PHARMACOKINETICS OF NXL104 100 MG IN NORMAL SUBJECTS AND PATIENTS WITH VARYING DEGREES OF RENAL IMPAIRMENT NXL104/1003- AMENDMENT N°1.0

Protocol Number: NXL104/1003
EudraCT Number: 2008-001408-22

CLINICAL STUDY PROTOCOL

Investigators

Sponsor's responsible medical expert

Date of issue: Final version 2.0

TABLE OF CONTENTS

PROTOCOL OUTLINE

Study number	NXL104/1003

Pharmacokinetics of NXL104 100 mg in normal subjects and patients with varying degrees of renal impairment

Investigator(s), study site(s)

Study duration	18 months:	**Phase**	I

Objectives

Primary objective:
To characterize the pharmacokinetics of NXL104 administered as a 100 mg single dose over 30 minutes intravenous infusion in normal subjects and patients with varying degrees of renal impairment.

Secondary objective:
To investigate the safety, tolerability of NXL104 administered as a 100 mg, single dose over 30 minutes intravenous infusion in normal subjects and patients with varying degrees of renal impairment.

Study design

The study will be conducted as an open-label, parallel group design with a study session duration of approximately 4 days.

Population

Patients with renal impairment, between 18 and 90 years old assigned to 4 renal function groups based on 24-hour creatinine clearance determined at screening as follows:

Group 1: Subjects with normal renal function (creatinine clearance >80ml/min)

Group 2: Patients with mild to moderate renal impairment (creatinine clearance 50 to 79ml/min)

Group 3: Patients with moderate renal impairment (creatinine clearance 30 to 49ml/min)

Group 4: Patients with severe renal impairment (creatinine clearance <30 ml/min) but not requiring hemodialysis or peritoneal dialysis

Group 5: Patients with end-stage renal failure requiring hemodialysis. Patients will participate in two randomized sessions: dialysis session and inter dialysis session separated by a washout period of 7 to 14 days All hemodialysed patients should have the same duration and same interval of dialysis (4hours, 3 times a week) and if possible the same equipment for dialysis with the same blood flow rate (300 to 360mL/min) and constant dialysate flow (500mL/min;)during dialysis.

Number of subjects

A total of 30 volunteers (6 in each group) from the investigators' patient population will be enrolled. Dropouts will be replaced.

Inclusion criteria

Patients meeting all of the following criteria will be considered for admission to the study:

- Healthy adults of any race aged between 18 and 90 years, inclusive, with good health determined by past medical and surgical history, physical examination, vital signs, electrocardiogram, and laboratory tests at screening and matched by age (+/- 5years) and weight (+/-5kg) with renal impairment patients

  Or

- Patients with renal impairments of any race aged between 18 and 90 years, inclusive, reasonably stable renal function as assessed from serum creatinine concentration and/or urinary creatinine monitoring.
  Good health excluding renal disease and associated controlled illnesses such as hypertension, diabetes mellitus, etc (based on medical history, physical examination, electrocardiograms (ECG, and clinical laboratory tests),

  AND FOR WOMEN

  Women are authorized to participate in this clinical study if they meet the following criteria:

  - Has been surgically sterilized or has been postmenopausal for at least one year OR
  - Is of childbearing potential, and all of the following conditions are met:

- had normal menstrual periods for the 3 months prior to study entry, and
- has a negative serum pregnancy test (serum β-hCG) prior to enrollment.
- must be willing to practice double barrier methods of birth control during treatment and for at least 28 days after dosing with study medication. Oral contraceptives should not be used as the sole method of birth control, because the effect of NXL104 on the efficacy of oral contraceptives has not yet been established.

AND FOR ALL, the following:

- Able to execute written informed consent
- Be available for treatment as inpatients for up to 3 days following treatment, especially for anuric patients on hemodialysis
- Negative serology: HIV antibody, hepatitis B surface antigen, hepatitis C antibody,
- Negative urine drug screen for cannabis, opiates, cocaine metabolite, amphetamines, barbiturates, benzodiazepines (except for hemodialysis patients) and alcohol breath test
- Laboratory values will be within normal ranges (with the exception of those criteria associated with renal disease and any associated illness)
- Patients should maintain their chronic treatment if treatments are stable for at least one month

Inclusion of any patients with laboratory test values outside of normal limits (excluding those laboratory test values associated with renal disease and associated controlled illnesses) must be approved by both the sponsor or his designee and the clinical investigator before study initiation. The prestudy laboratory tests, ECG (12-leads), and physical examinations will be initiated within 28 days and completed at least 1 day prior to the start of the study and documented on the case report forms (CRF). Some exceptions may be made to patients schedules and physical examinations may occur up to 28 days prior to dosing. The sponsor must approve those exceptions. During prestudy examination, 24-hour creatinine clearance (Cockcroft equation) will be determined for assignment into a particular renal function group. On day 1, the 24-hour creatinine clearance determination will be repeated.(Note: if patient are anuric, they will be assigned to Group 5 and the prestudy and/or day two 24-hour creatinine clearance determination will not be required). Patients will fast for at least 4 hours prior to any laboratory tests. The principal investigator will provide normal ranges for all clinical laboratory test and the name, address, and accreditation of the testing facility.

Written informed consent must be obtained for all subjects before enrollment in the study

Exclusion criteria

Subjects presenting with any of the following will not be included in the study:

For healthy subjects:

- Any condition requiring the regular use of any medication;
- Symptoms of a clinically significant illness in the 3 months before the study;
- Presence or sequelae of gastrointestinal, liver or kidney disease, or other conditions known to interfere with the distribution, metabolism, or excretion of drugs;

And FOR ALL, the following:

- Use within 14 days (or 5 half-lives, whichever is longer) prior to the first study dose or intended use of any over-the-counter (including St. John's Wort or any herbal products) with the exception of paracetamol (≤ 1000mg/day) or new prescription medication including those that are known to cause pharmacokinetic drug interaction, unless approved by the sponsor;
- Presence of QTc≥ 430 ms or a pronounced sinus bradycardia (<40 bpm) or hypokalemia (<3.2mEq/L) or family history of long QT syndrome or familial history of unexplained sudden death or sick sinus syndrome or any clinically relevant unstable cardiovascular disease or cardiovascular event (e.g. myocardial infarction, cerabrovascuar accident…) which occurred less than 6 months ago ;
- Participation in another study with any investigational drug in the 3 months prior to the first study dose;
- Be in the exclusion period of previous study;
- Evidence of human immunodeficiency virus (HIV) infection and/or positive human HIV antibodies;
- Evidence of hepatitis C and/or positive hepatitis C antibody and/or positive hepatitis B surface antigen;
- History of hypersensitivity to drugs with a similar chemical structure;
- History of significant allergic disease and acute phase of allergic rhinitis in the previous 2 weeks before randomization or food allergy;
- Blood or plasma donation of more than 500 mL during the previous 1 month before randomization;
- Smoker of more than 5 cigarettes/ day during the previous 3 months;
- Heavy caffeine drinkers defined by a regular intake of more than 5 cups of coffee (or equivalent in xanthine-containing beverages) per day;
- Current evidence of drug abuse or history of drug abuse within one year of randomization, and/or positive findings on urinary drug screening;
- Subjects who have an average weekly alcohol intake that exceeds 2 units per day or subjects unwilling to stop alcohol consumption for the duration of the study (1 unit = 12 oz or 360 mL of beer; 5 oz or 150 mL of wine; 1.5 oz or 45mL of distilled spirits);
- Mental condition rendering the subject incapable to understand the nature, scope, and possible consequences of the study;
- Adults under guardianship and people with restriction of freedom by administrative or legal decisions;
- Unlikely to comply with the clinical study protocol; e.g. uncooperative attitude, shift-worker, inability to return for follow-up visits, or unlikely to complete the study;
- Women who are pregnant or breastfeeding, or fertile women not practicing adequate methods of contraception (as defined in inclusion criteria); women planning to become pregnant within 1 month of the study.
- Any other condition that, in the opinion of the Investigator, would preclude participation in the study.

Any waiver of these inclusion and exclusion criteria must be approved by the investigator and the sponsor on a case-by-case basis prior to enrolling the subject. This must be documented by both the sponsor and the investigator. No subject will be allowed to enroll in this study more than once.

Treatments

NXL104 concentrated solution for infusion

Formulation	Vial containing 1000 mg of NXL104 lyophilized powder to be reconstituted with 10mL of water for injection to obtain a 100mg/mL solution for IV infusion
Doses	100 mg, single dose
Route	Intravenous infusion in volume of 50mL 5 % glucose for 30 minutes
Manufacturer	

NXL104 will be administered by intravenous route in a constant volume of 50mL 5% glucose for 30minutes. A 200 mg/100ml will be prepared and 50mL of this preparation will be infused during 30 minutes The concentration of the solution will be 2 mg/mL(see section 17.2 for details)

Pharmacokinetic Data

Blood collection: 4 mL of blood will be withdrawn in 2 separate 2mL BD Vacutainer® FX tubes (ref Nb 368920) containing sodium fluoride (5mg) and potassium oxalate (4 mg), then plasma will be stored at –80°C until shipment in dry ice.

- T0 = start of the infusion
- Sampling Times:
- Group 1 predose, 15 min (during infusion), 30 min (end of infusion), 45min, 1h, 1h30min 2h, 3h, 4h, 6h, 8h, 12h, 24h after the start of infusion (13 blood samples)
- Groups 2 to 4 additional samples at 48h after dosing
- Hemodialysis patients (group 5): 2 periods, two-way crossover:

  Treatment A: Day of dialysis: start of infusion **1:00h before dialysis session start H0=start of infusion**

  - **Predose: H-1:00**(before infusion),then **H1:00**(just before dialysis) then arterial and venous blood samples at 30min intervals during hemodialysis (4 hours dialysis) **H1.5**(30min dialysis); **H2:00**(1:00dialysis), **H2.5**(1:30dialysis), **H3:00**(2:00dialysis), **H3.5**(2:30dialysis), **H4:00**(3:0dialysis), **H4.5**(3:30dialysis), **H5:00**(4:00dialysis), (**10** arterial and venous samples)

  Treatment B: Interdialysis period: start of infusion 1 h after dialysis session.

  - Blood sampling: venous blood sampling same as for group 1 after infusion+ additional sampling just before next dialysis (14 samples)

Urine collection: 10mL urine should be collected on acetic acid and obtained before infusion = blank urine; then urine collection at **]0-6h],]6-12h],]12-24h],** post dosing, on day 1 after single administration..
The exact collection times and weights of urine will be recorded in the CRF. Samples will be stored at approx -80 °C until shipment in dry ice.

Analytes, media and methods: Unchanged NXL104 will be measured in plasma and urine by a validated LC-MS/MS assay method.

Estimated total blood volume taken per subject

Group 1 A total of 139 to 159 mL (around maximum 160 ml) will be drawn from each subject as follows:

- max 52 mL for PK sampling (13*4ml), + possibly 20ml for additional samples if needed
- 87 mL for safety sampling (including 27mL for screening (7mL for serology), 20mL D1 and, 20mL (D2 after last infusion and EOS visits) should be adapted to the center use

Groups 2 to 4 A total of 143 to 163 mL (around maximum 165 ml) will be drawn from each subject as follows:

- max 56 mL (14*4ml) for PK sampling, + possibly 20 ml for additional samples if needed
- 87 mL for safety sampling (including 27mL for screening (7mL for serology), 20mL D1 and, 20mL (D2 after last infusion and EOS visits) should be adapted to the center use

Group 5 A total of 249 to 269 mL (around maximum 270 ml) will be drawn from each subject as follows:

- dialysis max 80 mL (20*4ml) for PK sampling,(10 samples arterial +10 samples venous) + possibly 10ml for additional samples if needed

  Interdialysis max 56 mL (14*4ml) for PK sampling, + possibly 10ml for additional samples if needed - 107 mL for safety sampling (including 27 mL for screening (7mL for serology), and 20mL x2 for D-1(at each period), 20mL x2 (after last infusion), and EOS visits), should be adapted to the center use.

- 2.7ml x2 for Hematocrit sampling

Pharmacokinetic analysis: The following plasma pharmacokinetic parameters will be calculated using non-compartmental analysis in all non-dialyzed patients, and in dialyzed patients outside dialysis sessions: Cmax, Tmax, Clast, Tlast, AUC0-t, AUC, fractional AUCs, percent of extrapolated AUC, λ_Z, $t_{1/2,Z}$, boundaries of the terminal phase, number of data points in the terminal phase, Vss, CL, MRT. From urine data, the following pharmacokinetic parameters will be calculated: fractional and cumulated urinary excretion (amount), fractional and cumulated urinary recovery (%dose) fractional and cumulated renal clearance.

Within dialysis sessions, the following plasma pharmacokinetic parameters will be calculated: extraction ratio, hemodialysis clearance, AUC0-t, and amount removed by dialysis.

Pharmacodynamic data

Not applicable

Efficacy data

Not applicable

Safety data

Safety will be evaluated on the basis of:

- Adverse events reported
- Local tolerance
- Vital signs: supine and standing (10 minutes and 3 min) blood pressure, heart rate
- Body weight
- Parameters from ECGs performed during and post infusion: QRS, PR, RR, HR, QT, corrected QT (Bazett and Fridericia formula)

- Biochemistry
- Hematology
- Urinalysis and evaluation of diuresis (D1).

Volunteers will remain in the clinic from check-in on day-1 until the 24-hour blood sampling is drawn.

Statistical analysis

Safety analysis

Adverse events will be listed on an individual basis. Frequencies of treatment-emergent adverse events classified by MedDRA system organ class and preferred terms will be tabulated by group.

All safety data will be tabulated for each group and measuring time together with standard descriptive statistics, if appropriate. Laboratory data will be compared to the normal ranges given by the laboratory. Findings will further be evaluated using pre-defined change criteria.

ECG: Listings of all variables will be provided by time and group. Descriptive analysis will be presented for all ECG variables and their changes from baseline (Δ) by time and group. In addition, ECG will be evaluated using a system of predefined changes ($30 < \Delta QTc \leq 60$ ms; $\Delta QTc > 60$ ms; QTc > 450 ms; QTc > 480ms; QTc > 500ms).

Pharmacokinetic analysis

The effect of renal impairment will be evaluated for AUC0-∞, AUC0-t and Cmax using an ANOVA model applied to log transformed pharmacokinetic parameters with a class effect for the renal function group (but dialyzed patients during a dialysis session). For each parameter, a point estimate for the ratio of central values (renal patient/normal control) will be obtained by calculating the difference of least square means on the logarithmic scale and subsequent back transformation with the anti-log function. Likewise, 90% confidence intervals for the ratios will be obtained. In addition, total clearance of NXL104 will be plotted as a function of creatinine clearance and data will be evaluated by linear correlation analysis.

Interim analysis

No interim analysis will be performed.

Safety considerations for clinical studies (see investigator brochure for complete details)

- Local tolerance at the injection site should be assessed but good tolerability was observed in previous human studies.

- Based on animal data, adverse events such as vomiting and transient increase in arterial blood pressure may occur. Serum cholesterol, triglycerides, serum sodium, blood urea, haemoglobin content, packed cell volume and RBC, WBC, neutrophils, lymphocytes and basophils counts/levels should be monitored during treatment with NXL104.

- Based on phase I single and multiple dose studies in human, adverse events such as infusion site erythema or swelling, abdominal pain, orthostatic hypotension, drowsiness or anxiety may occur.

Study Schedule

Table 1- Overall Flowchart (Groups 1 to 4)

	Screening	***Hospitalization D-1 to 24h post start of infusion***			***End of Study***
Study days	***D-28 to D-2***	***D-1 Baseline***	***D1***	***D2***	***D3 to 7***
Informed consent	*X*				
Admitted to unit		*< - -*	*- -*	*- -*	
Check inclusion/exclusion criteria	*X*				
Review entry criteria		*X*			
Prior medication recording	*X*	*X*			
Medical/surgical history	*X*	*X*			
HIV, HBV, HCV tests	*X*				
Urine Drug screen and alcohol breath test	*X*	*X*			
Full laboratory test	*X*	*X*		*X*	*X*
Physical examination	*X*	*X*		*X*	*X*
Height	*X*				
Weight	*X*	*X*			*X*
Infusion of study drug			*T0- T30'*		
Local tolerance (Infusion site)			*X*	*X*	*X*
Blood pressure and pulse	*X*	*X*	*X*	*X*	*X*
12-lead ECG	*X*	*X*	*X*	*X*	*X*
Blood sampling for PK			*X*	*X*	*X*
Urine collection			*X*	*X*	
Creatinine clearance			*X*	*X*	
Concomitant medication	*< - - >*	*< - -*	*- -*		*- - >*
Adverse events recording	*< - -*	*< - -*	*- -*		*- - >*

1: BP+ pulse (10 min supine and 3 min standing): screening, D-1, 30 min (end of infusion in supine position), then, 1h, ,4h, 6h, 12h, 24h, post start of infusion

2: 12 lead ECG: screening, D-1, D1, D2 and EOS

3: Blood PK: Predose (before infusion); 15min(during infusion), 30min, (end of infusion), 45min, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h, 12h, 24h, post start of infusion, on D3 (48h postdose) for groups 2 to 4

4: Urine collection: Predose (10 mL urine = blank, before infusion) then,]0-6h],]6-12h],]12-24h] post start of infusion

5: Urine drug screen: cannabis, opiates, cocaine, metabolite, amphetamines, barbiturates, benzodiazepines

Study Schedule

Table 2- Overall Flowchart (Group 5)

	Screening	***2 periods with Hospitalization D1 to 24h post start of infusion***			***End of Study***
Study days	***D-28 to D-2***	***D-1 Baseline***	***D1***	***D2***	***D3 to 7 after 2nd dosing***
Informed consent	*X*				
Admitted to unit		*< - -*	*- -*	*- -*	
Check inclusion/exclusion criteria	*X*				
Review entry criteria		*X*			
Prior medication recording	*X*	*X*			
Medical/surgical history	*X*	*X*			
HIV, HBV, HCV tests	*X*				
Alcohol breath test	*X*	*X*			
Full laboratory test	*X*	*X*		*X*	*X*
Hematocrit			*X*		*X*
Physical examination	*X*	*X*		*X*	*X*
Height	*X*				
Weight	*X*		*X*		*X*
Infusion of study drug			*T0- T30'*		
Local tolerance			*X*	*X*	*X*
Blood Pressure and pulse	*X*	*X*	*X*	*X*	*X*
12-lead ECG	*X*	*X*	*X*	*X*	*X*
Blood sampling for PK			*X*	*X*	*X*
Concomitant medication	*< - - >*	*< - -*	*- -*		*- - >*
Adverse events recording	*< - -*	*< - -*	*- -*		*- - >*

1: BP+ pulse (10 min supine and 3 min standing): screening, D-1,30 min (end of infusion in supine position), then, 1h , 2h, EOS (during dialysis session); screening, D-1,30 min before infusion, 30 min (end of infusion in supine position), then, 1h, 4h, 6h, 1 h, 24h, EOS (inter dialysis period)

2: 12 lead ECG: screening, D-1, D1, D2 (interdialysis period) and EOS

3: Blood PK (interdialysis session): -30min, 15min, 30min, 45min, 1h, 1.30h, 2h, 3h, 4h, 6h, 8h, 12h, 24h and 48h post start infusion only for interdialysis treatment

Blood PK (dialysis session): before infusion, before start dialysis, 30min, 1h, 1.30h, 2h, 2.30h, 3h, 3.30h, 4h

4: Hematocrit after start and at end of dialysis.

5: Full laboratory test at screening, D-1 (dialysys and interdialysis session), after dialysis, and after interdialysis session, EOS.

ABBREVIATIONS AND DEFINITIONS

a m.	*Ante meridiem*
AE	Adverse event
ALAT (SGPT)	Alanine aminotransferase
Alk Phos	Alkaline Phosphatase
ALQ	Above the upper limit of quantification
ATC	Anatomical therapeutic chemical
ASAT (SGOT)	Aspartate aminotransferase
AUC_{0-t}	Area under the plasma concentration versus time curve from time zero to the time (t) corresponding to the last quantifiable concentration
$AUC_{0-\infty}$	Area under the plasma concentration versus time curve from time zero to infinity
AV	Atrioventricular
BE	Bioequivalence
BMI	Body mass index
BP	Blood pressure
°C	Degree Celsius
Cm	Centimetre
C_{max}	Maximum plasma concentration
CRF	Case report form
CRO	Contract research organization
CS	Clinically significant
CSALV	Clinically significant abnormal laboratory value
CV	Curriculum vitae
CV%	Coefficient of variation
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DIC	Delayed intraventricular conduction
e.g.	*Exempla gratia*
ECG	Electrocardiogram
EOS	End of study
G	Gram(s)
g	Gravity value
GCP	Good clinical practices
GGT	Gamma-glutamyl transferase
GLP	Good laboratory practices
H / hrs	Hour(s)
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
i.e.	*Id est*
I.U.	International unit
i.v.	Intravenous
ICF	Informed consent form including sample volunteer information sheet
ICH	International conference on harmonization of technical requirements for registration of

	pharmaceuticals for human use
Ke	Terminal rate constant
Kg	Kilogram
L	Litre
LBBB	Left bundle branch block
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLN	Lower limit of normal
LOQ	Limit of quantification
LPCA	Last on-treatment predefined change abnormal
M	Metre
Max	Maximum
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MD	Medical doctor
MedDra	Medical dictionary for drug regulatory affairs
Mg	Milligram
Min	Minimum
Min	Minute(s)
mL	Millilitre
mmHg	Millimetre mercury
Mmol	Millimole
µmol	Micromole
MRT	Mean residence time
MRSA	Methicillin resistant *Staphylococus aureus*
MRSE	Methicillin resistant *Staphylococus epidermidis*
Ms	Millisecond
N.A.	Not applicable
N.C.	Not calculated
NCS	Not clinically significant
Ng	Nanogram
N.R.	Not reported
Od	Once daily
P	Probability on null hypothesis
PC	Personal computer
PCA	Predefined change abnormal
PCSA	Potentially clinical significant abnormality
p m.	*Post meridiem*
Po	Per os
PK	Pharmacokinetics
PP	Per protocol
PR (interval)	Interval between beginning of P wave and beginning of Q or R waves
QAU	Quality assurance unit
QC	Quality control(s)
QT-interval	Interval between Q and T waves
QTc	QT interval corrected for heart rate

RBBB	Right bundle branch block
RBC	Red blood cells
S	Second
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of mean
SOP	Standard operating procedure(s)
SSRI	Selective serotonin reuptake inhibitor
TEAES	Treatment emergent adverse events
t_{max}	Maximum time concentration
$t_{1/2,z}$	Terminal half-life
ULN	Upper limit of normal
WBC	White blood cells
WHO-DRL	World health organization-drug reference list
WPW	Wolf-Parkinson-White

1 INTRODUCTION AND STUDY RATIONALE

1.1 Background on the product

1.1.1 Problem statement and Justification for development

It is estimated that 2 million patients per year in the United States acquire infections while in hospitals, approximately 350,000 (10–20%) of these infections involve the bloodstream, and 90,000 (4.5%) are fatal [1-4]. Gram-negative pathogens are responsible for nosocomial infections in 42%, 46% and 63 % of ICUs, non-ICUs, and out patient wings, respectively [5]. Gram-negative pathogens are also responsible for a substantial proportion of infections in the community.

Among the Gram-negative pathogens, Coliform (*Escherichia, Klebsiella, Enterobacter, Serratia,* and *Citrobacter*) and *Proteus* bacilli currently cause 29 % of nosocomial infections in the United States. This group of nosocomial pathogens is responsible for 46% of UTIs, 24% of surgical site infections, 17% of bacteraemia cases, and 30% of pneumonia cases.

Among Community-Acquired Infections, *Escherichia coli* is the major cause of UTIs, including prostatitis, pyelonephritis (hospitalization due to pyelonephritis) and septicemia. *Proteus*, *Klebsiella,* and *Enterobacter* species are also common urinary tract pathogens. *Proteus mirabilis* is the most frequent cause of infection-related kidney stones [6].

The prevalence of multidrug resistance (MDR) strains among Gram-negative bacilli is increasing [resistance to at least 3 different antibiotic groups] [1,7-9]. Compared to infections due to antimicrobial-susceptible Gram-negative bacilli, infections due to MDR Gram-negative bacilli lead to longer hospital stays, increased mortality, and greater costs of hospitalization [3,10].

Resistance to β-lactam drugs in Gram-negative bacteria is most commonly attributed to chromosomal or plasmid-borne β-lactamase production. Chromosomally mediated β-lactamase (Ambler class C) production is mainly through expression of the *ampC* gene, which is either constitutive or inducible and is found among the *Enterobacteriaceae* and *Pseudomonas aeruginosa* [13]. Class C β-lactamases are resistant to marketed β-lactamase inhibitors (clavulanic acid, tazobactam, sulbactam). In *Enterobacter*, the expression of the *ampC* gene is repressed, but derepression can be induced by β-lactams. These mutants are highly resistant to most β-lactam antibiotics except carbapenems [5].

Serratia, Morganella, Providencia, Enterobacter, Citrobacter freundii and P. aeruginosa have similar, although not identical, chromosomal *ampC* β-lactamase genes that are inducible [5, 13]. Plasmid-encoded AmpC enzymes have been reported from *Klebsiella spp.* and *E. coli* isolates. Ampicillin and amoxicillin, first- and second-generation cephalosporins, and cephamycins are strong AmpC β-lactamase inducers. They are also rapidly inactivated by these β-lactamases; thus, resistance is readily documented *in vitro* [5].

The most common mechanism of resistance to β–lactam antibiotics among Gram-negative pathogens is due to ESBLs: These enzymes are plasmid-mediated β-lactamases of predominantly Ambler class A. ESBLs represent a major group of β-lactamases that are now found in a significant percentage of *E.coli, Klebsiella pneumoniae* and other *Enterobacteriacae* species including *Enterobacter, Citrobacter, Proteus, Morganella morganii, Serratia marcescens, Shigella dysenteriae, P. aeruginosa,* and *Burkholderia cepacia* [11,12]. ESBL-producing bacteria often show cross-resistance to other groups of antibiotics, such as fluoroquinolones, aminoglycosides, tetracyclines and trimethoprim/ sulphamethoxazole.

ESBLs are capable of efficiently hydrolyzing penicillins, narrow spectrum cephalosporins, many extended-spectrum cephalosporins, cephalosporins containing an oxyimino group (cefotaxime, ceftazidime), and monobactams (aztreonam). The majority of ESBL-producing organisms produce more than one β-lactamase and strains producing multiple ESBLs are being reported. Different strains vary in the actual amount of each β-lactamase produced [6].

Infections due to ESBL-producing organisms present a major therapeutic dilemma, as the choice of antibiotics is extremely limited.
Clinical outcome is poor when 3rd generation cephalosporins are used to treat ESBL producing organisms. These organisms may appear susceptible at a standard inoculum of 10^5 but at higher inocula of 10^7 or 10^8 they have elevated MICs, indicating resistance. Bacteria producing ESBLs should be considered resistant to all generations of cephalosporins, all penicillins, and to the monobactams (aztreonam), even if the *in vitro* susceptibilities are in the sensitive range [5]. Even though cefepime exhibits more stability to hydrolysis by ESBLs than the 3rd generation cephalosporins, a positive clinical outcome from treatment with this antibiotic has not been established. Like the 3rd generation cephalosporins, MICs for cefepime rise substantially when the inoculum of infecting organisms rises [15-18].

Published clinical experience with the use of β-lactam/ β-lactamase inhibitor combinations for serious infections by ESBL-producers is limited, but several reports have described clinical failures with mortality rates exceeding 50%. β-lactam/ β-lactamase inhibitor combinations are also subject to rising MICs as the inoculum rises. In addition hyperproduction of β-lactamases, or combination of TEM and SHV enzymes, can also lead to reduction in activity of β-lactam/ β-lactamase inhibitor combinations [6, 15, 17, 19]. Thus, these combinations have limitations in the treatment of severe infections [13,15].

Clinical failures with aminoglycosides for the treatment of infections caused by ESBL-producers that showed elevated MICs to aminoglycosides (but within the susceptibility range) have been reported. Many ESBL-producing isolates are resistant to gentamicin because of the co-transfer of aminoglycoside resistance and β-lactamase genes on plasmids [6]. A practical issue is the concern for nephrotoxicity in severely ill patients who have acquired ESBL producing strains, making aminoglycoside therapy an undesirable option [19].

Quinolone resistance is found with high frequency (18% to 56%) in ESBL producing isolates [14, 20-26]. Although quinolone resistance commonly results from chromosomal mutation, plasmid pMG252, the first plasmid found to carry *qnr*, and which also carries the gene for AmpC-type β-lactamase FOX-5 is causing concern for the potential of further spread of fluoroquinolone resistance [15,19,27-29]. The newer fluoroquinolones are unlikely to confer added benefits.

Although the TEM and SHV ESBLs do not effectively hydrolyze the cephamycins, cefoxitin and cefotetan, some ESBL-producing strains have acquired a plasmid mediated *ampC* gene. The AmpC-type β-lactamase effectively hydrolyzes the cephamycins [30-33]. Emergence of resistant isolates while on cephamycin therapy can also occur probably caused by porin deficiency [19,34,35]. Clinical experience with these drugs is still lacking.

Carbapenems are the drugs of choice for serious infections caused by ESBL-producing organisms. Carbapenems are the only reliable β-lactam drugs for the treatment of severe *Enterobacter* infections. Resistance to carbapenems is rare but has been reported for imipenem in strains of *Enterobacter cloacae* [5]. Hyper-production (stable derepression) of AmpC β-lactamases, in association with some decrease in permeability to the carbapenems, may also cause resistance to these agents. Carbapenems are strong AmpC β-lactamase inducers, but have so far remained very stable to the action of these β-lactamases. Widespread use of carbapenems may lead to the emergence of carbapenem resistant *Acinetobacter baumannii* and *P.aeruginosa*, *Stenotrophomonas maltophilia* and Vancomycin resistant enterococci [15].

NXL104 is a novel, non β-lactam, β-lactamase inhibitor with a spectrum of activity encompassing both class A and class C β-lactamases, which include enzymes of profound clinical importance. NXL104 when associated with ceftazidime has also been shown to be active against strains, which express a combination of β-lactamase types, as well as strains, which are concomitantly resistant to other antibacterial classes such as fluoroquinolones. NXL104 acts by forming a very stable, practically irreversible (longer binding half life) binding to these enzymes with a lower inhibition IC_{50} as compared to currently marketed β-lactamases inhibitors clavulanic acid, tazobactam and sulbactam. In addition NXL104 is a potent inhibitor of class C enzymes whereas clavulanic acid, tazobactam and sulbactam lack any activity. Unlike currently available β-lactamases inhibitors, NXL104 does not induce β-lactamase production.

The potent *in vitro* activity of the NXL104 and ceftazidime combination against *Enterobacteriaceae* producing class A, and importantly class C, β-lactamases has been confirmed *in vivo* in murine pneumonia, septicemia and pyelonephritis models.

Currently, the options for the treatment of Gram-negative infections, especially multi drug resistant (MDR) strains including ESBL producers, are extremely limited. There are no new investigational compounds under early or late development specifically targeted to combat these organisms. Hence availability and development of new agents to treat these infections will be a welcome addition to the existing treatment(s).

1.1.2 ANTIMICROBIAL ACTIVITY

NXL104 [redacted] is a novel non β-lactam β-lactamase inhibitor with a spectrum covering both Ambler class A [Extended spectrum β-lactamases (ESBLs)] and class C (Amp C) enzymes. β-lactamase inhibition is effected through the formation of a stable covalent carbamoyl linkage in the acyl enzyme complex.

***in vitro* activity against β -lactamases**

NXL104 displays potent inhibition of both class A and class C enzymes. Purified TEM-1, SHV-4, KPC-2 and P99 were inactivated at low IC_{50} values, with low turnover numbers and long covalent intermediate half-lives were demonstrated with TEM-1 and P99.

***in vitro* antibacterial activity of ceftazidime / NXL104 combination**

NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects β -lactams from hydrolysis in a variety of class A and class C producing strains, including ESBL producers.

NXL104 restored ceftazidime activity against ceftazidime-resistant strains expressing various class A β -lactamases including strains producing the KPC carbapenemases. In addition, NXL104 protected ceftazidime activity from β -lactamase hydrolysis in strains expressing class C enzymes, including plasmid-borne and chromosomal, inducible or derepressed AmpC enzymes.

Finally, NXL104 combination remained active in bacterial isolates having a high hydrolytic activity due to production of multiple β -lactamases, as well as in isolates for which carbapenem resistance is conferred by a combination of impermeability plus cephalosporinase production.

Comparable MIC values were obtained when the ceftazidime / NXL104 combination was used with the constant antibiotic / inhibitor ratio of 4/1 or with the constant inhibitor concentration of 4 mg/L. The latter was the preferred susceptibility testing method, since it allowed measuring antibiotic activity with significant enzyme inhibition. In contrast to the potent *ampC* induction seen with cefoxitin and clavulanate in *E. cloacae*, NXL104 did not induce *ampC* expression.

No antagonism of ceftazidime *in vitro* antibacterial activity has been observed when combined with NXL104.

NXL104 *in vitro* pharmacodynamics were shown to be time-dependent in an hollow fiber infection model.

***in vivo* antibacterial activity of ceftazidime / NXL104 combination**

The ceftazidime / NXL104 combination showed therapeutic activity in mice infected by ceftazidime-resistant *Enterobacteriaceae* strains (class A and class C β -lactamase producers). According to the activity observed, the preferred ceftazidime/NXL104 dosing ratio was 4/1 w/w.

Ceftazidime / NXL104 has demonstrated

- Effective bacterial clearance in the lung in mouse pneumonia model
- Effective bacterial clearance in cerebrospinal fluid in the rabbit meningitis model
- Significant survival improvement in murine pyelonephritis model
- Significant survival improvement in murine septicemia model

1.1.3 SAFETY / GENERAL PHARMACOLOGY

The results of receptor binding assays showed that NXL104 had no affinity for the majority of the receptor binding sites studied. NXL104 displayed a low affinity for regulatory processes, neurotransmitters / neurotransmitter uptake processes, and neuropeptide receptors. NXL104 showed no significant inhibition or stimulation of selected (twelve) human serine proteases up to 1mM.

Following single intravenous administrations of 100, 300 and 1000 mg/kg of NXL104 there was no significant effect on general behaviour in Sprague-Dawley rats. In Wistar rats, there were dose dependent depressant effects (decreased reactivity to touch and decreased muscle tone) at all doses.

NXL104 showed no substantial effects on respiratory function up to the maximum administered single dose of 1000 mg/kg.

Following single intravenous administrations of 100, 300 and 1000 mg/kg of NXL104 in Wistar rats, no effects on urinary volume, pH, potassium and creatinine excretion were observed. However, a dose dependent increase in sodium excretion (significant only at 1000 mg/kg dose) was observed.

The exposures (AUC 0 to 24), in rats, at 100, 300 and 1000 mg/kg were 55.7, 188.4 and 787.7 mg.h/L, respectively and the maximum plasma concentrations (Cmax) were 73.6, 290.9 and 1234 mg/L, respectively.

NXL104 had no effect on hERG current, in HEK293 cells, at the highest tested concentration of 1000 µM (approximately 270 mg/L). In CHO cells it weakly blocked hERG currents with an IC50 of more than 300 µM.

In the single-dose (1000 mg/kg) intravenous infusion study in conscious normotensive rats, there was no effect on heart rate but there were slight and transient increases in blood pressure within 2 minutes from the end of administration. In telemetered conscious dogs, at doses up to 1000 mg/kg, there were no substantial effects on heart rate, blood pressure, the PR, QT and QTc intervals and there was no arrhythmia or change in the morphology of ECG tracings.

The exposures (AUC 0 to 24), in dogs, at 100, 300 and 1000 mg/kg were 363.3, 1063 and 3646 mg h/L, respectively and the Cmax were 362.5, 1026 and 3474 mg/L, respectively.

Following a single oral administration of NXL104 in rats (125, 500 and 2000 mg/kg), there was a moderate delay in mean intestinal transit time at 2000 mg/kg when compared to controls.

1.1.4 NON-CLINICAL PHARMACOKINETICS

Absorption: NXL104 was orally bioavailable in male rats. The absolute bioavailability of an oral solution in rats was estimated to be 5.4% at 45 mg/kg, and 12.1% at 500 mg/kg. NXL104 is likely to be orally bioavailable in man. However, the intended mode of administration in man is initially by intravenous infusion.

Distribution: The *in vitro* plasma protein binding of [14C]-NXL104 was less than 22.1% in animal species and apparently concentration-dependent. In contrast, protein binding to human plasma was concentration-independent, and low: 5.7 to 8.2%. NXL104 is unlikely to be involved in drug-drug interactions caused by protein binding displacement.

In rat and dog, the blood to plasma partitioning suggested little affinity towards blood cells.

In rat, NXL104-related radioactivity was distributed in a large variety of tissues and organs. Only the kidneys, the bladder and whole blood were more exposed than plasma (132% to 165% of plasma AUC). All other tissues, including endocrine glands, were less exposed than plasma (11% to 58% of plasma AUC).

The exposure of pigmented skin was nearly half the exposure of non-pigmented skin. NXL104 was distributed in a volume 0.6-0.7 L/kg in rat and 0.3-0.7 L/kg in dogs. The predicted steady-state volume of distribution in man is 11.8 L.

Metabolism: *in vitro* in liver microsomes, slow metabolism of NXL104 (13% parent drug depletion in 90 minutes) was detected only in rat material. In contrast, NXL104 was metabolically stable in mouse, rabbit, dog and human material. *In vivo*, after intravenous dosing in rats and dogs, the NXL104 metabolites formed resulted from mono- and di-hydroxylation of NXL104. The carboxamide side chain of mono-hydroxy-NXL104 was further hydrolysed to form the corresponding carboxylic acid derivative. In plasma at the end of infusion, the parent drug was the most abundant circulating entity. The metabolic clearance in healthy humans is predicted to be approximately 20% of total clearance. NXL104 is unlikely to cause, or to be sensitive to, clinically relevant metabolic drug-drug interactions, as long as renal function is normal.

Ceftazidime/NXL104 interaction: After single and repeated administrations to rats and dogs, NXL104 did not influence exposure to ceftazidime. In contrast, ceftazidime decreased NXL104 exposure (AUC0-24) by approx. 20 to 30% on average in both species, at steady state as well as after a single administration. The mechanism of this interaction is unknown.

Excretion: After a single intravenous infusion of [14C]-NXL104, about 73% (rat) and 82% (dog) of the dose was excreted via the urine, with excretion virtually complete within 168 h. Most of the dose was recovered in the first 24 h. Prolonged radioactivity terminal half-lives were estimated in rat and dog plasma (51.8 h and 167 h, respectively). Low total clearances were obtained (0.79-0.84 L/h/kg and 0.22-0.36 L/h/kg in rats and dogs, respectively). Resulting apparent terminal half-lives were 1.33-1.44 h in rats and 3.38-3.88 h in dogs. NXL104 may be classified as a low hepatic extraction drug in rats and dogs. The total clearance predicted in man is 7.9 L/h or 132 mL/min.

1.1.5 Toxicology

After a single intravenous administration of NXL104 the Highest Non-Lethal Dose (HNLD) was 2000 mg/kg in mice and rats. The main adverse effects observed at this dose were haematoma and crusts at the injection site (tail) in both species. There was no macroscopic abnormality at necropsy.

After a single oral (gavage) administration of NXL104 the No Observable Adverse Effect Level (NOAEL) and the Highest Non-Lethal Dose (HNLD) was 2000 mg/kg (highest dose tested) both in mice and in rats.

In the 7-day dose range finding intravenous toxicity study in rats with NXL104, the NOAEL was 167 mg/kg/day. A slight decrease in body weight gain and reduced RBC (red blood cell) counts in females were the only adverse events observed in animals treated with 500 mg/kg/day. Injection site intolerance (damaged tails, perivascular subacute inflammation and mural thrombi), reduced food consumption, slight decrease in mean body weight gain, increased urea level in males and decreased red blood cell counts, haemoglobin content and packed cell volume in females were reported at 1500 mg/kg/day.

In the 2-week oral (gavage) toxicity study in rats with NXL104, the NOAEL was 2000 mg/kg/day (highest tested dose).

In the 4-week intravenous toxicity study in rats with NXL104 alone, the NOAEL was 167 mg/kg/day corresponding to a mean Cmax value of 169.2 mg/L, and a mean AUC (0-24) value of 108.7 mg h/L. The main adverse effect at 500 mg/kg/day was poor tolerance at the injection sites (haematoma). Therefore 500 mg/kg/day was considered the NOAEL for systemic toxicity. The main adverse effects at 1000 and 1200 mg/kg/day consisted of vocalizations, dose dependent haematoma at the injection site (associated with findings such as blackish colour, shortened tail, scabs, reddish discharge, dryness, loss of tail and/or wound) and low body weight gain. Microscopic examination of the injection site showed subacute/chronic inflammation in the vascular structure, the subcutis and the overlying epithelium in all treated and control animals. No target organ has been identified.

In the second 4-week intravenous toxicity study in rats NXL104 (500 mg/kg/day) administered alone was compared to CAZ alone (2000 mg/kg/day) and to NXL104+CAZ (500+2000 mg/kg/day). Injection site lesions such as haematoma, dryness and scabs were observed in all treatment groups and were associated with wounds or cracks in the CAZ alone and the NXL104+CAZ groups. In general, local lesions were more severe in groups that received CAZ alone or CAZ+NXL104 as compared to the NXL104 alone group. The histopathological findings at the injection sites showed a similar trend. No target organ has been identified on histopathology examination in any of the treatment groups.

In the 2-phase exploratory intravenous (30-min infusion) dose range finding study in dogs, there was no clinically noteworthy finding at 500 mg/kg/day. There was vomiting at 1000 mg/kg/day and above during both phases of the study. In addition, during the first phase, one female showed dilated pelvis of the left kidney, a greenish nodule in the mucosa of the bladder and a grey/white area on the ventral surface of the spleen at necropsy (the significance of these findings are not known). During the second phase, the animals receiving 2000 mg/kg/day experienced, in addition to vomiting, ptyalism and decreases in neutrophils, lymphocytes, basophils and total WBC (white blood cell) counts.

In the 4-week intravenous toxicity study in dogs, with NXL104 alone, the exposure (mean AUC (0-t)) at the NOAEL dose of 500 mg/kg/day on day 28 was 2433 mg.h/L and the mean Cmax was 2039 mg/L. There was no mortality and no local/injection site reaction at all dose levels (250, 500 and 1000 mg/kg/day). There were no ophthalmological findings, no effect on body weight or food consumption, no treatment related changes in blood pressure, ECG parameters, haematology, blood chemistry and urinalysis. No systemic toxicity (no target organs) has been identified on necropsy and

histopathology. However, lesions at the injection site (as those commonly observed after repeated and prolonged intravenous injection) in all groups (including control) and a minimal irritant effect at 1000 mg/kg/day were recorded.

In the second 4-week intravenous toxicity study in dogs CAZ alone (1000 mg/kg/day) was compared to NXL104+CAZ (125+500 or 250+1000 mg/kg/day). There was no mortality, no ophthalmological findings, no effect on body weight or food consumption, and no treatment related changes in blood pressure, ECG parameters and haematology. There were dose related vomiting, salivation and increase in triglyceride and cholesterol values in all treated groups.

The increases in liver weight associated with minimal to slight centrilobular liver hypertrophy in groups receiving NXL104+CAZ (250+1000) seem to be due to CAZ as similar findings were observed in CAZ alone treated animals but were not seen in NXL104 alone treated animals. Lesions at the injection site (both in control and treated animals) were related to mechanical injury and not considered of toxicological importance.

In the local tolerance study using New Zealand white rabbits, NXL104 administered at the concentrations of 5 and 20 mg/mL in 5% (w/v) glucose and NXL104+CAZ administered at 5+20 mg/mL, respectively, in 5% (w/v) glucose were well tolerated locally and systemically. NXL104 either given alone or in combination with CAZ had no haemolytic activity on human whole blood.

NXL104 gave negative results in the *in vitro* bacterial reverse mutation tests, *in vitro* unscheduled DNA synthesis tests in the rat liver cells, *in vitro* micronucleus test in mouse lymphoma cells and in *in vivo* intravenous micronucleus test in rat bone marrow. NXL104 induced a moderate increase in chromosomal aberrations *in vitro* in cultured human lymphocytes at dose-levels ≥ 1500 mg/L, without metabolic activation (S9 mix) only and following a 44-hour treatment. This result is not considered as a safety issue as no chromosomal damage/aberration was detected in the *in vivo* (doses up to 2000 mg/kg i.v.) and *in vitro* micronucleus tests (concentrations up to 1000 mg/L).

Embryo/fetal development studies (including teratogenic potential assessment) were performed in rats and rabbits. In mated female Sprague-Dawley rats, NXL104 slightly decreased mean food consumption at 1000 mg/kg/day; there was no significant effect on embryo/fetal development at any dose selected. In pregnant rabbits, maternal toxicity was evidenced by effects on body weight gain and/or food consumption from 250 mg/kg/day. Evidence of embryo-fetal toxicity included an increase in post-implantation loss at 1000 mg/kg/day.

A male fertility and early embryonic development toxicity study has also been completed in rats. The study evaluated the effect of NXL104 on gonadal function, mating behavior, and reproductive performance when administered during spermatogenesis and mating. In male Sprague-Dawley rats, intravenous administration with NXL104 at doses of 250, 500 or 1000 mg/kg/day was considered likely to have caused mortality, poor local tolerance at the implantation site and slightly reduced body weight gain at all dose-levels, though not in a dose-related manner. Despite these effects, there was no effect on mating performance or fertility, including an assessment of sperm counts and motility, and no adverse macroscopic or weight changes associated with the reproductive organs in any group. The no observed effect level (NOEL) for gonadal function, mating behaviour and reproductive performance in the male Sprague-Dawley rat was 1000 mg/kg/day.

More details can be found in the investigator brochure.

1.1.6 Human experience - Phase I

Single Dose Study (104/1001)

There is one completed Phase I study evaluating the safety, tolerability and pharmacokinetics of single doses of NXL104. This study was a double blind, randomized, placebo-controlled study in healthy adult male subjects who were administered single doses of NXL104 intravenously alone (50, 100, 250, 500, 1000, 1500 and 2000 mg) or in combination with ceftazidime (1000 and 2000 mg ceftazidime for the 250 and 500 mg NXL104 cohorts, respectively, after a 7-day washout period). NXL104 was administered by intravenous route over 30 minutes in a constant volume of 250 mL.

No serious Adverse Event (SAE) was reported in this study and no subjects discontinued due to adverse events. There were no severe adverse events reported. No treatment emergent adverse events (TEAEs) were reported in any of the subjects belonging to 50, 100, 1000, and 1500 mg cohorts, nor were any TEAEs reported in the two combination cohorts. A total of 6 TEAEs were reported from 4 subjects treated with 250, 500, and 2000 mg doses. Of the 6 TEAEs reported, 5 were of mild severity (consisting of abdominal pain, sense of oppression, somnolence, anxiety and postural dizziness); 1 was of moderate intensity and consisted of orthostatic hypotension. There was no dose dependency in the number of AEs or in their intensity, and all subjects recovered from the adverse events without sequelae.

In this study,both C_{max} and $AUC_{(0-t)}$ appeared to increase in direct proportion to dose within the dose range of 50 to 1000 mg. When considering the whole dose range from 50 to 2000 mg, the relationships marginally deviated from strict proportionality, with a doubling in dose resulting in 2.07- and 2.08-fold increases in C_{max} and $AUC_{(0-t)}$, respectively. The decrease in plasma concentrations with time was characterized by 2 or 3 successive phases. The terminal half-life, which was better estimated at 1000 mg and above, ranged between approx. 2.2 and 2.7 h. Unlike the animal situation, ceftazidime at 1000 mg and 2000 mg did not alter the C_{max} or AUC of NXL104 at 250 and 500 mg, respectively.

Multiple Dose Study (104/1002)

This was a double blind, randomized, placebo-controlled study in healthy adult male subjects to evaluate the safety, tolerability, and pharmacokinetics of repeated intravenous doses of NXL104 alone and in combination with ceftazidime and to evaluate the absolute bioavailability of a single oral dose of NXL104. In Part A of the study, NXL104 was to be administered intravenously at doses of 500mg, 750mg, and 1000mg every 8 hours for 5 days. A separate group of 8 subjects was to receive 500mg NXL104 plus 2000mg ceftazidime IV every 8 hours for 10 days. In Part B of the study, 8 subjects were to receive single 500mg doses of NXL104 intravenously and orally in a two way cross-over.

NXL104 was generally well tolerated with no serious adverse events and no subject discontinued due to adverse events. None of the adverse events observed was considered related to NXL104 treatment. One adverse event each related to local tolerability was reported in the placebo and 750 mg intravenous NXL104 cohorts (consisting of moderate erythema at the infusion site). Three adverse events were reported in the 500mg NXL104 + 2000mg ceftazidime cohort (consisting of 2 mild to moderate hematoma above the injection site in 2 separate subjects, and mild left ankle arthralgia in a 3rd subject). In the 1000mg group, no adverse event was reported.

The moderate erythema occurred on Day 6 and Day 3 of dosing in the 500mg IV NXL104 (subject no.106) and placebo (subject no.105) subjects, respectively. In the 500mg NXL104 + 2000mg ceftazidime IV combination cohort, mild to moderate hematoma was observed on Day 8 (subject no.404) and Day 11 (subject no.406). The erythema and hematoma at the infusion site resolved without specific treatment; no other associated signs or symptoms were reported. Mild left ankle arthralgia was observed on Day 6 in a 3rd subject who received NXL104 and ceftazidime (subject no.402) in this same cohort; the arthralgia resolved in less than 3 days. No laboratory, physical examination, or ECG abnormalities of clinical significance were noted in the study.

Effect of Age and Gender (104/1004)

A study to evaluate the effect of age and gender on the pharmacokinetics and safety of NXL104 in healthy volunteers (NXL104/1004) was completed in the USA. This was a single dose study of 500mg NXL104 in 33 healthy subjects in 4 cohorts [young men (N=9), young women (N=8), elderly men (N=8) and elderly women (N=8)]. The mean age of subjects enrolled was similar among the two young cohorts [mean age in young men and women was 28.7 (range 20-37) and 30.9 (range 23-44), respectively]; likewise, the mean age of subjects enrolled was similar among the two elderly cohorts [mean age in elderly men and women was 68.8 (range 65-74) and 69.1 (range 65-76), respectively]. There were no SAEs or AEs leading to discontinuation. The incidence of treatment-emergent AEs was 11% (1/9) in young men, 37.5% (3/8) in young women, 25% (2/8) in elderly men, and 50% (4/8) in elderly women. Treatment-emergent AE that occurred in more than one subject (regardless of relationship to study drug) were application site bruising (4/33; 12%) and headache (2/33; 6%). Overall, 3 subjects experienced 6 treatment-

emergent adverse events considered to be drug-related, including dry mouth, feeling hot, feeling jittery, dysgeusia, headache, and hyperhidrosis; each event was mild in intensity. There were no clinically relevant changes in lab values in any cohort.

Table 1 provides the PK parameters across the age and gender cohorts. It appears that the PK (total plasma clearance, Cmax, AUCs, and half-life) of NXL104 is similar in the cohorts, regardless of age and gender.

Table 1. Comparison of PK parameters across the age and gender cohorts in Protocol 1004

	Young men N=9	Young women N=8	Elderly men N=8	Elderly women N=8
Cmax (µg/mL)				
Mean	33.83	36.86	26.45	38.41
SD	4.24	9.31	5.73	15.51
T½ (hours)				
Mean	2.09	1.71	3.17	2.43
SD	0.64	0.09	0.65	0.47
Cl (L/hr)				
Mean	10.16	10.34	9.82	7.98
SD	1.23	1.82	1.81	2.22
AUC (0-inf) (µg·hr/mL)				
Mean	49.86	49.75	52.4	66.23
SD	6.27	9.1	9.38	14.97

1.2 Rationale for the STUDY:

- This is a 100 mg single dose, parallel group, open-labeled study in patients with renal Impairment. A 100 mg dose has being studied in Phase I trials, was well tolerated and has been chosen for this study. The multiple ascending dose study (NXL104/1002) shown that, when given alone at doses of 500 mg, 750 mg or 1000 mg every 8 hours, the steady-state pharmacokinetics of NXL104 was predictable from a single dose. This supports conducting the present study after a single dose administration. A single dose in patients with renal impairment is thought to minimize their potential risk and to adequately characterize the impact of renal impairment on the disposition of NXL104.

- A control group with normal renal function and matched by age (+/- 5) and weight (+/-5Kg) to the renally impaired patients will be included in this study as suggested by the Guidance for Industry titled "Pharmacokinetics and Pharmacodynamics in Patients with Impaired Renal Function”

1.3 Rationale for the dose selection:

The choice of the NXL104 100 mg IV is based on safety, tolerability data and pharmacokinetic obtained in the ascending single dose study (NXL104/1001) in which 2000 mg of NXL104 was administered over 30 minutes.

Compared to a normal subject, the total clearance of NXL104 is expected to decrease 10- to 20-fold in an anuric patient, corresponding to the loss of the renal clearance. Accordingly, a 100 mg dose in an anuric patient would result in an AUC comparable to those observed at 1000 mg to 2000 mg in normal subjects. No treatment emergent adverse events (TEAEs) were reported in any of the subjects belonging to the 1000 and 1500 mg cohorts. One TEAE (orthostatic hypotension of moderate intensity) was reported from one subjects treated with 2000 mg. All subjects recovered from the adverse events without sequelae.

A 100 mg dose of NXL104 corresponds to 0.38 millimole and would therefore restrict the sodium intake to 8-9 milligrams. In addition, the proposed infusion volume is limited to 50 mL to help restricting the water intake. The final concentration of 2 mg/mL is compatible with local tolerance.

Finally, a 100 mg dose in healthy subjects resulted in measurable NXL104 plasma concentrations for up to 12 hours, allowing for a proper characterization of pharmacokinetics over a multiple of the terminal half-life (more than 6-fold).

2 STUDY OBJECTIVES

2.1 Primary objective

To characterize the pharmacokinetics of NXL104 administered as a 100 mg single dose over 30 minutes intravenous infusion in normal subjects and patients with varying degrees of renal impairment.

2.2 Secondary objective

To investigate the, tolerability of NXL104 administered as a 100 mg, single dose over 30 minutes intravenous infusion in normal subjects and patients with varying degrees of renal impairment.

3 STUDY DESIGN, DURATION AND DATES

3.1 Study design

The study will be conducted using an open-label stratified design in patients with different degrees of renal impairment as described below. The renal function groups should be comparable to each other with respect to age and gender.

Group1: Patients with normal renal function (creatinine clearance >80ml/min)

Group2: Patients with mild to moderate renal impairment (creatinine clearance 50 to 79ml/min)

Group3: Patients with moderate renal impairment (creatinine clearance 30 to 49ml/min)

Group4: Patients with severe renal impairment (creatinine clearance <30 ml/min) but no requiring hemodialysis or peritoneal dialysis

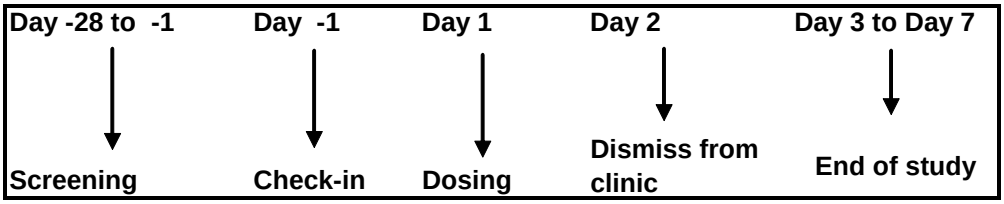
Group5: Patients with end-stage renal failure requiring hemodialysis. Patients will participate in two randomized sessions: dialysis session and inter dialysis session separated by a washout period of 7 to 14 days All hemodialysed patients should have the same duration and same interval of dialysis (4hours, 3 times a week)

and if possible the same equipment for dialysis with the same blood flow rate (300 to 360mL/min) and constant dialysate flow (500mL/min during dialysis).

All patients will receive a single 50 mL IV infusion over 30 min. of 5% glucose containing 100 mg NXL104.

The following diagram shows the series of events for:

Group 1, 2, 3 and 4 treatment period.



Group 5 Hemodialysis patients

Period 1: Treatment A or Treatment B; Period 2: Treatment B or Treatment A.

Screening	**Treatment A** **Dialysis day**		**Wash-out** 7 to 14 days	**Treatment B** **Interdialysis Session**				**End of Study**
D-28 to D-1	Day -1	Day 1		Day -1	Day 1	Day 2	Day 3	D3 to D7
Screening	Check (at end of previous Dialysis)	Dosing before dialysis follow up to end of dialysis		Check (at end of preceding the start of infusion)	Dosing after dialysis session hospitalisation till H24 post dosing	Dismiss from clinic at H24 post dosing	Last Blood sampling before next dialysis session	End of Study Visit

Subjects and/or patients of groups 1 to 4 will receive one single 100mg I.V. administration of NXL104. Subjects will participate in a 28-day screening period, followed by a baseline period (Day-1, start of hospitalization), followed by single I.V. dose treatment (D1) and 24 hours post dosing follow-up (hospitalization up to 24h post dosing). An End of Study (EOS) visit will be performed 3 to 7 days after dosing.

Hemodialysed patients (group5) will participate in 2 randomly allocated periods separated by a washout period of 7 to 14 days.

One treatment will be the dialysis day starting with dialysis to end of dialysis session. As theses subjects are regularly followed there will be no hospitalization for D-1 as for the other groups. The D-1check will be done after the previous dialysis preceding the start of infusion.

The other treatment (after a wash-out of 7 to 14 days) will be the interdialysate period starting with end of dialysis. The D-1check for this period will done after the dialysis preceding the start of infusion.

Day 1 = day of dosing: start of infusion after end of dialysis. End of Study visit will be performed at the end of next dialysis.

3.2 Study duration and dates

The duration of this study is expected to be 6 months, with subject recruitment proposed to start in. The actual overall study duration or subject recruitment period may vary.

4 SELECTION OF SUBJECTS

4.1 Number of subjects

As calculated in *Section 11.5*, 30 subjects should be enrolled and treated in this study. It is planned to recruit this sample in two centers. Enrollment into the screening or randomization phase of the study will be stopped when the anticipated or actual subject numbers have been achieved.

4.2 Inclusion criteria

Subjects and/or Patients meeting all of the following criteria will be considered for admission to the study:

- Healthy adults of any race aged between 18 and 90 years, inclusive, with good health determined by past medical and surgical history, physical examination, vital signs, electrocardiogram, and laboratory tests at screening and matched by age (+/- 5years) and weight (+/-5kg) with renal impairment patients

  Or

- Patients with renal impairments of any race aged between 18 and 90 years, inclusive, reasonably stable renal function as assessed from serum creatinine concentration and/or urinary creatinine monitoring
  Good health excluding renal disease and associated controlled illnesses such as hypertension, diabetes mellitus, etc (based on medical history, physical examination, eletrocardiograms (ECG, and clinical laboratory tests),

  AND FOR WOMEN

  Women are authorized to participate in this clinical study if they meet the following criteria:

  - Has been surgically sterilized or has been postmenopausal for at least one year OR
  - Is of childbearing potential, and all of the following conditions are met:
    - had normal menstrual periods for the 3 months prior to study entry, and
    - has a negative serum pregnancy test (serum β-hCG) prior to enrollment.
    - must be willing to practice double barrier methods of birth control during treatment and for at least 28 days after dosing with study medication. Oral contraceptives should not be used as the sole method of birth control, because the effect of NXL104 on the efficacy of oral contraceptives has not yet been established.

  And FOR ALL, the following

- Able to execute written informed consent

- Be available for treatment as inpatients for up to 3 days following treatment. especially for anuric patients on hemodialysis
- Negative serology: HIV antibody, hepatitis B surface antigen, hepatitis C antibody,
- Negative urine drug screen for cannabis, opiates, cocaine metabolite, amphetamines, barbiturates, benzodiazepines (except for hemodialysis patients) and alcohol breath test
- Laboratory values will be within normal ranges (with the exception of those criteria associated with renal disease and any associated illness.)
- Patients should maintained their chronic treatment if treatments are stable for at least one month

Inclusion of any patients with laboratory test values outside of normal limits (excluding those laboratory test values associated with renal disease and associated controlled illnesses) must be approved by both the sponsor or his designee and the clinical investigator before study initiation. The prestudy laboratory tests, ECG (12-leads), and physical examinations will be initiated within 7 days and completed at least 1 day prior to the start of the study and documented on the case report forms (CRF). Some exceptions may be made to patients schedules and physical examinations may occur up to 28 days prior to dosing. The sponsor must approve those exceptions. During prestudy examination, 24-hour creatinine clearance will be determined for assignment into a particular renal function group. On day 2, the 24-hour creatinine clearance determination will be repeated.(Note: if patient are anuric, they will be assigned to Group5 and the prestudy and/or day two 24-hour creatinine clearance determination will not be required). Patients will fast for at least 4 hours prior to any laboratory tests. The principal investigator will provide normal ranges for all clinical laboratory test and the name, address, and accreditation of the testing facility.

Written informed consent must be obtained for all subjects before enrollment in the study
(see *Section 12.3*).

4.3 Exclusion criteria

Subjects presenting with any of the following will not be included in the study:

For healthy subjects:

- Any condition requiring the regular use of any medication;
- Symptoms of a clinically significant illness in the 3 months before the study;
- Presence or sequelae of gastrointestinal, liver or kidney disease, or other conditions known to interfere with the distribution, metabolism, or excretion of drugs;

And FOR ALL, the following:

- Use within 14 days (or 5 half-lives, whichever is longer) prior to the first study dose or intended use of any over-the-counter (including St. John's Wort or any herbal products) with the exception of paracetamol (≤ 1000mg/day) or new prescription medication including those that are known to cause pharmacokinetic drug interaction, unless approved by the sponsor;
- Presence of QTc≥ 430 ms, or a pronounced sinus bradycardia (<40 bpm) or hypokalemia (<3.2mEq/L) or family history of long QT syndrome or familial history of unexplained sudden death or sick sinus syndrome or any clinically relevant unstable cardiovascular disease or cardiovascular event (e.g. myocardial infarction, cerabrovascuar accident…) occurred less than 6 months ago;

- Participation in another study with any investigational drug in the 3 months prior to the first study dose;
- Be in the exclusion period of previous study;
- Evidence of human immunodeficiency virus (HIV) infection and/or positive human HIV antibodies;
- Evidence of hepatitis C and/or positive hepatitis C antibody and/or positive hepatitis B surface antigen;
- History of hypersensitivity to drugs with a similar chemical structure;
- History of significant allergic disease and acute phase of allergic rhinitis in the previous 2 weeks before randomization or food allergy;
- Blood or plasma donation of more than 500 mL during the previous 1 month before randomization;
- Smoker of more than 5 cigarettes/ day during the previous 3 months;
- Heavy caffeine drinkers defined by a regular intake of more than 5 cups of coffee (or equivalent in xanthine-containing beverages) per day;
- Current evidence of drug abuse or history of drug abuse within one year of randomization, and/or positive findings on urinary drug screening;
- Subjects who have an average weekly alcohol intake that exceeds 2 units per day or subjects unwilling to stop alcohol consumption for the duration of the study (1 unit = 12 oz or 360 mL of beer; 5 oz or 150 mL of wine; 1.5 oz or 45mL of distilled spirits);
- Mental condition rendering the subject incapable to understand the nature, scope, and possible consequences of the study;
- Adults under guardianship and people with restriction of freedom by administrative or legal decisions;
- Unlikely to comply with the clinical study protocol; e.g. uncooperative attitude, shift-worker, inability to return for follow-up visits, or unlikely to complete the study;
- Women who are pregnant or breastfeeding, or fertile women not practicing adequate methods of contraception (as defined in inclusion criteria); women planning to become pregnant within 1 month of the study.

- Any other condition that, in the opinion of the Investigator, would preclude participation in the study.

Any waiver of these inclusion and exclusion criteria must be approved by the investigator and the sponsor on a case-by-case basis prior to enrolling the subject. This must be documented by both the sponsor and the investigator. No subject will be allowed to enroll in this study more than once.

4.4 Subjects of reproductive potential

Not applicable

5 STUDY TREATMENTS

5.1 Details of study treatments

Table 3 - Study treatments

Formulation	Vial containing 1000 mg of NXL104 lyophilized powder to be reconstituted with 10mL of

	water for injection to obtain a 100mg/mL solution for IV infusion
Doses	100 mg, single dose
Route	Intravenous infusion in volume of 50mL 5 % glucose for 30 minutes
[redacted]	[redacted]

NXL104 will be administered by intravenous route in a constant volume of 50mL 5% glucose for 30minutes. A 200 mg/100ml will be prepared and 50mL of this preparation will be infused during 30 minutes. The concentration of the solution will be 2 mg/mL (see section 17 Appendix A for details)

5.2 Dosage schedule

NXL104 will be administered in the morning 30 minutes after breakfast.

Two mL (2mL) of the reconstituted solution for infusion of NXL104 (100mg/mL) will be diluted in 100ml of 5% glucose by the pharmacist at the center, following the randomization as described in Appendix A section 17

Given the investigational nature of the product and to provide the subjects with the maximum level of safety in case of an unexpected event, the following requirements must be fulfilled before any administration of the investigational product can start:

- Immediate access to appropriate resuscitative equipment or treatment must be secured throughout the study.
- Personnel appropriately qualified and trained to use the above equipment or treatment must be available throughout the study.
- At least one physician must be present during the administration period and for at least one hour after the end of administration (see *Section 12.2* for delegation of investigator duties).

5.3 Treatment assignment

A patient will be given a screening number after signing the informed consent. The treatment numbers will be randomly assigned within each group.
The renally compromised patients should be known to the investigator and ideally should have been followed long enough to establish the fact that they have reasonab1y stable renal function as assessed from serum creatinine concentration and/or urinary creatinine clearance monitoring. Preferably, the serum creatinine concentration should not have varied by more than approximately 0.4 mg/dl over a 3-month period prior to the initial screening. However, documentation of stable serum creatinine concentration and/or creatinine clearance up to 3 months prior to the initial screening will a1so be acceptable.

The patient and the treatment number will be the same. Once assigned, subject numbers will not be reused within the study site, even if the subject fails to meet protocol criteria for continuing. Note that subjects will be identified by their subject numbers throughout the entire course of the study.

A randomization assessing the session A (dialysis) or session B (interdialysis) list will be prepared for group 5 by the Biometrics department of SGS using a validated program (SAS software, SAS Inc., Cary, NC, USA).

Subject will be assigned the following treatment number:

- Group 1: treatment number from 101 to 106 (replacement 5101 to 5106),
- Group 2: treatment number from 201 to 206 (replacement 5201 to 5206),
- Group 3: treatment number from 301 to 306 (replacement 5301 to 5306),
- Group 4: treatment number from 401 to 406 (replacement 5401 to 5406).

- Group 5: treatment number from 501 to 506 (replacement 5501 to 5506).

Subjects and/or Patients withdrawn from the study retain their subject number, if already given. New subjects must always be allotted a new subject number/treatment number (replacement treatment number).

5.4 Packaging, and Labeling

The investigational products will be packaged by , according to the appropriate randomization plan.

The content of the labeling is in accordance with the local regulatory specifications and requirements and translated as appropriate.

Each vial will be labeled as follows: sponsor company name, address and telephone number, study number, subject or patient N° and site, investigator name, reconstitution of the lyophilized powder and the direction for use, batch number, expiry date, the storage condition information, “for clinical trial use only”, use under strict medical supervision, to be used according to clinical protocol instructions.

The subject vials will be packaged into treatment dose group boxes. The box for the study medication will be labeled as follows.

Sponsor company name, address and telephone number, study number, drug name, the dose and the randomization numbers, number of vials, formulation, instructions for reconstitution, route of administration, the storage condition information, batch number and expiry date “for clinical trial use only”, investigator’s name, use under strict medical supervision, to be used according to clinical protocol instructions.

For replacement treatment, the supplies will be labeled with the same information as indicated above except that the replacement randomization number; the dose will be left blank. The information corresponding to the replacement subject will be added to the label in handwriting. Replacement wording will also be printed on the label.

The investigator or the hospital pharmacist is responsible for the appropriate storage of the investigational product packages at the research sites.

5.5 Specific Restrictions/Requirements

The subjects and/or patients will attend the CRU prior to treatment for informed consent and baseline tests for each study period.

The subjects (group1) and patients (groups 2 to 4) will be admitted to the CRU, 1 day prior to dosing day for a baseline (blood sampling will be done after 4 hours fasting) for 24 hours and will consume no food or caloric beverage after 21:30 hours the day before the dosing day until breakfast (within 30 min before dosing) of the dosing day. Subject will stay in the CRU till 24h post dosing, except for group 5 (see section 7.2.2).

Subjects will consume only the food provided by the research staff during the CRU visit. Sharing of food with other subjects, visitors, or staff will not be permitted.

5.6 Supplies and accountability

The 100 mL 5% glucose infusion bag will be supplied by the facility.

The pharmacist will inventory and acknowledge receipt of all shipments of the investigational products. The investigational products must be kept in a locked area with restricted access. The investigational products must be stored and handled in accordance with the manufacturer's instructions (section 17). The investigator or pharmacist will also keep accurate records of the quantities of the investigational products dispensed. At the end of the study, the study

monitor will check the supplies of investigational products held by the investigator or pharmacist to verify accountability of all investigational products used. At the conclusion of the study, all unused investigational products and all medication containers will be returned to the sponsor unless other arrangements have been approved by the sponsor. The sponsor will verify that a final report of drug accountability to the unit dose level is prepared and maintained in the investigator study file.

5.7 Compliance

The investigational product will be administered by the investigator or sub investigator. Any delegation of this responsibility must follow the terms set forth in *Section 12.2.*

6 PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

6.1 Prior and concomitant illnesses

All patients recruited for Groups 2, 3, 4 and 5 of this study will be patients with stable renal disease. It is therefore understood that these patients may also have other controlled illnesses associated with renal disease such as hypertension, diabetes mellitus, etc.

Additional illnesses present at the time informed consent is given are regarded as concomitant illnesses and will be documented on the appropriate pages of the case report form.

Illnesses first occurring or detected during the study are to be regarded as adverse events and must be documented as such in the case report form (see *Section 8*).

6.2 Prior and concomitant treatments

Concomitant medications should be kept to a minimum during the study. For patients recruited for Groups 2, 3 4 and 5 (renally impaired patients), medications essential for the patients' well-being while on the study and which are unlikely to interfere with the study medication will be continued at the investigator’s discretion. A patient who requires emergency treatment with prescription or non prescription medication within 7 days of the study start shall contact the investigator. Should this occur, the medication will be reviewed by the study monitor and investigator prior to NXL104 administration. The exclusion of a patient's study data because of non study medications taken during the study shall be determined by the sponsor on a case-by-case basis.
No concomitant treatments are permitted for the duration of the study, except 1-2 tablets of acetaminophen (≤ 1000 mg/day) in case of headache. Renal impaired patients should maintain their chronic treatment without modifications

Any treatment that might have to be given in addition to the investigational product during the study is regarded as a concomitant treatment and must be documented on the appropriate pages of the case report form.

Relevant previous treatments taken within 2 weeks before the study must also be documented in the case report form.

7 STUDY PROCEDURES AND SCHEDULE

7.1 Overview of data collection

Safety:

- Adverse events;
- Local tolerance;
- Vital signs: blood pressure and pulse (10 min supine, and 3 min standing);
- Height and weight;
- 12-lead ECG;
- Hematology: hemoglobin, hematocrit, red blood cells, WBC total and differential count, platelets;
- Clinical chemistry: fasting glucose, urea, creatinine, Na, K, Cl, Ca, P, total cholesterol, triglycerides, Alk phos, ASAT, ALAT, Gamma-GT, total protein, total and direct bilirubin;
- Urinalysis (by dipstick: pH, glucose, ketones, leukocytes, blood, protein) and diuresis;
- Hepatitis B surface antigen, hepatitis C antibodies, and HIV screen;
- Qualitative urine drug test (cannabis, opiates, cocaine metabolite, amphetamines, barbiturates, benzodiazepines) and alcohol breath test.

Pharmacokinetics

- Concentrations of NXL104 in plasma and urine from groups 1 to 4 patients;
- In patients from group 5 in their dialysis session: blood flow through the dialyzer, ultrafiltration rate, NXL104 concentration in plasma (arterial and venous), total proteins.

The schedule of data collection is described in Section 7.2, the methods of data collection are described in Section 7.3, and the analysis variables are given in Section 11.1.

7.2 Description of study days

7.2.1 Screening

Results of screening tests will be recorded on the case report form. In case of screen failures, only demographic data and reason of screen failure will be recorded in the case report form.

Each potential patient will be examined before the start of the study to determine his eligibility for participation. These tests are to be conducted no more than 4 weeks before the start of the study. The following examinations will be performed:

- Inclusion/exclusion criteria;
- Physical examination and relevant medical/surgical history, including date of birth, sex and race; only findings relevant to the study are to be documented;
- Height and weight;
- Previous medication recording;
- Full laboratory test: Hematology, clinical chemistry, and urinalyses (Table 4), section 7.3.1.1 and Table 5 , section 7.3.1.2);
- Blood pressure and pulse (10 min supine, and 3 min standing) (Table 6, Section 7.3.2);
- 12-lead ECG;

- Hepatitis B surface antigen, hepatitis C antibodies, and HIV screen;(to be discussed for Hemodialyses patients);
- Qualitative urine drug test (cannabis, opiates, cocaine metabolite, amphetamines, barbiturates, benzodiazepines except for dialysis patients) and alcohol breath test;
- Creatinine clearance using Cockroft formula: (mL/min) =

CL(ml/min) = (140-age) x weight (kg) / creatininemia (µmol/L) x 1.23 OR

CL (mL/min) = (140-age) x weight (kg) / creatininemia (mg/dL) x 72

For group 5, the laboratory tests will be done immediately after the dialysis.

Written Informed consent obtained for all subjects

7.2.2 Study days for subjects of group 1 and patients of groups 2, 3, 4

All subjects will participate in a single period receiving I.V. infusion for 30 minutes.

The evaluations will be the same in each period.

Day-1:

The subjects will be admitted to the clinical research unit in the morning in a fasting state. Subjects will be asked to confirm that there have been no relevant changes in their physical condition since screening and that they have complied with the restrictions (see section 7.4).

The following examinations will be performed:

- Entry criteria reviewed and concomitant medication documented;
- Physical examination, medical/surgical history;
- Previous medication recording;
- Screening for drug abuse in urine + alcohol breath test;
- 12- lead ECG (Table 6, Section 7.3.2);
- Blood pressure, heart rate (10 min supine, and 3 min standing) (Table 6, Section 7.3.2);
- Full laboratory test: Hematology, clinical chemistry, and urinalysis (Table 4, section 7.3.1.1 and Table 5, section 7.3.1.2).

Subjects will stay overnight at the unit.

Day 1:

Day 1 will follow day-1. There will be no day 0.

Within 1 hour before dosing:

- Subjects should take their breakfast at least 30 min before infusion start
- Record 12-lead resting ECG (Table 6, Section 7.3.3);

- Blood pressure, heart rate (10 min supine, and 3 min standing) (Table 6, Section 7.3.2);
- Insert cannula into a forearm vein, for blood samples;
- Insert cannula into the other forearm vein, for infusion (local tolerance will be followed as long as infusion is performed);
- Blood sample for bioanalysis (Table 4, section 7.3.1.1);

Time "zero" (T0) will be defined as the time of start of infusion.

After administration (I.V.) D1:

- Blood samples for bioanalysis (Table 4, section 7.3.1.1);
- 12-lead ECG: (Table 6, Section 7.3.2);
- Blood pressure and heart rate (min supine, and 3 min standing) (Table 6, Section 7.3.2);
- Adverse event questioning;
- Urine collection time]0h to 6h],]6h to 12h],]12h to 24h] (Table 5);

The cannula used for infusion should be removed after end of infusion and the cannula used for blood sample will be removed after the 12h blood sample

The subjects will stay overnight at the unit.

Day 2:

24 hours after dosing

- Physical examination;
- 12-lead ECG (Table 6, Section 7.3.2);
- Blood pressure and heart rate (10 min supine, and 3 min standing) (Table 6, Section 7.3.2);
- Blood sample for bioanalysis (Table 4, section 7.3.1.1);
- Full laboratory test: Hematology, clinical chemistry, and urinalysis (Table 4, section 7.3.1.1 and Table 5, section 7.3.1.2);
-- Adverse event questioning;

Subjects will be allowed to leave the clinical research unit on day 2 after completion of all examinations and if there are no prohibitive findings.

7.2.3 End of study (day 3 to 7 after last dosing) for groups 1 to 4

- Physical examination,
- Weight,
- Full laboratory test: Hematology, clinical chemistry, and urinalysis (Table 4, section 7.3.1.1 and Table 5, section 7.3.1.2),
- 12-lead ECG: (Table 6, Section 7.3.2),
- Blood pressure and heart rate (10 min supine, and 3 min standing) (Table 6, Section 7.3.2),
- Adverse event questioning,

Provided there are no safety concerns arising from the final examination, the subjects' participation in the study will be completed.

7.2.4 Study days for subjects of group 5

Hemodialysed patients (group 5) will participate in 2 randomly allocated periods separated by a washout period of 7 to 14 days.

During each period, the patients will receive an I.V infusion of NXL104 100mg (50 ml of a 5%glucose solution containing 2mg/ml NXL104 during 30 min according to one of the following treatment:

Treatment A: IV infusion will be done 1 hour before start of dialysis.

Treatment B: IV infusion will be done 1 hour after of dialysis.

Dialysis procedure should be as standard as possible.

Hemodialysis procedure

All hemodialysis sessions to be carried out in this study must have a duration of 4 hours, ideally from 8 am to 12 noon.

Blood flow (Qbl) should be in the range of 250-300 mL/min and the dialysate flow (Qd) should be maintained constant at 500 mL/min throughout the hemodialysis sessions. Routine anticoagulation medication with normal- or low molecular weight heparin will be applied as a single pre-dialysis dose in order to avoid clotting in the extracorporeal circulation (supplemented with continuous infusion of unfractionated heparin if appropriate). The total volume of dialyzed blood (and heparin) will be recorded in the CRF.

The equipment used will be kept the same during the two dialysis sessions. The choice of the type and surface of the membrane is to be done by the investigator on an individual basis. However, the membrane must be a highflux one, with a surface from 1.1 m^2 to 1.8 m^2. The type, surface of the membrane and type of solutions used must be recorded in the CRF.

The pH of the solution used should be equal to 7.40 ± 0.1.

At the considered time points, 4 ml blood samples (simultaneously collected from the inflow and outflow tract of the extracorporeal blood circuit) and aliquot fractions of 20 ml of dialysate fluid (if closed circuit) will be taken simultaneously for pharmacokinetic purposes.

During dialysis, the following parameters will be recorded in the CRF:

- Weight of the subject at the beginning (Ws) and at the end (We) of the dialysis.
- Blood pressure, measured during the dialysis session.
- Rate of ultrafiltration (Quf) measured every 30 minutes during the dialysis session.
- Weight of fluid added during the dialysis session (Wfl) : beverages and perfusion.
- Blood flow through the dialyser (Qbl) measured every 30 minutes during the dialysis session.

The hematocrit will be measured on the first and the last samples (2.7 mL of blood sample per assay) taken during dialysis.

Treatment A: Dialysis period

The evaluation of D-1will be done at the end of the dialysis preceding the start of infusion. (i.e if infusion is planned on the Wednesday , the D-1 examination should be done after end of the Monday's dialysis)

The following examinations will be performed:

- Entry criteria reviewed and concomitant medication documented;

- Physical examination, medical/surgical history;
- Previous medication recording;
- Alcohol breath test ;
- 12- lead ECG (Table 6a, Section 7.3.2);
- Blood pressure, heart rate (10 min supine, and 3 min standing) (Table 6a, Section 7.3.2);
- Full laboratory test: Hematology, clinical chemistry (Table 4a, section 7.3.1.1).

Day 1 corresponds to the dialysis session

Within 1 hour before dosing:

- Subjects should take their breakfast at least 30 min before infusion start;
- Record 12-lead resting ECG (Table 6a, Section 7.3.2);
- Blood pressure, heart rate (in supine position) (Table 6a, Section 7.3.2);
- Insert cannula into the forearm vein (arm not used for dialysis) , for infusion (local tolerance will be followed as long as infusion is performed);
- Blood sample for bioanalysis (Table 4a, section 7.3.1.1).

Time "zero" (T0) will be defined as the time of start of infusion. (1hour before dialysis started)

After administration (I.V.)

- Blood samples for bioanalysis (Table 4a, section 7.3.1.1);
- 12-lead ECG (Table 6a, Section 7.3.2);
- Blood pressure and heart rate (10 min supine, and 3 min standing) (Table 6a, Section 7.3.2).

Treatment B: Inter dialysis period

The evaluation of D-1will be done after the dialysis session

The following examinations will be performed:

- Entry criteria reviewed and concomitant medication documented;
- Physical examination, medical/surgical history;
- Previous medication recording;
- alcohol breath test ;
- 12- lead ECG (Table 6b, Section 7.3.2)
- Blood pressure, heart rate (10 min supine, and 3 min standing) (Table 6b, Section 7.3.2)
- Full laboratory test: Hematology, clinical chemistry (Table 4b, section 7.3.1.1).

Day 1

Within 1 hour before dosing:

- Subjects should take their breakfast at least 30 min before infusion start;
- Record 12-lead resting ECG (Table 6b, Section 7.3.2);
- Blood pressure, heart rate (in supine position) (Table 6b, Section 7.3.2);
- Insert cannula into the forearm vein (arm not used for blood samples) , for infusion (local tolerance will be followed as long as infusion is performed);
- Blood sample for bioanalysis (Table 4b, section 7.3.1.1).

Time "zero" (T0) will be defined as the time of start of infusion.

After administration (I.V.)

- Blood samples for bioanalysis (Table 4b, section 7.3.1.1);
- 12-lead ECG (Table 6b, Section 7.3.2);
- Blood pressure and heart rate (10 min supine, and 3 min standing) (Table 6a, Section 7.3.2).

Day 2:

24 hours after dosing

- Physical examination;
- 12-lead ECG (Table 6a & b, Section 7.3.2);
- Blood pressure and heart rate (10 min supine, and 3 min standing) (Table 6a & b, Section 7.3.2);
- Blood sample for bioanalysis (Table 4a & b, section 7.3.1.1);
- Full laboratory test: Hematology, clinical chemistry, and urinalysis (Table 4a & b, section 7.3.1.1);
- Adverse event questioning.

Subjects will be allowed to leave the clinical research unit on day 2 after completion of all examinations and if there are no prohibitive findings.

7.2.5 End of study (day 3 to 7 after last dosing) for group 5

- Physical examination;
- Weight;
- Full laboratory test: Hematology, clinical chemistry, and urinalysis (Table 4a, section 7.3.1.1);
- 12-lead ECG (Table 6a, Section 7.3.2);
- Blood pressure and heart rate (10 min supine, and 3 min standing) (Table 6a, Section 7.3.2);
- Adverse event questioning;

Provided there are no safety concerns arising from the final examination, the subjects' participation in the study will be completed.

7.3 Methods

Hematology: hemoglobin, hematocrit, red blood cells (RBC), white blood cell (WBC) total and differential count, platelets.

Clinical chemistry: fasting glucose, urea, creatinine, Na, K, Cl, Ca, P, total cholesterol, triglycerides, Alk phos, ASAT, ALAT, Gamma-GT, total protein, total and direct bilirubin.

HBV, HCV, and HIV testing: Hepatitis B virus surface antigen (HBs Ag), Hepatitis C virus (HCV) antibody and Human immunodeficiency virus (HIV 1 and 2) antibody presence will be tested. If the subject is positive for any one of these antigen or antibodies, subject will not be included.

Urinalysis by dipstick: pH, glucose, ketones, leukocytes, blood, and protein.(except for dialysis patients)

Qualitative urine drug test: cannabis, opiates, cocaine metabolite, amphetamines, barbiturates, benzodiazepines (except for dialysis patients)

Alcohol breath test.

Creatinine clearance.

7.3.1 Collection schedule for biological samples

7.3.1.1 Blood:

Blood specimens 2*2 mL for pharmacokinetics and 20 mL for safety laboratory tests will be collected in Vacutainer FX and heparinized tubes (+ 7 mL for serology) respectively as scheduled in Table 4 below.
Two additional samples (2x 2.7 ml) will be collected for hematocrit measurement in Group 5.

Table 4 – Collection schedule for blood samples

Groups 1 to 4

Day	Sampling time relative to dosing (h:min)	Lab. Safety (20mL)	Serology (7mL)	PK (2x2mL)
Screening		X	X	
Day –1		X		
Day 1	- 0:30min			X
	00:15			X
	00:30			X
	00:45			X
	01:00			X
	01:30			X
	02:00			X
	03.00			X
	04:00			X
	06:00			X
	08:00			X
	12.00			X
D2 before discharge	24:00	X		X
D3 to D7 (End of study) Ambulatory in groups 2 to 4	48:00	X		X

1: PK also on Day 3 (except Group 1)

Should an infusion be prematurely stopped, the stopping time will be accurately recorded, and every attempt will be made to carry-on blood collection for PK evaluation at the end of infusion then as planned (see Table 4).

Table 4a – Collection schedule for blood samples Group 5 during dialysis session

Day	Sampling time relative to dosing (h:min)	Lab. Safety (20mL)	Serology (7mL)	PK Arterial and venous (2x2mL)
Screening		X	X	
Day –1(end of previous dialysis)		X		
Day 1(day of dialysis)	**H-1:00** (just before infusion)			X
Infusion 1h before dialysis start				
	H1.00 Before start dialysis		X Only hematocrit	X
	H1.5 30min dialysis			X
	H2.00 1:00h dialysis			X
	H2.5 1:30h dialysis			X
	H3.00 2:00h dialysis			X
	H3.5 2:30h dialysis			X
	H4.00 3:00h dialysis			X
	H4.5 3:30h dialysis			X
	H5.00 4:00h dialysis	X	X Only hematocrit	X

Screening for Group 5 is only performed once (before dialysis session or interdialysis session period).
Samples for hematocrit should be collected as soon as possible after the start of dialysis and at the end of dialysis.

Table 4b – Collection schedule for blood samples Group 5 during interdialysis session

Day	Sampling time relative to dosing (h min)	Lab. Safety (20mL)	Serology (7mL)	PK (2x2mL)
Screening		X	X	
Day –1		X		
Day 1(after dialysis session)	- 0:30min			X
	00:15			X
	00:30			X
	00:45			X
	01:00			X
	01:30			X
	02:00			X
	03.00			X
	04:00			X
	06:00			X
	08:00			X
	12.00			X
D2 before discharge	24:00	X		X
D3 Just before next dialysis End of study		X		X

Screening for Group 5 is only performed once (before dialysis session or interdialysis session period)

7.3.1.2 Urine:

The urine collection will be performed at the following periods:

Table 5 - Collection of urine samples (groups 1 to 4)

Days	Sampling time relative to dosing (h:min)	Drug screen	Urinalysis (dispstick)	Creatinine clearance	Pk analysis
Screening		X	X	X (cockroft)*	
Day-1		X	X		
Day1	Before infusion T0				10 mL as blank matrix
	0h-6h				X
	6h- 12h				X
	12h-24h			X	X
End Of Study			X		

***Use**: Cockroft formula for men : CL (mL/min) = (140-age) x weight (kg) / creatininemia (µmol/L) x 1.23 or
CL (mL/min) = (140-age) x weight (kg) / creatininemia (mg/dL) x 72

The label on each tube will state: Urine, study number, subject number, randomization number, collection period, and study day.

The exact collection times and volume of urine will be recorded in the CRF.

Urine samples for urinalysis will be collected at pre study screening, before dosing with study medication and at the end of the study visit. In addition, a urine sample will be taken to screen for drugs of abuse at screening and about 12 hours before dosing.
For bioanalytics, four urine fractions will be collected (predose, 0-6h, 6-12h, 12-24h) into recipients containing acetic acid. The volume of acetic acid will be adjusted according to the size of the urine container: 0.1 mL in a container of 1 liter. Two aliquots of 10 mL of each urine sample will be stored at **–70°C** in polypropylene tubes at the study site.
For creatinine clearance determination, urines will be pooled as to obtain 0-24h sample which will be forwarded to the "laboratory of the unit ".

7.3.1.3 Sample handling and shipment:

Refer to Appendix B

7.3.2 Measurement schedule for other study variables

Table 6 - Measurement schedule for other study variables (groups 1to 4)

	Time relative to dosing (h:min)	Vital signs[a]	12lead ECG	AE questioning	Local tolerance	Physical examination
Screening		X	X			X
Day –1		X	X			X
Day 1	-0 :30 (predose)	X	X		X	
Start Infusion = TO						
End infusion = 0:30		X	X		X	
	1:00	x	x		X	
	4:00	X				
	6:00	X				
	12:00	X				
Day 2	24:00	X	X		X	X
End of study D 3 to D 7 post last dosing		X	X			X

Vital signs will be measured 10 min supine, 3 min standing.

Table 6a - Measurement schedule for other study variables (groups 5 during dialysis session)

	Time relative to dosing (h:min)	Vital signs[a]	12lead ECG	AE questioning	Local tolerance	Physical examination
Screening		X	X			X
Day –1		X	X			X
Day 1	-0 :30 (predose)	X	X		X	
Start Infusion = TO						
End infusion = 0:30		X	X		X	
	1:00	x	x		X	
	2:00	x				
	3:00					
	4:00					
End of study D 3 to D7 post last dosing		X	X			X

During dialysis, vital signs will be measured in supine position only.

Table 6b - Measurement schedule for other study variables (group 5 during interdialysis period)

	Time relative to dosing (h:min)	Vital signs[a]	12lead ECG	AE questioning	Local tolerance	Physical examination
Screening		X	X			X
Day –1		X	X			X
Day 1	-0 :30 (predose)	X	X		X	
Start Infusion = TO						
End infusion = 0:30		X	X		X	
	1:00	x	x		X	
	4:00	X				
	6:00	X				
	12:00	X				
Day 2	24:00	X	X		X	X
End of study D 3 to D7 post last dosing		X	X			X

Screening is only performed once (before dialysis session or interdialysis session).
Vital signs will be measured 10 min supine, 3 min standing.

Medical/ surgical history and Physical examination

Subject will have a complete medical/surgical and concomitant therapy history recorded at screening. Significant medical conditions that have occurred within the past 2 years or conditions that are ongoing (i.e., headache, indigestion) are to be recorded.

The physical examination will include an assessment of general appearance, skin, eyes, ears, nose, throat, heart, chest, abdomen, reflexes, lymph nodes, spine and extremities. All deviations from normal will be recorded in the medical history or in the adverse section of the CRF depending of the study period (see section 8.2).

Vital signs

Vital signs consist of systolic and diastolic blood pressure, and pulse rate. They will be measured after 10 minutes rest in supine position and after 3 minutes in standing position (except during dialysis time where the vitals signs will be measured in supine position only Table 6). In case where vital signs measurements are scheduled at the same time as collection of laboratory or pharmacokinetic sample, the blood sampling will always be withdrawn prior to measurement of vital signs.

Blood pressure (diastolic and systolic) and heart rate will be measured using an automated blood pressure monitoring (dynamap or equivalent) recorder after 10 minutes rest in supine position and after 3 minutes in standing position.

Electrocardiograms

Standard 12-lead ECG will be recorded in a supine position after 10 minutes rest (speed of recording 25 mm/s, calibration at 1 cm/mV), using a digital recording device.

Heart rate, PR, QRS, QT and QTc values will be recorded in the CRF. Baseline values (Day 1H0, predose) for the analysis will be the 12-lead resting ECG recorded before dosing.

The investigator should provide a comment on normality/abnormality. Any clinically relevant abnormality will be recorded as an adverse event and will be followed to resolution/satisfaction.

7.3.3 Methods of evaluation

7.3.3.1 Pharmacokinetics/ Pharmacodynamics

The concentration of NXL104 in plasma samples and postdose urine samples will be analyzed using validated LC-MS/MS methods at the laboratory (the method and the shipment details are described in Appendix B section 17.3).

7.3.3.2 Safety

Adverse event data will be collected via non-directive questioning and spontaneous reporting by the subjects.

Physical examination and weight measurement: will be conducted at the CRU using standard procedures.

Local tolerance: The site of infusion will be examined for the presence of inflammatory signs such as pain (spontaneous or after palpation), erythema, swelling, induration and palpation of the venous cord during and after the infusion. Any symptoms rated as moderate or severe will be reported as adverse event.

Safety profile: hematology, clinical chemistry, and urinalysis will be carried out according to standard operating procedures by the validated laboratory of the clinical research laboratories.

Drug screen in urine and urinalysis will be performed at the CRU.

Abnormal, clinically significant results will be verified to rule out laboratory error. Persistent relevant abnormal values must be followed up until the cause is determined or until they return to the premedication value.

7.4 General and dietary restrictions

The subjects will not take any non-trial medication, including over-the-counter remedies, throughout the study and in the two weeks before the study without consulting the investigator in advance with the exception of emergency cases (see *Section 10.2*).

Subjects will abstain from strenuous physical activity, alcohol, stimulating beverages containing xanthine derivatives (tea, coffee, Coca Cola-like drinks, chocolate) and grapefruit or grapefruit juice from 7 days before until 24 hours after last administration of the investigational product. Subjects must abstain from smoking for during confinement in the hospital.

A 100 mg dose of NXL104 will bring approximately 8 to 9 milligrams of sodium.

On the screening day, subjects will come to the unit after an overnight fast of at least 4 hours. At day-1 the subject will come to the unit in the morning and will stay till 24h post dosing morning of D2 (D-1 to T 24h post dosing). Subjects should sleep for at least 7 hours a night before the study days.

On Day 1(for groups 1 to 4), the Pre-dose urine collection will stop at around 07:00; a large glass of water will be taken. The treatment will be administered to the volunteers 30 min after breakfast. The subjects will have lunch 4h post dose. Water will be *ad-libitum* but not less than 1500 mL/24h. The subjects will be discharged on Day 2 T 24h with recommendations for next visit, instructions and a card with emergency numbers.

No additional food (sweets, cookies, etc...) is allowed for the whole in-hospital periods. On the other days, meals should be served at customary times. Plain water will be supplied *ad libitum* (starting after 2 hours following drug administration). No other beverages are allowed.
Subjects should be in a sitting or supine position for at least up to 1 hour after drug administration.

Note: Time of meals and total fluid intake should be documented.

8 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse event

The term **adverse event** covers any unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the period of observation in the clinical study. Clinically relevant abnormal results of diagnostic

procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be adverse events.

Worsening of a sign or symptom of the condition under treatment will normally be measured by efficacy parameters. However, if the outcome fulfils the definition of "serious adverse event", it must be recorded as such (see *Section 8.1.2*).

The adverse event may be:

- A new illness;
- Worsening of a concomitant illness;
- An effect of the study medication, including comparator;
- A combination of two or more of these factors.

No causal relationship with the study medication or with the clinical study itself is implied by the use of the term "adverse event".

Adverse events fall into the categories "non serious" and "serious" (see *Section 8.1.2*).

Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an adverse event, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the condition(s) leading to these measures are not adverse events, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

8.1.2 Serious adverse event

A serious adverse event is one that at any dose (including overdose):

- Results in death;
- Is life-threatening[1];
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity[2];
- Is a congenital anomaly or birth defect;
- Is an important medical event[3].

[1]"Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2]"Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3]Medical and scientific judgments should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood

dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A diagnosis of cancer during the course of a treatment should be considered as medically important. The List of Critical Terms (1998 adaptation of WHO Adverse Reaction Terminology Critical Terms List, provided in the "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form") should be used as guidance for adverse events that may be considered serious because they are medically important.

Clarification of the difference in meaning between "severe" and "serious". The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache).

This is not the same as "serious", which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.1.3 Alert terms and other reasons for expedited reporting to Pharmacovigilance

No special events are subject to reporting as alert terms in this study.

However, cases in which a "significant overdose" of the investigational product was taken and a non-serious adverse event or no adverse event occurred are to be reported to the sponsor in an expedited manner on a "Serious Adverse Event/Expedited Report from a Clinical Trial" form.

8.2 Period of observation

For the purposes of this study, the period of observation for collection of adverse events extends from the signature of the informed consent until the follow-up visit. However, pre-existing medical conditions noted during screening will be recorded as medical history.

If the investigator detects a serious adverse event in a study subject after the end of the period of observation, and considers the event possibly related to prior study treatment, he or she should contact the sponsor to determine how the adverse event should be documented and reported.

8.3 Documentation and reporting of adverse events by investigator

All adverse events that occur during the observation period set in this protocol (see *Section 8.2*) must be documented on the pages provided in the case report form in accordance with the instructions for the completion of adverse event reports in clinical studies. These instructions are provided in the investigator's study file and/or in the case report form itself.

The following approach will be taken for documentation:

- **All adverse events** (whether serious or non-serious, or considered as an alert term) must be documented on the "Adverse Event" page of the case report form.
- If the adverse event is serious (see *Section 8.1.2*), the investigator must complete, in addition to the "Adverse Event" page in the case report form, a "Serious Adverse Event/Expedited Report from a Clinical Trial" form at the time the serious adverse event is detected. This form must be sent to the sponsor, who will forward it to the sponsor's Pharmacovigilance department.
- When a "significant overdose" of the investigational product occurs without an adverse event or in other situations where the sponsor requires an expedited report without an adverse event (see *Section 8.1.3*), the

investigator should only complete a "Serious Adverse Event/Expedited Report from a Clinical Trial" form. Instructions on where to send this form will be provided by the sponsor. In this case, there is no need to complete the "Adverse Event" page in the case report form.

Every attempt should be made to describe the adverse event in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

All subjects who have adverse events, whether considered associated with the use of the investigational products or not, must be monitored to determine the outcome.

The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

All questions on the completion and supply of adverse event report forms and any further forms issued to the investigator at a later date to clarify unresolved issues should be addressed to the sponsor.

8.4 Immediate reporting by investigator to sponsor

Serious adverse events and adverse events that fulfill a reason for expedited reporting to Pharmacovigilance (alert term and/or "significant overdose", as defined in *Section 8.1.3*) must be documented on a **"**Serious Adverse Event/Expedited Report from a Clinical Trial" form in accordance with the "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form". This form must be completed and supplied to the sponsor within 24 hours, or at the latest on the following working day. Information to the Ethic Committee has to be sent. The **"**Serious Adverse Event/Expedited Report from a Clinical Trial" form and the instructions are provided in the investigator's study file and in the Trial Master File.

The investigator must also inform the study manager/monitor in all cases. The CRO monitor will ensure that all legal reporting requirements are met.

The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s).

Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up **"**Serious Adverse Event/Expedited Report from a Clinical Trial" forms.

The "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form" give more detailed guidance on the reporting of serious adverse events, adverse events that comply with alert terms, and adverse events initially reported as non-serious that become serious. In the latter situation, when a non-serious event becomes serious, details must be forwarded immediately to the sponsor on a "Serious Adverse Event/Expedited Report from a Clinical Trial" form.

9 WITHDRAWALS

9.1 Withdrawal of subjects

Subjects may be withdrawn from the study (i.e. from any further study medication or study procedure) for the following reasons:

- At their own request;
- If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being;
- At the specific request of the sponsor.

In all cases, the reason for and date of withdrawal must be recorded in the case report form and in the subject's medical records. The subject must be followed up to establish whether the reason was an adverse event, and, if so, this must be reported in accordance with the procedures in *Section 8*.

As far as possible, all examinations scheduled for the final study day must be performed on all subjects who receive the investigational product but do not complete the study according to protocol.

The investigator must make every effort to contact subjects lost to follow-up. Attempts to contact such subjects must be documented in the subject’s records (e.g., times and dates of attempted telephone contact, receipt for sending a registered letter).

9.2 Replacement of subjects

Subjects who do not complete their study period will be replaced, unless there is a safety concern for administering the study medication at that specific dose level.

In case of moderate or severe adverse event causing the subject to withdraw, a joint decision between the sponsor and the investigator will be taken on appropriateness to replace the subject or not.

10 EMERGENCY PROCEDURES

10.1 Emergency sponsor contact

In emergency situations, the investigator should contact the sponsor by telephone at the number given on the title page of the protocol.

10.2 Emergency treatment

During and after a subject's participation in the trial, the investigator and/or institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator and/or institution should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

11 STATISTICAL PROCEDURES

Complete details of the statistical analyses and methods, including data conventions, will be contained in a separate statistical analysis plan that will be finalized before the database is locked. This section of the protocol outlines the general principles that will be used in the analyses.

The PK parameters will be derived using WinNonlin software version 5.0.1 (Pharsight corporation) or an equivalent piece of software.

The statistical analysis on PK parameters and safety variables will be performed using SAS software (SAS Institute).

11.1 Analysis variables

11.1.1 Safety

Safety will be evaluated on the basis of:

- Adverse events reported;
- Blood pressure and pulse rate (after 10 min supine and 3 min in standing position);
- 12-lead ECG parameters QRS, PR, RR, QT, QTcB (QT corrected with Bazett's formula), QTcF (QT corrected with Fridericia's formula);
- Physical examination, body weight;
- Laboratory data (including hematology, clinical chemistry and urine analysis).

11.1.2 Pharmacokinetics

NXL104 concentrations will be determined in plasma (all time-points) and postdose urine samples. Pharmacokinetics parameters for NXL104 will be determined *using a non-compartmental method.*

For pharmacokinetic evaluation, the actual blood sampling times will be taken into account only if they deviate by more than ±5% from the nominal sampling times.

Data permitting, the following pharmacokinetic parameters will be determined from NXL104 in **plasma**:

- Cmax Maximum observed plasma concentration,
- Tmax Time to reach maximum plasma drug concentration,
- Clast Last measurable concentration,
- Tlast Time associated with Clast,
- AUC Area under the concentration-time curve, from time zero to infinity,
- AUC_{0-t} Area under the concentration-time curve for time 0 to the time tlast calculated using log-linear trapezoidal method
- Partial AUCs AUC over the successive urine collection periods: 0-6h, 6-12h, 12-24h,
- %Extrap Percent of extrapolated AUC, related to infinite AUC
- CL: Total plasma clearance of drug will be calculated as dose/ AUC

- $t_{½,z}$ Apparent terminal half-life. The terminal rate constant (λz) will be determined by linear regression of the terminal portion of the natural log (ln) concentration time curve. The t1/2z will be calculated as ln(2)/ λz.
- Boundaries of the terminal phase and number of data points in the terminal phase
- MRT Mean residence time, calculated as AUMC/AUC – 0.5*infusion time (when applicable)
- Vss: Apparent volume of distribution at steady state will be calculated as CL*MRT

Fractional and cumulated urinary excretion (Ae), fractional and cumulated urinary recovery (% administered dose) and fractional and cumulated renal clearance (CLr) will be calculated. Non renal clearance will be calculated as CL – CLr.

In patients from group 5 during a dialysis session, the following pharmacokinetic parameters will be calculated:

Concentrations of protein and plasma flow entering the dialyser are needed.:

- QPL Plasma flow trough the dialyzer, calculated as
- $Q_{PL}=Q_B *(1-H)$
- Where Q_B is the blood flow and H is the mean hematocrit during dialysis

- AUC_{1-5} Area under the concentration-time curve for time 1 to time 5 h, calculated using log-linear trapezoidal method from NXL104 plasma concentrations measured at the *arterial* side of the dialyzer
- E Extraction coefficient of NXL104 by the dialyzer, taken as the arterio-venous difference of NXL104 plasma concentrations, divided by the arterial concentration. Venous concentrations of NXL104 will be corrected for the hemoconcentration, using total proteins as a marker:

$$E = \frac{[NXL104]_A - [NXL104]_V \cdot (Prot_A/Prot_V)}{[NXL104]_A}$$

- CL_{HD} Hemodialysis clearance, calculated as the product of the above extraction coefficient by the measured *plasma* flow *entering* the dialyzer.
- Removal Amount of NXL104 removed by a 4-hour dialysis session, and calculated as $CL_{HD} \cdot AUC_{0-4}$ and expressed as a fraction of dose.

11.2 Analysis populations

The study populations for the statistical analyses will be as follows:

11.2.1 Included Population

All subjects included in the study.

11.2.2 Population for the safety and tolerability analysis

Data will be summarized for all subjects who received at least one dose of study medication.

11.2.3 Population for the pharmacokinetic analyses

All subjects who have received the complete study treatment and for whom pharmacokinetic data are considered interpretable by the sponsor will be included in the pharmacokinetic analysis.

11.3 Statistical methods

Complete details of the statistical analyses and methods, including data conventions, will be contained in a separate statistical analysis plan, which will be finalized before the database is locked.

The statistical analysis will be performed in a descriptive and exploratory fashion.

Descriptive summary statistics will be provided for all safety, and pharmacokinetic variables. For continuous variables, the number of subjects, mean (geometric only for PK parameters, arithmetic), standard deviation, standard error of the mean, minimum, median, and maximum will be calculated. For categorical variables, frequency distributions, including percent values, will be provided. Graphical presentations will be given where appropriate.

In the event of treatment miss-assignment, subjects will be analyzed according to the treatment that they received.

A description of the included population will be performed for all parameters recorded at screening and D-1.

11.3.1 Safety investigations

Safety data will be analyzed according to the Novexel safety analysis standards. The safety population will be used to address issues of safety and tolerability. All safety variables will be tabulated per group and measuring time together with descriptive statistics for each variable, if appropriate.

AEs will be coded using the Medical Dictionary for Regulatory Affairs (MedDRA).

All AEs will be listed on an individual basis. AEs with any of the following will be counted as treatment emergent adverse events:

- Time of onset between start of dosing and 48 hours after start of dosing

- Event started before dosing and continued and worsened in intensity (severity or frequency) after dosing

Frequency tables will be generated for all treatment emergent AEs per group based on system organ class and preferred terms.

Laboratory parameters: Listings of all variables will be provided by group and time In addition, variables of laboratory findings will be evaluated using a system of predefined changes, and clinically significant abnormal values (according to the Novexel guidelines), taking into account the investigator's normal ranges and checked for clinically relevant changes from baseline.

Local tolerance: Listings of all variables will be provided by group and time.

ECG. Listings of all variables will be provided by group and time. Descriptive analysis will be presented for all ECG variables and their changes from baseline (Δ) by group and time. In addition, ECG will be evaluated using a system of predefined changes according to the recommendations by the Committee for Proprietary Medicinal Products for QT interval and ICH ($30<\Delta QTc\leq 60$ ms; $\Delta QTc > 60$ ms; $QTc>450$ ms; $QTc>480$ ms; $QTc>500$ ms.

Vital signs: data will be presented per group for each measuring time, together with descriptive statistics. In addition, data will be compared to reference ranges to be specified in the Statistical Analysis Plan and checked for clinical relevant changes from baseline.

Physical examination, body weight will be presented for each measuring time with descriptive statistics.

11.3.2 Pharmacokinetics

All subjects who will complete the study and for whom the concentrations of NXL104 in plasma are considered to be sufficient and interpretable will be included in the pharmacokinetic analysis.

Individual plasma concentrations of NXL104 will be tabulated together with standard descriptive statistics (mean and SD). Individual and mean profiles will be presented graphically.

Individual and cumulative amounts of NXL104 excreted in urine will be tabulated, and plotted by dose levels.

Descriptive statistics (mean, SD, min, median, max, CV% and geometric mean) for pharmacokinetics parameters will be calculated by subject groups.

The effect of renal impairment will be evaluated for AUC0-∞, AUC0-t and Cmax using an ANOVA model applied to log transformed pharmacokinetic parameters with a class effect for the renal function group (but dialyzed patients during a dialysis session). For each parameter, a point estimate for the ratio of central values (renal patient/normal control) will be obtained by calculating the difference of least square means on the logarithmic scale and subsequent back transformation with the anti-log function. Likewise, 90% confidence intervals for the ratios will be obtained. In addition, total clearance of NXL104 will be plotted as a function of creatinine clearance and data will be evaluated by linear correlation analysis.

Complete details of the statistical analyses and model will be contained in a separate statistical analysis plan.

11.4 Interim analysis

No interim analysis is planned for this study.

11.5 Sample size justification

The sample size is therefore based on currently accepted standards for this type of investigation and safety and pharmacokinetic results from the single tolerability study. Consequently, 6 subjects per subgroup of subjects will be included.

12 ETHICAL AND LEGAL ASPECTS

12.1 Good clinical practice

This study is to be conducted according to globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice, 1 May 1996), in agreement with the Declaration of Helsinki and in keeping with local regulations.

12.2 Delegation of INVESTIGATOR DUTIES

The investigator should ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions.

The investigator should maintain a list of sub investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

Should the investigator delegate the supervision of the investigational product administration to a designated person, this individual must have the appropriate medical qualifications to effectively conduct or supervise any potential resuscitation procedures.

12.3 Subject information and informed consent

Prior to start of screening, subjects must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to them.

An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. This document will contain all the elements required by the ICH E6 Guideline for Good Clinical Practice and any additional elements required by local regulations. The document must be in a language understandable to the subject and must specify who informed the subject. Where required by local law, the person who informs the subject must be a physician.

After reading the informed consent document, the subject must give consent in writing. The subject's consent must be confirmed at the time of consent by the personally dated signature of the subject and by the personally dated signature of the person conducting the informed consent discussions.

One signed original consent document must be given to the subject, the second signed original consent document will be retained by the investigator.

The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

The investigator should inform the subject’s primary physician about the subject’s participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

12.4 Confidentiality

Subject names will not be supplied to the sponsor. Only the subject number and subject initials will be recorded in the case report form, and if the subject name appears on any other document (e.g., laboratory report), it must be obliterated on the copy of the document to be supplied to the sponsor.

Study findings stored on a computer will be stored in accordance with local data protection laws. The subjects will be informed that representatives of the sponsor, independent ethics committee (IEC)/ institutional review board (IRB), or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

12.5 Protocol amendments

Neither the investigator nor the sponsor will alter this clinical study protocol without obtaining the written agreement of the other. Once the study has started, amendments should be made only in exceptional cases. The changes then become part of the clinical study protocol.

12.6 Approval of the clinical study protocol and amendments

Before the start of the study, the clinical study protocol, informed consent document, and any other appropriate documents will be submitted to the IEC/IRB **with a cover letter or a form listing the documents submitted, their dates of issue, and the site (or region or area of jurisdiction, as applicable) for which approval is sought**. If applicable, the documents will also be submitted to the authorities, in accordance with local legal requirements.

Investigational products can only be supplied to the investigator once the sponsor has received documentation on **all** ethical and legal requirements for starting the study. This documentation must also include a list of the members of the IEC/IRB and their occupation and qualifications. If the IEC/IRB will not disclose the names of the committee

members, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP. Formal approval by the IEC/IRB should preferably mention the study title, study code, study site (or region or area of jurisdiction, as applicable), amendment number where applicable, and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member.

Before the first subject is enrolled in the study, all ethical and legal requirements must be met.

The IEC/IRB and, if applicable, the authorities must be informed of all subsequent protocol amendments and administrative changes, in accordance with local legal requirements. Amendments must be evaluated to determine whether formal approval must be sought and whether the informed consent document should also be revised.

The investigator must keep a record of all communication with the IEC/IRB and, if applicable, between a coordinating investigator and the IEC/IRB. This also applies to any communication between the investigator (or coordinating investigator, if applicable) and the authorities.

12.7 Ongoing information for independent ethics committee/ institutional review board

Unless otherwise instructed by the IEC/IRB, the investigator must submit to the IEC/IRB:

- Information on serious or unexpected adverse events from the investigator's site, as soon as possible

12.8 Closure of the study

The study must be closed at the site on completion. Furthermore, the sponsor or the investigator has the right to close this study site at any time. As far as possible, premature closure should occur after mutual consultation. Depending on local legislation, it may be necessary to inform IEC/IRB and the regulatory authorities when the study site is closed.

Study materials must be returned, disposed of or retained as directed by the sponsor.

12.9 Record retention

The investigator must obtain approval in writing from the sponsor before destruction of any records.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, because of international regulatory requirements, the sponsor may request retention for a longer period.

Essential documents include:

During the course of the study, the following documents will be filled in the Trial Master File:

- Signed informed consent documents for all subjects;
- Subject identification code list*, screening log (if applicable) and enrollment log;
- Record of all communications between the investigator and the IEC/IRB;
- Composition of the IEC/IRB (or other applicable statement as described in *Section 12.6*);
- Record of all communications between the investigator and sponsor (or CRO).

List of sub investigators and other appropriately qualified persons to whom the investigator has delegated significant trial-related duties, together with their roles in the study and their signature.

As for the others, they will be stored in clinical/pharmacy files under the investigator's responsibility:

- Copies of case report forms and of documentation of corrections for all subjects;
- Investigational product accountability records;
- Record of any body fluids or tissue samples retained;
- All other source documents (subject medical records, hospital records, laboratory records, etc.);
- All other documents as listed in section 8 of the ICH E6 Guideline for Good Clinical Practice (Essential Documents for the Conduct of a Clinical Trial).

Normally, these records will be held in the investigator's archives. If the investigator is unable to meet this obligation, he or she must ask the sponsor for permission to make alternative arrangements. Details of these arrangements should be documented.

*EU legislation requires this list to be maintained for a minimum of 20 years.

12.10 Liability and insurance

Liability and insurance provisions for this study are given in separate agreements.

12.11 Financial disclosure

Not applicable to this study.

13 STUDY MONITORING AND AUDITING

Monitoring and auditing procedures developed or endorsed by the sponsor will be followed, in order to comply with GCP guidelines. Direct access to the on-site study documentation and medical records must be ensured.

13.1 Study monitoring and source data verification

Monitoring will be done by personal visits from a representative of the sponsor (study monitor) who will check the case report forms for completeness and clarity, and crosscheck them with source documents. In addition to the monitoring visits, frequent communications (letter, telephone, and fax), by the study monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements.

Study closeout will be performed by the study monitor upon closure of the study.

13.2 On-site audits

Domestic and foreign regulatory authorities, the IEC/IRB, and an auditor authorized by the sponsor may request access to all source documents, case report forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that subject names are obliterated on the copies to ensure confidentiality.

14 DOCUMENTATION AND USE OF STUDY FINDINGS

14.1 Documentation of study findings

A case report form will be provided for each subject.

The investigator must enter all protocol-required information collected during the study, or designated representative, in the case report form. Details of case report form completion and correction will be explained to the investigator. If the investigator authorizes other persons to make entries in the case report form, the names, positions, signatures, and initials of these persons must be supplied to the sponsor.

The investigator, or designated representative, should complete the case report form pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

A source data location list will be prepared prior to study start. This list will be filed in both the trial master file and the investigator study file and updated as necessary.

The completed case report form must be reviewed and signed by the investigator named in the clinical study protocol or by a designated sub investigator.

The sponsor will retain the originals of all case report forms. The investigator will retain a copy of all completed case report form pages.

14.2 Use of study findings

All information concerning the product as well as any matter concerning the operation of the sponsor, such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the sponsor and are unpublished, are confidential and must remain the sole property of the sponsor. The investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from the sponsor is obtained.

The sponsor has full ownership of the original case report forms completed as part of the study.

By signing the clinical study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. The authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

If applicable the sponsor will involve the investigator in the review process of any publication that might arise from this study.

The sponsor will ensure that a final report on the study is prepared.

The investigator will be required to sign a statement that he or she confirms that, to the best of his or her knowledge, it accurately describes the conduct and results of the study.

All materials, documents and information supplied by the sponsor to the investigator, and all materials, documents and information prepared or developed in the course of the study to be performed under this protocol, shall be the sole and exclusive property of the sponsor. Subject to obligations of confidentiality, the investigator reserves the right to publish only the results of the work performed pursuant to this protocol, provided, however, that the investigator provides an

authorized representative of the sponsor with a copy of any proposed publication for review and comment at least 45 days in advance of its submission for publication. In addition, if requested, the investigator will withhold publication an additional 90 days to allow for filing a patent application or taking such other measures as sponsor deems appropriate to establish and preserve its proprietary rights.

The manner and timing in which publications will be generated will be agreed upon by sponsor and investigator prior to initiation of the study, provided that in no event shall any publication occur until the study is completed and the results are unblinded and analyzed.

15 DECLARATIONS OF SPONSOR AND INVESTIGATOR

15.1 Declaration of sponsor

This clinical study protocol was subject to critical review and has been approved by the sponsor. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product;
- The moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of GCP as described in ICH Guidelines E6.

The investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Clinical Pharmacologist

Date: _____ Signature: _____

Name (block letters):_____

15.2 Declaration of investigator

I have received the following:

Investigator's Brochure with details of clinical and non-clinical data on the investigational product that are relevant to the study of the product in human subjects, 4th edition final version 1 [redacted]; Serious adverse event forms and instructions for reporting.

I have been adequately informed about the development of the investigational product to date. I will confirm the receipt of updated investigator's brochures. I have read this clinical study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol.

I will not enroll the first subject in the study until I have received approval from the appropriate IEC and until all legal requirements in my country have been fulfilled.

The study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the GCP guidelines of ICH E6 (CPMP/ICH/135/95) and the French code of Public Health.

I agree to obtain, in the manner described in this clinical study protocol, written informed consent to participate for all subjects enrolled in this study.

I agree to make all trial-related records, including source documents and medical records, available for direct access to the monitor, auditor, IEC/IRB or regulatory authority upon request.

I am aware of the requirements for the correct reporting of serious adverse events, and I undertake to document and to report such events as requested.

I agree to supply the sponsor with evidence of current laboratory accreditation, the name and address of the laboratory, and a list of normal values and ranges.

I agree with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals.

I agree to keep all source documents and case report forms as specified in *Section 12.9* of this protocol.

I will provide curriculum vitae before the study starts, which may be submitted to regulatory authorities.

Investigator

Date: _____ Signature: _____

Name (block letters):_____

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17 APPENDICES

17.1 Appendix A: Preparation of infusion

The study medication is a NXL104 1000mg lyophilised powder to be reconstituted with 10mL of water for injection to obtain the concentration of 1000mg/10mL

Solutions of NXL104 for intravenous infusion will be prepared for each individual subject under aseptic conditions no more than 24h before administration. The solutions will be stored at +2°C to +8°C temperature

The study medication is a NXL104 1000mg lyophilised powder to be reconstituted with 10mL of water for injection to obtain the concentration of 100mg/mL, 2ml of this reconstitution will be added to the 100 mL 5% glucose infusion bag.= concentration of 2mg/mL of which 50 ml will be infused

Before adding the adequate amount of NXL104 to be infused (2ml), it is requested to withdraw, the same amount of 5% glucose from the 100 mL infusion bag. Then, the appropriate amount (2 ml) of NXL104 will be transferred into the infusion bag and the volume adjusted (if required) to 100 mL with 5% glucose according to the following table:

Table 7 – Preparation of the final solution to be administered

Group	Dose NXL104 (mg)	Number of NXL104 vials required/subject	Concentration of NXL 104 to be prepared (mg/mL) to infused 50mL in 30 minutes	Preparation of the 100mL 5% glucose solution	
				solution of 100mg/ml of NXL104(mL)	Volume adjusted to 100ml 5 % Glucose (mL)
all	100	1	2	2,00	98,00

50 ml of the above solution will be infused

The preparation should be labeled as follows:

- Study code NXL104/1003
- Randomization n°/patient n°
- For I.V infusion use only
- Date
- Time of preparation

After administration, collect two 5mL aliquots of the administered solutions from each infusion bag (preferably collect the amount at the end of the infusion line), transfer them into labeled polypropylene tubes and store them at -20°C. The label on each tube will state: study no., subject no., randomization no., 2mg/mL, Solution for infusion, and date. They will be stored until the final PK analysis. In case of paradoxical a PK response, these aliquots will be available for a concentration check at , where the assay method has been validated.

17.2 Compatibility and Stability of NXL104 in 5% Glucose

References 17
Compatibility and stability studies were performed with 250 and 500 mL polyethylene Ecoflac® 8C820 containing a 5% glucose solution:
- Study A061540 ()
The compatibility study and the stability study was split into two investigations:

Stability of NXL104 alone and a mixture of NXL104/ Ceftazidime for 24h at *ca.* +5°C: Sampling at 0.1, 6 and 24 hours.
Stability of NXL104 alone and a mixture of NXL104/ Ceftazidime for 30 min at room temperature: Sampling at 0, 10, 20 and 30 minutes.

Four concentrations were tested:
NXL104 at 0.2mg/ml and 8.0mg/ml.
NXL104/ Ceftazidime at ¼ mg/ml and 2/8 mg/ml (ratio).

Two conditions were evaluated:
Ambient light (only for tests performed at room temperature).
Protected from light.

Each individual HPLC method was specific to the test substance of interest.

Conclusion:

Each solution described above can be left in contact with a 500ml polyethylene ecoflac 8C820 (containing a 5% glucose solution) IV administration set for up to 24 hours at ca. +5°C or 30 minutes at room temperature (ambient light or protected from light).

17.3 Appendix B: Sample collection and shipping procedures for pharmacokinetic samples

MATERIALS FOR PLASMA SAMPLING

The Facility will provide the following materials:

- Vacutainer blood collection tubes (lithium heparinate: 5 mL for biochemistry, EDTA: 3 mL for haematology, FX for pharmacokinetics)
- Polypropylene specimen stabilization/storage tubes (13 x 75 mm)
- Vacutainer collection needles and needle holders or via an indwelling intravenous catheter
- Centrifuge capable of achieving 3000 rpm and refrigerated at approximately + 4 °C
- Tube rack for handling of specimens during collection and storage
- Freezer capable of maintaining temperature at –80 ° C or colder, with routine temperature monitoring and documentation
- The facility supplied specimen labels containing the following information: study number (NXL104/1003), subject number, nominal time in relation to study medication administration, and identification of analyte, “NXL104 assay”.

PLASMA COLLECTION FOR PK:

• Collect all blood samples (2x2 mL) for NXL104 assay in tubes containing sodium fluoride (5mg)/potassium oxalate (4mg) (Vacutainer FX reference number 368920). Immediately after collection, mix the whole blood with the anticoagulant by gently inverting the tube at least five times and then place it on ice (+4°C) until centrifugation.

• Centrifuge for 10 minutes at 2000g at a temperature of +4°C,

• Divide the plasma into two aliquots of equal quantity. Store immediately in sealed, labeled polypropylene tubes at –80°C. The whole procedure should not exceed 45 minutes.

• The label on each tube will state: NXL104/1003, subject no., group number, period number (only for group 5), Sampling site (arterial or venous for group 5 only), Plasma, T°, sampling time (given in hours pre and post dose).

URINE SAMPLES FOR PK

• Add 0.1mL of pure acetic acid per liter of empty recipient for urine collection. After addition of acetic acid, weigh each recipient before urine collection

• Weigh each recipient at the end of urine collection.

• Mix well before collecting 2 aliquots.

• For bio analytics, two aliquots of 10 mL of each urine sample will be stored at – 80°C in polypropylene tubes at the study site.

• The label on each tube will state: NXL104/1003, subject no., group number, Urine, T°, collection interval (given in hours pre and post dose,).

SAMPLE LABELLING, STORAGE, PACKING AND SHIPMENT

Specific "Plasma sample collection form" and “Urine sample collection form” included in the CRF will be filled out.

For plasma, record the study number (NXL104/1003), subject randomization number, initials and the actual blood sample withdrawal time (24:00h clock) and the date (mm/dd/yy) on the “Plasma sample collection form”. This is the only place the actual collection date and time are recorded for the sponsor. Record also on the CRF page “study medication administration - dosing time” the date and actual time (start and end) of NXL104 administration and the actual dose.

SPECIMEN STORAGE PRIOR TO SHIPMENT (for plasma and urine samples)

Prior to sample collection, the storage freezer (or temporary freezer) must be identified and checked for acceptability by monitoring the temperature for five consecutive working days using a calibrated thermometer. Samples must be stored at –80 °C or colder until ready for shipment. If a calibrated freezer is not available, the samples must be stored on dry ice. Documentation of freezer monitoring throughout the study will be recorded in the Freezer Temperature Log and must include date and time of temperature reading, temperature value, and initials of the individual taking the reading. Use the Freezer Temperature Log to record any transfers of stored specimens to another device (e.g., temporary storage in an insulated container with dry ice, relocation to a different freezer meeting the storage acceptance criteria listed above), and any maintenance or repairs of the freezer unit. Temperature measurements must be obtained using a digital thermometer or equivalent recording device that is calibrated against NIST traceable devices at least once a year.

The sponsor will advise the investigator of the frequency of shipping. Contact the sponsor if additional information concerning temperature monitoring or an actual monitoring device is needed. Note: Samples must be tightly capped to prevent desiccation (i.e. loss of fluid) that can occur during storage.

SAMPLE SHIPMENT

Shipping Documentation

Each sample shipment must be accompanied with a copy of “Study medication administration - dosing time”, “Plasma sample collection form" and “Urine sample collection form”.

Shipping Procedures

Plasma and urine samples for NXL104 assay will be shipped frozen in dry ice by express service to:

Shipment

Study samples will be transferred at end of study by the facility using a container filled with enough dry ice. During their shipment, the study samples will be kept at about -80°C.

In addition, the following information should be provided on the outside of the package:

Description: FROZEN HUMAN PLASMA AND /OR URINE SAMPLES

PERISHABLE (KEEP FROZEN),

Biohazard sticker,

Dry ice sticker (with weight of dry ice),

Correct address label,

Origin of samples,

Study (protocol) number,

Name of person shipping samples.

The laboratory will be notified by fax at the time of shipment. This fax will include protocol number, subjects number, quantity of samples being shipped, number of boxes, date of shipment, carrier and commercial invoice. A copy of this fax will be sent to the study manager.

17.4 Appendix C: Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002

Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements.

No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the

subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best-proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

17.5 Appendix D: Decision chart

NEUTROPENIA

Neutrophils < 1500/mm^3

Repeat immediately a full blood count if value close to 1500/mm^3

Neutrophils < 1500/mm^3 confirmed with signs of infection	18	Neutrophils < 1500/mm^3 confirmed with no sign of infection
1. DISCONTINUE Investigational Product, hospitalization should be considered. 2. PERFORM laboratory investigations for infection		1. DISCONTINUE Investigational Product 2. INVESTIGATE for infection

in both situations

3. INFORM the local monitor
4. INVESTIGATE previous treatments, particularly long-term, even a long time ago, exposure to toxic agents, e.g. benzene, X-rays, etc.
5. PERFORM and collect the following investigations (results):
   - RBC and platelet counts
   - Serology: EBV, (HIV), mumps, measles, rubella
6. DECISION for bone marrow aspiration: to be taken in specialized unit
7. FREEZE serum (5 mL x 2) on Day 1 (cessation of Investigational Product) and Day 5
8. MONITOR the leukocyte count 3 times per week for at least one week, then twice a month until it returns to normal,

THROMBOCYTOPENIA

Platelets < 100 000/mm^3
(rule out EDTA-induced pseudo-thrombocytopenia)

Repeat immediately the count
(rule out EDTA anticoagulant in the sample)

Platelets < 100 000/mm^3 confirmed with bleeding	19	Platelets < 100 000/mm^3 confirmed with no bleeding
1. DISCONTINUE Investigational Product 2. HOSPITALIZATION should be considered		1. DISCONTINUE Investigational Product 2. INVESTIGATE for bleeding

in both situations

3. INFORM the local monitor
4. QUESTION about last intake of quinine (drinks), alcoholism, heparin administration
5. PERFORM or collect the following investigations:
   - Complete blood count, schizocytes, creatinine
   - Bleeding time and coagulation test (fibrinogen, PT, aPTT), Fibrin Degradation Product
   - Viral serology: EBV, HIV, mumps, measles, rubella
6. FREEZE serum (5 mL x 2) on Day 1 (end of treatment) and Day 5 to test for drug-induced antiplatelet antibodies
7. DECISION for bone marrow aspiration: to be taken in specialized unit
   - On Day 1 in the case of associated anemia and/or leukopenia
   - On Day 8 if the platelets remain < 50 000/mm^3.
8. MONITOR the platelet count every day for at least one week and then regularly until it returns to normal

INCREASE IN AMINOTRANSFERASES

(Expressed as a multiple of the upper limit of normal (ULN) for the laboratory performing the assay)

Increase in aminotransferases
Either ALT (SGPT)
or AST (SGOT) and CPK normal

≤ 3ULN

Investigational Product administration may be continued

Repeat the measurement
- Twice during the first week
- Then once weekly until return to normal or at least one month after the end of the trial

>3ULN

Repeat immediately the count if confirmed

≤ 3 ULN

1. DISCONTINUE administration of the Investigational Product 2. HOSPITALIZATION should be considered if ALT > 10 ULN and/or jaundice or coagulation disorder (PT <50% with factor V <50%) or signs of hepatic encephalopathy 3. INFORM the local monitor 4. INTERVIEW patient again about consumption of alcohol, drugs and herbals received *before* and *during* the trial and possible contamination by non-A , non-B, non-C virus in the last six months (blood or blood product transfusion, travel to Africa, Asia, intravenous drug addiction) 5. INVESTIGATE for illness and/or hypotension and/or episode or arrhythmia in the previous 48 hours	1. PERFORM the following examinations: • Complete blood count • Serum creatinine • Anti-HIV IgM, anti HBc IgM, anti-HCV IgM, anti-CMV IgM Specific serologic markers of recent infection with ***** EBV, herpes viruses and toxoplasma (depending on the clinical context) ***** hepatobiliary ultrasonography 2.FREEZE serum (5 mL x 2) 3.MONITOR aminotransferases every 3 days for the first week then once weekly until return to normal or for at least 3 months

ACUTE RENAL FAILURE

Rapid increase in serum creatinine over 150 µmol/L

Can be rapidly reversed:

- by volume repletion
- or relief of urinary tract obstruction (according to etiology)

Cannot be rapidly reversed:

- Occurrence/aggravation of life threatening symptoms of ARF: anemia, hyperkalemia, hyperuricemia, metabolic acidosis, cardiac insufficiency, pulmonary edema, arrhythmia, DIC, etc.
- and/or predominant elimination of Investigational Product by renal route

1. Investigational Product may be continued.
2. MONITOR serum creatinine until return to baseline level

1. DISCONTINUE Investigational Product administration
2. HOSPITALIZATION should be considered and seek for nephrologic advice
3. INFORM the local monitor
4. PERFORM the following examinations
   - BP, HR, hydration status, ECG
   - Blood count
   - Liver function tests + CPK
   - Biochemistry
   - Urinalysis
5. FREEZE serum (5 mL x 2)
6. MONITOR renal function until return to baseline level (everyday at the beginning, then every week)

AMENDMENT N°1.0 TO STUDY PROTOCOL

PHARMACOKINETICS OF NXL104 100 MG IN NORMAL SUBJECTS AND PATIENTS WITH VARYING DEGREES OF RENAL IMPAIRMENT NXL104/1003

Investigators

Sponsor's responsible medical expert

Signatory of the sponsor

Date of issue: Final version 2.0

1 -Reason for amendment:

Based on the results of the phase I study NXL104/1004 (age and gender), the enrollment in this study can be extended to women and to patients aged between 18 and 90 years old.

Based on the results of the male fertility and early embryonic development toxicity study in rats, the requirement for male patients to abstain from sexual intercourse without the use of a condom/spermicide while on study drug and for 90 days post-treatment was removed from the protocol.

Typographic errors have been corrected.

2- Updated sections

Section 1.1.2 Antimicrobial Activity

Reason for change: Update with new data available

Previous text:

NXL104 [redacted] is a novel non β lactam β lactamase inhibitor with a spectrum covering both Ambler class A [Extended spectrum β lactamases (ESBLs)] and class C (Amp C) enzymes. β lactamase inhibition is effected through the formation of a stable covalent carbamoyl linkage in the acyl enzyme complex.

***in vitro* activity against β -lactamases**

NXL104 displays potent inhibition of both class A and class C enzymes. Purified TEM 1 and P99 were inactivated at low IC_{50} values, with low turnover numbers and long covalent intermediate half lives.

***in vitro* antibacterial activity of ceftazidime / NXL104 combination**

NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects β lactams from hydrolysis in a variety of class A and class C producing strains, including ESBL producers.

NXL104 restored ceftazidime activity against ceftazidime resistant strains expressing various class A β lactamases. In addition, NXL104 protected ceftazidime activity from β lactamase hydrolysis in strains expressing class C enzymes, including plasmid borne and chromosomal, inducible or derepressed AmpC enzymes.

In contrast to the potent *ampC* induction seen with cefoxitin and clavulanate in *E. cloacae*, NXL104 did not induce *ampC* expression.

No antagonism of ceftazidime *in vitro* antibacterial activity has been observed when combined with NXL104.

***in vivo* antibacterial activity of ceftazidime / NXL104 combination**

The ceftazidime / NXL104 combination showed therapeutic activity in mice infected by ceftazidime resistant *Enterobacteriaceae* strains (class A and class C β lactamase producers).

Ceftazidime / NXL104 has demonstrated

- Effective bacterial clearance in the lung in mouse pneumonia model
- Effective bacterial clearance in cerebrospinal fluid in the rabbit meningitis model
- Significant survival improvement in murine pyelonephritis model

New text:

NXL104 [redacted] is a novel non β lactam β lactamase inhibitor with a spectrum covering both Ambler class A [Extended spectrum β lactamases (ESBLs)] and class C (Amp C) enzymes. β lactamase inhibition is effected through the formation of a stable covalent carbamoyl linkage in the acyl enzyme complex.

***in vitro* activity against β -lactamases**

NXL104 displays potent inhibition of both class A and class C enzymes. Purified TEM 1, SHV 4, KPC **2** and P99 were inactivated at low IC_{50} values, with low turnover numbers and long covalent intermediate half lives were demonstrated with TEM 1 and P99.

***in vitro* antibacterial activity of ceftazidime / NXL104 combination**

NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects β lactams from hydrolysis in a variety of class A and class C producing strains, including ESBL producers.

NXL104 restored ceftazidime activity against ceftazidime resistant strains expressing various class A β lactamases including strains producing the KPC carbapenemases. In addition, NXL104 protected ceftazidime activity from β lactamase hydrolysis in strains expressing class C enzymes, including plasmid borne and chromosomal, inducible or derepressed AmpC enzymes.

Finally, NXL104 combination remained active in bacterial isolates having a high hydrolytic activity due to production of multiple β lactamases, as well as in isolates for which carbapenem resistance is conferred by a combination of impermeability plus cephalosporinase production.

Comparable MIC values were obtained when the ceftazidime / NXL104 combination was used with the constant antibiotic / inhibitor ratio of 4/1 or with the constant inhibitor concentration of 4 mg/L. The latter was the preferred susceptibility testing method, since it allowed measuring antibiotic activity with significant enzyme inhibition. In contrast to the potent *ampC* induction seen with cefoxitin and clavulanate in *E. cloacae*, NXL104 did not induce *ampC* expression.

No antagonism of ceftazidime *in vitro* antibacterial activity has been observed when combined with NXL104.

NXL104 *in vitro* pharmacodynamics were shown to be time dependent in an hollow fiber infection model.

***in vivo* antibacterial activity of ceftazidime / NXL104 combination**

The ceftazidime / NXL104 combination showed therapeutic activity in mice infected by ceftazidime resistant *Enterobacteriaceae* strains (class A and class C β lactamase producers). According to the activity observed, the preferred ceftazidime/NXL104 dosing ratio was 4/1 w/w.

Ceftazidime / NXL104 has demonstrated

- Effective bacterial clearance in the lung in mouse pneumonia model
- Effective bacterial clearance in cerebrospinal fluid in the rabbit meningitis model
- Significant survival improvement in murine pyelonephritis model
- Significant survival improvement in murine septicemia model

Section 1.1.5 Toxicology

Reason for change: update with new data available in toxicology.
The following text has been added.

Additional text:

Embryo/fetal development studies (including teratogenic potential assessment) were performed in rats and rabbits. In mated female Sprague Dawley rats, NXL104 slightly decreased mean food consumption at 1000 mg/kg/day; there was no significant effect on embryo/fetal development at any dose selected. In pregnant rabbits, maternal toxicity was evidenced by effects on body weight gain and/or food consumption from 250 mg/kg/day. Evidence of embryo fetal toxicity included an increase in post implantation loss at 1000 mg/kg/day.

A male fertility and early embryonic development toxicity study has also been completed in rats. The study evaluated the effect of NXL104 on gonadal function, mating behavior, and reproductive performance when administered during spermatogenesis and mating. In male Sprague Dawley rats, intravenous administration with NXL104 at doses of 250, 500 or 1000 mg/kg/day was considered likely to have caused mortality, poor local

tolerance at the implantation site and slightly reduced body weight gain at all dose levels, though not in a dose related manner. Despite these effects, there was no effect on mating performance or fertility, including an assessment of sperm counts and motility, and no adverse macroscopic or weight changes associated with the reproductive organs in any group. The no observed effect level (NOEL) for gonadal function, mating behaviour and reproductive performance in the male Sprague Dawley rat was 1000 mg/kg/day.

Section 1.1.6 Human experience – Phases I

Reason for change: update with final results of study 104/1002 and study 104/1004.

Previous text:

Multiple Dose Study (104/1002)

This was a double blind, randomized, placebo controlled study in healthy adult male subjects to evaluate the safety, tolerability, and pharmacokinetics of repeated intravenous doses of NXL104 alone and in combination with ceftazidime and to evaluate the absolute bioavailability of a single oral dose of NXL104. In Part A of the study, NXL104 was to be administered intravenously at doses of 500mg, 750mg, and 1000mg every 8 hours for 5 days. A separate group of 8 subjects was to receive 500mg NXL104 plus 2000mg ceftazidime IV every 8 hours for 10 days. In Part B of the study, 8 subjects were to receive single 500mg doses of NXL104 intravenously and orally in a two way cross over.

Preliminary blinded safety data are available for this study. NXL104 was generally well tolerated with no serious adverse events and no subject discontinued due to adverse events. One adverse event each related to local tolerability was reported in the 500 and 750 mg intravenous NXL104 cohorts (consisting of moderate erythema at the infusion site), and in the 500mg NXL104 + 2000mg ceftazidime cohort (consisting of moderate hematoma 20cm above the injection site).

The events occurred on Day 2 of dosing in the 500 and 750mg IV NXL104 cohorts, and on Day 8 of dosing for the IV combination 500mg NXL104 + 2000mg ceftazidime cohort. The moderate erythema at the infusion site and the moderate hematoma resolved within 72 hours and 5 days, respectively, without specific treatment; no other associated signs or symptoms were reported. In the 1000mg group, no adverse event was reported. No laboratory, physical examination, or ECG abnormalities of clinical significance were noted.

Plasma concentrations reached a maximum at the end of infusion in most individuals, then declined in several phases. Concentrations were still measurable in every subject dosed with NXL104 eight hours after the start of infusion. The average plasma clearance was approximately 10 to 12 L/h and NXL104 had a steady state volume of distribution of ca. 16 to 21 L. The terminal half life determined up to the 8 hour time point ranged between 1.4 and 1.7 h, resulting in little or no accumulation upon repeated dosing. The steady state AUC over a dosing interval on day 5 was similar to infinite AUC after the first dose on day 1. This indicates that, whenever given alone, steady state pharmacokinetics of NXL104 is predictable from a single dose.

New text:

Multiple Dose Study (104/1002)

This was a double blind, randomized, placebo controlled study in healthy adult male subjects to evaluate the safety, tolerability, and pharmacokinetics of repeated intravenous doses of NXL104 alone and in combination with ceftazidime and to evaluate the absolute bioavailability of a single oral dose of NXL104. In Part A of the study, NXL104 was to be administered intravenously at doses of 500mg, 750mg, and 1000mg every 8 hours for 5 days. A separate group of 8 subjects was to receive 500mg NXL104 plus 2000mg ceftazidime IV every 8 hours for 10 days. In Part B of the study, 8 subjects were to receive single 500mg doses of NXL104 intravenously and orally in a two way cross over.

NXL104 was generally well tolerated with no serious adverse events and no subject discontinued due to adverse events. None of the adverse events observed was considered related to NXL104 treatment. One adverse event each related to local tolerability was reported in the placebo and 750 mg intravenous NXL104 cohorts (consisting of moderate erythema at the infusion site). Three adverse events were reported in the 500mg NXL104 + 2000mg ceftazidime cohort (consisting of 2 mild to moderate hematoma above the injection site in 2 separate subjects, and mild left ankle arthralgia in a 3rd subject). In the 1000mg group, no adverse event was reported.

The moderate erythema occurred on Day 6 and Day 3 of dosing in the 500mg IV NXL104 (subject no.106) and placebo (subject no.105) subjects, respectively. In the 500mg NXL104 + 2000mg ceftazidime IV combination cohort, mild to moderate hematoma was observed on Day 8 (subject no.404) and Day 11 (subject no.406). The erythema and hematoma at the infusion site resolved without specific treatment; no other associated signs or symptoms were reported. Mild left ankle arthralgia was observed on Day 6 in a 3rd subject who received NXL104 and ceftazidime (subject no.402) in this same cohort; the arthralgia resolved in less than 3 days. No laboratory, physical examination, or ECG abnormalities of clinical significance were noted in the study.

Effect of Age and Gender (104/1004)

A study to evaluate the effect of age and gender on the pharmacokinetics and safety of NXL104 in healthy volunteers (NXL104/1004) was completed in the USA. This was a single dose study of 500mg NXL104 in 33 healthy subjects in 4 cohorts [young men (N=9), young women (N=8), elderly men (N=8) and elderly women (N=8)]. The mean age of subjects enrolled was similar among the two young cohorts [mean age in young men and women was 28.7 (range 20 37) and 30.9 (range 23 44), respectively]; likewise, the mean age of subjects enrolled was similar among the two elderly cohorts [mean age in elderly men and women was 68.8 (range 65 74) and 69.1 (range 65 76), respectively]. There were no SAEs or AEs leading to discontinuation. The incidence of treatment emergent AEs was 11% (1/9) in young men, 37.5% (3/8) in young women, 25% (2/8) in elderly men, and 50% (4/8) in elderly women. Treatment emergent AE that occurred in more than one subject (regardless of relationship to study drug) were application site bruising (4/33; 12%) and headache (2/33; 6%). Overall, 3 subjects experienced 6 treatment emergent adverse events considered to be drug related, including dry mouth, feeling hot, feeling jittery, dysgeusia, headache, and hyperhidrosis; each event was mild in intensity. There were no clinically relevant changes in lab values in any cohort.

Table 1 provides the PK parameters across the age and gender cohorts. It appears that the PK (total plasma clearance, Cmax, AUCs, and half life) of NXL104 is similar in the cohorts, regardless of age and gender.

Table 1. Comparison of PK parameters across the age and gender cohorts in Protocol 1004

	Young men N=9	Young women N=8	Elderly men N=8	Elderly women N=8
Cmax (µg/mL)				
Mean	33.83	36.86	26.45	38.41
SD	4.24	9.31	5.73	15.51
T½ (hours)				
Mean	2.09	1.71	3.17	2.43
SD	0.64	0.09	0.65	0.47
Cl (L/hr)				
Mean	10.16	10.34	9.82	7.98
SD	1.23	1.82	1.81	2.22
AUC (0 inf) (µg·hr/mL)				
Mean	49.86	49.75	52.4	66.23
SD	6.27	9.1	9.38	14.97

Section 3.2 Study duration and dates

Reason for change: increase of study duration to complete study recruitment.

Previous text:

The duration of this study is expected to be 6 months, with subject recruitment proposed to start in. The actual overall study duration or subject recruitment period may vary

New text

The duration of this study is expected to be 14 months, with subject recruitment proposed to start in. The actual overall study duration or subject recruitment period may vary

Section 4.2 Inclusion criteria

Reason for change: Update based on final results from phase I study NXL 104/1004 (age and gender).

Previous text:

- Healthy adults males of any race aged between 18 and 65 years, inclusive, with good health determined by past medical and surgical history, physical examination, vital signs, electrocardiogram, and laboratory tests at screening and matched by age (+/ 5years) and weight (+/ 5kg) with renal impairment patients

  Or

- Patients with renal impairments males of any race aged between 18 and 65 years, inclusive, reasonably stable renal function as assessed from serum creatinine concentration and/or urinary creatinine monitoring
  Good health excluding renal disease and associated controlled illnesses such as hypertension, diabetes mellitus, etc (based on medical history, physical examination, eletrocardiograms (ECG, and clinical laboratory tests),

  And FOR ALL, the following

- Able to execute written informed consent
- Be available for treatment as inpatients for up to 3 days following treatment. especially for anuric patients on hemodialysis
- Negative serology: HIV antibody, hepatitis B surface antigen, hepatitis C antibody,
- Negative urine drug screen for cannabis, opiates, cocaine metabolite, amphetamines, barbiturates, benzodiazepines (except for hemodialysis patients) and alcohol breath test
- Laboratory values will be within normal ranges (with the exception of those criteria associated with renal disease and any associated illness.)

Patients should maintained their chronic treatment if treatments are stable for at least one month

New text:

Subjects and/or Patients meeting all of the following criteria will be considered for admission to the study:

- Healthy adults of any race aged between 18 and 90 years, inclusive, with good health determined by past medical and surgical history, physical examination, vital signs, electrocardiogram, and laboratory tests at screening and matched by age (+/ 5years) and weight (+/ 5kg) with renal impairment patients

  Or

- Patients with renal impairments of any race aged between 18 and 90 years, inclusive, reasonably stable renal function as assessed from serum creatinine concentration and/or urinary creatinine monitoring
  Good health excluding renal disease and associated controlled illnesses such as hypertension, diabetes mellitus, etc (based on medical history, physical examination, eletrocardiograms (ECG, and clinical laboratory tests),

  AND FOR WOMEN

  Women are authorized to participate in this clinical study if they meet the following criteria:

  - Has been surgically sterilized or has been postmenopausal for at least one year OR
  - Is of childbearing potential, and all of the following conditions are met:
    - had normal menstrual periods for the 3 months prior to study entry, and
    - has a negative serum pregnancy test (serum β hCG) prior to enrollment.
    - must be willing to practice double barrier methods of birth control during treatment and for at least 28 days after dosing with study medication. Oral contraceptives

should not be used as the sole method of birth control, because the effect of NXL104 on the efficacy of oral contraceptives has not yet been established.

4.3 Exclusion criteria

Reason for change : Update based on the results of the male rat fertility study described in Section 1.1.5 toxicology.
The exclusion criteria concerning cardiac disease has been clarified.

Previous text:

- Male subjects who are not willing to abstain from sexual intercourse without use of a condom/spermicide while taking the study drug and for at least 28 days after treatment with study drug.
- Presence of QTc≥ 430 ms, or a pronounced sinus bradycardia (<40 bpm) or hypokalemia (<3.2mEq/L) or family history of long QT syndrome or familial history of unexplained sudden death or sick sinus syndrome or any clinically relevant cardiovascular disease;

New text:

- ~~Male subjects who are not willing to abstain from sexual intercourse without use of a condom/spermicide while taking the study drug and for at least 28 days after treatment with study drug.~~
- Presence of QTc≥ 430 ms, or a pronounced sinus bradycardia (<40 bpm) or hypokalemia (<3.2mEq/L) or family history of long QT syndrome or familial history of unexplained sudden death or sick sinus syndrome or any clinically relevant unstable cardiovascular disease or cardiovascular event (e.g. myocardial infarction, cerabrovascular accident…) occurred less than 6 months ago;
- Women who are pregnant or breastfeeding, or fertile women not practicing adequate methods of contraception (as defined in inclusion criteria); women planning to become pregnant within 1 month of the study.

Section Protocol Outline – Study schedule - Footnote of table 1 Overall flowchart (groups 1 to 4)

Reason for change : typographic error

Previous text:

3: Blood PK: Predose (before infusion); 10min(during infusion), 30min, (end of infusion), 40min, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h, 12h, 24h, post start of infusion, on D3 (48h postdose) for groups 2 to 4

New text:

3: Blood PK: Predose (before infusion); 15min(during infusion), 30min, (end of infusion), 45min, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h, 12h, 24h, post start of infusion, on D3 (48h postdose) for groups 2 to 4

Section 7.3.1 Collection schedule for biological samples -Table 4- Collection schedule for blood samples

Reason for change: typographic error. End of study visit is done between D3 and D7.

Previous text:

Table 4 – Collection schedule for blood samples

Groups 1 to 4

Day	Sampling time relative to dosing (h:min)	Lab. Safety (20mL)	Serology (7mL)	PK (2x2mL)
Screening		X	X	
Day 1		X		
Day 1	0:30min			X
	00:15			X
	00:30			X
	00:45			X
	01:00			X
	01:30			X
	02:00			X
	03.00			X
	04:00			X
	06:00			X
	08:00			X
	12.00			X
D2 before discharge	24:00	X		X
D5 to D7 (End of study) Ambulatory in groups 2 to 4	48:00	X		X

1: PK also on Day 3 (except Group 1)

New text:

Table 4 – Collection schedule for blood samples

Groups 1 to 4

Day	Sampling time relative to dosing (h:min)	Lab. Safety (20mL)	Serology (7mL)	PK (2x2mL)
Screening		X	X	
Day 1		X		
Day 1	0:30min			X
	00:15			X
	00:30			X
	00:45			X
	01:00			X
	01:30			X
	02:00			X
	03.00			X
	04:00			X
	06:00			X
	08:00			X
	12.00			X
D2 before discharge	24:00	X		X
D3 to D7 (End of study) Ambulatory in groups 2 to 4	48:00	X		X

1: PK also on Day 3 (except Group 1)

Section 7.3.2 Measurement schedule for other study variables –table 6

Reason for change: Typographic error. Local tolerance assessment has to be performed at 24h.

Previous text

Table 6 - Measurement schedule for other study variables (groups 1to 4)

	Time relative to dosing (h:min)	Vital signs[a]	12lead ECG	AE questioning	Local tolerance	Physical examination
Screening		X	X			X
Day –1		X	X			X
Day 1	-0 :30 (predose)	X	X		X	
Start Infusion = TO						
End infusion = 0:30		X	X		X	
	1:00	x	x		X	
	4:00	X				
	6:00	X				
	12:00	X				
Day 2	24:00	X	X			X
End of study D 3 to D 7 post last dosing		X	X			X

Vital signs will be measured 10 min supine, 3 min standing.

New text

Table 6 - Measurement schedule for other study variables (groups 1to 4)

	Time relative to dosing (h:min)	Vital signs[a]	12lead ECG	AE questioning	Local tolerance	Physical examination
Screening		X	X			X
Day –1		X	X			X
Day 1	-0 :30 (predose)	X	X		X	
Start Infusion = TO						
End infusion = 0:30		X	X		X	
	1:00	x	x		X	
	4:00	X				
	6:00	X				
	12:00	X				
Day 2	24:00	X	X		X	X
End of study D 3 to D 7 post last dosing		X	X			X

Vital signs will be measured 10 min supine, 3 min standing.

Section 7.4 General and dietary restrictions

Reason for change: Typographic error.

Previous text
On the screening day, subjects will come to the unit after an overnight fast of at least 10 hours

New text
On the screening day, subjects will come to the unit after an overnight fast of at least 4 hours