipenem/cilastatin (N = 2) n (%) m
Subjects with at least 1 TEAE	8 (80.0%) 12	2 (100.0%) 3
Nervous system disorders	5 (50.0%) 5	0 (0.0%) 0
Dysgeusia	2 (20.0%) 2	0 (0.0%) 0
Headache	2 (20.0%) 2	0 (0.0%) 0
Tension headache	1 (10.0%) 1	0 (0.0%) 0
Infections and infestations	2 (20.0%) 2	1 (50.0%) 1
Upper respiratory tract infection	1 (10.0%) 1	1 (50.0%) 1
Vulvovaginal candidiasis	1 (10.0%) 1	0 (0.0%) 0
Gastrointestinal disorders	1 (10.0%) 1	1 (50.0%) 1
Abdominal pain	0 (0.0%) 0	1 (50.0%) 1
Diarrhea	1 (10.0%) 1	0 (0.0%) 0
Musculoskeletal and connective tissue disorders	1 (10.0%) 1	1 (50.0%) 1
Arthralgia	0 (0.0%) 0	1 (50.0%) 1
Musculoskeletal stiffness	1 (10.0%) 1	0 (0.0%) 0
General disorders and administration site conditions	1 (10.0%) 1	0 (0.0%) 0
Fatigue	1 (10.0%) 1	0 (0.0%) 0
Metabolism and nutrition disorders	1 (10.0%) 1	0 (0.0%) 0
Polydipsia	1 (10.0%) 1	0 (0.0%) 0
Skin and subcutaneous tissue disorders	1 (10.0%) 1	0 (0.0%) 0
Rash pruritic	1 (10.0%) 1	0 (0.0%) 0

Note: Adverse events are classified into system organ class and preferred term using MedDRA Version 19.1. TEAEs are sorted in descending order of total system organ class frequency across all groups and then total preferred term frequency across all groups. Percentages were based on the number of subjects within each cohort.

IV = intravenous; m = number of events occurring in n subjects; MedDRA = Medical Dictionary for Regulatory Affairs; N = number of subjects per cohort; n = number of subjects with event; TEAE = treatment-emergent adverse event.

5.3.2 Study CS2514-2017-0001

The safety of ETX2514SUL has been evaluated in the study, "A Phase 1 Study to Determine and Compare Plasma, Epithelial Lining Fluid, and Alveolar Macrophage Concentrations of Intravenous ETX2514 and Sulbactam Administered to Healthy Adult Subjects" (Study CS2514-2017-0001).

No deaths or SAEs occurred during this study. Of the 30 subjects enrolled in the study, 4 (13.3%) subjects experienced 5 adverse events; 4 events were considered unrelated to study drug, and 1 TEAE of infusion site pain was considered related to study drug. Three of the 5 events were considered mild in severity, and 2 were considered moderate in severity. The TEAE of mild hepatic enzyme increased was considered unrelated to study drug. The event occurred on Follow-up Day 13, with an ALT elevation of 94 IU/L (normal range: 5 to 46 IU/L) and aspartate aminotransferase (AST) of 233 IU/L (normal range: 10 to 41 IU/L). Both values returned to normal upon recheck in 7 days. Bilirubin and alkaline phosphatase remained within normal limits.

There were no clinically significant abnormalities in clinical chemistry, hematology and coagulation, or urinalysis parameters. There were no clinically significant abnormalities noted in vital signs, physical findings, or ECG results. Mean changes from baseline in clinical laboratory, vital signs, or ECG results were not appreciably different when measured at different time points, and no trends were noted.

Overall, administration of ETX2514 and sulbactam was found to be safe and well tolerated in healthy adult subjects in Study CS2514-2017-0001.

5.3.3 Study CS2514-2017-0002

The safety of ETX2514SUL has been evaluated in the Phase 2 study, "A Phase 2 Study to determine the plasma and urine pharmacokinetics and safety of ETX2514SUL in healthy adult subjects and adult patients with renal insufficiency" (Study CS2514-2017-0002).

Overall, the infusion of ETX2514SUL was well tolerated in 30 enrolled subjects; there were no deaths or SAEs. No adverse events were reported among the normal healthy volunteers or the patients with mild renal insufficiency. Among patients with moderate or severe renal insufficiency and patients receiving hemodialysis, only 1 TEAE was reported in more than 1 patient (nausea [6% of patients]) and was thought to be related to study drug. The safety profile of ETX2514SUL was comparable to that of prior studies. Other than the increased creatinine and laboratory and ECG abnormalities associated with underlying disease and

renal insufficiency, there were no changes of clinical significance in laboratory parameters, vital signs, or ECG parameters relative to baseline.

ETX2514SUL was found to be safe and well tolerated in patients with renal insufficiency including those receiving hemodialysis.

5.3.4 Study CS2514-2017-0003

The safety of ETX2514SUL with imipenem/cilastatin has been evaluated in the study, "A Double-blind, Randomized, Placebo-controlled Study to Evaluate the Safety and Efficacy of Intravenous Sulbactam-ETX2514 in the Treatment of Hospitalized Adults With Complicated Urinary Tract Infections, Including Acute Pyelonephritis" (Study CS2514-2017-0003).

Overall, treatment with ETX2514SUL and imipenem/cilastatin was well tolerated, with no deaths or SAEs. Table 5–22 presents drug-related TEAEs by SOC and preferred term. The safety profile was similar to that in prior studies. Infusion reactions (pain and phlebitis) were more frequent in the ETX2514SUL recipients. WBC and neutrophil counts from patients in this study were similar between the ETX2514SUL and placebo treatment groups at all time points, with patients in the ETX2514SUL arm having slightly higher baseline values. The change from baseline of total WBC and absolute neutrophil counts in the ETX2514SUL recipients was greater at all time points post baseline compared to the placebo arm. However, the differences were small, and no patients developed neutropenia. The clinical significance of this finding, in the setting of an acute infection with high WBC and neutrophil counts at baseline, is unclear. There were no adverse events of leukopenia or neutropenia reported.

Mild increases in ALT, AST, and alkaline phosphatase were observed in both arms of the study. No values over 3 × the upper limit of normal were observed in any of these enzymes, and there were no concurrent increases in bilirubin. One TEAE of ALT increased was reported for Patient 302-003. This 45-year-old male experienced an increased ALT of 83 U/L on Day 5 (normal range: 6 to 41 U/L), with elevated gamma-glutamyl transferase (GGT) of 391 U/L (normal range: 11 to 52 U/L). Subsequent values were within the normal range until the TOC Visit 1 week after completion of therapy, when ALT was elevated at 92 U/L and GGT was elevated at 367 U/L. At the Last Follow-Up Visit 1 week later, both values had improved: ALT was 52 U/L and GGT was 64 U/L. Bilirubin and alkaline phosphatase remained normal throughout the study. No significant changes in ECG or vital sign parameters were observed.

Table 5–22. Summary of study drug-related TEAEs (≥2 patients in any treatment group) by system organ class and preferred term – Study CS2514-2017-0003 (Safety Population)

System Organ Class Preferred Term	ETX2514SUL + IMI (N = 53) n (%)	Placebo + IMI (N = 27) n (%)	Total (N = 80) n (%)
Patients with any study drug-related TEAE	12 (22.6)	4 (14.8)	16 (20.0)
Gastrointestinal disorders	4 (7.5)	2 (7.4)	6 (7.5)
Nausea	2 (3.8)	1 (3.7)	3 (3.8)
Diarrhea	2 (3.8)	0 (0.0)	2 (2.5)
Nervous system disorders	3 (5.7)	1 (3.7)	4 (5.0)
Headache	3 (5.7)	1 (3.7)	4 (5.0)
Vascular disorders	2 (3.8)	0 (0.0)	2 (2.5)
Vascular pain	2 (3.8)	0 (0.0)	2 (2.5)

Percentage was calculated using the number of patients in the column heading as the denominator.
A TEAE was defined as an adverse event occurring on or after the administration of the first dose of study drug.
ETX2514SUL = sulbactam/ETX2514; IMI = imipenem/cilastatin; n = number of observations; N = number of patients; TEAE = treatment-emergent adverse event.

5.3.5 Analysis of adverse events in clinical studies

Overall analysis of adverse events observed in healthy volunteers and in patients are tabulated below in Table 5–23 and Table 5–24, respectively. To delineate the effects of ETX2514SUL, the adverse event analysis has been done among healthy volunteers (Study CS2514-2016-0001, Study CS2514-2017-0001, and Cohort 1 of Study CS2514-2017-0002) and among patients with renal insufficiency or cUTI (Cohorts 2 through 5 of Study CS2514-2017-0002 and Study CS2514-2017-0003). Catheter/infusion site reactions were the most common adverse events seen among healthy subjects and patients, with most of these reactions being characterized as mild or moderate in severity. There was no observed trend of a dose relationship of systemic adverse events. Comparing adverse events in the ETX2514-exposed groups to placebo groups, there was no evidence of an emergent safety signal.

Table 5–23. Adverse events reported in >1 subject among healthy volunteers – Studies CS2514-2016-0001, CS2514-2017-0001, and CS2514-2017-0002

Adverse Events in >1 Subject in Either Arm	Number of Subjects (%) Any ETX2514 Exposure N = 132	Number of Subjects (%) Placebo N = 30
Catheter/infusion site reactions [1]	18 (14)	5 (17)
Headache [2]	15 (11)	4 (13)
Dizziness	4 (3)	1 (3)
Upper respiratory tract infection	4 (3)	2 (7)
Nasal congestion	3 (2)	0 (0)
Nausea	3 (2)	1 (3)
Somnolence	2 (2)	1 (3)
Back pain	2 (2)	0 (0)
Pruritus	2 (2)	1 (3)
Vulvovaginal candidiasis	2 (2)	0 (0)
Dysgeusia	2 (2)	0 (0)
Arthralgia	1 (1)	2 (7)
Pain in extremity	1 (1)	2 (7)
Oropharyngeal pain	1 (1)	2 (7)

1. Includes additional terms: catheter site extravasation, catheter site rash, catheter site pain, catheter site phlebitis, phlebitis, catheter site bruise, catheter site inflammation, infusion site pain, infusion site reaction, and injection site pain.
2. Includes additional terms: head discomfort and tension headache.

N = Number of subjects.

Table 5–24. TEAEs reported in >1 patient among patients with renal insufficiency or cUTI – Studies CS2514-2017-0002 and CS2514-2017-0003

TEAEs in >1 Patient	Number of Subjects (%) ETX2514SUL ± IMI N = 79	Number of Subjects (%) Placebo + IMI N = 27
Catheter/infusion site reactions [1]	7 (9)	1 (4)
Nausea	4 (5)	1 (4)
Headache	5 (6)	2 (7)
Diarrhea	2 (3)	0 (0)
Vomiting	2 (3)	0 (0)

1. Includes additional terms: infusion site reaction, phlebitis, and vascular pain.

cUTI = complicated urinary tract infection; ETX2514SUL = sulbactam/ETX2514; IMI = imipenem/cilastatin; N = number of patients; TEAE = treatment-emergent adverse event.

5.3.6 SAEs and deaths

There have been no drug-related SAEs or deaths reported to date in the development program. One SAE has been reported thus far in the program in Study CS2514-2016-0001. The SAE involved a severe anaphylactic reaction in Subject 210003, a 33-year-old Black male with an ongoing medical history of allergy to Brazil nuts that was undisclosed at the time of screening. This event occurred on Study Day 6, resolved the same day, and was considered unrelated to ETX2514 (subject had eaten a dessert containing Brazil nuts in the clinic that day). The subject was treated in the emergency room, recovered, and remained in the study, but study drug was discontinued. Table 5–25 presents the cumulative summary of SAEs.

Table 5–25. Cumulative summary of SAEs

System Organ Class Preferred Term	ETX2514SUL	Blinded	Active Comparator	Placebo
Immune system disorders				
Anaphylaxis	1	0	0	0
ETX2514SUL = sulbactam/ETX2514; SAE = serious adverse event.				

5.3.7 Adverse events leading to discontinuation

In the FIH study (Study CS2514-2514-2016-0001), 3 subjects discontinued study drug due to an adverse event. In the MAD portion of the study, 1 subject (Subject 211001) withdrew on Day 1 after receiving only 13.4 mL of the 1 g/100 mL dose of ETX2514 due to infusion site reaction (considered very likely/certain to be ETX2514-related) and somnolence (considered unlikely to be ETX2514- related). Two additional subjects completed the study but had study drug withdrawn. Subject 210002 in the 0.5 mg dosing group received 3 doses on Day 1 and 1 dose on Day 2; study drug was discontinued due to nausea and somnolence. Subject 210003 in the 0.5 mg dosing group received all doses on Days 1 through 5, and 2 doses on Day 6; study drug was discontinued due to anaphylactic reaction to Brazil nuts.

In the cUTI study (Study CS2514-2017-0003), 2 patients discontinued the study due to TEAEs. Patient 103-004 developed mild urticaria that led to discontinuation of ETX2514SUL in combination with imipenem/cilastatin on Day 2. Patient 303-003 had a mild increase of serum creatinine to 138 mmol/L on Day 6 that required a discontinuation of imipenem/cilastatin per the protocol.

5.3.7.1 Analysis of laboratory parameters, vital signs, and ECG parameters

Details of laboratory parameters, vital signs, and ECG parameters from each study are described in the previous sections. Key findings that were seen in more than 1 study include:

- A trend toward reduction in total WBC and neutrophil counts in healthy subjects dosed with multiple doses of ETX2514 (MAD escalation and long-term therapy cohorts of Study CS2514-2016-0001) and in patients in the Phase 2 study (Study CS2514-2017-0003). WBC and neutrophil counts from patients in the Phase 2 study were similar between the ETX2514SUL and placebo treatment groups at all time points, with patients in the ETX2514SUL arm having slightly higher baseline values. The change from baseline of total WBC and absolute neutrophil counts in the ETX2514SUL recipients was greater at all time points post baseline compared to the placebo arm. However, the differences were small, and no patients developed neutropenia. The clinical significance of this finding, in the setting of an acute infection with high WBC and neutrophil counts at baseline, is unclear. There were no adverse events of leukopenia or neutropenia reported.

- Multiple dosing of ETX2514SUL (but not ETX2514) showed sporadic low grade increases in hepatic transaminases that were asymptomatic, transient, and not accompanied by increases in bilirubin. This is well described as an effect of sulbactam.

- There were no trends in vital signs or ECG parameters reported in clinical studies to date. Mild, asymptomatic, and reversible increases that were still within normal ranges, in blood pressure were noted in the elderly cohort of the FIH study (Study CS2514-2016-0001).

5.3.8 Sulbactam

The clinical safety of sulbactam has been established by nearly 30 years of experience with Unasyn (ampicillin/sulbactam). Unasyn is extensively prescribed on a worldwide basis and is regarded as generally safe and well tolerated. The dose of sulbactam, 1 g q6h (maximum daily dose of 4 g), that will be used in combination with ETX2514 is the top dose approved for human use (Unasyn Product Label). The most frequently reported adverse experiences with Unasyn, derived from product circulars, are listed in Table 5–26. Sulbactam is a penicillin derivative and, as such, should not be used in subjects with a history of penicillin hypersensitivity. Additional data on adverse experiences can be found in the US and

European product labels (Unasyn Product Label, Ampicillin/sulbactam Summary of Product Characteristics).

Table 5–26. Adverse events associated with ampicillin/sulbactam (Unasyn™).

System Organ Class	Frequency	Undesirable Effects
Gastrointestinal	Common	Nausea, vomiting, diarrhea
General disorders of administration site	Common	Phlebitis or pain and injection site
Nervous system	Uncommon	Headache, dizziness
Skin and subcutaneous tissue disorders	Uncommon	Rash, pruritus, and other cutaneous reaction
Hepatobiliary disorders	Very rare	Transient and reversible increases in transaminases ALT, AST, and bilirubin
Blood and lymphatic system disorders	Very rare	Hematological changes such as reversible and hematological anemia, thrombocytopenia, eosinophilia, and leukopenia
Common (≥1/100 to <1/10). Uncommon (≥1/1000 to ≤1/100). Very rare (≤1/10,000). ALT = alanine aminotransferase; AST = aspartate aminotransferase.		

5.3.9 Conclusions with respect to clinical safety

- ETX2514 was generally safe and well tolerated when given alone or with sulbactam and/or imipenem/cilastatin.
- To date, there have been no serious adverse drug reactions or deaths in clinical studies.
- The most common adverse events seen have been headache and infusion site reactions, including phlebitis and vascular pain at the infusion site.
- Mild reductions in total WBC and absolute neutrophil counts have been observed with multiple doses of ETX2514SUL, although no events of leukopenia or neutropenia have been observed.
- Asymptomatic, transient increases in hepatic enzymes, unaccompanied by increases in bilirubin, have been observed with ETX2514SUL, and these are thought to be related to known effects of sulbactam.

- Allergic reactions including urticaria have been observed at rates <1%, with ETX2514SUL, and all have been mild or moderate.

- No clinically significant changes in vital signs or ECG parameters have been observed with ETX2514SUL in patients. Transient increases in systolic blood pressure within normal ranges were observed in healthy elderly subjects dosed with ETX2514SUL.

5.4 Marketing experience

5.4.1 ETX2514 and ETX2514SUL

ETX2514 and ETX2514SUL have not yet been approved or marketed in any country at this time.

5.4.2 Sulbactam

Sulbactam is a penicillin derivative and is used widely as an inhibitor of β-lactamases. Although sulbactam is available as a standalone product in a small number of countries (e.g., Combactam, Germany; Sulbactam Summary of Product Characteristics), the vast majority of human use is in combination with β-lactams (e.g., Unasyn, ampicillin/sulbactam; Unasyn Product Label, Ampicillin/sulbactam Summary of Product Characteristics). Unasyn, is approved by regulatory authorities in the United States, Europe, and the Asia-Pacific region.

5.5 Emerging safety profile

5.5.1 ETX2514 and ETX2514SUL

Section 4.4 outlines the observed finding in the repeat-dose studies completed in rodents and nonrodent species. The adverse experience profile of ETX2514 in man, either alone or in combination with sulbactam, is summarized in Section 5.3. ETX2514, either alone or in combination with sulbactam, has been generally well tolerated in man. The observations from the repeat-dose studies in rodents and nonrodent species and from the clinical studies will serve as the basis for monitoring in future clinical studies in man.

5.5.2 Sulbactam

The adverse experiences for sulbactam are based on a review of product information labels for Unasyn (Unasyn Product Label). The most frequently reported adverse experiences, derived from product circulars, are listed in Table 5–26.

5.6 References

Ampicillin/sulbactam 1 g/0.5 g and 2 g/1 g powder for solution for injection or infusion. Summary of Product Characteristics (date of revision of text 07 July 2012). Strides Arcolab International Limited. Accessed at http://www.mhra.gov.uk/home/groups/par/documents/websiteresources/con183914.pdf on 11 July 2016.

Sulbactam 0.5 g and 1.0 g powder for solution for injection/infusion. Summary of Product Characteristics (date of revision of text June 2016). Accessed at https://www.pfizer.de/fileadmin/produktdatenbank/pdf/007628_freigabe.pdf.

Unasyn® ampicillin sodium and sulbactam sodium injection, powder, for solution. (date of revision 12/2014). Accessed at www.Pfizer.com on 10 July 2016.

6 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

6.1 ETX2514

ETX2514 is a BLI being developed in combination with sulbactam for the treatment of infections caused by *A. baumannii*. ETX2514 is not a β-lactam. ETX2514, either alone or in combination with sulbactam, has been studied in 3 clinical studies.

6.1.1 Nonclinical data for ETX2514

Nonclinical studies of ETX2514 revealed no significant test article–related adverse findings. ETX2514 was not carcinogenic or cardiotoxic. In consideration of mean ETX2514 clinical exposures observed in the Phase 2 study, the lowest exposure at the NOAEL (rat) represents a 6.2-fold AUC margin and a 36-fold C_{max} margin. ETX2514 was not genotoxic, and no safety pharmacology signals were noted in cardiac, CNS, or pulmonary studies. When combined with sulbactam and dosed for 28 days in rats, there was a finding of nonspecific, mild to moderate inflammatory exudate without evidence of necrosis in the liver, lung, and spleen that was not observed in the animals sacrificed 14 days after the end of dosing, indicating reversibility of the findings. C_{max} values of ETX2514 in the combination arms were 3.6- to 6.5-fold higher than human exposures, and C_{max} of sulbactam ranged from 3.4- to 6.8-fold higher than human exposures. Exposure margins based on AUC_{0-24}, compared to human exposures, ranged from 0.4- to 1.0-fold for ETX2514 and 0.8- to 1.8-fold for sulbactam.

The NOAEL for fertility, maternal toxicity, and EFD was considered 1000 mg/kg/day.

6.1.2 Clinical data for ETX2514

ETX2514 demonstrates linear PK, with very little accumulation over time. There is no DDI with sulbactam or with imipenem/cilastatin. ETX2514 is not metabolized by the CYP hepatic enzymes and is predominantly excreted renally. Dose adjustments are necessary for patients with moderate or severe renal insufficiency and for patients on dialysis. ETX2514SUL demonstrates good penetration into the ELF at 40% and 81% relative to unbound plasma concentrations of drug, thereby making it a good choice for treatment of pneumonia. Efficacy of ETX2514SUL was demonstrated in a Phase 2 study, where it showed clearance of imipenem nonsusceptible Gram-negative infections in the blood and urine.

Studies in healthy adult and elderly subjects and in patients with renal impairment or cUTI have revealed no serious adverse reactions (SARs) or deaths to date, and the withdrawal rate due to adverse events has been low (1%). Adverse events have generally been mild to

moderate in severity. The most common TEAEs in both healthy elderly subjects and in patients have included injection site reactions/pain and headache; nausea, diarrhea and vomiting were also common TEAEs in patients. Overall, ETX2514SUL has been well tolerated, with no deaths or SARs and only 1 drug-unrelated SAE reported to date. Doses of ETX2514SUL must be adjusted in patients with impaired renal function as detailed in Section 5.1.3.1.

Mild decreases from baseline in mean WBC and neutrophil counts have been observed in subjects receiving ETX2514 alone or multiple doses of ETX2514SUL with imipenem/cilastatin. Similar findings had been observed in the 14-day repeat-dose toxicity study in dogs receiving ETX2514 2000 mg/kg/day. Mild increases in ALT were observed in subjects receiving ETX2514SUL. These findings are in line with well described sulbactam-related hepatic effects. However, WBC counts and hepatic function will be monitored closely in ongoing clinical studies. Increases from baseline in blood pressure and/or heart rate have been observed in healthy elderly subjects receiving ETX2514 and in healthy adults receiving ETX2514 at the highest dose administered (twice the proposed dose). No clinically significant changes in vital signs or ECG parameters have been observed in patients treated with ETX2514SUL with or without imipenem/cilastatin.

The safety profile of ETX2514SUL in patients with renal impairment or cUTI requiring hospitalization was comparable to that of healthy adult volunteers. No deaths, SARs, or SAEs were reported.

Overall, ETX2514SUL with or without imipenem/cilastatin has been well tolerated in healthy adults including the elderly, and in patients with renal insufficiency or cUTI.

6.1.3 Possible risks and adverse reactions

Identified risks include infusion site reactions (including extravasation, bruise, rash, phlebitis, and/or inflammation) and infusion site pain. In healthy volunteers, 1 or more of these events has been reported in 14% of subjects exposed to ETX2514, and in 17% of subjects receiving placebo (with or without sulbactam and/or imipenem/cilastatin). Events were generally mild to moderate in severity; 1 subject was withdrawn from Study CS2514-2514-2016-0001 due to an infusion site reaction. Clinical observation and local symptomatic treatment as needed should be adequate to address these risks.

Mild decreases from baseline in WBC and absolute neutrophil counts have been observed in healthy adults and patients with cUTI, without evidence of leukopenia or neutropenia. As noted above, similar findings were noted in dogs receiving 2000 mg/kg/day in the 14-day

repeat-dose toxicity study. This is considered a potential risk, since values have remained within normal range in all subjects and/or patients. Additionally, baseline values tend to be elevated in patients with cUTI, and the significance of a greater decline with ETX2514SUL therapy is unclear. Close monitoring of hematologic parameters appears sufficient to further evaluate any potential significance of these findings.

Increased hepatic transaminases have been observed in subjects and patients receiving ETX2514SUL, but not in those receiving ETX2514 alone in the SAD and MAD cohorts of the FIH study (Study CS2514-2016-0001). Transaminases increased is listed as a common adverse event in the Combactam Summary of Product Characteristics (SmPC) (Sulbactam Summary of Product Characteristics) and has been correlated with hepatic glycogen deposits in nonclinical species. Since ETX2514 will always be administered in combination with sulbactam, this event is considered a potential risk for ETX254SUL. Increases from baseline in hepatic transaminases experienced by subjects and/or patients (with normal baseline values) were all low grade and not accompanied by hyperbilirubinemia. Close monitoring of hepatic transaminases and other hepatic function parameters appears sufficient to further evaluate the potential clinical significance of these findings.

Mild increases in blood pressure or heart rate relative to baseline and greater than placebo have been observed in healthy elderly subjects receiving single 1 g doses of ETX2514 and in healthy adults who received twice the proposed dose of ETX2514 in the MAD study cohort. In elderly subjects in the SAD portion of the study, the maximum systolic blood pressure was 141 mmHg at 3 hours post-infusion on Day 1; the maximum diastolic blood pressure was 93 mmHg at 3 hours post-infusion on Day 1. In subjects receiving 2 g ETX2514 in the MAD portion of the study, the maximum systolic blood pressure was 148 mmHg at 12 hours post-infusion on Day 3; the maximum diastolic blood pressure was 90 mmHg at 12 hours post-infusion on Day 3. No clinically significant changes in vital signs or ECG parameters have been observed in any of the studies to date, and 1 TEAE of increased blood pressure was reported in Study CS2514-2514-2017-0003 (maximum value of 122/78 that normalized). Although this finding is unlikely to be of concern, it will be considered a potential risk until further clinical data has been accumulated. Patients in clinical studies will have monitoring of vital signs.

Hypersensitivity reactions are listed as an uncommon adverse event in the Combactam SmPC but would not be unexpected in association with antibiotic therapy. To date there has been 1 report of mild urticaria in a subject receiving ETX2514SUL in combination with imipenem/cilastatin. The event resulted in the subject's withdrawal from the study. The product information for imipenem/cilastatin also lists hypersensitivity reactions as an adverse

drug reaction. Review of the medical history for β-lactam allergies and close observation of patients receiving ETX2514SUL, with discontinuation at the first sign of hypersensitivity, will be utilized to minimize this risk.

Skin disorders including rash have been reported in association with antibiotic therapy, including sulbactam. No serious skin reactions have been reported to date with ETX2514SUL. Rash and rash pruritic have been reported in 2 subjects receiving ETX2514SUL, and pruritus has been reported in 2 subjects receiving ETX2514SUL.

Review of the patient's medical history for previous drug-related serious cutaneous adverse reactions with other β-lactams and close observation of patients receiving ETX2514SUL, with immediate evaluation of the need to discontinue therapy if rash and/or pruritus occurs, will be utilized to minimize this risk. Clinical studies will exclude patients with history of serious and severe hypersensitivity to other β-lactams.

Superinfections, including infections due to *Clostridium difficile*, may be expected with any antibiotic, and such adverse events are listed in the Combactam SmPC. To date there have been 2 reports of vulvovaginal candidiasis in healthy subjects receiving ETX2514SUL, and 1 report of dysbacteriosis in a patient receiving placebo in combination with imipenem/cilastatin. Close observation of patients receiving ETX2514SUL therapy and limiting treatment to the shortest appropriate duration will be utilized to minimize this risk, or to allow early treatment as needed.

6.1.3.1 Contraindications

No possible contraindications have been identified for ETX2514; patients with a history of severe allergic or hypersensitive reactions to β-lactams should not be given ETX2514SUL. Sulbactam should be used with caution in patients with a prior history of cholestatic jaundice or hepatic dysfunction associated with Unasyn.

6.1.3.2 Possible interactions

ETX2514 and sulbactam have a low potential for DDIs. Probenecid decreases clearance of both drugs and concurrent use is not advisable.

6.1.4 General precautions, warning, and special action

Reductions in absolute neutrophil count and monocyte count were reported in the dog repeat-dose toxicity studies and will be monitored by routine hematology assessments in human subjects.

6.2 Sulbactam

Sulbactam is a penicillin derivative and is prescribed widely as an inhibitor of β-lactamases. Although sulbactam is available as a standalone product in a small number of countries (e.g., Combactam, Germany; Sulbactam Summary of Product Characteristics), the vast majority of human use is in combination with β-lactams (e.g., Unasyn, ampicillin/sulbactam; Unasyn Product Label, Ampicillin/sulbactam Summary of Product Characteristics). Unasyn, is approved by regulatory authorities in the United States, Europe, and the Asia-Pacific region. The clinical safety of sulbactam has been established by nearly 30 years of experience with Unasyn. Based on the product label, Unasyn is generally safe and well tolerated. Up-to-date information on sulbactam can be found in US and European Unasyn product labels (Unasyn Product Label, Ampicillin/sulbactam Summary of Product Characteristics).

6.2.1 Contraindications

Sulbactam is a penicillin derivative and, as such, should not be used in subjects with a history of β-lactam hypersensitivity.

Ampicillin/sulbactam is contraindicated in patients with a previous history of cholestatic jaundice/hepatic dysfunction associated with Unasyn.

6.2.2 Possible risks and adverse reactions

The most common adverse events reported with ampicillin/sulbactam are nausea, vomiting, diarrhea, pain at the injection site, and skin rash. Hepatic dysfunction, including hepatitis and cholestatic jaundice has been associated with the use of ampicillin/sulbactam. Hepatic toxicity is usually reversible; however, deaths have been reported. Routine monitoring of liver function will be conducted at baseline, during and after administration of ETX2514 and/or sulbactam in human subjects.

C. difficile associated diarrhea (CDAD) has been reported with use of nearly all antibacterial agents, and may range in severity from mild diarrhea to fatal colitis. Treatment with antibacterial agents alters the normal flora of the colon leading to overgrowth of *C. difficile*. *C. difficile* produces toxins A and B which contribute to the development of CDAD. Hypertoxin producing strains of *C. difficile* cause increased morbidity and mortality, as these infections can be refractory to antimicrobial therapy and may require colectomy. CDAD must be considered in all patients who present with diarrhea following antibacterial drug use. If CDAD is suspected or confirmed, ongoing antibacterial drug use not directed against *C. difficile* may need to be discontinued. Appropriate fluid and electrolyte management,

protein supplementation, antibacterial treatment of *C. difficile*, and surgical evaluation should be instituted as clinically indicated.

6.2.3 Possible interactions

Sulbactam has demonstrated limited potential for DDIs. Probenecid decreases the renal tubular secretion of sulbactam; as such, concurrent use of probenecid may result in increased and prolonged blood levels of sulbactam.

6.3 Sulbactam/ETX2514

ETX2514 and sulbactam have been dosed in combination in subjects in the FIH Phase 1 study. Based on preliminary blinded data, ETX2514SUL was generally well tolerated. The general safety profile of ETX2514 is unchanged when co-administered with sulbactam. There is no consistent pattern of local infusion site reactions to suggest a specific concern.

6.4 Reference safety information

This section is the reference safety information for ETX2514SUL and for sulbactam. See Table 6–1 for a summary of previous and ongoing risks due to ETX2514SUL. Since ETX2514 will always be administered with sulbactam, the known risk profile of sulbactam (Combactam) is included in the Summary of Important Risks. The adverse events described in this section will be used in determination of "expectedness" for safety reporting purposes. In this section, an adverse reaction is a drug-related TEAE with a relationship to study drug of "Very Likely/Certain," "Probable," or "Possible."

Table 6–1. Summary of previous and ongoing important risks due to ETX2514SUL

Risk	Nonclinical Data	Clinical Data	Actions
Identified risks			
Injection site reactions		Injection site reactions (extravasation, bruise, rash, phlebitis, inflammation) are the most frequently reported TEAEs.	Clinical monitoring of injection site, treatment as appropriate.
Injection site pain		Reported more frequently with ETX2514 than placebo. Injection site pain associated with sulbactam.	Clinical monitoring of injection site, symptomatic treatment as appropriate.
Potential risks			
Decreased mean WBC and neutrophil counts	14-day study in dogs: slightly reduced sternum bone marrow cellularity without evidence of changes in peripheral hematologic parameters.	Mild decreases noted in healthy adults and patients; no values outside normal limits. Leukopenia, neutropenia associated with sulbactam.	Frequent monitoring of total WBC and differential count during treatment.
Increased hepatic transaminases		Mild increases noted in healthy adults and patients; no concurrent increase in bilirubin. Transaminase increased and blood bilirubin increased associated with sulbactam.	Frequent monitoring of hepatic enzymes during treatment.
Increased blood pressure and/or heart rate		Observed only in healthy elderly adults or at doses above proposed dose.	Frequent monitoring of vital signs during treatment.
Hypersensitivity reactions		Urticaria reported in single subject with ETX2514SUL. Hypersensitivity reactions including anaphylactic shock associated with sulbactam.	Monitor and treat as appropriate.
Skin reactions		Rash and pruritus reported with ETX2514 and sulbactam. Severe SCAR reported in sulbactam SmPC.	Monitor and treat as appropriate. Avoid treating patients with history of SCAR related to beta-lactams.
Superinfections		Vulvovaginal candidiasis reported with ETX2514SUL. Sulbactam associated with oral candidiasis and *Clostridium difficile* infections.	Monitor and treat as appropriate.

ETX2514SUL = sulbactam/ETX2514; SmPC = Summary of Product Characteristics; SCAR = serious cutaneous adverse reaction; TEAE = treatment-emergent adverse event; WBC = white blood cell.

Other known adverse drug reactions listed in the Combactam SmPC (sulbactam; Sulbactam Summary of Product Characteristics) that are considered risks of ETX2514SUL include nausea, vomiting, diarrhea, decreased appetite, increased flatulence, headache, dizziness, seizures, eosinophilia, thrombocytopenia, thrombocytosis, leukocytosis, anemia, bleeding time increased, petechial bleed into skin and mucosa, muscle pains, blood creatinine increased, and interstitial nephritis. Additional precautions in the Combactam SmPC include the following:

- To date, there is no sufficient experience for safe use of sulbactam in pregnant women. Sulbactam diffuses readily into most body tissues and fluids in the human and crosses the placental barrier. Therefore, Combactam should be used during pregnancy only if the potential benefits outweigh the potential risks.
- Breastfeeding should be discontinued during sulbactam therapy.
- Reproductive toxicology studies with ETX2514 are incomplete at this time and all clinical study participants will be asked to take precautions against conception, and pregnant and lactating women will not be permitted in clinical studies.

6.5 References

Sulbactam 0.5 g and 1.0 g powder for solution for injection/infusion. Summary of Product Characteristics (date of revision of text June 2016). Accessed at https://www.pfizer.de/fileadmin/produktdatenbank/pdf/007628_freigabe.pdf.

Unasyn® ampicillin sodium and sulbactam sodium injection, powder, for solution. (date of revision 12/2014). Accessed at www.Pfizer.com on 10 July 2016.