

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

206494Orig1s000

PHARMACOLOGY REVIEW(S)

comments on NDA 206494 AVYCAZ

From: Abby Jacobs

Date: Feb 20, 2015

1. I concur that there are no pharm-tox related approval issues.

I have conveyed a number of other comments to the supervisor and she will address them as appropriate.

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/s/

ABIGAIL C JACOBS
02/20/2015

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 206494
Supporting document/s: 1
Sponsor's letter date: 6/25/2014
CDER stamp date: 6/25/2014
Product: AVYCAZ (avibactam and ceftazidime)
Indication: Treatment of infections caused by susceptible isolates of the designated micro-organisms causing complicated intra-abdominal infections (used in combination with metronidazole), and complicated urinary tract infections, including acute pyelonephritis.
Sponsor: Cerexa
Review Division: Division of Anti-infective Products
Reviewer: Wendelyn Schmidt
Armand Balboni
Secondary Reviewer: Amy Ellis
Division Director: Sumathi Nambiar
Project Manager: Carmen DeBellas

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1 Executive Summary

1.1 Introduction

AVYCAZ is an intravenous combination drug consisting of ceftazidime (CAZ), a cephalosporin antibiotic, and avibactam (AVI), a beta lactamase inhibitor, at a 4:1 ratio.

1.2 Brief Discussion of Nonclinical Findings

Most of the nonclinical studies submitted to the NDA addressed the toxicity of the AVI alone, as CAZ was previously found to be safe and effective in NDA 50634, NDA 50646, and NDA 50578.

The nonclinical toxicities of CAZ were described in the package insert label and in the literature. In the one month rat study, doses of 300 and 900 mg/kg/day yielded changes suggesting liver and kidney changes (increased serum cholesterol, proteinuria, and liver kidney organ weight increases) which were reversible. CAZ was negative in genotoxicity assays, and had no teratogenic effects in mice at doses up to 6.5 g/kg/day. Dogs were reported to show no toxicity at doses up to 540 mg/kg/day for 30 days (route of administration not specified) Capel-Edwards et al., J. Antimicrob. Chemother, (1981)8suppleB 237-239.

In the intravenous toxicity studies in rats and dogs, AVI was minimally toxic. No safety pharmacology signal was noted in cardiac, renal, CNS, or gastrointestinal studies. The protein binding in multiple species, including humans was low and effects on cytochrome P450 enzymes minimal, so drug interactions should be negligible. Rats were particularly susceptible to injection site damage, which included inflammation, thrombi, venous collagenous and myo-degeneration; vascular necrosis, and phlebitis.

In the 1 month rat and dog studies with the combination of CAZ-AVI at 4:1, a minimal increase (not synergistic or even additive) was noted in the damage at the injection site in either species. No new toxicities were noted with the combination, and no changes in the pharmacokinetic profile of either drug were seen.

AVI was not genotoxic. AVI was not teratogenic in the rat or rabbit, although a slight increase in late resorptions was noted in the rabbit at a minimally maternotoxic dose (2 fold the human exposure). Fertility was unaffected by administration of AVI to males or females prior to mating. The peri and post natal rat study showed an increase in the litter incidence of dilated pelvis and dilatation of the ureter, but no other significant changes.

1.3 Recommendations:

From a pharmacology/toxicology perspective, AVYCAZ can be approved.

1.3.2 Additional non-clinical recommendations

None at this time.

1.3.3 Labeling:

Section 8.1 initially read as follows. The deletions are underlined while the insertions are italicized.

8.1 Pregnancy

Pregnancy Category B

Animal reproductive toxicity studies have been conducted with ceftazidime and with avibactam. However, there are no adequate and well-controlled studies of AVYCAZ, ceftazidime, or avibactam in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used in pregnancy only if clearly needed.

Ceftazidime

Reproduction studies have been performed in mice and rats at doses up to 40 times the human dose and showed no evidence of ^{(b) (4)} harm to the fetus due to ceftazidime.

Avibactam

Reproductive studies performed during early pregnancy in ^{(b) (4)}

Avibactam was not teratogenic in rats or rabbits. In the rat, intravenous studies showed no embryofetal toxicity at doses of 1000 mg/kg/day, approximately 9 times the human dose based on exposure (AUC). In a rat pre- and postnatal study at ^{(b) (4)} human exposure ^{(b) (4)} based on AUC), there were no effects on pup growth and viability. A dose-related increase in the incidence of renal pelvic and ureter dilatation was observed in female weanling pups that was not associated with pathological changes to renal parenchyma or renal function, with renal pelvic dilatation persisting after female weanling pups became adults.

Reproductive studies performed during early pregnancy in rabbits showed no effects on embryofetal development at doses of 100 mg/kg, twice the human exposure (AUC). ^{(b) (4)} At higher doses, ^{(b) (4)} increased post-implantation loss, lower mean fetal weights, and slightly delayed ossification of several bones were observed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Ceftazidime and avibactam were each evaluated for mutagenic potential in several in vitro and in vivo assays. Ceftazidime was negative for mutagenicity in a mouse micronucleus test and an Ames test. Avibactam was negative for (b)(4) in the Ames assay, unscheduled DNA synthesis, chromosomal aberration assay, and a rat micronucleus study. Avibactam had no adverse effects on fertility of male and female rats given up to 1 g/kg (approximately 20 fold higher than the recommended clinical dose (b)(4)). There was a (b)(4) dose-related increase in the percentage of pre- and post-implantation loss relative to controls, resulting in a (b)(4) lower mean litter size at doses of 0.5 g/kg and greater with intravenous administration to female rats beginning 2 weeks prior to mating.



The label conveyed to the sponsor reads as follows:

8.1 Pregnancy

Pregnancy Category B

Animal reproductive toxicity studies have been conducted with ceftazidime and with avibactam. However, there are no adequate and well-controlled studies of AVYCAZ, ceftazidime, or avibactam in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used in pregnancy only if clearly needed.

Ceftazidime

Reproduction studies have been performed in mice and rats at doses up to 40 times the human dose and showed no evidence of harm to the fetus due to ceftazidime.

Avibactam

Avibactam was not teratogenic in rats or rabbits. In the rat, intravenous studies showed no embryofetal toxicity at doses of 1000 mg/kg/day, approximately 9 times the human dose based on exposure (AUC). In a rat pre- and postnatal study at up to (b) (4) mg/kg/day intravenously (11 times the human exposure (based on AUC), there were no effects on pup growth and viability. A dose-related increase in the incidence of renal pelvic and ureter dilatation was observed in female weanling pups that was not associated with pathological changes to renal parenchyma or renal function, with renal pelvic dilatation persisting after female weanling pups became adults.

Reproductive studies performed during early pregnancy in rabbits showed no effects on embryofetal development at doses of 100 mg/kg, twice the human exposure (AUC). At higher doses, increased post-implantation loss, lower mean fetal weights, delayed ossification of several bones and other anomalies were observed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Ceftazidime and avibactam were each evaluated for mutagenic potential in several in vitro and in vivo assays. Ceftazidime was negative for mutagenicity in a mouse micronucleus test and an Ames test. Avibactam was negative for genotoxicity in the Ames assay, unscheduled DNA synthesis, chromosomal aberration assay, and a rat micronucleus study.

Avibactam had no adverse effects on fertility of male and female rats given up to 1 g/kg/day (roughly 20 fold higher than the recommended clinical dose on a body surface area basis). There was a dose-related increase in the percentage of pre- and post-implantation loss relative to controls, resulting in a lower mean litter size at doses 0.5 g/kg and greater with intravenous administration to female rats beginning 2 weeks prior to mating.

2 Drug Information

2.1 Drugs

Avibactam and Ceftazidime (Fortaz® or Tazicef®)

CAS Registry Numbers

Avibactam: 1192491-61-4

Ceftazidime: 78439-06-2

Code Name: Avibactam: NXL104, RU84726, P135 (AVE1330A)

Chemical Names:

Avibactam: 1, 6, diazabicyclo[3.2.1]octane-2-carboxamide, 7-oxo-6-(sulfoxy)-, monosodium salt, (1R,2S,5R)

Ceftazidime: pentahydrate of pyridinium, 1-[[7-[[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-en-3-yl]methyl]-hydroxide, inner salt, [6R-[6 α , 7 β (Z)]]

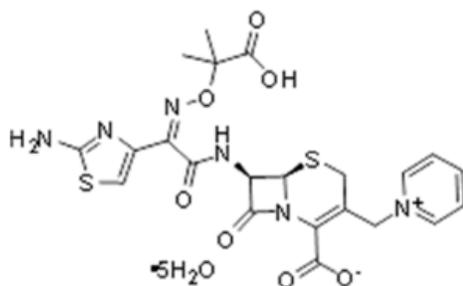
Molecular Formulas/Molecular Weights

Avibactam: C₇H₁₀N₃O₆SNa, mw = 287.2

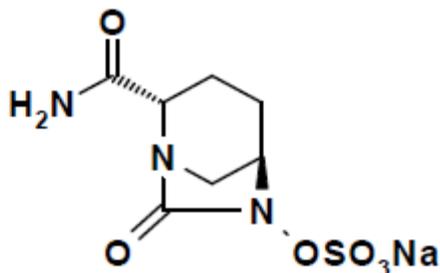
Ceftazidime: C₂₂H₃₂N₆O₁₂S₂; mw = 636.6

Structures:

Chemical Structure of Ceftazidime Pentahydrate



Chemical Structure of Avibactam



Pharmacologic Classes

Ceftazidime: third generation cephalosporin antibacterial agent

Avibactam: β -lactamase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

Ceftazidime: NDA 50634, NDA 50646, NDA 50578, DMF (b) (4)

Combination: IND 101307

2.3 Drug Formulation

(b) (4) avibactam sodium and 2635 (b) (4) ceftazidime pentahydrate/sodium carbonate (total weight (b) (4))

2.4 Comments on Novel Excipients

There are no excipients.

2.5 Comments on Impurities/Degradants of Concern:

(b) (4) was qualified at (b) (4) % for drug substance and (b) (4) % for drug product based on the 4 week dog study. (b) (4) at (b) (4) % was also considered qualified in the 4 week dog study. The PDE for (b) (4) at (b) (4) (b) (4) was acceptable. The PDE for (b) (4) of (b) (4) was also considered acceptable based on ICH Q3D. (b) (4) were all below the limit of detection and therefore acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed population is for treatment of patients age 18 or older, with complicated urinary tract infection (including acute pyelonephritis) or complicated intra-abdominal infection when used in combination with metronidazole. The dose will be 2.5 g (2 g ceftazidime and 0.5 g avibactam) intravenously over 2 hours administered every 8 hours, generally for 10-14 days.

2.7 Regulatory Background

Ceftazidime was approved in several NDAs (including NDAs 50646, 50634 and 50578) in the late 1980s. The sponsor is relying on the Agency's findings of safety and efficacy for CAZ to support this NDA. The combination of CAZ and AVI was studied in non-clinical efficacy models as well as in several Phase 2 clinical studies and an ongoing phase 3 study. The toxicology of avibactam alone was studied primarily in rats and dogs. One month studies in rats and dogs were conducted with the CAZ/AVI combination.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacodynamics: Reviewed by Clinical Microbiology

Secondary Pharmacodynamics:

1. 1-1023624-0. To evaluate, in enzyme assays, the activity of the test compound NXL104 (PT# 1078951). 2006
2. NXL104/DS0037 [CRO Study 9920321] In vitro pharmacology. 2003.
3. NXL104. NXL 104 effect on serine protease activity. 2006

Safety Pharmacology:

1. DSE2003-1095: A00211330A: Intravenous exploratory general behavior (Irwin Profile) in male rats. 2004.
2. NXL104/DS0010 {CRO Study 05.624/4} Safety pharmacology study for NXL104 in the Irwin test in the rat (intravenous administration). 2007.
3. DSE 2002-1730:A000211330A. Effects on the clone human cardiac potassium channel hERG. 2002.
4. NXL104/DS0012 [CRO study 05.626/4]. hERG current (I_{Kr}) expressed in human embryonic kidney (HEK) cells. 2007.
5. DSE 2003-1094: A000211330A. Effects on blood pressure and heart rate after a single intravenous administration in conscious rats. 2003.
6. NXL104/DS0011 [CRO Study 05.625/4]. Safety pharmacology study for NXL104 on cardiovascular risk in the conscious dog monitored by telemetry. 2007.
7. NXL104/DS009[CRO study 05.623/4. Safety pharmacology study for NXL104 on diuresis and urinary electrolyte excretion in the rat. 2007.
8. NXL104/DS0030 [CRO study AA40059]. NXL104: charcoal propulsion test in the conscious rat after on single oral administration. 2007.
9. NXL104/DS0008 [CRO study 05.622/4. Safety pharmacology study for NXL104 on respiratory function in the conscious rat (whole body plethysmography). 2007.

Pharmacokinetics:

Absorption

1. Drugs 1985; 29:105-161. Ceftazidime: a review of its antibacterial activity, pharmacokinetics, properties and therapeutic use.
2. Antimicrob Agents Chemother 1984; 26:204-207. A comparative pharmacokinetics of YM13115, ceftriaxone and ceftazidime in rats, dogs, and rhesus monkeys.
3. Study NXL104PK0014. NXL104—pharmacokinetics of NXL104 in plasma and urines in male CD1 mice after a single 25 mg/kg IV and o0ral administration. 2009.
4. Study A051310. Pharmacokinetics and excretion in urine of NXL104 in Sprague-Dawley rats after single oral and intravenous administration of NXL104. 2006.

5. Study A051236. Blood and plasma radioactivity kinetics and NXL104 plasma pharmacokinetics in male Sprague-Dawley rats after single 45 mg/kg intravenous administration. 2006.
6. Study A051237. Radioactivity mass balance of ¹⁴C-NXL104, blood and plasma radioactivity kinetics and NXL plasma pharmacokinetics in male beagle dogs after single 15 mg/kg/ intravenous administration 2006.
7. Study A051132. In vitro extent of binding of NXL104 to mouse, rat, rabbit, dog and human plasma. 2005.
8. Study NXL/03. NXL104: further investigations into the in vitro plasma protein binding in mouse, rat, rabbit, dog and human. 2009.
9. Dtsch tierarztl Wschr 2001; 108:168-171. Pharmacokinetics, intramuscular bioavailability, and tissue residue profiles of ceftazidime in a rabbit model.
10. Antimicrob Agents Chemother 1995; 39:2729-2731. Analysis of ceftriaxone and ceftazidime distribution in cerebrospinal fluid of and cerebral extracellular space in awake rats by in vivo microdialysis.

Distribution

1. Biopharm Drug Dispos 1998; 19: 473-478. Distribution of ceftazidime in rat tissues.
2. Study A051238. Distribution of ¹⁴C-NXL104 in rat after single 45 mg/kg intravenous administration. 2006.
3. Study NXL104/PK0007. Effect of NXL 104, a novel non-B-lactam b-lactamase inhibitor in combination with ceftazidime in a rabbit meningitis model. 2007.
4. Study NXL104/PK0009. NXL104—pharmacokinetics of NXL104 and ceftazidime in plasma and bronchoalveolar lavage fluid in normal and *K. Pneumoniae* infected female Swiss OF1 mice after single subcutaneous administration of 150 mg/kg ceftazidime and 37.5 mg/kg NXL104. 2008.
5. Study CAZ-AVI-M1-065. Pharmacokinetics of ceftazidime and avibactam in plasma and lung epithelial lining fluid of female neutropenic CD-1 mice with a thigh infection of experimental pneumonia. 2013.
6. Study CAZ-AVI M1-062. Efficacy of ceftazidime-avibactam against *Pseudomonas aeruginosa* in the murine lung infection model. 2012.

7. Study 194174. NXL104: The placental transfer of total radioactivity in the rat following intravenous administration of ¹⁴C-NXL104. 2012.
8. Study 194169. NXL104: the placental transfer of total radioactivity in the rabbit following intravenous administration of ¹⁴C-NXL104. 2012.

Metabolism:

1. Study PR6633/CC2109. In vitro interspecies comparison of NXI104 metabolism rat on mouse, rat, rabbit, dog, and human liver microsome preparations. 2005.
2. Study A051131. Qualitative metabolite profiles of ¹⁴C-NXL104 after in vitro incubation with rat, rabbit, dog, monkey and human liver microsomes. 2006.
3. Study A051246. Metabolic profiles and identification of NXL104 metabolites in biological specimens from male Sprague-Dawley rats after a single intravenous administration of ¹⁴C-NXL104 at 45 mg/kg. 2006.
4. Study A051248. Metabolic profiles and identification of NXL104 metabolites in biological specimens from male beagle dogs after a single intravenous administration of ¹⁴C-NXL104 at 15 mg/kg. 2006.

Excretion

1. Study A051240. Radioactivity mass balance of ¹⁴C-NXL104 in male Sprague-Dawley rats after single 45 mg/kg intravenous administration. 2006.
2. Study 194195: NXL104: The disposition of ¹⁴C-NXL104 in the female rabbit following intravenous administration. 2013.

Interactions:

1. Study PR6634/CC2108. Evaluation of human cytochrome P450 inhibitory properties of NXL 104: in vitro experiments. 2006.
2. Study 300205. NXL104: inhibition of CYP2C9 determination of the apparent K_i in human liver microsomes. 2007.
3. Study ADME-AZSwave3-130910. Assessment of the cytochrome P450 2B6 (CYP2B6) inhibition potential of avibactam in human liver microsomes. 2013.
4. Study 100236. NXL104: MTT cytotoxicity testing with human hepatocytes and cytochrome P450 induction in human hepatocytes. 2012.
5. Study 30110555. Evaluation of induction potential of CYP1A2, CYP2B6, and CYP3A4 by ceftazidime in human hepatocytes. 2012.

6. Study 301113129. Evaluation of inhibition of catalytic activity of uridine 5'-diphosphoglucuronosyl transferases (UGT) 1A1 by the test substances, NXL104 and ceftazidime in human liver microsomes. 2011.
7. Study 9316. Assessment of NXL104 for substrate and or inhibition potential and ceftazidime for inhibition potential for the human transport proteins MDR1, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MRP4, and BSEP. 2012.

General Toxicology:

Acute Toxicology:

1. NXL104/DS0006 [CRO study 30574TAS] Acute intravenous (infusion) toxicity in mice. 2006.
2. NXL104/DSo00031 [CRO study AA40060] NXL104-single dose toxicity study by the oral route (gavage) in the mouse. 2008.
3. NXL/DS0005 [CRO study 30573TAR] Acute intravenous (infusion) toxicity study in rats. 2006.
4. NXL104/DS0032 [CRO study AA40061] NXL104 Single dose toxicity study by the oral route (gavage) in the rat. 2008.
5. AA21246. NXL –7 day intravenous (30 minute injection) exploratory toxicity study in the rat. 2005.

Subchronic Toxicology

1. NXL104/DS0007 [CRO study 30052 TSR] 4 week toxicity study by intravenous route (infusion) in rats followed by a 2-week treatment-free period. 2007.
2. NXL104/DS0027 [CRO study AA39561] NXL104 13 week intravenous (30 minute infusion) toxicity study in the rat followed by a 4 week treatment free period. 2010.
3. NXL104/DS0013 [CRO study 30900 TSR] 4-week toxicity study by intravenous route in rats. 2006.
4. NXL104/DS0017 [CRO study 30054 TSC] 28 day toxicity study by intravenous route (30 minute infusion) in beagle dogs followed by a 14 day treatment free period. 2007.
5. NXL104/DS0029 [CRO study AA39562] NXL104 13 week intravenous (30 minute infusion) toxicity study in the beagle dog followed by a 4 week treatment free period. 2010.

6. NXL104/DS0014 [CRO study 30901 TSC] NXL104 and ceftazidime: 28 day toxicity study by intravenous route in beagle dogs. 2007;

Reproductive Toxicology:

1. NXL104/DS0025 [CRO study AA 39555]. NXL104: Male fertility and early embryonic development toxicity study by the intravenous route (30 minute infusion) in the rat. 2009.
2. NXL104/DS0026 [CRO study AA39559]. NXL140: Fertility and early embryonic development toxicity study in the intravenous route in the female rat. 2009.
3. NXL104/DS0020 [CRO study AA39553]. NXL104—Dose range-finding study by the intravenous route in the pregnant rat. 2008.
4. NXL104/DS0021 [CRO study AA39554]. NXL104—embryo-fetal development toxicity study by the intravenous route (30 minute infusion) in the rat. 2008.
5. NXL104/DS0022 [CRO study AA39550]. NXL104—Determination of the maximum tolerated dose by the intravenous route in the non-pregnant rabbit. 2008.
6. NXL104/DS0024 [CRO study 39552]. NXL104 embryo-fetal development toxicity study by the intravenous route (30 minute infusion) in the rabbit. 2008.
7. 3225WR [CRO study AB04834]. Avibactam (NXL104): Pre- and postnatal development study by the intravenous route (30 minute infusion) in the surgically implanted Sprague-Dawley rat. 2013.

Genotoxicology:

1. NXL104/DS0002 [CRO study 30484 MMO] Bacterial reverse mutation test. 2006.
2. HMR memo26/06/99 Exploratory in vitro unscheduled DNA synthesis.
3. DSE2002-0649. RU84726 Exploratory in vitro unscheduled DNA synthesis (UDS) test in rat liver cells in primary culture. 2004.
4. DSE 2003-0771. A00211330A (RU84726): Exploratory micromethod of the in vitro micronucleus test in mouse lymphoma cells (L5178Y) 2003.
5. NXL104/DS0003 [CRO study 30485 MLH]. In vitro mammalian chromosome aberration test in cultured human lymphocytes. 2006.
6. NXL104DS0018 [CRO study 31872 MAR]. Bone marrow micronucleus test by intravenous route (30 minute infusion) in rat. 2006.

7. 2814BV: AZ13591372. Genetic toxicity evaluation using a bacterial reverse mutation test. 2012.

Special Toxicology:

1. NXL104/DS0016 [CRO study 31394TAL]. NXL104 and ceftazidime: evaluation of the venous and perivenous local tolerance after a single administration in rabbits. 2007.
2. NXL104/DS0038 [CRO study AA82616]. 4 week intravenous (do minute infusion) toxicity study in the Sprague-Dawley rat with an immunotoxicological endpoint. 2010.
3. NXL104/DS0039 [CRO study 8214776]. Evaluation of in vitro phototoxicity of Balb/c 3T3 fibroblasts using the neutral red uptake assay. 2010.
4. NXL104/DS0015 [CRO study 259739]. Evaluation of the hemolytic potential of formulations of NXL104 in human blood in vitro. 2006.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary and Pharmacology

Avibactam is a beta lactamase inhibitor, while ceftazidime is a cephalosporin antibacterial.

4.2 Secondary Pharmacology

A series of radioligand binding assay and enzyme assays (including receptors, ion channels and transporters as well as serine proteases) were exposed to AVI at up to 100 uM (serine proteases at up to 1000 uM. No significant inhibition at relevant concentrations was noted (defined as less than 50% inhibition at the high concentration).

4.3 Safety Pharmacology

NXL-104 was examined in an extensive panel of neurological, cardiovascular, pulmonary, renal and gastrointestinal GLP safety pharmacology studies.

Gastrointestinal Safety Pharmacology

1. **Effect of NXL-104 on total intestinal transit time in rat** (Study NXL104/DS0030): No acute mortality was observed at 2000 mg/kg (high dose, HD), 500 mg/kg (mid-dose, MD), 125 mg/kg (low dose, LD) or vehicle (5% glucose aqueous solution). There was a statistically significant delay in mean % of the total intestinal tract distance achieved by charcoal test meal (transit time) at the HD (54.5% versus vehicle 71.9%). Intestinal transit time was not significantly affected at the MD or LD compared to the vehicle group. Reference substance (morphine 20 mg/kg) significantly delayed intestinal transit time (18.4% versus 71.9% vehicle).

Neurological Safety Pharmacology

1. **Effects of Intravenous Administration of NXL 104 on General Behavior (Irwin profile) in Rats** (Study DSE 2003-1095): Single administration of NXL 104 at 1000 mg/kg (HD), 300 mg/kg (MD) and 100 mg/kg (LD) had no effect on autonomic symptoms (respiratory rate, lacrimation, urination/diarrhea, salivation, piloerection, change in skin color and palpebral ptosis), alertness and reactivity (hypo reactivity, hyper reactivity, aggression and vocalization), motor activity (hypo activity, hyper activity, catalepsy and stereotypy), tone and coordination (hypo tonicity, ataxia, tremor and seizure).
2. **Effects of Intravenous Administration of NXL 104 in the Irwin test in the Rat** (Study NXL104/DS0010): Formulation sample instability at stored temperature of -20° C precluded an exact measurement of NXL 104 administered to animals during this study. Complimentary sample analyses provided nominal dosage information as follows: "100 mg/kg" (100 mg/kg), "300 mg/kg" (240-300 mg/kg), "1000 mg/kg" (800-1000 mg/kg). Single administration of NXL 104 provoked a dose-dependent depressant effect (decreased muscle tone and reactivity to touch) at approximately 100 mg/kg (Low dose), 300 mg/kg (Intermediate dose), and 1000 mg/kg (High dose). Induced defecation/diarrhea occurred in 3 of 4 HD animals.

Reviewer's note: the sample instability may have led to the very different toxicity profile from both the safety pharmacology and toxicology studies.

Cardiovascular Safety Pharmacology

1. **Effects of NXL104 on the Cloned Human Cardiac Potassium Channel HERG** (Study DSE 2002-1730): NXL104 at 300 μ M (HD) blocked HERG currents (**Avg. 20.7%**; range 8.6-23.3% - **IC₅₀ >300 μ M**). Peak tail currents of HERG were inhibited at concentrations of 100 μ M (**Avg. 15.4%**; range 6.4-22.7%) and 30 μ M (**Avg. 3.9%**; range 0-7%).

Reviewer's note: The findings suggest a large margin of safety.

2. **Effects of a Single Intravenous Administration of NXL104 on Blood Pressure and Heart Rate in Conscious Rats** (Study DSE2003-1094): At 1000 mg/kg (HD) blood pressure was transiently increased for approximately 2 minutes (10-14% versus 5% glucose solution). Heart rate was not affected.
3. **Effects of a Single Intravenous Administration of NXL 104 on Cardiovascular Function in Conscious Telemetrized Beagle Dogs** (Study NXL 104/DS0011): Formulation sample instability at stored temperature of -20° C precluded an exact measurement of NXL 104 administered to animals during this study. Complimentary sample analyses provided nominal dosage information as follows: “100 mg/kg” (240-300 mg/kg), “1000 mg/kg” (1000 mg/kg) producing nominal doses of 100, 300, 1000 mg/kg. NXL104, evaluated at the nominal doses of 100, 300 and 1000 mg/kg (i.v. infusion of 30 minutes) had no significant effects on arterial blood pressure, heart rate, and the PR, the QT and the QTc (Fridericia’s and van de Water’s formulae) intervals. No arrhythmia or other changes in the morphology of the electrocardiogram were observed at any dose level.

Pulmonary Safety Pharmacology

1. **Effects of a Single Intravenous Administration of NXL 104 on Respiratory Function as Measured by Whole Body Plethysmography in the Conscious Rat** (Study NXL104/DS0008): Formulation sample instability at stored temperature of -20° C precluded an exact measurement of NXL 104 administered to animals during this study. Complimentary sample analyses provided nominal dosage information as follows: “100 mg/kg” (100 mg/kg), “300 mg/kg” (240-300 mg/kg), “1000 mg/kg” (800-1000 mg/kg). Nominal doses of NXL 104 at 100, 300, and 1000 mg/kg had no significant effects on respiratory function compared to vehicle (5% glucose solution) or reference substance (Theophylline 16 mg/kg).

Renal Safety Pharmacology

1. **Single Intravenous Infusion of NXL 104 and Effects on Diuresis and Urinary Electrolyte Excretion in Rats** (Study NXL104/DS0009): Formulation sample instability at stored temperature of -20° C precluded an exact measurement of NXL 104 administered to animals during this study. Complimentary sample analyses provided nominal dosage information as follows: “100 mg/kg” (100 mg/kg), “300 mg/kg” (240-300 mg/kg), “1000 mg/kg” (800-1000 mg/kg). At the nominal doses of 100, 300 and 1000 mg/kg NXL104 had no significant effects on urinary volume, urinary pH and potassium and creatinine excretion. A dose dependent increase in sodium excretion was observed reaching statistical significance at 1000 mg/kg (0.246 ± 0.026 mmol/100 g body weight) versus vehicle control (0.130 ± 0.026 mmol/100 g body weight) and reference substance (Furosemide 8 mg/kg i.v. bolus: 0.549 ± 0.014).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The PK studies generally were not conducted under GLP regulations with the exception of the placental transfer studies in rats and rabbits and the toxicokinetic studies. The detection methods were either ¹⁴C-radiolabel or LC MS/MS.

In Study PK0003, male Sprague Dawley rats were administered an intravenous infusion of avibactam at 45 mg/kg over 30 minutes and the plasma kinetics were followed. Note that in table 10, measurements were via radiolabel while Table 11 used a LC/MS/MS technique and unchanged NXL was approximately 50% of the plasma radioactivity. Bioavailability after oral administration was quite low (approximately 5%), but only intravenous administration will be used in the clinic.

Rat Pharmacokinetics:

Table 10: Mean Pharmacokinetic parameters of total radioactivity in blood and plasma

Pharmacokinetic parameters	Plasma	Blood
T _{max} (h)	0.5	0.5
C _{max} (µg eq.g ⁻¹)	110.98	59.33
C _{last} (µg eq.g ⁻¹)	0.02	0.13
t _{1/2} (h)	51.79	176.38
AUC _{0-t} (µg eq.h.g ⁻¹)	103.88	108.40
AUC _{tot} (µg eq.h.g ⁻¹)	105.37	141.48
AUC _{extra} (%)	1.4	23.4

Rat Pharmacokinetics:

Table 11: Pharmacokinetic parameters of unchanged of NXL104 (total and free) in plasma

Pharmacokinetic parameters	Total NXL104	Free NXL104
T _{max} (h)	0.5	0.5
C _{max} (µg.mL ⁻¹)	67.11	22.90
C _{last} (µg.mL ⁻¹)	0.01	0.01
t _{1/2} (h)	1.27	0.71
AUC _{0-t} (µg.h.mL ⁻¹)	53.32	17.58
AUC _{tot} (µg.h.mL ⁻¹)	53.34	17.59
AUC _{extra} (%)	0.02	0.06
Cl (L.h ⁻¹ .kg ⁻¹)	0.84	2.56
Vd (L.kg ⁻¹)	1.55	2.64
Vss (L.kg ⁻¹)	0.66	1.90
MRT (h)	0.78	0.74

Table 7: Oral and iv pharmacokinetic parameters of NXL104 in mouse plasma and oral to iv ratios of exposure parameters

PARAMETERS	UNIT	ORAL ROUTE	Iv ROUTE	ORAL/iv RATIO
C _{max}	ng/mL	596	45466	0.013
t _{max}	h	0.5	0.083	nc
C _{last}	ng/mL	92	31.6	2.91
t _{last}	h	6	5	nc
AUC _{last}	h.ng/mL	1043	10642	0.098
AUC _{0-∞}	h.ng/mL	2580	10729	0.24
t _{1/2,z}	h	11.54	1.89	nc
%Extrap	%	59.5	0.8	nc

nc: no calculated

Plasma binding in all species was low (human 6-8%, mouse 7-12%, dog 13-22%, rabbit 5-19% and rat 2-22%) using an ultracentrifugation method. There was minimal correlation between binding and concentration. In a second study, anti-coagulants (sodium fluoride/potassium oxalate or lithium heparin) made no difference in the minimal plasma protein binding of NXL-104. Partitioning into polymorphonuclear cells was less than 10%.

In the rat, 1/100th of the plasma levels were found in the brain. The highest levels of NXL104 were found in the kidneys and bladder during the first few hours. In female Swiss mice injected subcutaneously with 37.5 mg/kg AVI and 150 mg/kg CAZ, epithelial lining fluid (ELF) accounted for 0.17 of the plasma exposure or C_{max} of AVI. CAZ and AVI levels in the mouse plasma did not differ significantly with infection. ELF levels were about a third lower in mice infected with *K. pneumonia*, but still showed 5-6 log reductions in bacterial load. Using radiolabeled NXL104 (1000 mg/kg in pregnant rats on gestation day 17, sampled at 0.5, 1, 2, and 6 hours post-administration), fetal levels of radioactivity only exceeded plasma levels at 6 hours and the maximum level in the fetus was 0.25% of the total administered dose. When 1000 mg/kg of NXL104 was administered on gestation day 20 to rabbits, 0.08% of the total dose was found in the fetus.

Metabolism of NXL104 was investigated in vitro and in vivo in multiple species. With human, mouse, rabbit, and dog hepatic microsomes, NXL104 was not depleted with up to 200 uM concentrations and 90 minutes at 37°C; with rat microsomes and 200 uM NXL104, concentration was depleted by 13% after 90 minutes. Further qualification in the liver microsome preparation with radiolabel showed up to 5 small peaks, 3/5 were noted in the human (individually less than 6%) as well as rat, rabbit dog and monkey. One metabolite was seen only in dog, and the other in rat and rabbit. Peaks were identified as decarbonyl-NXL104, dihydroxy NXL104, and monohydroxy NXL104. Another study with fresh rat and dog and frozen human liver sections incubated with 10 uM NXL104 for up to 180 minutes showed that the small peaks which eluted early (< 5 minutes) corresponded to peaks which formed with drug in buffer alone. Only rat gave a measurable metabolite by HPLC-MS, not humans or dogs. In whole animal studies of metabolism where plasma, urine and fecal levels of metabolites were monitored, rat showed the highest levels of metabolites in the urine, with dog as an intermediate and humans with greater than 93% of the dose excreted in urine as parent compound (see table below). Based on the metabolic profiles, both rat and dog are appropriate models for testing.

Disposition of NXL104 was studied using ¹⁴C-NXL104 following a single intravenous administration through 168 hours. The primary route of excretion, in dog, rat and rabbit, was urine. Most of the drug had been eliminated within the first 24 hours after administration. After 168 hours, less than 0.2% remained in the carcass in rats and rabbits. Fecal elimination accounted for a maximum of 17% of the dose in rats, 5% in dogs, and <1% in rabbits. Data is shown in the table below. Elimination via respiration was measured only in the rats and accounted for less than 0.2% of the dose.

Species	Dose	% in urine	% in feces	% in cage wash	Total recovery
Dog (male)	15 mg/kg	82%	5%	11%	97%
Rabbit (female)	1000 mg/kg	78%	0.6%	6%	85%
Rat (male)	45 mg/kg	73%	17%	12%	102%

Table 1.4-1. Pharmacokinetics: Metabolism In Vivo - Study Numbers A051246, A051248, and Avibactam KMX001

Species:				Rat	Dog	Human											
Gender (M/F)/Number of animals:				M / 3	M / 3	M / 6											
Feeding condition:				Fed	Fed	Fed											
Vehicle/Formulation:				5% w/v glucose in water	5% w/v glucose in water	0.9% saline											
Route of Administration:				Intravenous infusion	Intravenous infusion	Intravenous infusion											
Method of Administration:				Infusion for 30 min	Infusion for 30 min	Infusion for 60 min											
Dose (mg/kg):				45	15	500 mg/subject											
Radionuclide:				[¹⁴ C]	[¹⁴ C]	[¹⁴ C]											
Specific Activity (µCi/kg):				250	50	4.3 (300 µCi/subject)											
Analytical method(s):				Liquid scintillation counting	Liquid scintillation counting	Liquid scintillation counting											
Study number:				A051246	A051248	Avibactam KMX001											
Species	Sample	Sampling period	% of dose in sample	% of Compound in sample												Total % recovery	
				Parent	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11		M12
Rat	Urine	0-24 h	71.84	58.70	5.53	0.32	-	0.22	-	-	4.65	-	1.49	-	0.93	-	100
Rat	Faeces	0-48 h	17.63	15.05	0.53	-	0.52	-	-	0.62	0.22	-	-	0.69	-	-	88.1
Dog	Urine	0-24 h	79.08	72.14	-	2.82	0.87	-	-	0.76	0.36	-	-	1.96	-	0.17	100
Dog	Faeces	0-48 h	10.29	7.66	-	0.30	0.63	-	0.23	0.42	-	-	-	0.74	-	0.31	68 - 95
Human	Urine	0-96 h	97.01 ± 0.99	93.0	7.0	-	-	-	-	-	-	-	-	-	-	-	100
Human	Faeces	0-96 h	0.20 ± 0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	Not analyzed
Additional information: Due to low levels of total radioactivity in human feces, it was not analyzed for any metabolites																	
Study conducted by the applicant: No																	
If no, indicate the name and address of the institute that conducted the study: (b) (4)																	
Study in compliance with GLP: No																	

Table 1.4-6. Pharmacokinetics: Possible Metabolic Pathways



The effects of NXL104 and ceftazidime on human cytochrome P450 enzymes and human transporter proteins were investigated in vitro. NXL104 and ceftazidime did not significantly inhibit or induce cytochrome P450s. Similarly, at relevant concentrations, NXL104 and ceftazidime did not affect transporter proteins such as OAT1, OCT3, OCT2, BSEP, and BCRP.

6 General Toxicology

6.1 Single-Dose Toxicity

Study Type/number	NXL-104	N/sex/dose	Results	NOAEL
TAR acute I.V. (infusion) in rats/ NXL104/DS0005	0 and 2000 mg/kg	5/sex/dose	No mortality and no clinical signs were observed at 0 or 2000 (high-dose) mg/kg.	2000 mg/kg/day
TAS acute I.V. (infusion) in mice/ NXL104/DS0006	0 and 2000 mg/kg	5/sex/dose	No mortality and no clinical signs were observed at 0 or 2000 (high-dose) mg/kg.	2000 mg/kg/day
Single oral dose (gavage) study in CD1 mice/ NXL104/DS00031	0 and 2000 mg/kg	5/sex/dose	No mortality and no clinical signs were observed at 0 or 2000 (high-dose) mg/kg.	2000 mg/kg/day
Single oral dose (gavage) in rats/ NXL104/DS0032	0 and 2000 mg/kg	5/sex/dose	No mortality and no clinical signs were observed at 0 or 2000 (high-dose) mg/kg.	2000 mg/kg/day

6.2 Repeat-Dose Toxicity

The sponsor conducted a 7 day intravenous exploratory study (OECD GLP) in the rats at 0, 167, 500, and 1500 mg/kg/day at 5 mL/kg/day of NXL 104 (avibactam). The vehicle control was 5% glucose. All animals survived to scheduled sacrifice. Some damage to the injection site was noted at the high dose. Decrements in RBC parameters and BUN were noted at the HD as well as decrements in heart and kidney weights in the absence of histopathologic correlates. The sponsor considered the HD (1500 mg/kg/day) to be a maximum tolerated dose, 500 mg/kg to be a NOAEL, and 125 mg/kg/day to be a NOEL.

Study title: 4-week toxicity study by intravenous route (infusion) in rats followed by a 2 week treatment free period.

Study no.: NXL104/DS0007
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: October, 2005
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: P135 (AVE1330A)batch # 0500012675, 99.7% pure

Key Study Findings: The major finding was injection site damage which included inflammation, thrombi, venous collagenous and myo-degeneration; vascular necrosis, and phlebitis. The NOAEL was 500 mg/kg.

Methods

Doses: Males: 0, 167, 500, 1200 mg/kg/day;
 Females: 0, 167, 500, 1000 mg/kg/day
 0, 1000 mg/kg/day males to replace 1200 mg/kg/day group
 Frequency of dosing: Daily for 28 consecutive days
 Route of administration: intravenous
 Dose volume: 10 mL/kg/day for males @167, 500 mg/kg/day, 15 mL/kg/day for females and 1000 mg/kg/day males
 Formulation/Vehicle: 5% aqueous solution of glucose
 Species/Strain: Sprague Dawley; Crl CD(SD) IGS BR rats
 Number/Sex/Group: 10/sex/dose
 Age: 6 weeks
 Weight: M: 175-213 g, F: 134-167 g
 Satellite groups: 6/sex/control and HD for recovery
 6/sex/dose for TK (2/sex/control group)
 6/sex/dose for immune response
 Unique study design: Immune response (see "Special Evaluation")
 Deviation from study protocol: Males at 1200 mg/kg/day were euthanized on day 16 due to intolerance at the injection site; a new group of males was treated with control and 1000 mg/kg/day and treated for 28 consecutive days with appropriate recovery, TK and immune response groups.

Observations and Results

Mortality (twice daily): One male each at 1200, 1000, 500 (satellite) and 167 (satellite) mg/kg died on days 2, 18, 9 and 6. The 2 satellite animals died during restraint. The 1200 mg/kg animal was euthanized due to tail (injection site)

lesions. The 1000 mg/kg animal died during restraint. All but the 1200 mg/kg animal were considered by the sponsor to be related to restraint.

Clinical Signs (once daily, detailed clinical examinations once weekly): There was a dose dependent increase in damage to the injection site (tail) which included hematomas, scabs, wounds, and dryness. Damage at the HD was noted as early as the first week of dosing. Abnormal vocalization was noted in the HD (1200 mg/kg in males, 1000 mg/kg in females). Damage resolved by the end of the recovery period.

Body Weights (weekly): There were no significant effects on body weight with treatment with NXL 104; however, in the satellite recovery group for females at 1000 mg/kg, there was a roughly 10% decrease in body weight from day 11 through 43.

Feed Consumption (weekly): There were no remarkable differences in food consumption with treatment.

Ophthalmoscopy (pretest [all], prior to necropsy in control and HD): There were no remarkable findings in any group.

Hematology (pretest, pre-necropsy): There were no remarkable dose dependent changes in hematologic parameters.

Clinical Chemistry (pretest, pre-necropsy): ALP levels in the 1200 mg/kg males were increased roughly 5 fold at day 16; however, no concomitant controls were available. No remarkable changes were noted in the treated rats at day 28 or at recovery.

Urinalysis (pre-necropsy): There were no remarkable differences between groups with treatment.

Gross Pathology (D16 for HD males, D28 or D 42): Damage at the injection site was seen primarily at the 1200 mg/kg males (sacrificed at day 16) and at a lower incidence, 1000 mg/kg males and females. This damage included reddish/ purple/black color, hematoma, and scabs.

Organ Weights: In the males, there were decrements at the 500 mg/kg group (approximately 10% in the spleen and thymus), but no changes in the 1000 mg/kg group that was treated at a later time. In the females at 1000 mg/kg, there were roughly 10% decrements in the liver and thymus weights at the end of the treatment period, but no changes at the end of the recovery period. At the end of the recovery period, the testes weight in the males was increased by approximately 10%. All of these findings are of dubious toxicologic significance but should be monitored closely in the 13 week study.

Histopathology (all control and HD animals, all LD and MD tissues with macroscopic lesions)

Adequate Battery-- Yes

Peer Review--Yes

Histological Findings—Microscopic damage at the injection site included inflammation, collagenous degeneration of the venous wall and subcutis, hemorrhage, vascular necrosis, perivenous and subcutis fibrosis, thrombi, and at a lesser incidence or only at the 1200 mg/kg dose, phlebitis, myodegeneration, hyperkeratosis, epidermal hyperplasia, infarction, neovascularization and congestion. Other minor findings included granulocyte infiltration in the lung, congestion in the lymph nodes, and increased hematopoiesis in the spleen.

Special Evaluation (immune response: D 22 administered 1 mL of KLH (keyhole limpet hemocyanin) at 2.5 mg/mL s.c; at D29, sample blood for ELISA for anti-KLH IgG): Mean antibody levels did not differ significantly between groups, suggesting a minimal potential for immunoresponse.

Toxicokinetics (day 1, end of treatment: 33 minutes [3 minutes after end of infusion] , 1, 3, 5, 7, and 24 hours after start of infusion):

Dose-level (mg/kg/day)	Sex	Study day	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₄ (µg/mL.h)	t _{1/2} (h)	CL (L/h/kg)	R _{AC}
167	female	1	149000	0.55	117.8	*	1.418**	0.8191
		29	132900	0.55	96.49	4.634	1.731	
	male	1	101000	0.55	86.09	*	1.940**	
		29	205500	0.55	121.0	*	1.380	
500	female	1	500800	0.55	323.4	4.604	1.545	1.703
		29	750500	0.55	550.6	5.084	0.9081	
	male	1	660400	0.55	428.9	3.427	1.166	
		29	762300	0.55	483.7	3.726	1.034	
1000	female	1	1217000	0.55	783.8	4.301	1.276	0.8385
		29	882800	0.55	657.2	5.032	1.522	
	male	1	1700000	0.55	1025	4.814	0.9751	
		29	1659000	0.55	1051	4.701	0.9515	
1200	male	1	135300	1.00	141.2	*	*	-

*: Parameter could not be calculated due to insufficient data points in the terminal phase of the profile

** : Clearance = dose/AUC₀₋₄ (where on day 1 AUC_{0-∞} could not be calculated)

Lower Limit Of Quantification (as free acid): 10 ng/mL

Dosing Solution Analysis: All dosing solutions were within 10% of the intended concentration.

Study title: 13-week intravenous (30 minute infusion) toxicity study in the rat followed by a 4-week treatment-free period.

Study no.: NXL104/DS0027
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: August, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NXL-104, Batch # 063508002, 98.9% pure

Key Study Findings: The major toxicity to the rats was injection site damage. The NOAEL could not be determined due to catheter related infections. However, no new toxicities were noted, so repetition is not necessary.

Methods

Doses: Males/Females: 0, 65, 125, 250 mg/kg/day
Frequency of dosing: Daily for 91 days
Route of administration: Intravenous
Dose volume: 15 mL/kg
Formulation/Vehicle: 5% aqueous solution of glucose
Species/Strain: Sprague Dawley; CrI CD(SD) rats
Number/Sex/Group: 12/sex/dose (Terminal phase)
Age: 9 weeks
Weight: M: 297-368 g, F: 185-231 g
Satellite groups: 5/sex from 0, 250 mg/kg/day (Recovery phase),
6/sex/dose for TK
Unique study design: None
Deviation from study protocol: Due to high morbidity/mortality occurring during the study in active and control groups, several of the moribund animals (especially at the HD) were moved from the recovery group to the main study, while healthier main study animals were switched to recovery.

Observations and Results**Mortality (twice daily):**

Early deaths are summarized in the following table.

Rats found dead or moribund sacrifice				
	Males		Females	
	# dead	Day of death	# dead	Day of death
Control	1	83	4	66 (2), 69, 87
65 mg/kg	2	65, 70	2	48, 82
125 mg/kg	2	70, 88	2	47,66
250 mg/kg	2	43, 84	6	40, 45, 66, 69, 77 (2)

Masses, swelling and induration at the implantation site were associated with most deaths. Respiratory distress was sporadically noted and did not correlate with dose.

Clinical Signs (Once-daily: Treatment period; Once-weekly: Recovery period):

Macroscopic indurations/edematous aspects were commonly observed at the injection site and dark or pale raised areas were noted on the lungs secondary to the implanted catheter. Findings along the catheter site were noted across all animal groups. Other clinical signs, such as hair-loss, scabs or sores, were observed for all animals and were probably due to the administration process (e.g. use of jacket).

Body Weights (weekly): There were no significant effects on body weight with treatment with NXL 104.

Feed Consumption (weekly): There were no remarkable differences in food consumption with treatment.

Ophthalmoscopy (All: day -3; Terminal phase group 1 and 4 animals: M: day 88; F: day 87): A likely spontaneous corneal opacity was noted in one control animal and two treatment animals. There were no treatment-related ophthalmological findings.

Hematology (Terminal phase animals: M: day 91; F: day 91; Recovery phase animals: M: day 120; F: day 119): Changes to hematology values (low red blood cell parameters and high red cell distribution widths; high white blood cell parameters; high activated partial thromboplastin time) were noted in two males dosed at 250 mg/kg/day.

Clinical Chemistry (Terminal phase animals: day 91; Recovery phase animals: M: day 120; F: day 120): No remarkable changes were noted in the treated rats at day 91 or at recovery.

Urinalysis (Terminal phase animals: day 91; Recovery phase animals: M: day 120; F: day 120): No treatment-related variations in urinary parameters were observed during the study.

Gross Pathology (Terminal phase animals (sacrifice): day 91; Moribund animals: as discovered): Prominent and widespread lesions or abscesses were noted in all animals at premature death. Similarly, abscesses were noted across all groups but not in all animals of terminal sacrifice animals including recovery animals. Considering the similar incidence and severity in the distribution of the abscesses and their associated lesions across the groups including controls and the noted poor clinical conditions of the housed animals, it is unclear whether NXL104 may have contributed to the development of these lesions or their exacerbation. All other histological findings noted in the study are considered to be incidental or part of the background findings seen in rats of this age.

Organ Weights: A statistically significant increase in the absolute and relative weight of the spleen was noted in males at 250 mg/kg/day and also a higher mean relative weight in males at 65 mg/kg/day ($p \leq 0.01$). A statistically significant mean absolute and relative pituitary gland weight was noted in males (65, 250 mg/kg/day) versus controls ($p \leq 0.01$). There were no noted histological changes in the pituitary glands of those affected animals. The poor clinical condition and widespread presence of abscesses across all groups in this study together with the lack of clear histopathological findings (below) in the affected animals make it difficult to determine whether such changes were related to NXL 104 treatment.

Histopathology:

Adequate Battery--Yes

Peer Review--Yes

As noted above, a number of animals presented with severe hematological findings at day 91 (such as low hemoglobin concentration and packed cell volume, high fibrinogen concentration). These animals had severe histological lesions (especially abscesses) in the tissues/organs (principally associated with the injection site, liver, lungs, and kidneys). Marked but less severe histological lesions were also observed in similar anatomical locations across all groups, including the control group. Therefore, while noted for completeness of the review, histological findings were considered to be due to the presence of bacteria from indwelling catheters and not likely due to NXL 104 treatment.

Special Evaluation: During the course of the study, a poor general clinical condition was observed in animals from all groups, including the control group, followed by death or sacrifice due to a moribund condition. Macroscopically, indurations and abscesses were observed at the injection site and systemically in significant numbers of animals. These findings were considered by the supervising

veterinary pathologist to be secondary to probable bacterial infection due to the duration of the implanted catheter (13 weeks). *Pseudomonas aeruginosa* was detected in organ samples and blood samples from a number of animals and may have contributed to the indurations and abscesses observed.

Toxicokinetics (6 satellite animals per sex in all groups including controls on the first day of dosing (day 0) and on day 85): The toxicokinetics have been investigated in male and female rats after intravenous doses of NXL104. The increases seen in C_{max} and AUC_{0-24h} were approximately linear to increase in dose on both Day 0 and Day 85 (between 3-fold and 5-fold increases for a 4-fold increase in dose). There was negligible accumulation between Day 0 and Day 85. There was no apparent gender effect.

Occasion	Dose (mg/kg/day)	Sex	C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	AUC_{0-24h} ($\mu\text{g}\cdot\text{h/mL}$)
Day 0	65	Male	77.3	0.55	58.0
		Female	78.8	0.55	59.8
	125	Male	145	0.55	124
		Female	129	0.55	99.6
	250	Male	326	0.55	315
		Female	259	0.55	182
Day 85	65	Male	108	0.55	92.5
		Female	70.3	0.55	76.6
	125	Male	187	0.55	175
		Female	176	0.55	275
	250	Male	427	0.55	366
		Female	296	0.55	350

Dosing Solution Analysis: The dosing solutions were between -2 and +4.5% of the intended concentrations.

Study title: 28 –day toxicity study by intravenous route (30 minute infusion) in beagle dogs followed by a 14-day treatment-free period.

Study no.: NXL104/DS0017
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: July 2006
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104, batch # 0600032422, 97.8% pure; batch # 0600042423, 95.5% pure

Key Study Findings:

Findings with avibactam administration up to 1000 mg/kg/day for 28 consecutive days were minimal. The HD, 1000 mg/kg/day could be considered a NOAEL. Observations to follow in a 13 week study were potential for elevated BUN and injection site damage, only injection site damage was significant in the longer study.

Methods

Doses: 0, 250, 500, 1000 mg/kg/day
Frequency of dosing: Daily for 28 consecutive days
Route of administration: Intravenous (alternating cephalic or external saphenous veins)
Dose volume: 5 mL/kg/30 minutes
Formulation/Vehicle: 5% aqueous glucose
Species/Strain: Beagle dogs
Number/Sex/Group: 3/sex/dose
Age: 8 months old
Weight: M: 82.-10.5 kg; F: 6.8 -8.9 kg
Satellite groups: 2/sex/dose for control, HD for recovery
Unique study design: none
Deviation from study protocol: None that were significant

Observations and Results

Mortality (twice daily): All dogs survived to scheduled sacrifice.

Clinical Signs (twice daily): Vomiting and salivation were increased in the HD males, only vomiting increased in incidence in the HD females.

Body Weights (twice weekly): There were no significant differences in body weights or body weight gains with treatment.

Feed Consumption (daily): There were no remarkable differences in food consumption with treatment.

Ophthalmoscopy (pretest, end of treatment): There were no treatment related effects on the eyes.

ECG (pretest, end of treatment “during the quarter following the end of infusion”, also includes BP): There were no remarkable differences between groups in ECG parameters or blood pressure (systolic or diastolic). The measurement period would be expected to include the peak plasma levels of drug.

Hematology (pretest, end of treatment, includes marrow): There were no noteworthy changes in hematologic values with treatment.

Clinical Chemistry (pretest, end of treatment): BUN was nearly doubled in a single HD male and increased by approximately 40% in 2/5 HD females. No histopathologic correlate was noted. This should be monitored in the 13 week study.

Urinalysis (pretest, end of treatment): There were no remarkable changes with treatment.

Gross Pathology: At necropsy, hematomas at the injection sites were noted in the MD and HD males. Brownish areas were seen in a single male and female at each dose.

Organ Weights: While prostate weights (absolute and relative) appeared to be nearly doubled in most of the treated males, the individual weights and sexual maturity level were highly variable. Male thymus and thyroid weights, absolute and relative, increased dose dependently to a maximum of 15 and 40% of the controls, respectively. In females, ovary weights at the HD were doubled as compared to controls; no apparent correlation with estrus cycling was noted. Female thymus weight was not significantly affected and thyroid weights increased in all treated groups by approximately 40% at the HD. At the end of the recovery period, thyroid weights were still increased (nearly doubled) in the HD males as compared to controls. In the females at recovery, ovary weights were still increased.

Histopathology

Adequate Battery: yes

Peer Review: yes

Histological Findings: Injection site findings included inflammation, hemorrhage, phlebitis, fibrosis, granuloma, and collagen degeneration. Severity and incidence did not always correlate with dose at the separate sites. No microscopic findings correlated with the increased thyroid and thymus weights.

Toxicokinetics (Day 1, day 28; samples taken at 0.5 [end of infusion], 2, 4, 8, 12, 24 hours after start of infusion):

NXL did not accumulate between day 1 and day 28. No consistent differences were noted between males and females. AUC was less than linear with dose.

NXL104									
Validated Range (as free acid)		10-5000 ng/mL							
LLOQ (as free acid)		10 ng/mL							
Calibration Model		Linear weighted 1/x							
Precision (%CV) Inter-assay		Less than 4.8%							
Accuracy (%RE) Inter-assay		Less than ±2.8%							
Mean Toxicokinetic Parameter Summary									
Dose (mg)	Sex	Study Day	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-t} (µg/mL.h)	t _{1/2} (h)	CL (L/h/kg)	V _{ss} (L/kg)	R _{AC}
250	female	1	741700	0.5	786.1	7.332	0.3337	0.4537	1.359
		28	953000	0.5	1018	8.796	0.2480	0.2994	
	male	1	955500	0.5	1045	6.670	0.2394	0.2713	1.088
		28	933000	0.5	1134	6.780	0.2210	0.2873	
500	female	1	1521000	0.5	1890	5.766	0.2675	0.3176	1.247
		28	1856000	0.5	2377	7.837	0.2182	0.2556	
	male	1	1832000	0.5	2103	8.177	0.2380	0.2903	1.194
		28	2222000	0.5	2489	7.503	0.2049	0.2343	
1000	female	1	2931000	0.5	3206	6.346	0.3279	0.3677	1.003
		28	2696000	0.5	3131	7.189	0.3208	0.3683	
	male	1	3411000	0.5	3454	6.873	0.3041	0.3188	1.134
		28	3335000	0.5	3696	7.129	0.2593	0.3108	
Equipment									
Sciex API4000									

Dosing Solution Analysis: All of the dosing solutions were within 10% of the intended concentrations.

Study title: 13-week intravenous (30 minute infusion) toxicity study in the beagle dog followed by a 4-week treatment free period

Study no.: NXL104/DS0029
 Study report location: EDR
 Conducting laboratory and location:
 Date of study initiation: January, 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NXL104 Batch # 08120739, 92% pure (correction factor 1.087)

Key Study Findings: Injection site damage (intimal thickening and perivascular irritation) with a minimal dose association was the major toxicity seen in this study.

Methods

Doses: Males/Females: NXL104 0, 65, 125, 250 mg/kg/day (Group 1-4)
Frequency of dosing: Daily for 91 days (13-weeks)
Route of administration: Intravenous (continuous saline between doses 1mL/h/animal; dosed 30-minute infusion via implanted catheter in anterior vena cava)
Dose volume: 5 mL/kg/day over 30-minute infusion period
Formulation/Vehicle: 5% aqueous solution of glucose
Species/Strain: Beagle dog
Number/Sex/Group: 4/sex/dose for main study
Satellite groups: 2/sex/dose in control, HD for recovery
Age: 8 months
Weight: M: 6-11 kg F: 5-10 kg
Unique study design: None
Deviation from study protocol: None

Observations and Results

Mortality (twice daily): All dogs survived to scheduled sacrifice.

Clinical Signs (twice pretest, weekly for first 5-weeks, termination of treatment and treatment free periods): There were no noteworthy differences in observations between groups.

Body Weights (weekly): There were no significant differences in body weight or body weight gains with treatment.

Feed Consumption (daily): Food consumption was not affected by treatment with NXL104.

Ophthalmoscopy (pretest, day 89): There were no treatment-related ophthalmological findings noted at any dose level.

Hematology (pretest, days 21, 84, 113): There were no relevant differences in hematology in any of the groups following treatment with NXL104.

Clinical Chemistry (pretest, days 21, 84, 113): There were no relevant differences in serum clinical chemistry in any of the groups following treatment with NXL104.

Urinalysis (day 85): There were no relevant differences in urinalysis noted for any of the groups following treatment with NXL104.

Gross Pathology (End treatment period, M: day 91, F: day 92; End treatment free period: day 120): There were no remarkable observations that correlated with treatment.

Organ Weights: No significant differences in organ weights were noted between treatment/pre-treatment animal groups/sex.

Histopathology: Injection site intimal thickening and perivascular inflammation consistent with intravenous studies were noted in treatment and control animals. No other histopathological findings were noted other than those commonly associated spontaneous lesions in beagle dog (i.e., hypospermatogenesis in 1 high dose male; testicular atrophy in 1 control male).

Adequate Battery--Yes

Peer Review—Yes

Special Evaluation: None

Toxicokinetics (days 0, 84): C_{max} and AUC_{0-24h} were dose-proportional between 65 and 250 mg/kg/day.

Occasion	Dose (mg/kg/day)	Sex	C _{max} (µg/mL)	T _{max} (h)	AUC _{0-24h} (µg.h/mL)
day 0	65	Male	137	0.517	208
		Female	150	0.517	200
	125	Male	271	0.517	382
		Female	283	0.517	387
	250	Male	531	0.517	741
		Female	609	0.517	801

Occasion	Dose (mg/kg/day)	Sex	C _{max} (µg/mL)	T _{max} (h)	AUC _{0-24h} (µg.h/mL)
day 84	65	Male	122	0.517	178
		Female	147	0.517	190
	125	Male	239	0.517	328
		Female	279	0.517	377
	250	Male	475	0.517	635
		Female	594	0.517	767

Dosing Solution Analysis: Concentrations of NXL104 formulated at 13, 25 and 50 mg/mL were within -6.0 % to 1.3 % of the intended concentrations

Study title: 4-week toxicity study by intravenous route in rats.

Study no.: NXL104/DS0013
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: March, 2006
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NXL104 Batch # 0500012675;
 CEFTAZIDIME Batch # 44701503, 99.8%
 pure

Key Study Findings: No new toxicities were noted with the combination of CAZ and AVI. The main toxicity noted was injection site (tail vein) damage which included inflammation, edema, necrosis, myodegeneration and thrombi. The incidence was greatest in the 2000/500 CAZ/AVI group, followed by the CAZ alone group. No NOAEL was observed with the combination. Slight hematologic changes were noted with the combination (decreased WBC #, increased thrombocytes). CAZ did not affect AVI plasma levels and AVI did not affect CAZ plasma levels.

Methods

Doses: G1: vehicle, G2: 666 mg/kg/167 mg/kg CAZ/AVI; G3: 2000 mg/kg/500 mg/kg CAZ/AVI; G4: 2000 mg/kg CAZ; G5: 500 mg/kg AVI
 Frequency of dosing: Daily for 28 day. Due to tail vein damage, 2000/500 CAZ/AVI and 2000 CAZ groups were terminated at day 14.
 Route of administration: Intravenous (30-minute infusion)
 Dose volume: 15 mL/kg
 Formulation/Vehicle: 5% aqueous solution of glucose
 Species/Strain: Sprague Dawley; CrI CD(SD) IGS BR rats
 Number/Sex/Group: 10/sex/day for main study, 2/sex/control, 6/sex/dose for satellite toxicokinetic groups
 Age: 6 weeks
 Weight: M: mean 173 g (range: 152-194 g) F: mean 160 g (135-174 g)
 Unique study design: None
 Deviation from study protocol: Following poor tolerance of the test item treatment in the tails of the animals, the dosing period was shortened for high dose (group 3 and 4 animals): treatment stopped on day 14 and necropsy performed on day 15. For these groups, all examinations initially scheduled in week 4 were carried out on days 13, 14 or 15

Observations and Results

Mortality (Treatment: Twice daily): Following the poor condition of the tails after one week of dosing, the three satellite females given 2000/500 CAZ/AVI were euthanized prematurely on day 7. During the week preceding euthanasia, wounds, scabs and hematoma on tail were observed, and these were also noted at necropsy. Furthermore, blackish tail was noted for one out of the three females. One AVI alone male was euthanized on day 22. And

Clinical Signs (Once daily): Injection site (tail) discoloration, scabs, desquamation, dryness, and hematomas were noted most frequently in the 2000/500 CAZ/AVI with slightly lesser incidence in the 2000 CAZ group. Damage was also noted in the 666/167 CAZ/AVI and to a minimal extent, AVI alone. Changes occurred earlier (day 1-8) in the 2000/500 CAZ/AVI group than the lower combination dose. Piloerection was also noted in 8/10 2000/500 CAZ/AVI males.

Body Weights (Pre-randomization, Day 0, once weekly): Body weight was decreased by approximately 10% in the 2000/500 CAZ/AVI and 2000 CAZ males at week 2 as compared to controls. Females, and males treated with the 666/167 CAZ/AVI, AVI alone, showed no difference.

Feed Consumption (Once weekly): Food consumption in males was consistently lower than control in the 2000/500 CAZ/AVI group and the CAZ 2000 alone groups by approximately 10%.

Ophthalmoscopy (Main study animals: Day 0, Once weekly): There were no treatment-related ophthalmological findings.

Hematology (Group 3/4: Day 15; Surviving animals: Day 28): RBC # and hemoglobin were decreased approximately 20% in the males and females treated with CAZ and the combination of CAZ/AVI at 2000/500. Decrements were slightly increased in the combination. Similarly, neutrophils, leukocytes, and platelets were all increased in CAZ treated groups with a maximal increase in the HD combination.

Haematological parameters affected by treatment

Sex	Male					Female				
	Treatment	Vehicle	CAZ/NXL104	CAZ	NXL104	Treatment	Vehicle	CAZ/NXL104	CAZ	NXL104
Dose-level (mg/kg/day)	0	666/167	2000/500	2000	500	0	666/167	2000/500	2000	500
RBC (T/L)	8.05	7.66**	6.18µ	6.53 µ	7.73*	7.17	6.99	5.76 µ	5.89 µ	7.57
% from controls		(-5)	(-23)	(-19)	(-4)			(-20)	(-18)	(+6)
HB (g/dL)	15.9	14.8**	12.5 µ	13.4 µ	15.3*	14.1	13.8	11.9 µ	12.6 µ	15.3*
% from controls		(-7)	(-21)	(-16)	(-4)			(-16)		(+9)
PCV (L/L)	0.46	0.43**	0.38 µ	0.40 µ	0.45*	0.40	0.39	0.34 µ	0.36 µ	0.43*
% from controls		(-7)	(-17)	(-13)	(-2)			(-15)		(+8)
RDW (%)	12.1	13.4**	14.4 µ	13.2 µ	12.5	12.4	13.7	15.0 µ	15.5 µ	11.7
% from controls			(+19)		(+3)			(+21)	(+25)	(-6)
RTC (%)	2.90	4.32**	10.00 µ	6.86 µ	4.18*	3.77	5.35	8.91 µ	9.17 µ	3.12
% from controls			(+245)	(+136)	(+44)			(+136)	(+143)	(-17)
WBC (G/L)	10.17	11.6	9.40 µ	7.72 µ	10.98	9.71	8.84	10.56 µ	8.41 µ	7.69
% from controls										
N (G/L)	0.79	1.52	2.41 µ	1.63 µ	1.15*	0.88	1.71	3.13 µ	2.36 µ	0.81
% from controls			(+205)	(+106)	(+46)			(+256)	(+168)	
L (G/L)	9.04	9.71	6.61 µ	5.76 µ	9.40	8.38	6.73	7.05 µ	5.77 µ	6.55
% from controls			(-27)	(-36)	(+4)			(-28)	(-16)	(-31)
PLT (G/L)	1217	1250	1491 µ	1259 µ	1197	1064	1272*	1744 µ	1570 µ	1087
% from controls			(+23)					(+20)	(+64)	(+48)
FIB (g/L)	3.30	4.23**	6.95 µ	6.09 µ	3.52	3.11	3.66*	5.70 µ	4.96 µ	3.05
% from controls		(+28)	(+111)	(+84)			(+94)	(+83)	(+59)	

Statistically different from control: *: p<0.05, **: p<0.01. No statistical analyses were made for the groups treated at 2000/500 or at 2000 mg/kg/day. µ: no statistical analysis from controls as no control group value was recorded on day 15.

Clinical Chemistry (Group 3/4: Day 15; Surviving animals: Day 28): Serum chemistry parameters were not significantly affected by treatment with NXL 104 alone, Ceftazidime, or any NXL 104/Ceftazidime combination.

Urinalysis (Group 3/4: Day 15; Surviving animals: Day 28): A minimal increase in urine color was noted in males and females given CEFTAZIDIME alone or in the high-dose combination.

Gross Pathology (Principal animals: Premature euthanized: Day 15; Surviving animals: Day 28; Satellite animals: Day 13): Severe lesions on the tail (injection sites) were observed in all test item-treated groups. Observations included hematoma, dryness redness, and scabs with the highest incidence in

the 2000/500 CAZ/AVI group, followed by the 2000 CAZ alone group. Pale kidneys were noted in the 2000/500 CAZ/AVI and 2000 CAZ alone males and females.

Organ Weights: No significant changes noted.

Histopathology:

Adequate Battery--Yes

Peer Review—Yes

Injection site damage showed the greatest incidence in the 2000/500 CAZ/AVI group followed by the 2000 CAZ group, and consisted of a combination of inflammation, hemorrhage, vascular necrosis, myodegeneration, collagenous degeneration, epidermal necrosis, thrombus, edema, and follicular/venous ectasia. There were no dose dependent correlates with pale kidneys from the macroscopic observations.

Toxicokinetics (Euthanized (prematurely): Day 7; Surviving animals: Days 1, 13):

CAZ and AVI pharmacokinetics did not differ with gender. Neither drug showed accumulation after 13 days of dosing. The AUC and C_{max} of AVI were not significantly affected by CAZ and vice versa. The half-life for NXL104 ranged from 3.2 to 7.9 hours, while the half-life of CAZ ranged from 3.0 to 9.5 hours.

			NXL104				Ceftazidime			
Validated Range (as free acid)			10–5000 ng/mL				50–3000 ng/mL			
LLOQ (as free acid)			10 ng/mL				50 ng/mL			
Calibration Model			Linear weighted 1/x				Linear weighted 1/x			
Precision (%CV) Inter-assay			≤ 7.4%				≤ 6.5%			
Accuracy (%RE) Inter-assay			≤ ±5.9%				≤ ±6.9%			
Mean Toxicokinetic Parameters			C _{max} (ng/mL)	t _{max} (h)	AUC _(0-t) (µg/mL.h)	CL (L/h/kg)	C _{max} (ng/mL)	t _{max} (h)	AUC _(0-t) (µg/mL.h)	CL (L/h/kg)
Group 2	F	1	38980	0.55	52.9	3.157*	264700	1	366.4	1.818*
		13	143800	0.55	91.84	1.818	822700	0.55	520.8	1.279
	M	1	201000	0.55	122.3	1.365*	1090000	0.55	705.2	0.9444*
		13	147800	0.55	93.25	1.791	759400	0.55	620.9	1.073
Group 3	F	1	327400	0.55	278.1	1.797	1180000	0.55	1123	1.781
		13	555000	0.55	341.4	1.465	2209000	0.55	1348	1.484
	M	1	362600	0.55	316.2	1.58	2402000	0.55	1868	1.071
		13	323900	0.55	328.8	1.521	1033000	0.55	1411	1.417
Group 4 (ceftazidime)	F	1	462200	0.55	352.5	1.418	1757000	0.55	1840	1.087*
		13	465600	0.55	366.9	1.363	2496000	0.55	1586	1.261
Group 5 (NXL104)	M	1	856400	0.55	516.3	0.9681	1855000	0.55	1282	1.559
		13	552400	0.55	413	1.211	2366000	0.55	1715	1.166

Dosing Solution Analysis: All dosing solutions were within 10% of the intended concentration.

Study title: NXL104 and Ceftazidime: 28 day toxicity study by intravenous route in beagle dogs.

Study no.:	NXL/DS0014
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 2006
GLP compliance:	Yes, OECD
QA statement:	Yes
Drug, lot #, and % purity:	NXL 104 batch # 0500024396, 98.2% pure; ceftazidime batch # 44701503, 99.8% pure

Key Study Findings:

There were no new findings with the combination of CAZ-AVI that were not seen with CEF alone. The major observations were changes in cholesterol/triglycerides and hepatocellular hypertrophy at 1000 mg/kg CAZ with or without AVI. Injection site damage (phlebitis, fibroplasia, perivenous inflammation, perivenous hemorrhage, venous wall necrosis) was noted in all dogs and were considered procedurally related. Small thymus and lymphoid depletion were noted in the 1000 mg/kg CAZ dogs with or without AVI.

Methods

Doses:	1) Vehicle, 2) 500/125 mg/kg CAZ/AVI, 3) 1000/250 mg/kg CAZ/AVI and 4) 1000 mg/kg CAZ
Frequency of dosing:	Daily for 28 consecutive days
Route of administration:	Intravenous over 30 minutes via cephalic or saphenous veins
Dose volume:	5 mL/kg/30 minutes
Formulation/Vehicle:	5% aqueous glucose
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/sex/dose
Age:	7 months
Weight:	M: 8.1-9.4 kg; F: 6.8 -8.8 kg
Satellite groups:	---
Unique study design:	CAZ and NXL104 were combined at a 4:1 ratio and prepared on a weekly basis
Deviation from study protocol:	None significant.

Observations and Results

Mortality (twice daily): All dogs survived to scheduled sacrifice.

Clinical Signs (twice daily): Salivation, soft feces and vomiting was noted primarily with 1000 mg/kg CAZ (with or without AVI).

Body Weights (twice weekly): There were no remarkable differences in body weight between dose groups in males or females.

Feed Consumption (daily): There were no noteworthy differences in food consumption between dose groups.

Ophthalmoscopy (pretest, end of treatment): There were no changes in observations in any group with treatment.

ECG (pretest, end of treatment, also looked at BP): There were no remarkable differences between heart rate, QT interval or systolic/diastolic blood pressure between control and treated dogs at the end of the infusion period when plasma levels of drugs should be at a maximum.

Hematology (Pretest, end of treatment): There were no remarkable changes in hematologic parameters with treatment.

Clinical Chemistry (Pretest, end of treatment): There were no statistically significant changes in serum chemistry; however, there was a trend toward an increase in cholesterol and triglycerides in the male and female dogs treated with 1000 mg/kg CAZ, with or without AVI. In the 1000/250 mg/kg females, urea values were doubled as compared to controls, but no changes were noted in the 1000 mg/kg CAZ alone.

Urinalysis (pretest, end of treatment): Specific gravity and pH did not differ markedly from control values.

Gross Pathology: Hematomas at the injection site were the prevalent finding and did not correlate with dose. Pale liver was seen in one 100/250 CAZ-AVI male and one CAZ only male.

Organ Weights: Absolute and relative liver weights in males were increased above control by approximately 50% in the CAZ-AVI group but only increased by approximately 25% in the CAZ alone males.

Reviewer's note: Both the increases in cholesterol/triglycerides and increases in liver weights (in the absence of cytochrome P450 induction suggests hepatic changes; however, no histopathologic correlate was noted.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: Injection site findings (phlebitis, fibroplasia, perivenous inflammation, perivenous hemorrhage, venous wall necrosis) did not differ significantly in incidence or severity between dose groups (includes controls). Liver hepatocellular

hypertrophy was noted in the G3 and G4 dogs at similar severity and incidence. Thymic lymphoid depletion was noted with increasing severity at all CAZ-AVI groups, with the greatest incidence and severity in the CAZ only treated dogs.

Toxicokinetics (Day 1, 27 or 28; 0.5, 2, 4, 8, 12, 24 h after start of infusion): The NXL exposure was linear with dose and no accumulation after 1 month was noted. No gender differences in PK parameters were observed. The values are shown in the table below. Similarly, there were no remarkable differences in exposure to CAZ at day 1 and 28 or by gender. There was a linear dose response for AUC for CAZ as well. AVI did not affect the exposure to CAZ and vice versa as shown by similar AUCs regardless of whether the drugs were given alone or in combination.

Mean toxicokinetic parameters			NXL104					
			C _{max} (ng/mL)	t _{max} (h)	AUC ₍₀₋₂₄₎ (µg/mL.h)	CL (L/h/kg)	V _{ss} (L/kg)	R _{ac}
Group 2 125 mg/kg/d NXL104 and 500 mg/kg CEFTAZIDIME	Day 1	Female	280700	0.5	308.9	0.4037	0.4797	-
		Male	313000	0.5	394.8	0.3216	0.4138	-
	Day 28*	Female	369600	0.5	333.6	0.3761	0.4065	1.081
		Male	373600	0.5	374.2	0.3347	0.3688	0.971
Group 3 250 mg/kg/d NXL104 and 1000 mg/kg CEFTAZIDIME	Day 1	Female	773000	0.5	754.5	0.3419	0.3482	-
		Male	621300	0.5	718.6	0.3502	0.4076	-
	Day 28*	Female	561500	0.5	603.1	0.4377	0.5340	0.799
		Male	752700	0.5	721.7	0.3493	0.3742	1.019
Group 4 0 mg/kg/d NXL104 and 1000 mg/kg CEFTAZIDIME	Day 1	Female	-	-	-	-	-	-
		Male	-	-	-	-	-	-
	Day 28*	Female	-	-	-	-	-	-
		Male	-	-	-	-	-	-

Lower Limit Of Quantification (as free acid): 10 ng/mL

* final sampling day in male animals was day 27

-: not applicable

Mean toxicokinetic parameters			CEFTAZIDIME					R _{ac}	
			C _{max} (ng/mL)	t _{max} (h)	AUC ₍₀₋₂₄₎ (µg/mL.h)	CL (L/h/kg)	V _{ss} (L/kg)		
Group 2 125 mg/kg/day NXL104 and 500 mg/kg/day CEFTAZIDIME	Day 1	Female	868900	0.5	1034	0.4876	0.5846	-	
		Male	839300	0.5	1073	0.4767	0.5973	-	
	Day 28*	Female	1556000	0.5	1502	0.3362	0.3210	1.479	
		Male	1099000	0.5	1138	0.4407	0.4785	1.082	
	Group 3 250 mg/kg/day NXL104 and 1000 mg/kg/day CEFTAZIDIME	Day 1	Female	3859000	0.5	3639	0.2874	0.2718	-
			Male	2996000	0.5	3518	0.2874	0.3290	-
Day 28*		Female	2653000	0.5	2868	0.3618	0.4215	0.794	
		Male	4067000	0.5	4183	0.2425	0.2337	1.195	
Group 4 0 mg/kg/day NXL104 and 1000 mg/kg/day CEFTAZIDIME	Day 1	Female	3448000	0.5	3506	0.2875	0.2784	-	
		Male	4036000	0.5	4586	0.2189	0.2389	-	
	Day 28*	Female	3228000	0.5	3228	0.3134	0.3097	0.920	
		Male	3628000	0.5	3513	0.2848	0.2928	0.772	

Lower Limit Of Quantification (as free acid): 50 ng/mL

* final sampling day in male animals was day 27

-: not applicable

Dosing Solution Analysis: The dosing solution was stable over a 9 day period and the drug levels were within 10% of the intended concentrations.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: NXL104: Bacterial Reverse Mutation Test

Study no.: NXL104/DS0002
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Oct. 2005
 GLP compliance: Yes, OECD
 QA statement: Yes
 Drug, lot #, and % purity: NXL104, batch # 0500012675, 99.7% pure

Key Study Findings: NXL 104 was not mutagenic, but was toxic to the background lawn at concentrations > 50 ug/plate, so this assay is not appropriate for the assessment of AVI for genotoxicity.

Methods

Strains: TA 1535, TA1537, TA98, TA 100, TA102
Concentrations in definitive study: 0, 1.56, 3.13, 6.25, 12.5, 25, and 50 ug/plate
Basis of concentration selection: Toxicity to the background lawn
Negative control: water
Positive control: Sodium azide, 9-aminoacridine, nitrofluorene, mitomycin C, Anthramine (all but mitomycin C dissolved in DMSO)
Formulation/Vehicle: water
Incubation & sampling time: 48 to 72 hours

Study Validity: Based on similarity to previous vehicle controls and significant increases in revertants with positive controls (minimum of 5 fold increases in the TA 100 strain), the study was valid.

Results: There were no increases in the number of revertants with increasing concentration of NXL104 up to 50 ug/plate. Concentrations of drug >50 ug/plate were toxic to the background lawn.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: (b) (4) memo 26/6/99 exploratory. In vitro unscheduled DNA synthesis.

Study no.: None-memo
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/24/99
GLP compliance: no
QA statement: no
Drug, lot #, and % purity: Not provided

Key Study Findings: No evidence of DNA repair was noted with NXL104.

Methods

Strains: Liver cells from male Sprague Dawley rat
Concentrations in definitive study: 0, 500, 1000 ug/mL
Basis of concentration selection: Solubility (up to 1000 ug/mL), cytotoxicity (>1000 ug/mL), steatosis at 500 ug/mL
Negative control: DMSO
Positive control: 2 AAF
Formulation/Vehicle: DMSO, water
Incubation & sampling time: 20 hrs

Study Validity: The positive control increased the grains/nuclei (indicative of repair) by approximately 4 fold above the negative control.

Results: The number of grains incorporated (indicative of repair) were similar between the control and NXL-104 groups.

Study title: RU 84726: Exploratory in vitro unscheduled DNA synthesis (UDS) test in rat liver cells in primary culture.

Study no.: DSE2002-0649.
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: March, 2002
GLP compliance: No
QA statement: Yes
Drug, lot #, and % purity: RU84726, Batch 31088-017-E

Key Study Findings: RU84726 did not induce DNA repair in cultured rat hepatocytes.

Methods

Cell line: Liver cells from male Sprague Dawley rat
Concentrations in definitive study: 0, 250, 500, 1000, 2000 ug/mL
Basis of concentration selection: Assume solubility
Negative control: Culture medium
Positive control: 2-aminofluorene (2-AF)
Formulation/Vehicle: Culture medium
Incubation & sampling time: 18 hours

Study Validity: A positive response was a nuclear grain count > 5 and more that 20% of the cells in repair. Cytotoxicity was evaluated by the decrease in thymidine incorporation or altered morphology as compared to controls.

Results: The net # of grains did not differ significantly between control and up to 2000 ug/mL RU84726 in treated cells. The percent of cells in repair was < 5% for all concentrations of test drug. The positive control increased grain count by approximately 10 fold, and all the cells were in a state of repair.

Study title: RU84726: Exploratory micromethod of the in vitro micronucleus test in the mouse lymphoma cells (L5178Y).

Study no.:	DSE 2003-0771
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	May 2003
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	Not provided.

Key Study Findings: The study appeared to be valid. RU84726 was not clastogenic in this model.

Methods

Cell line: Mouse L5178Y cells
Concentrations in definitive study: 0, 700, 850 and 1000 ug/mL
Basis of concentration selection: Not provided, assume cell survival.
Negative control: DMSO + culture medium
Positive control: Mitomycin C, benzo(a)pyrene
Formulation/Vehicle: Culture medium
Incubation & sampling time: 3 hours + metabolic activation, 24 h without.

Study Validity: The positive control showed a significant decrease in survival as compared to controls, and increased the number of micronucleated cells by more than 25 fold in the non-metabolic activated cells and by 9 fold in the metabolically activated cultures.

Results: There were no statistically significant increases in micronucleated cells with or without metabolic activation at up to 1000 ug/mL test article.

Study title: NXL104: In vitro mammalian chromosome aberration test in cultured human lymphocytes.

Study no.: NXL104/DS0003
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: October, 2005
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104, batch 0500012675, 86.44% of content

Key Study Findings: NXL104 increased structural aberrations in the absence of metabolic activation at concentrations \geq 1500 ug/mL. The study was valid.

Methods

Cell line: Human lymphocyte primary cultures
Concentrations in definitive study: 0-5000 ug/mL
Basis of concentration selection: pH, osmolality, solubility, toxicity
Negative control: water
Positive control: Mitomycin C, cyclophosphamide
Formulation/Vehicle: water
Incubation & sampling time: 3 hours incubation, sample 20, 44 hours after start of treatment

Study Validity: The study was valid based on the performance of the negative and positive controls (increased the % of cells with aberrations by approximately 30X).

Results: There were no increases in cellular aberrations with NXL-104 in the presence of S9 mix. In the studies where no S9 metabolic activation was added, chromosomal aberrations were increased at doses of NXL-104 greater than or equal to 1500 ug/mL where decreases in mitotic index were also noted (maximum approximately 3 fold above controls). The mitomycin C increased aberration frequency by approximately 13 to 30X.

Study title: In vitro mammalian chromosome aberration test in cultured human lymphocytes.

Study no.: NXL104/DS0018
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: Dec., 2006
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104 batch # 0600032422

Key Study Findings: The study was valid and negative for chromosomal aberrations at NXL104 concentrations up to 5000 ug/mL.

Methods

Cell line: Primary human lymphocyte cultures
Concentrations in definitive study: 0-5000 ug/mL
Basis of concentration selection: pH, osmolality and solubility, cytotoxicity
Negative control: Water
Positive control: Mitomycin C, cyclophosphamide
Formulation/Vehicle: Water
Incubation & sampling time: 3 hours incubation, 20 or 44 hours before harvest

Study Validity: The study was valid based on control values and substantial increases in aberrations in positive controls.

Results: There were no statistically significant increases in the % of cells with chromosomal aberrations (including gaps) with NXL 104 treatment. Positive controls did increase aberrations by > 10X.

Reviewer's note: The prior study did show a small elevation (approximately 2-3 fold above controls of aberrations), while this study did not. The prior study may have been an aberration in itself or could be called "equivocal".

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Bone marrow micronucleus test by intravenous route (30 minute infusion) in rat

Study no: NXL140/DS0004.
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: Nov. 2005
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104, batch # 0500012675, 99.7% pure

Key Study Findings: The study was valid and negative.

Methods

Doses in definitive study: 0, 500, 1000, 2000 mg/kg/day
Frequency of dosing: Twice (2 doses, 24 hours apart)
Route of administration: Intravenous
Dose volume: 10 mL/kg
Formulation/Vehicle: 5% glucose
Species/Strain: Sprague Dawley rats
Number/Sex/Group: 5/sex/dose
Satellite groups: none
Basis of dose selection: Limit dose based on ICH guidance
Negative control: vehicle
Positive control: cyclophosphamide

Study Validity: 2000 mg/kg was the maximum dose administered as per the ICH guidance as no toxicity was noted.

Results: All rats survived to scheduled sacrifice 24 hours after the second drug administration. There were no significant increases in the number of micronucleated polychromatic erythrocytes with NXL 104 treatment. Cyclophosphamide increased micronucleated cells by > 10 fold.

8 Carcinogenicity

Not needed based on short duration of intended clinical use.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: NXL104—Male fertility and early embryonic development toxicity study by the intravenous route (30 minute infusion) in the rat.

Study no.: NXL104/DS0025
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 2008
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104, batch # 0635 08 001, 88.9% pure (used correction factor of (b) (4) for salt and impurities) (pH 7.01-7.10, osmolality 426-791 mOsmol/kg for test article)

Key Study Findings: The NOAEL for fertility was the highest dose tested, 1000 mg/kg/day.

Methods

Doses: 0, 250, 500, 1000 mg/kg/day
 Frequency of dosing: Males only treated, once daily, 29 days prior to mating, through mating for up to 64 or 65 days.
 Dose volume: 15 mL/kg/day (30 minute infusion)
 Route of administration: Inclusion via catheter in posterior vena cava via femoral vein
 Formulation/Vehicle: 5% glucose
 Species/Strain: Sprague Dawley rats, M: 9 weeks old, 304-376 g; F: 11 weeks old, 235-305 g
 Number/Sex/Group: 20 males /dose (20 females/dose untreated)
 Satellite groups: None
 Study design: Males terminated at day 64/65, females at GD 13.
 Deviation from study protocol: None sufficiently significant to affect the interpretation of the study.

Observations and Results

Mortality (twice daily): The following table describes the early deaths. The sponsor noted that all deaths might be attributed to drug. However, the lack of a dose response in combination with an indwelling catheter suggests procedural related mortality. The early death animals had irregular breathing, subdued behavior, and piloerection. Cause of death was associated with changes in the abdominal cavity and respiratory tract changes.

Dose	#dead	Day (s) of death
250 mg/kg	4	53, 58, 59, 63
500 mg/kg	2	53, 58
1000 mg/kg	2	56, 57

Clinical Signs (daily): There were no clinical signs in the rats that survived to scheduled sacrifice that appeared related to NXL-104.

Body Weight (twice weekly): Mean body weights in the LD, MD, and HD males were decreased by 9%, 7% and 5% in the high, mid, and low dose NXL-104 groups compared to controls. Female body weights during gestation were not remarkably different between dose groups.

Feed Consumption (weekly): There were no dose related changes in food consumption with treatment.

Ophthalmology (Day 58): There were no differences in the incidence of ophthalmoscopic findings with treatment.

Dosing Solution Analysis: The dosing solutions were within 5% of the intended concentrations.

Necropsy: The mean weight of the testes, prostate, and epididymis were similar across dose groups. At the injection site, adhesions, edema and induration were common and increased slightly with dose.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, implantations, resorption sites, dead embryos): All of the males copulated with the females within a similar time frame. A single rat was not pregnant in the control, LD, and HD groups, 5 rats (25%) were not pregnant in the MD group. There were no statistically significant differences between groups in corpora lutea, implantations, viable embryos, % implantation, or pre- post-implantation loss.

Sperm analysis: Sperm counts and sperm motility were similar across test groups.

Study title: NXL104—Fertility and early embryonic development toxicity study by the intravenous route in the female rat.

Study no.:	NXL104/DS0026
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 2008
GLP compliance:	Yes, OECD
QA statement:	Yes
Drug, lot #, and % purity:	NXL104, batch # 0635 08 001, 88.9% pure (used correction factor of (b) (4) for salt and impurities) pH 7.11 to 7.27, osmolality was 417-784 mOsm/kg

Key Study Findings: The NOAEL for fertility was the HD, 1000 mg/kg/day. Based on pre and post implantation losses and decreases in live embryos, the NOAEL for early embryonic development was the LD, 250 mg/kg/day.

Methods

Doses:	0, 250, 500, 1000 mg/kg/day
Frequency of dosing:	Only females were dosed; once daily, starting 14 days before mating, through mating until GD 7
Dose volume:	15 mL/kg/day
Route of administration:	Intravenous infusion over 30 minutes via implanted catheter (posterior vena cava via femoral artery)
Formulation/Vehicle:	5% glucose
Species/Strain:	Sprague Dawley rats, females 9 weeks old, 174-249 g; males 13 weeks old, 398-464 g
Number/Sex/Group:	20/group
Satellite groups:	None
Study design:	See frequency of dosing, a single virgin male was housed with a single virgin female during the mating period. Females were housed singly prior to mating and during gestation.
Deviation from study protocol:	None that affected the interpretation of the study

Observations and Results

Mortality (Twice daily): One LD female died after day 1 post-coitum with no apparent cause of death. One MD female was killed due to complications during re-implantation of the catheter.

Clinical Signs (once daily): There were no noteworthy changes in clinical signs with treatment.

Body Weight (twice weekly, GD0, 4, 8, 13): There were no significant differences in body weights between treated and control rats either prior to mating or during gestation.

Feed Consumption (twice weekly): There were no remarkable differences between treated and control rats in food consumption.

Estrus Cycle: There were no remarkable differences in estrus cycle length, or percentage of estrus days between treated and control females.

Stability and Homogeneity: All solutions were within 10% of the intended concentrations of drug.

Necropsy (GD 13, gross pathology and ovarian weights): There were no noteworthy differences in ovarian weights between treatments. There were no remarkable gross observations that differed with treatment.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, Live embryos, resorptions, implantation sites): All but 1 MD rat copulated. There were 20, 18, 16 and 18 rats pregnant in the control, LD, MD and HD groups respectively. Both pre-implantation loss and post-implantation loss were increased significantly in the MD and HD groups (doubled and more than tripled as compared to controls in the MD and HD groups, see table below).

Study No. : AA39559F	NXL104 - FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TOXICITY STUDY BY THE INTRAVENOUS ROUTE IN THE FEMALE RAT.			
	SUMMARY OF CAESAREAN SECTION DATA			
	Group 1 Control 0 mg/kg/day	Group 2 Low dose 250 mg/kg/day	Group 3 Intermed. dose 500 mg/kg/day	Group 4 High dose 1000 mg/kg/day
Number of dams	20	18	16	18
Corpora lutea per group	336	296	265	321
per dam: mean	17 d	16	17	18
st. dev.	3	2	4	4
Implantations per group	315 f	276	223**	257**
% of Corpora lutea per dam: mean	93.8	93.2	84.2	80.1
st. dev.	16 k	15	14	14
	2	2	4	4
Viable Embryos per group	306	255	204	225
per dam: mean	15 k	14	13	13
st. dev.	2	2	5	5
% of Implantations per group	97.1 f	92.4*	92.7	90.4**
per dam: mean	97 k	92	86	80
st. dev.	5	7	25	32
Postimplantation Loss total per group	9 f	21*	19*	32**
% of implantations per dam: mean	2.9	7.6	8.5	12.5
st. dev.	0 k	1	1	2
No. of dams affected	1	12	10	2
	6	12	10	11
Preimplantation Loss per group	21 f	20	42**	64**
% of Corpora lutea per dam: mean	6.3	6.8	15.8	19.9
st. dev.	1 k	1	3	4
No. of dams affected	2	2	5	4
	12	12	10	14

Statistical key: d-Anova/Dunnett test f-Chi2/Fisher Exact test k-Kruskal-Wallis/Dunn test * - p<0.05 ** - p<0.01

9.2 Embryonic Fetal Development

Three non-GLP dose ranging studies were conducted, one in non-pregnant rabbits, and one each in pregnant rats and rabbits. The dose ranging study in non-pregnant rabbits tested doses of 0, 125, 250, 500, 1000 and 2000 mg/kg/day for 5 consecutive days. Rabbits at the highest dose had severe injection site damage and were terminated early. In the pregnant rabbits, using doses of 0, 250, 500, and 1000 mg/kg/day from Gestation Day (GD) 6 through 19, toxicities included a slight increase in early resorptions at 1000 mg/kg/day. The sponsor concluded that the doses tested would be 0, 250, 500 and 1000 mg/kg/day in the definitive study. In the rat, the 28 day toxicity study had a high dose of 1000 mg/kg/day based on injection site issues (tail vein, not catheterized). The dose ranging study in pregnant rats used doses of 0, 250, 500, and 1000 mg/kg via the tail vein on GD 6-15. There were no significant toxicities at any dose.

Study title: NXL104: Embryo/fetal development toxicity study by the intravenous route (30 minute infusion) in the rabbit

Study no.: NXL104/DS0024
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: June , 2007
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104, batch # 0500024396 and 0600032422, 86.53 and 83.53% pure, respectively. Dosed based on corrections of 1.16 and 1.2. pH 7.1, osmolality

Key Study Findings: The dosing was adequate based on abortion and injection site damage seen at the highest dose. There were no teratogenic findings. There were decreases in fetal weights at the HD. The sponsor considered the LD, 100 mg/kg to be the NOAEL

Methods

Doses: 0, 100, 300, 1000 mg/kg/day
Frequency of dosing: Daily on GD 6-19
Dose volume: 5 mL/kg/day
Route of administration: Intravenous via the ear vein
Formulation/Vehicle: 5% glucose
Species/Strain: New Zealand White Rabbit , aged 17-19 weeks at mating, 3-4 kg weight
Number/Sex/Group: 22 females/dose
Satellite groups: None
Study design: Standard
Deviation from study protocol: One HD female was not dosed from GD 13 through 19, but was kept on study; perivenous dosing in 1 LD on GD6, HD on GD 19; subcutaneous dosing 2 MD females on days 13 and 16. None considered to have affected the conclusions of the study.

Observations and Results

Mortality (twice daily): One 1000 mg/kg doe was euthanized after aborting on GD 23. She was not eating and had lost weight prior to abortion, and at necropsy had fluid in the stomach and intestines. The sponsor attributed the death to maternal toxicity.

Clinical Signs (daily): Colored urine (pale to dark orange) were seen in all treatment groups. At the 1000 mg/kg dose, additional signs included injection site necrosis.

Study No.: AA39552 NXL104 - EMBRYO/FETAL DEVELOPMENT TOXICITY STUDY BY THE INTRAVENOUS ROUTE (30-MIN INFUSION) IN THE RABBIT.

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 100 mg/kg/day	Group 3 Intermed. dose 300 mg/kg/day	Group 4 High dose 1000 mg/kg/day
Pregnant	N	21	21	22	18
Dams with no Viable Fetuses	N	0	0	0	1
Dams with Viable Fetuses	N	21	21	22	17
Corpora Lutea	TOTAL	224	227	259	208
No. per animal	MEAN	10.7 d	10.8	11.8	11.6
	S.D.	2.5	2.4	2.7	2.5
Implantation Sites	TOTAL	214	209	224	189
No. per animal	MEAN	10.2 d	10.0	10.2	10.5
	S.D.	2.7	2.8	3.2	3.3
Preimplantation Loss	TOTAL	13	18	35	19
No. per animal	MEAN	0.6 d	0.9	1.6	1.1
	S.D.	1.1	1.4	2.0	1.8
% per animal	MEAN*	5.6 k	9.7	14.9	10.0
	S.D.	10.3	15.0	21.2	19.0
Live Fetuses	TOTAL	189	180	203	158
No. per animal	MEAN	9.0 d	8.6	9.2	8.8
	S.D.	2.8	3.4	2.9	3.4
Males	TOTAL	92	82	102	67
	MEAN*	47.3 k	42.4	48.3	46.0
	S.D.	15.3	20.3	20.2	21.5
Females	TOTAL	97	98	101	91
	MEAN*	52.7 k	57.6	51.7	54.0
	S.D.	15.3	20.3	20.2	21.5

Statistical key: d-Anova/Dunnett test k-Kruskal-Wallis/Dunn test

Study No.: AA39552 NXL104 - EMBRYO/FETAL DEVELOPMENT TOXICITY STUDY BY THE INTRAVENOUS ROUTE (30-MIN INFUSION) IN THE RABBIT.

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 100 mg/kg/day	Group 3 Intermed. dose 300 mg/kg/day	Group 4 High dose 1000 mg/kg/day
Postimplantation Loss	TOTAL	25	29	21	31
No. per animal	MEAN	1.2 d	1.4	1.0	1.7
	S.D.	1.5	2.5	1.3	2.0
% implants per animal	MEAN*	11.4 k	13.2	8.5	16.7
	S.D.	14.7	21.8	10.1	23.4
Dead Fetuses	TOTAL	0	0	0	0
No. per animal	MEAN	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
% of implants per animal	MEAN*	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Resorptions: Early	TOTAL	15	24	12	12
No. per animal	MEAN	0.7 k	1.1	0.5	0.7
	S.D.	1.4	2.3	1.0	1.7
% of implants per animal	MEAN*	7.0 k	11.0	5.1	8.4
	S.D.	13.4	20.3	7.8	23.5
Resorptions: Late	TOTAL	10	5	9	19
No. per animal	MEAN	0.5 k	0.2	0.4	1.1
	S.D.	0.7	0.5	1.0	1.5
% of implants per animal	MEAN*	4.4 k	2.2	3.4	8.4
	S.D.	6.1	5.2	8.0	11.4

Statistical key: d-Anova/Dunnett test k-Kruskal-Wallis/Dunn test

Study No.: AA39552 NXL104 - EMBRYO/FETAL DEVELOPMENT TOXICITY STUDY BY THE INTRAVENOUS ROUTE (30-MIN INFUSION) IN THE RABBIT.

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 100 mg/kg/day	Group 3 Intermed. dose 300 mg/kg/day	Group 4 High dose 1000 mg/kg/day
Petal Body Weight (g)	MEAN	37.4 d	36.5	34.1	32.5*
	S.D.	4.9	4.9	6.9	4.7
	N	21	21	22	17
Male Fetuses	MEAN	38.6 d	36.2	33.5**	32.5**
	S.D.	5.4	4.2	5.9	5.9
Female Fetuses	MEAN	36.4 d	35.8	33.4	32.1
	S.D.	5.3	5.5	7.1	4.2

Statistical key: d-Anova/Dunnett test * - p<0.05 ** - p<0.01

Offspring (Malformations, Variations; all fetuses examined externally, viscera, skeletally with alizarin red, half of each litter were decapitated and examined after serial sectioning): There were no dose dependent changes in incidence of external malformations or variations with treatment. Viscerally, the incidence of missing azygous lung lobes (variation) increased dose dependently by both fetal and litter incidence to roughly 3-4 fold above control at the HD (increased 2-3 fold at MD). This incidence was only slightly higher than the historic control level. Porencephalic cysts and brain vacuole (both anomalies) were seen in 1 MD and 4 HD litters; the sponsor noted these findings to be of no major physiologic significance. There were no dose dependent skeletal malformations or variations that reached statistical significance. In light of these variations, only seen where evidence of maternal reactions to dosing, suggest that AVI is of low concern during pregnancy.

Study title: NXL104: Embryo/fetal development toxicity study by the intravenous route (30-minute infusion) in the rat.

Study no: NXL104/DS0021
Study report location: EDR
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: April, 2007
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104, batch # 0600032422, 89.7% pure, weight adjusted by [REDACTED] (b) (4) for salt/impurities.

Key Study Findings: There were no teratogenic or embryotoxic findings at doses up to 1000 mg/kg/day of NXL104. With the exception of 1 dam with injection site damage, there was no significant maternal toxicity at doses up to 1000 mg/kg/day.

Methods

Doses: 0, 250, 500, 1000 mg/kg/day for GD6 through GD 17
Frequency of dosing: Once daily as 30 minute infusion
Dose volume: 15 mL/kg/day
Route of administration: Intravenous, via tail vein
Formulation/Vehicle: 5% glucose
Species/Strain: Sprague-Dawley rats, age 10-13 weeks at mating, weight 204-246 g
Number/Sex/Group: 25 dams/dose
Satellite groups: 3 control, 9/dose treated for toxicokinetics
Study design: standard
Deviation from study protocol: None that affected the interpretation of the study results.

Observations and Results

Mortality (twice daily): One HD female was killed on GD10 due to local injection site reactions and inability to administer drug.

Clinical Signs (twice daily): One MD dam rat had a necrotic tail from the 2nd week of dosing on, while 2 HD dams had hair loss on the torso and paws.

Body Weight (GD 0, 6, 11, 15, 18, 20): There were no remarkable differences in body weights or body weight gains across dosing groups. Gravid uterus weight did not differ significantly between groups.

Feed Consumption (GD 0-6, 6-11, 11-15, 15-18, 18-20): Food consumption was decreased by approximately 10% at the MD and HD during the course of the study.

Toxicokinetics (GD 19 at 0.5, 2, 4, 7, 10, 24 hours after start of infusion, analyzed by LC-MS/MS technique, conducted by [REDACTED] (b) (4) [REDACTED]): While T_{max} and t_{1/2} were similar for all doses, there was no correlation between dose administered and AUC or C_{max}. It is possible that samples were mixed up, considering how the data otherwise parallels AUCs in other studies.

TABLE 12
Toxicokinetic parameters for NXL104

Dose (mg)	C _{max} (ng/mL)	T _{max} (h)	Az (1/h)	T _{1/2} (h)	AUC _(0-t) (µg/mL.h)	Clearance (L/h/kg)	V _{ss} (L/kg)	MRT (h)
250	593600	0.5000	0.08608	8.055	394.6	0.6336	0.4204	0.6645
500	1460000	0.5000	0.1182	5.868	1004	0.4990	0.3113	0.6257
1000	671700	0.5000	0.08864	7.820	454.0	2.203	1.730	0.7875

Stability and Homogeneity (first and last days of administration): The dosing solutions were within 10% of the intended concentrations.

Necropsy (GD 30)

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.) There were no significant differences between treated and control groups in the # of dams with viable fetuses, corpora lutea/dam, implantation sites, pre-implantation loss, live fetuses, ratio of male/female pups, or fetal body weights.

Offspring (Malformations, Variations; half of the feti examined for visceral malformation, other half stained with Alizarin red for skeletal exam.)

There were no noteworthy changes in the incidence of external malformations or variations with NXL104 treatment. Two high dose fetuses from 2 separate litters had anophthalmia ; this incidence is within historical controls. There were no dose dependent statistically significant increases in the incidence of skeletal malformations or variations.

9.3 Prenatal and Postnatal Development

A dose ranging study (AB04833) with toxicokinetics was conducted using avibactam in 0.9% saline vehicle. The top dose, based on solubility and volume was 825 mg/kg/day. No significant toxicities were noted at the HD. The toxicokinetics are shown in the following table. Levels in milk and pups at Day 7 are also measured.

Table 6.4.1-1. Mean Plasma Toxicokinetic Parameters for Avibactam in Sprague Dawley Rats (Study AB04833)

Day	Dose mg/kg/day	C_{max} ($\mu\text{g/mL}$)	AUC_{eoi-t} ($\mu\text{g}\cdot\text{h/mL}$)	T_{max} (hours)
		Females	Females	Females
Day 16 of gestation	120	203	104	0.5
	450	720	392	0.5
	825	1510	870	0.5

eOi – end of infusion

Table 6.4.1-2. Post-Natal Day 7. Mean Plasma Avibactam Concentrations in Sprague Dawley Rats (Study AB04833)

Day	Dose mg/kg/day	Females (ng/mL)		Milk (ng/mL)	Pup (ng/mL)s	
		0 hours	0.5 hours	0.5 hours	0 hours	0.5 hours
Post-natal Day 7	120	24	41900	7940	BLQ	14
	450	64	198000	32000	27	44
	825	88	374000	890000	34	63

BLQ = below limit of quantification

Study title: Avibactam(NXL104): Pre- and postnatal developmental study by the intravenous (30 minute infusion) in the surgically implanted Sprague Dawley rat.

Study no.: AB04834 (3225WR)
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: October, 2011
 GLP compliance: Yes, OECD
 QA statement: Yes
 Drug, lot #, and % purity: NXL104, batch # G266445, 99.6% pure (91.5% used for dose calculation), pH 6.1 to 6.4, osmolality at 304-389 mOsm/kg

Key Study Findings: The highest dose tested, 825 mg/kg was considered a NOAEL for maternal effects. There was an increase in dilatation of the kidney pelvis and ureters in the female F1 generation at the MD (450 mg/kg) and HD. The sponsor noted that there was no histopathologic evidence of hydronephrosis. The NOAEL for reproductive performance was the HD, 825 mg/kg.

Methods

Doses: 0, 120, 450, 825 mg/kg/day
Frequency of dosing: Daily from GD 6 through PND(post natal day) 20
Dose volume: 15 mL/kg/day
Route of administration: Intravenous (30 minute infusion)
Formulation/Vehicle: 0.9% saline
Species/Strain: Sprague Dawley mated females, 10-13 weeks old, 203-273 g, catheterized in the vena cava via the femoral artery.
Number/Sex/Group: 25/dose
Satellite groups: None
Study design: standard
Deviation from study protocol: None significant.

Observations and Results

F₀ Dams

Survival: (Twice daily): One LD dam was sacrificed moribund due to catheter complications on GD 18. One control dam was killed on PND 0 due to total litter death. There were no macroscopic findings in this rat.

Clinical signs: (daily): There were no remarkable differences in observations with dose.

Body weight: (Day 0, 6, 9, 12, 15, 18, 20 of gestation, D1, 4, 7, 10, 14, 17, 21 of lactation): There were no statistically significant differences in body weight between treated and control rats during gestation or lactation.

Feed consumption: GD 0-6, 6-9, 9-12, 12-15, 15-18, 18-20; LD 1-4, 4-7, 7-10, 10-14, 14-21): There was a 10% decrement in food consumption at the HD as compared to the controls between GD 6 and 20.

Uterine content: There were no dose dependent, statistically significant differences between groups in the numbers of pregnant females, females completing delivery, females with stillborn pups (8 control litters, 1-2 treated litters), gestation duration, or complete litter deaths.

Necropsy observation: There were no remarkable observations with dose.

Dosing Solution Analysis Ranged from 96 to 103% of intended concentrations

F₁ Generation

- Survival: (Once daily): One control dam was killed on PND due to total litter death. One LD dam was sacrificed due to catheter issues.
- Clinical signs: (Daily): There were no noteworthy differences between groups.
- Body weight: (Weekly, F₁ mated females on GD 0, 4, 8, 13): Pup weights did not differ significantly in males through day 91, and in females through day 56. There were no differences in the mated females during gestation days 0-13.
- Feed consumption: Not measured
- Physical development: (pinna unfolding, incisor eruption, eye opening, vaginal opening, balano preputial skinfold cleavage): None of the physical development milestones showed a difference in timing from controls.
- Neurological assessment: (surface righting reflex PND 8, gripping reflex PND 17, pupillary and auditory reflex PND 21, E shaped water maze at 5-6 weeks, open field at 7 weeks, ophthalmology at 9 weeks) : There were no remarkable differences in the water maze or open field tests between dosage groups. There were no remarkable ophthalmologic observations with treatment.
- Reproduction: There were no differences in time to mating, copulation index or fertility index for the F₁ generation. There were no differences in the numbers of corpora lutea, implantation sites, viable embryos, or pre-post-implantation loss.
- Other: In the weanling female pups, there was a dose dependent increase in kidney dilatated pelvis and ureter dilatation (see table below).

Table 5 Salient necropsy findings of weanling pups

Dose mg/kg	0		120		450		825	
Sex	M	F	M	F	M	F	M	F
Number of pups (litters)	68 (23)	69 (24)	60 (22)	69 (23)	71 (24)	63 (23)	70 (25)	74 (24)
Kidney(s): dilated pelvis	0	2 (2)	0	2 (2)	0	7 (4)	1 (1)	9 (7)
Ureter(s): dilatation	0	1 (1)	0	1 (1)	0	4 (3)	0	5 (4)
Total number of affected pups (litters)	0	2 (2)	0	2 (2)	0	7 (4)	1 (1)	9 (7)

M: Male.

F: Female.

10 Special Toxicology Studies

1. Evaluation of the venous and perivenous local tolerance after a single administration in rabbits. Study # NXL104/DS0016.

The study was conducted at [REDACTED] ^{(b) (4)} in 2006 according to GLP.

Three New Zealand white rabbits/sex/dose were administered vehicle (5% glucose), 5 mg/mL NXL104 (Batch 0600032422), 20 mg/mL NXL104, or 5 mg/mL NXL104 plus 20 mg/mL ceftazidime (CAZ) intravenous in the left ear (marginal vein) at 0.5 mL and perivenous to the marginal vein of the right ear at 0.2 mL. Rabbits were observed for mortality, clinical signs, body weight and local reactions for 5 days post-injection.

All rabbits survived to scheduled sacrifice without significant dose dependent effects on clinical signs or body weight gain. While one rabbit had crusts at the perivenous injection site of the CAZ-AVI combination, there were no other significant dose dependent changes in erythema or hematomas at the injection sites for intravenous or perivenous administration.

Study title: Evaluation of the hemolytic potential of formulations of NXL 104 in human blood *in vitro*

Study no.: NXL104/DS0015
 Study report location: EDR
 Conducting laboratory and location: [REDACTED] (b) (4)
 Date of study initiation: September, 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NXL104 Batch #0500012675;
 Ceftazidime Batch #44701503, 100%
 pure

Key Study Findings

NXL 104 solutions at 5 and 20 mg/mL in 5% w/v glucose had a hemolytic index <2% (%RBC hemolysis: non-hemolytic under ASTM standard practice for assessment of hemolytic potential of materials). Combination NXL 104/Ceftazidime 5/20 mg/mL in 5% w/v glucose had a hemolytic potential <2% (non-hemolytic). Positive control (Saponin, 80 g/L) had a mean hemolysis index of 76.4% (range: 72.3-79.0% - severely hemolytic). Negative control (154 mM NaCl) showed no hemolysis in any of the human whole blood samples.

Methods

Doses: Single: NXL104 5mg/mL; Ceftazidime 20 mg/mL
 Combination: NXL104/Ceftazidime 5/20 mg/mL;
 Positive hemolysis control: Saponin 80 g/L;
 Negative hemolysis control: NaCl 154 mM

Frequency of dosing: Incubation - 37°C 1hr
 Route of administration: 5mL conical test tube
 Dose volume: 3 mL (150 µL (Saponin, NaCl, NXL 104); 2.85 mL fresh whole blood)

Formulation/Vehicle: 5% w/v glucose
 Species/Strain: Whole human blood - fresh
 Number/Sex/Group: 3 human blood donors/dose
 Age: NA
 Weight: NA

Unique study design: *In vitro* hemolytic potential in fresh human whole blood

Deviation from study protocol: None that affected the achievement of the study objectives

Observations and Results

Hemolytic potential assay: Hemolysis in incubated supernatant was calculated from the absorbance values of NXL 104 or control treated samples of fresh human blood. All NXL 104 samples and saline treated samples were non-hemolytic. The positive hemolysis control sample (Saponin) was severely hemolytic even when diluted 1:200 (mean hemolysis index 76.4%). The data indicate that NXL 104 was non-hemolytic with a hemolytic index <2 (mean 0.00%).

Dosing Solution Analysis: Certificate of analysis of NXL104 Batch #0500012675 was provided (12/14/2004). Certificate of analysis of Ceftazidime Batch #44701503, 100% pure, was provided (12/14/2004).

Study title: 4-week intravenous (30 minute infusion) toxicity study in the Sprague Dawley rat with an immunotoxicological endpoint

Study no.: NXL104/DS0038
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: July, 2009
GLP compliance: Yes, OECD
QA statement: Yes
Drug, lot #, and % purity: NXL104 Batch # 08120739

Key Study Findings: There were no effects on B cell subpopulation, plaque forming cell assay, or immune organ pathology with up to 1000 mg/kg/day of AVI for 28 days.

Methods

Doses:	0, 250, 500, 1000 mg/kg/day
Frequency of dosing:	Daily for 28 days
Route of administration:	Continuous intravenous infusion via catheter (dose: 30-minute infusion)
Dose volume:	15 mL/kg/day
Formulation/Vehicle:	5% aqueous solution of glucose
Species/Strain:	Sprague-Dawley rats: Crl: CD (SD)
Number/Sex/Group:	10/sex/dose, 8/sex/dose for Plaque Forming Cell Assay (PFC)
Age:	9 weeks
Weight:	Main study: M: 246-368 g F: 176-221 g; Satellite animals PFC assay: M: 246-357 g F: 176-212 g
Unique study design:	Immunotoxicology endpoint; PFC assay – satellite group
Deviation from study protocol:	None that affected the achievement of the study objectives

Observations and Results

Immune cell phenotyping results indicate no depletions in CD3, CD4, CD8, NK cells (CD3-/CD161+) or NK T cell (CD3+/CD161+) populations. The CD45RA B-cell subpopulation was lower in males treated with test item at dose levels of 500 and 1000 mg/kg/day (day 28, group 3 and 4). However, this was not associated with a decrease in overall B-cell function using the Plaque Forming Cell assay. Accordingly, no depletion of immune function was observed by cytotoxicity and plaque forming cell assays. In addition, no histopathological changes were observed in the lymphoid organs of any treated animals.

Mortality (twice daily): There were no treatment-related deaths during the study. One satellite animal (no. 114) treated at the intermediate test item dose level (500 mg/kg/day) was sacrificed in a moribund condition on day 3. During the pretest period this animal lost weight (day -12: 269 g and day 0: 246 g) and this death was considered incidental and not treatment-related.

Clinical Signs (pretreatment, twice daily after dosing, except day 19 – once after dosing): There were no treatment-related clinical signs at any dose level.

Body Weights (pretreatment randomization, day 0 prior to dosing, once weekly): Body weight was unaffected by treatment at any dose level.

Feed Consumption (once weekly): Food consumption was unaffected by treatment at any dose level.

Ophthalmoscopy (pretreatment, post treatment necropsy): There were no treatment related ophthalmic events at any dose level.

Hematology (post treatment): There were decreased RBC counts, hemoglobin concentrations, and packed cell volumes observed in two males (500 and 1000

mg/kg/day) compared to mean values in controls. They appear to be isolated and were not considered to be attributable to NXL 104.

Clinical Chemistry: None

Urinalysis: None

Gross Pathology (day 28/29):

At necropsy, male no. 23 (low dose of NXL104) had an induration along the catheter which correlated histopathologically with marked chronic purulent and fibrosing inflammation. Control animal nos. 08 and 19 and treated animal nos. 41, 58 (intermediate dose) and no. 72 (1000 mg/kg/day) had cutaneous sores/crusts in the thoracic or axillary region correlating histopathologically with slight inflammatory changes of the skin. These included ulceration, pustule formation, fibrosing inflammation in the superficial dermis and epidermal hyperplasia. Based on lack of dose dependence, these observed changes do not appear to be related to NXL 104 treatment and were related to the procedure. .

Organ Weights: No significant differences in organ weights were noted between treatment/pre-treatment animal groups/sex.

Histopathology: There were no treatment-related changes observed with respect to organ histopathology.

Adequate Battery—Yes, for the purposes of this study; limited tissue panel examined at control, HD included adrenals, bone marrow, bronchi, cecum, colon, duodenum, ileum, injection site, jejunum, kidneys, liver, lungs, mandibular and mesenteric lymph node, Peyer's patches, skin, spleen, thymus and gross lesions.

Peer Review—Yes

Special Evaluation:

Toxicokinetics (days 0, 84): C_{max} and AUC_{0-24h} were dose-proportional between 65 and 250 mg/kg/day.

Cytotoxicity assay (day 28/29, spleen cell viability as evidenced by trypan blue exclusion): No differences were noted in the Cytotoxicity activity of cells between controls and the NXL 104 treatment groups (250, 500, 1000 mg/kg/day).

Plaque Forming Cell (PFC) assay (satellite animals day 28/29): No differences were noted in the number of Plaque Forming Cells between controls and the NXL 104 treatment groups (250, 500, 1000 mg/kg/day).

Lymphocyte subset analysis (day 28/29): A statistically significant but non-dose related decrease in B lymphocytes (CD45RA+) was noted on Day 28 in the males given 500 and 1000 mg/kg/day of NXL104 versus sex matched group 1 controls. No differences in CD45RA+ counts were observed in any females at any dose compared to controls.

Dosing Solution Analysis: NXL104 was discovered in control group blood samples obtained at day 28/29. Although quantifiable concentrations of NXL104 were

detected at exceedingly low levels (close to the detection limit of 10 ng/ml) in vehicle treated control animal samples, these findings were unexplained. Given the lack of pharmacokinetic and other correlates (e.g. very low levels of NXL104 detected, lack of Cmax behaviour or time point relationships in control animal samples etc.), such exposure does not appear to have altered the study objectives.

Study title: Evaluation of in vitro toxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay

Study no.: NXL104/DS0039
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September, 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NXL104 Batch
 #AFCH000707/07113P002

Key Study Findings

NXL 104 treatment of mouse fibroblast 3T3 cell cultures resulted in a < 50% cell loss at all tested concentrations in the absence and in presence of UV-A light. NXL 104 was therefore not phototoxic in this *in vitro* assay (Neutral Red Uptake assay) when tested up to 1000 µg/mL, the maximum recommended concentration according to OECD guidelines.

Methods

Doses: Chlorpromazine (+ control): 0.100 µg/mL; NXL 104 (active): 0.316, 1.000, 3.160, 10.00, 100.0, 316.0, 1000 µg/mL
 Frequency of dosing: Treatment: 60 min at 37 °C; UV-A: 93 min (5 J/cm²); Post UV-A incubation: 20±2 hrs at 37 °C
 Route of administration: 96 well plate - Incubation
 Dose volume: 100 µL
 Formulation/Vehicle: PBS
 Species/Strain: Mouse fibroblasts (Balb/c 3T3 clone A31)
 Number/Sex/Group: Duplicate samples
 Age: NA
 Weight: NA
 Unique study design: Phototoxicity via Neutral Red Uptake assay
 Deviation from study protocol: None that affected the achievement of the study objectives

Observations and Results

Neutral Red Uptake assay: Treatment of cultures with NXL 104 resulted in minimal decrease in cell survival, both in the absence and presence of UV-A light. The survival curves were generally similar and there was no significant difference in cell uptake of neutral red in the presence of UV-A compared to those cells not exposed to UV-A. The cell survival at the highest concentration (1000 µ/mL) was more than 50% thus IC₅₀ and PIF values could not be calculated. Positive controls (Chlorpromazine) had IC₅₀ values of 58.098 µg/mL (absence UV-A) and 0.891 µg/mL (within acceptable ranges) and a PIF 65.21 (>6 acceptable).

Dosing Solution Analysis: Duplicate samples (1 mL) of all test article concentrations, were taken from the primary dilution in purified water and from the final treatment dilutions in PBS for analysis of achieved concentrations. Vehicle control samples were similarly taken for analysis. The report, Analytical Determination of Achieved Concentrations, detailed five deviations, all of which did not affect the findings of no phototoxicity of NXL 104 at all tested concentrations detailed in this study.

11 Integrated Summary and Safety Evaluation

Ceftazidime/Avibactam (CAZ/AVI) is an intravenous combination of a cephalosporin and a beta lactamase inhibitor at a ratio (by weight) of 4:1. Most of the nonclinical studies addressed the toxicity of the AVI alone, as CAZ was previously found to be safe and effective in NDA approvals in the late 1980s. However, 28 day studies with the combination were conducted in rats and dogs. The proposed dose for CAZ/AVI is a premix of 2000 CAZ with 500 mg AVI over 30 minutes every 8 hours.

The clinical warnings for CAZ in the label for FORTAZ include hypersensitivity, CDAD, interaction with renal impairment, and prothrombin time decrements. CAZ was negative for genotoxicity and had no teratogenic effects in mice or rabbits at doses of 6.5 g/kg/day and 0.2 mg/kg/day respectively (Capel-Edwards et al., J. Antimicrob. Chemother, (1981)8suppleB 237-239). The review article offered little detail; however, liver and kidneys were a target in the rat with high doses over at least a month of dosing. The authors noted that intravenous and subcutaneous routes of dosing showed little difference in toxicity and toxicities were reversible. Dogs were reported to show no toxicity at doses up to 540 mg/kg/day for 30 days (route of administration not specified) Capel-Edwards et al., J. Antimicrob. Chemother, (1981)8suppleB 237-239.

At intravenous doses of up to 1000 mg/kg as a single administration, AVI had minimal effects on behavior, gastrointestinal transit, blood pressure, heart rate, QT interval, or neurologic, renal or respiratory function. A hERG assay was also negative. AVI did not

interact significantly with a series of receptors, proteases and proteins.

The ADME of AVI was investigated alone and in combination with CAZ. The half-life in rats and dogs in the toxicokinetic studies ranged between 3 and 10 hours. There were no significant gender effects on pharmacokinetics, and no accumulation over time. AVI plasma levels did not significantly affect CAZ plasma levels and CAZ did not significantly alter AVI plasma levels. Protein binding of AVI was low in humans, mice, rats, rabbits and dogs (all less than 25% by an ultracentrifugation method). Distribution was primarily into the kidney and bladder in the first few hours following injection. AVI penetration into the brain or across the placenta was minimal. AVI was minimally metabolized as determined in vitro by exposure to liver microsomal preparation or as measured in the urine and plasma of rats, dogs and humans after IV dosing. The primary route of excretion was urine; fecal elimination accounted for 17% of the dose in rats, 5% in dogs, and <1% in rabbits. AVI did not stimulate or inhibit cytochrome P450 enzymes or transporter proteins.

The toxicity of AVI was investigated in mice, rats, and dogs. In single dose studies, intravenous administration of up to 2000 mg/kg was a NOAEL in rats and mice. Not unexpectedly given a bioavailability of <10% by the oral route, a single oral gavage dose of 2000 mg/kg was also a NOAEL in rats and mice. AVI administered IV to rats or dogs for 4 or 13 weeks primarily caused damage to the injection site. The damage included thrombi, inflammation, and myodegeneration. The 13 week rat study was difficult to interpret due to presumed pseudomonas aeruginosa infection from the catheters with observations of multiple organ abscesses and induration. Dogs showed only injection site damage in the 13 week study. No new toxicities emerged with increased duration of dosing. Specialty toxicity studies included local tolerance in the rabbit, human blood hemolysis, immunotoxicology in the rat, and phototoxicity in 3T3 cells. These assays were negative for NXL104. It should be noted that the dose in the perivous/venous tolerance study was significantly lower than that in the toxicology studies.

In the 4 week combination studies of CAZ and AVI with a 4:1 ratio of drug, toxicity in the rat was again injection site damage, the damage was more severe with the highest dose combination than CAZ alone. No new toxicities were seen with the combination in the rat. In the dog, injection site damage was seen in the controls at the same rate as the CAZ/AVI treated dogs. Some hints at liver changes (increases in cholesterol/triglycerides and liver weight in the absence of P450 cytochrome induction) were noted with the high dose CAZ/AVI combination.

AVI had no effects on fertility in male or female rats at the highest dose tested (1000 mg/kg or approximately 20 fold greater than the human dose based on exposure (AUC)). However, pre and post implantation loss was increased in the females

administered AVI prior to mating at doses greater than or equal to 500 mg/kg (the NOAEL was 250 mg/kg or approximately equivalent to the human dose based on body surface area). Embryo-fetal developmental toxicity was not observed in rats at doses up to 1000 mg/kg/day (high dose limited by injection site damage). The highest dose tested did not show significant maternal toxicity or developmental malformations or variations in the fetuses. The AUC in the rats was approximately 9 fold higher than that in the humans at the recommended clinical dose.

In the definitive rabbit fetal development study, the high dose (1000 mg/kg) resulted in abortions in a single dam, which is likely related to maternal toxicity. An increase in late resorptions and decrement in fetal body weights were noted at the high dose of 1000 mg/kg/day. The AVI NOAEL was 100 mg/kg/day based on fetal variations at the MD of 300 mg/kg. Based on AUC, rabbit exposure was approximately 2 fold higher than that in humans.

The rat peri- and post-natal toxicity study showed an increase in the incidence of dilated pelvis and dilatation of the ureter at the high dose of 825 mg/kg/day, but there were no other significant changes.

Study	Embryofetal NOAEL	AUC @ NOAEL	Sponsor's AUC values	Ratio animal/human	Sponsor ratio
Rat Seg II	1000 mg/kg	454 ug.h/mL	1000 ug.h/mL	12	9
Rat Seg III	825 mg/kg (NOAEL for fertility, pup viability)	870 ug.h/mL	1250 ug.h/mL	23	11
Rabbit Seg II	100 mg/kg	272 ug.h/mL	272 ug.h/mL	7	2

After discussion with the sponsor (as well as the clinical pharmacologist) it was agreed that it was appropriate to use the single dose human PK AUC value X 3 for a daily exposure based on the proposed clinical dose (113 ug.h/mL). Further, the rat AUC level at 100 mg/kg for the Embryo-Fetal development study was significantly lower than the 500 mg/kg level, and did not agree with prior data from the 28 day study at 1000 mg/kg (although the rest of the data was close). While data in pregnant rats can differ from non-pregnant rats, and use of the AUC from the same study would be optimal, I concur with the sponsor that the 1000 ug.h/mL value better fits the overall dataset. The rat segment 3 study AUC value was mistakenly based on the end of infusion to t (eoi-t) value instead of the 0-t. Based on these arguments, the reviewer concurs with the sponsor's values for comparison of animal to human exposure.

The potential for AVI genetic toxicity was investigated in the Ames bacterial mutation, unscheduled DNA synthesis, mouse lymphoma clastogenicity, human lymphocyte chromosomal aberrations, and rat micronucleus assays. All were negative, although

based on the inhibition of the background lawn, the Ames test was inappropriate for AVI mutagenicity assessment. CAZ was previously investigated and labeled as negative in the Ames test and a mouse micronucleus assay. Carcinogenicity testing was not conducted based on the brief duration of use.

The majority of observations in the animals were relatively mild, generally confined to the injection site, and of minimal clinical concern. AVI is non-genotoxic, showed no signal in safety pharmacology studies or special toxicity studies, did not interfere with CYP enzymes for drug interactions or bind to plasma proteins. AVI and CAZ together did not cause synergistic even additive toxicity or interfere with each other's exposure levels. From the pharmacology/toxicology perspective, AVYCAZ can be approved.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WENDELYN J SCHMIDT
02/18/2015

AMY L ELLIS
02/18/2015