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# CLINICAL STUDY PROTOCOL

## A Prospective, Multicenter, Double-blind, Randomized, Comparative Study to estimate the safety, tolerability and efficacy of NXL104/ceftazidime plus metronidazole vs. meropenem in the treatment of complicated intra-abdominal infections in hospitalized adults

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**References :**

Sponsor Number : NXL104/2002

IND Number : 101,307

EudraCT Number : 2008-005604-54

**Multicenter study**

[redacted]

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|---|---|---|---|
| **Date of issue:** | 09/08/08 | **Document number:** | NXL104/2002<br>Version 1.0 |

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# PROTOCOL OUTLINE

**Study number** NXL104/2002

**Title**

A prospective, multicenter, double-blind, randomized, comparative study to estimate the safety, tolerability and efficacy of NXL104/ceftazidime plus metronidazole vs. meropenem in the treatment of complicated intra-abdominal infections (cIAI) in hospitalized adults

**Investigator(s), study site(s):** Multicenter

| **Indication** | Complicated Intra-abdominal Infection | **Phase** | II |
|---|---|---|---|

## Objectives

Primary Objective:

To estimate the efficacy of NXL104/ceftazidime plus metronidazole with respect to the clinical response in baseline microbiologically evaluable patients with cIAI at the Test of Cure (TOC) visit, 2 weeks post-treatment, compared to meropenem.

Secondary Objectives:

1. To evaluate the safety and tolerability profile of NXL104/ceftazidime plus metronidazole in the treatment of cIAI in adults.
2. To estimate the efficacy of NXL104/ceftazidime plus metronidazole with respect to the clinical response in baseline microbiologically evaluable patients with cIAI at the end of IV therapy and at the late follow-up visit at 4 to 6 weeks post-treatment compared to meropenem.
3. To estimate the clinical response of NXL104/ceftazidime plus metronidazole at the end of IV therapy, at the Test of Cure visit, and at the late follow up visit, 4 to 6 weeks post therapy compared to meropenem.
4. To estimate the microbiological response of NXL104/ceftazidime plus metronidazole in with cIAI at the end of IV therapy, at the Test of Cure visit, and at the late follow-up 4 to 6weeks post-therapy, compared to meropenem.

## Study design

This is a prospective, multicenter, double-blind, randomized, two arm, parallel group (1:1) study to estimate the efficacy, safety, and tolerability of NXL104/ceftazidime plus metronidazole vs. meropenem in the treatment of adults with cIAI. Complicated IAI are the infections requiring surgical intervention and which extend beyond the hollow viscus into the peritoneal space. (The

minimum duration of therapy is 5 days, and the suggested maximum duration of therapy is 14 days). Each patient is expected to complete the study, including follow-up, within approximately 8 weeks. The entire study duration is expected to be approximately 1 year.

Patients will be stratified by baseline severity of disease (Apache II score $\leq$ 10, and > 10 but < 25) and randomized 1:1 to ceftazidime/NXL104 plus metronidazole or meropenem. Study medication includes 500mg NXL104/2000mg ceftazidime plus metronidazole 500mg that will be given intravenously every 8hr and 1000mg meropenem that will be given intravenously every 8hr. In order to maintain blinding, a placebo to metronidazole (100mL 0.9% saline) will be administered every 8 hours to patients randomized to meropenem. After at least 5 days of parenteral therapy, if clinical improvement is clearly demonstrated (the patient is afebrile for $\geq$ 24 hours, WBC < 12,500/µL, and oral intake and bowel function has resumed) IV antimicrobial therapy may be discontinued at the discretion of the investigator. If antibiotic therapy is required beyond 14 days, the medical monitor should be contacted.

Approximately 200 hospitalized adult patients (18 to 65 years of age) with a presumed (preoperative) or definitive (intraoperative or postoperative) diagnosis of cIAI will be enrolled. Diagnosis of infection will be based on the patient's clinical syndrome and intraoperative findings, including intraoperative cultures. Operative intervention includes open laparotomy, laparoscopic procedure, and percutaneous drainage procedure. All patients will undergo a preliminary evaluation within the 24 hour period prior to initiation of intravenous study antibiotic therapy.

An overall clinical assessment, vital signs, and detailed abdominal assessment will be performed at baseline, daily during study therapy, at the discontinuation of study therapy, at the Test of Cure visit (2 weeks post-antibiotic therapy), and at the late follow-up (4 to 6 weeks post-antibiotic therapy). The investigator is responsible for assessing the patient's response to therapy, determining the appropriate duration of IV therapy, and assessing the relationship of adverse events to study therapy.

The primary efficacy assessment is the clinical response in the microbiologically evaluable population at the Test of Cure visit, 2 weeks post-therapy.

## Number of subjects

A total of approximately 200 patients will be enrolled, 100 patients in each treatment arm.

## Treatments

Following a diagnosis of cIAI, written informed consent will be obtained prior to any study related procedures being performed. Patients will be randomized to 1 of the 2 study regimens in a 1:1 ratio according to the central allocation/randomization schedule. Patients will be stratified for balance by disease severity (Apache II score) prior to randomization:

Stratum 1: $\leq$ 10, or
Stratum 2: > 10 and < 25

Study treatments:

- 500mg NXL104/2000mg ceftazidime IV q 8hr plus 500mg metronidazole IV q 8hr
- 1000mg meropenem IV q 8hr

## Efficacy data

- Clinical : Infection-related signs and symptoms
- Microbiological: cultures from intra-abdominal sites and blood cultures and in vitro susceptibility testing to NXL104/ceftazidime plus metronidazole and meropenem

## Safety data

Safety will be evaluated on the basis of:

- Adverse events based on spontaneous reporting, questioning by the study staff and review of the medical record
- Local tolerability
- Vital signs: blood pressure, heart rate.
- Physical examinations
- ECG parameters from 12 lead-ECGs: QRS, PR, RR, HR, QT, corrected QT (Bazett and Fridericia formula)
- Laboratory safety: biochemistry, hematology, and urinalysis

## Pharmacokinetic data

Blood samples for population pharmacokinetics will be collected. Five samples will be taken from each patient during the course of therapy within four pre-defined time windows.

## Statistical procedures

Microbiological and clinical outcome data will be summarized and compared by treatment group using descriptive statistics. Efficacy outcomes will be compared using the appropriate statistical tests at the 0.05 significance level. No adjustment for multiplicity will be made as this is an early phase study. Treatment emergent adverse events, laboratory abnormalities and all safety data will be summarized by treatment group with descriptive statistics as appropriate.

## Study Duration and Dates

The duration of this study is expected to be 12 months with recruitment to start in October 2008. The actual overall study duration and subject recruitment may vary.

| Procedures/Assessments | Eligibility screening | Study antibiotic treatment period | | Follow-up period | |
|---|---|---|---|---|---|
| | **< 24 hours prior to study therapy** | **During antibiotic therapy** | **End of IV therapy** | **2 wk follow-up** | **4 to 6 wk follow-up** |
| Informed consent | **X** | | | | |
| Inclusion/exclusion criteria | **X** | | | | |
| Randomization | **X** | | | | |
| Medical history | **X** | | | | |
| Description of operative procedures [a] | **X** | | | | |
| APACHE II score | **X** | | | | |
| Physical examination | **X** | | **X** | **X** | **X** |
| Vital signs and Temperature | **X** | **daily** | **X** | **X** | **X** |
| Abdominal signs and symptoms plus wound exam postoperatively | **X** | **daily** | **X** | **X** | **X** |
| Blood and urine for safety analysis | **X** | **Every 3 days** | **X** | **X** | **X** |
| ECG | **X** [b] | | **X** | **X** | |
| Serum β hCG for women of childbearing potential | **X** | | | **X** | **X** |
| Adverse events | **X** | **daily** | **X** | **X**[c] | |
| Local tolerability | | **daily** | **X** | | |
| Prior and concomitant medications | **X** | **Daily** | **X** | **X** | **X** |
| Culture from site of abdominal infection [d] | **X** | **As clinically indicated** | | | |
| Blood cultures | **X** | **As clinically indicated** | | | |
| Clinical outcome assessment | | | **X** | **X** | **X** |
| Investigator case summary/operative note/hospital discharge summary | | | | | **X** |
| Microbiological outcome assessment | | | **X** | **X** | **X** |
| Blood for PK analysis | | **X**[e] | **X** [e] | | |

a. with study entry and any subsequent procedure
b. ECG should be performed prior to dosing on day 1
c. monitor for adverse events for 14 days after the completion of study therapy
d. these assessments will be used to determine microbiological outcome
e. schedule for PK samples should be defined at study entry according to schedule in section 7.3

# ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| AE | Adverse event |
| AIDS | Acquired Immune Deficiency Syndrome |
| ALT | Alanine amino transferase |
| AP | Alkaline phosphatase |
| AST | Aspartate amino transferase |
| ATCC | American Type Culture Collection |
| AUC | Area under the curve |
| b-hCG | Beta Human Chorionic Gonadotrphin |
| BP | Blood pressure |
| CAZ | Ceftazidime |
| CE | Clinically evaluable |
| cIAI | Complicated intra-abdominal infection |
| CLSI | Clinical and Laboratory Standards Institute |
| CNS | Central Nervous System |
| CRF | Case Report Form |
| CT | Clinical Trial |
| ECG | Electrocardiogram |
| EOT | End of therapy |
| ESBL | Extended spectrum β-lactamase |
| GLM | Generalized linear model |
| HIV | Human Immunodeficiency Virus |
| HR | Heart Rate |
| IAI | Intra-Abdominal Infection |
| $IC_{50}$ | Concentration showing 50% inhibitory effect |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| IVRS | Interactive Voice Response System |
| ME | Microbiologically evaluable |
| MIC | Minimum inhibitory concentration |
| mITT | Modified intent-to-treat |
| mMITT | Microbiological modified intent to treat |
| MRI | Magnetic Resonance Image |
| MRSA | Methicillin Resistant Staphylococcus aureus |
| PK | Pharmacokinetics |
| PK/PD | Pharmacokinetics/Pharmacodynamics |
| PMN | Polymorphonuclear leukocyte |
| PP | Per protocol |
| PR | PR interval |
| PT | Prothrombin time |
| QC | Quality Control |

| | |
|---|---|
| QT | QT interval |
| RBC | Red blood cell |
| SAE | Serious adverse event |
| SGPT/ALT | Alanine Amino Transferase |
| SGOT/AST | Aspartate Amino Transferase |
| TEAE | Treatment Emergent Adverse Effect |
| TOC | Test of Cure |
| ULN | Upper Limit Normal |
| WBC | White blood cells |

# 1 INTRODUCTION AND STUDY RATIONALE

## 1.1 BACKGROUND ON THE PRODUCT

### 1.1.1 Problem statement and Justification for development

Complicated intra-abdominal infections are local or systemic infections that occur as a result of a perforation in the gastrointestinal tract or by necrotic gut wall into the peritoneal space, leading to abscess formation or generalized peritonitis. These infections require operative intervention or percutaneous drainage in conjunction with broad spectrum antibacterial therapy. Adequate surgical source control is critical to successful treatment of intra-abdominal infections. Other important determinants of outcome include age, nutritional status, underlying comorbidities (e.g., cardiovascular disease, diabetes, and malignancy), severity and extent of infection, and in particular APACHE II score [1, 2]. Almost all intra-abdominal infections are polymicrobial and are caused by organisms from the gastrointestinal tract, including aerobes and facultative and obligate anaerobes. Enterobacteriaceae are isolated most commonly.

The 2002 Guidelines from the Therapeutic Agents Committee of the Surgical Infection Society [1] and the 2003 Guidelines of the Infectious Diseases Society of America (IDSA) [2] recommend broad-spectrum single agent (beta lactam/beta lactamase inhibitor, carbapenem) or combination therapy regimens (metronidazole plus cephalosporin or aztreonam or fluoroquinolone). Specific regimens are recommended for higher risk patients with severe or postoperative nosocomial intra-abdominal infections where resistant pathogens such as Enterococcus or Pseudomonas may occur. Initial empiric therapy is critical because inappropriate treatment may be associated with delays in clinical response, increases in hospital stay, and an increased risk of mortality [3, 4].

The prevalence of multidrug resistance (MDR) strains among Gram-negative bacilli is increasing [resistance to at least 3 different antibiotic groups] [5, 11-13]. 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 [7, 14].

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* [17]. 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 [9].

*Serratia, Morganella, Providencia, Enterobacter, Citrobacter freundii and P. aeruginosa* have similar, although not identical, chromosomal *ampC* β-lactamase genes that are inducible [9-17]. 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* [9].

The most common mechanism of resistance to β–lactam antibiotics among Gram-negative pathogens is due to ESBLs (Extended Spectrum β-Lactamases): These enzymes are plasmid-mediated β-lactamases of

ses 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*, and non-fermenters *P. aeruginosa*, and *Burkholderia cepacia* [15, 16]. 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 [10].

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 [9]. 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 [19-22].

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* [9]. 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 [19].

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-lactamase 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 clinically useful 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 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).

NXL104 (formerly AVE1330) is a novel non β-lactam β-lactamase inhibitor with a spectrum covering both Ambler class A ESBLs and class C (AmpC) enzymes. β-lactamase inhibition is effected through the formation of a stable covalent carbamoyl linkage in the acyl enzyme complex.

### 1.1.2 Antimicrobial Activity

NXL104 (formerly AVE1330) 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.

***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 producing strains, including ESBL producers and strains producing 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.

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
- Effective bacterial clearance in murine pyelonephritis 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).

Details can be found in the investigator brochure (40).

### 1.1.6 Human experience - Phase I

Single Dose Study (NXL104/1001)
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.

Multiple

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

Effect of Age and Gender (NXL104/1004)

A study to evaluate the effect of age and gender on the pharmacokinetics and safety of NXL104 in healthy volunteers (NXL104/1004) is ongoing in the USA. This is a single dose study of 500mg NXL104 in 32 healthy subjects in 4 cohorts of 8 subjects each (young men, young women, elderly men and elderly women. Dosing and safety follow-up for the first 2 cohorts

young women) are complete and preliminary safety and PK data are availab

Pharmacokinetics of NXL104 in Normal Subjects and Patients with Varying Degrees of Renal Impairment (NXL104/1003)

This is an open-label, parallel group study designed to evaluate the pharmacokinetics of 100mg NXL104 in healthy subjects and 4 cohorts of patients with renal impairment (mild; moderate; severe but not on dialysis; and end stage renal disease on hemodialysis). A total of 30 subjects (6 in each cohort) will be enrolled. Patients on hemodialysis will participate in two randomized sessions, dialysis and inter-dialysis, separated by a washout of 7 to 14 days. The study is ongoing at 2 sites in Belgium but no data are available yet.

Phase II Study of NXL104/ceftazidime vs. imipenem cilastatin in the treatment of complicated urinary tract infections (cUTI) in adults (NXL104/2001)

This is the first Phase II study of NXL104/ceftazidime designed to evaluate the efficacy, safety and tolerability compared to imipenem cilastatin in cUTI. The study will include ~ 150 patients at 40 sites in the US and Lebanon. The primary efficacy endpoint is the microbiological response in the microbiologically evaluable population at 5-9 days post-therapy. This study is ongoing.

### 1.1.7 Rationale for the Study

NXL104 is a novel non-β lactam, β lactamase inhibitor with a spectrum of activity encompassing Ambler Class A and Class C β lactamases, including Class A carbapenemases such as KPC. It is being developed as an intravenous formulation in combination with ceftazidime for the treatment of Gram negative bacterial infections. The strategy of using β lactam, β lactamase inhibitor combinations has been successful against class A β lactamases in a variety of bacterial infections.

Gram negative pathogens, including those producing ESBLs, are important causes of intra-abdominal infections (cIAI). Infections are typically polymicrobial, involving anaerobes such as *B. fragilis* group. The spectrum of activity of NXL104/ceftazidime when combined with metronidazole is well suited to treatment of pathogens commonly responsible for cIAIs. Meropenem has been selected as the comparator, because it has excellent efficacy against the Gram positive, gram negative and anaerobic pathogens isolated in cIAIs, it is approved for and has been used widely for the treatment of cIAIs, and carbapenems are the drugs of choice against ESBL producing Gram negative pathogens.

### 1.1.8 Rationale for Dose Selection

NXL104 is a novel, non β-lactam, β-lactamase inhibitor with a spectrum of activity that includes enzymes of major clinical importance. NXL104 acts by forming a very stable, practically irreversible binding to these enzymes with a lower inhibition $IC_{50}$ (concentration needed to inhibit 50% of enzyme activity) than the currently marketed β-lactamase inhibitors clavulanic acid, tazobactam, and sulbactam. Unlike currently available β-lactamase inhibitors, NXL104 does not induce β-lactamase production. NXL104 has virtually no intrinsic antibacterial activity.

To determine the appropriate β-lactam for use in combination with NXL104, in vitro susceptibility testing was performed by standard CLSI methodology against panels of Gram-negative pathogens with known mechanisms of resistance. Agents tested in combination with NXL104 included ceftazidime, ceftriaxone, piperacillin, cefepime, meropenem and imipenem. NXL104 effectively reduced the MICs of the β-lactams tested against most of the isolates resistant to a given drug. However, susceptibility was more consistently restored with ceftazidime or ceftriaxone than piperacillin. There were fewer isolates with baseline resistance to cefepime or the carbapenems so the overall effect of adding NXL104 was less pronounced. In these studies, NXL104 when associated with ceftazidime was also shown to be active against strains that express a combination of β-lactamase types as well as strains that are concomitantly resistant to other antibacterial classes such as fluoroquinolones and aminoglycosides.

Other data supporting the selection of ceftazidime as the β-lactam partner for NXL104 include the similarity in PK profiles and the lack of interaction of ceftazidime on NXL104 pharmacokinetics. In addition, the PK/PD of NXL104 was evaluated in an in vitro hollow fiber model where varying dosing regimens of NXL104 were administered in the presence of a constant concentration of ceftazidime. Results with two ceftazidime-resistant *K. pneumoniae* strains and one resistant strain of *E. cloacae*

showed that NXL104 has time-dependent activity, further supporting use in a multiple daily dose regimen.

Another component of the *in vitro* susceptibility testing experiments was to evaluate the ratio of β-lactam antibiotic to NXL104. Ratios of 2/1 to 16/1 (β-lactam: NXL104) were tested. Most of the data were generated with ceftazidime. A small number of gram negative isolates with high baseline MICs were not restored to susceptible when the ratio of 16/1 was used; ceftazidime susceptibility was restored with both 8/1 and 4/1, with MICs generally marginally lower with the 4/1 ratio. In the experiments where 2/1 was tested, there did not appear to be any significant advantage of the 2/1 over the 4/1 ratio. Different ratios of β-lactam to β-lactamase inhibitor were also studied in murine models of systemic and localized infection with resistant Gram-negative pathogens. In these studies, ceftazidime plus NXL104 in a 4/1 ratio performed as well as or better than other ratios and other combinations.

In summary, across the studies performed NXL104 restored ceftazidime activity against ceftazidime-resistant strains expressing various class A and class C β-lactamases both *in vitro* and *in vivo* (murine pneumonia, septicemia and pyelonephritis, and rabbit meningitis). These experiments have also shown that the combination is effective at a ceftazidime / NXL104 weight/weight ratio of 4/1 and this is the ratio that will be evaluated in clinical trials.

The combination of NXL104 and ceftazidime is being developed for the treatment of serious Gram-negative infections, especially those due to multi-drug resistant strains and extended spectrum β-lactamase producers. The dose of ceftazidime approved for the treatment of serious Gram negative infections is 2g IV every 8 hours. Thus, for the Phase II study in complicated IAI the dosing regimen selected is 2000mg ceftazidime plus 500mg NXL104 IV every 8 hours.

Meropenem is approved for and widely used as treatment for complicated intra-abdominal infections and carbapenems are considered the drugs of choice for treating infectons due to ESBL-producing Gram negative bacilli. The usual dose of meropenem for adults with cIAI is 1g IV every 8 hours. This is the dosing regimen of meropenem that will be used in this study.

# 2 STUDY OBJECTIVES

## 2.1 PRIMARY OBJECTIVE

To estimate the efficacy of NXL104/ceftazidime plus metronidazole with respect to the clinical response in baseline microbiologically evaluable patients with cIAI at the Test of Cure (TOC) visit, 2 weeks post-treatment, compared to meropenem.

## 2.2 SECONDARY OBJECTIVES

1. To evaluate the safety and tolerability profile of NXL104/ceftazidime plus metronidazole in the treatment of cIAI in adults.
2. To estimate the efficacy of NXL104/ceftazidime plus metronidazole with respect to the clinical response in baseline microbiologically evaluable patients with cIAI at the end of IV therapy and at the late follow-up 4 to 6 weeks post-therapy compared to meropenem.
3. To estimate the clinical response of NXL104/ceftazidime plus metronidazole in clinically evaluable patients with cIAI at the end of IV therapy, at the Test of Cure visit, and at the late follow-up 4 to 6 weeks post therapy, compared to meropenem.
4. To estimate the microbiological response of NXL104/ceftazidime plus metronidazole in patients with cIAI at the end of IV therapy, at the Test of Cure visit, and at the late follow-up 4 to 6 weeks post therapy, compared to meropenem.

# 3 STUDY DESIGN, DURATION AND DATES

## 3.1 STUDY DESIGN

This is a multicenter, double-blind, randomized, two arm, parallel group (1:1) study to evaluate the efficacy, safety, and tolerability of NXL104/ceftazidime plus metronidazole vs. meropenem in adults with cIAI. cIAI are those requiring surgical intervention and which extend beyond the hollow viscus into the peritoneal space.

Approximately 200 hospitalized adult patients (18 to 65 years of age) with a presumed (preoperative) or definitive (intraoperative or postoperative) diagnosis of cIAI will be enrolled. Diagnosis of infection will be based on the patient's clinical syndrome and intraoperative findings, including intraoperative cultures. Operative intervention includes open laparotomy, laparoscopic procedure, and percutaneous drainage procedure. All patients will undergo a preliminary evaluation within the 24 hour period prior to initiation of intravenous study antibiotic therapy.

An overall clinical assessment, vital signs, and detailed abdominal assessment will be performed at baseline, daily during study therapy, at the discontinuation of study therapy, at the early follow-up or Test of Cure visit (2 weeks) post-antibiotic therapy, and at the late follow-up visit (4 to 6 weeks post-antibiotic therapy). The blinded investigator is responsible for assessing the patient's response to therapy, determining the appropriate duration of IV therapy, and assessing the relationship of adverse events to study therapy.

The primary efficacy assessment is the clinical response in the microbiologically evaluable population at the Test of Cure visit, 2 weeks post-therapy.

Duration

Each patient will complete the study, including follow-up, within approximately 8 weeks. The minimum duration of therapy is 5 days, and the suggested maximum duration of therapy is 14 days. After at least 5 days of parenteral therapy, if clinical improvement is clearly demonstrated (the patient is afebrile for > 24 hours, WBC < 12500/μL, and oral intake and bowel function have resumed) IV antimicrobial therapy may be discontinued at the discretion of the investigator. If antibiotic therapy is required beyond 14 days, the sponsor should be contacted. The overall study duration will be approximately 1 year.

Randomization

Patients will be stratified by baseline severity of disease (Apache II score $\leq$ 10, and > 10 but < 25) and randomized 1:1 to ceftazidime/NXL104 plus metronidazole or meropenenm. Study medication includes 500mg NXL104/2000mg ceftazidime plus 500mg metronidazole given intravenously every 8hr and 1000mg meropenem given IV every 8hr.

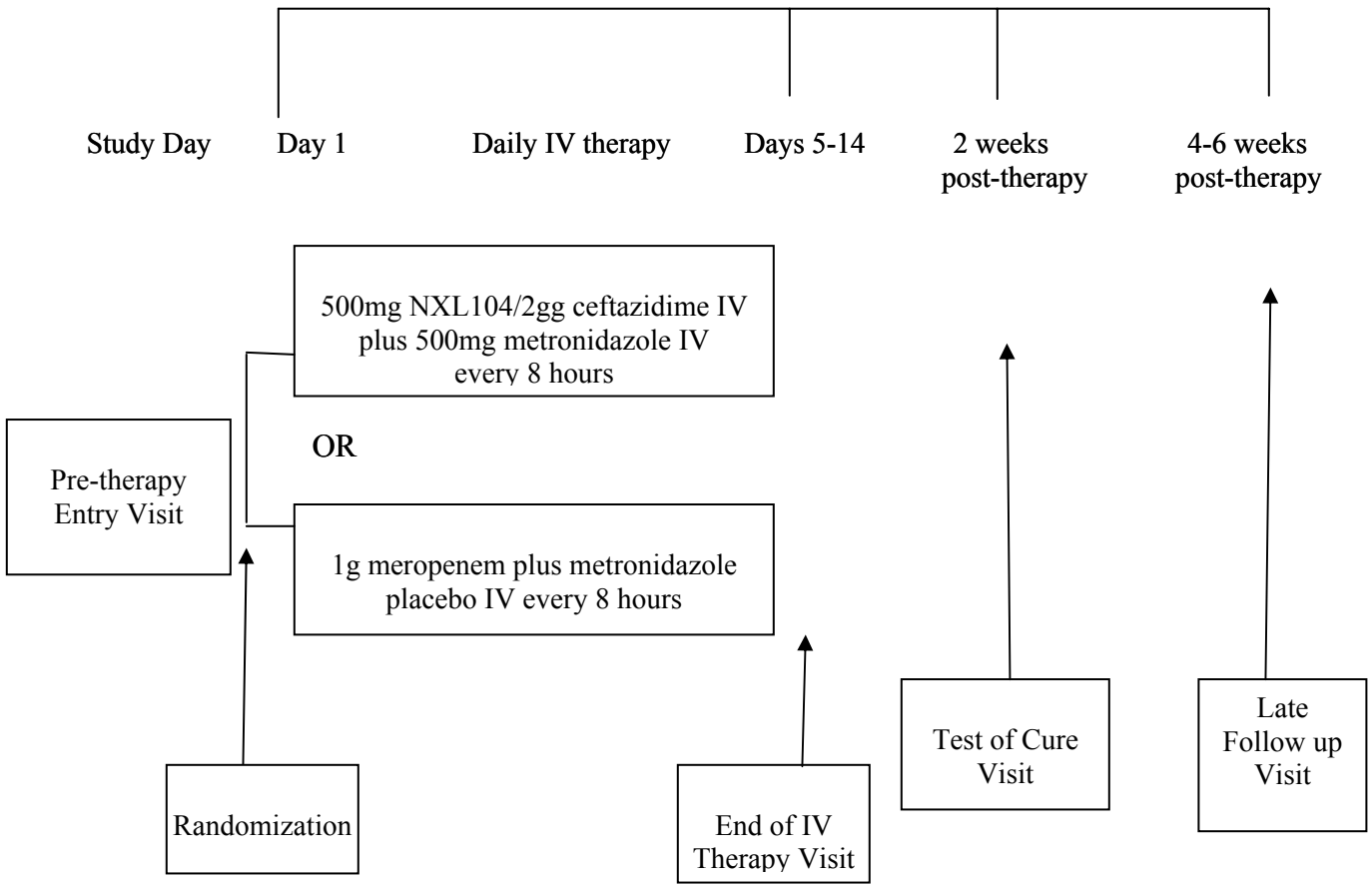
Blinding

This is a double-blind study where each patient will receive six infusions of study drug daily. To maintain blinding, the patients randomized to meropenem will receive a metronidazole placebo (100mL 0.9% saline) IV every 8 hr.

Primary Endpoint

The primary efficacy assessment is the clinical response in the microbiologically evaluable population at the Test of Cure visit, 2 weeks post-therapy.

The following diagram illustrates the study design:



## 3.2 STUDY DURATION AND DATES

The duration of this study is expected to be 12 months, with subject recruitment proposed to start in October 2008. The actual overall study duration and subject recruitment period may vary.

# 4 SELECTION OF SUBJECTS

## 4.1 NUMBER OF SUBJECTS

As described in *Section 11.5*, 200 subjects will be enrolled and treated in this study. It is planned to recruit this sample in approximately 40 centers.

Adult patients may be enrolled intra- or post-operatively on the basis of operative findings OR preoperatively on the basis of compelling preoperative clinical findings as described below:

## 4.2 INCLUSION CRITERIA

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

Subjects must meet all of the following criteria to be considered for admission to the study:

**1. 18 to 65 years of age**

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

- Has been surgically sterilized or post menopausal 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) within 1 day prior to enrollment.
  - must be willing to practice double barrier methods of birth control (e.g., condoms or diaphragms together with spermicidal foam or gel) 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.

**2. Intraoperative/postoperative enrollment**

Patients may be enrolled intraoperatively or postoperatively upon visual confirmation (presence of pus within the abdominal cavity) of an intra-abdominal infection. Surgical intervention includes open laparotomy, percutaneous drainage of an abscess, or laparoscopic surgery.

Diagnoses considered eligible for this study are those in which there is evidence of intraperitoneal infection. The patient must have one of the following diagnoses:

a. cholecystitis with gangrenous rupture or perforation or progression of the infection beyond the gallbladder wall
b. diverticular disease with perforation or abscess
c. appendiceal perforation or peri-appendiceal abscess
d. acute gastric and duodenal perforations, only if operated on > 24 hours after perforation occurs
e. traumatic perforation of the intestines, only if operated on > 12 hours after perforation occurs
f. secondary peritonitis (but not spontaneous bacterial peritonitis associated with cirrhosis and chronic ascites)
g. intra-abdominal abscess (including of liver and spleen)

AND

Specimens from the surgical intervention are sent for culture and susceptibility testing

AND

Infection is caused or presumed to be caused by mircroorganisms susceptible to the intravenous study medications (ceftazidime/NXL104 plus metronidazole or meropenem)

**Note:** 1) infections limited to the hollow viscus, such as simple cholecystitis and simple appendicitis, are not eligible. Ischemic bowel disease without perforation is not eligible. Acute suppurative cholangitis and acute necrotizing pancreatitis are not eligible. 2) Postoperative (or intraoperative) enrollment of patients is encouraged. If, however, preoperative data are available that strongly suggest an appropriate diagnosis for entry (e.g., rupture of intraperitoneal abscess on CT or MRI), then these patients may be enrolled preoperatively.

**3. For Preoperative Enrollment**

The following clinical criteria must be met, and the patient's infection must be confirmed by a surgical intervention within 24 hours of entry:

a. Evidence of systemic inflammatory response, with at least one of the following:
   1) Fever (temperature $\geq$ 37.8°C; $\geq$ 38°C tympanic; $\geq$ 38.3°C rectal; or hypothermia with a core body temperature < 35°C
   2) Elevated WBC ($\geq$ 10,500/mm$^3$)
   3) Drop in blood pressure (however, systolic BP must be $\geq$ 90 mm Hg without pressor support)
   4) Increased pulse (HR > 90) and respiratory rates (> 20)
   5) Hypoxemia
   6) Altered mental status

AND

b. Physical findings consistent with Intra-abdominal infection, such as:
   1) Abdominal pain and/or tenderness, with or without rebound
   2) Localized or diffuse abdominal wall rigidity
   3) Mass
   4) Ileus

AND

c. Supportive radiologic imaging findings of intra-abdominal infection such as perforated intraperitoneal abscess detected on CT scan, MRI, or ultrasound

AND

d. requirement for surgical intervention, including open laparotomy, percutaneous drainage of an abscess, or laparoscopic surgery;

AND

e. Specimens from the surgical intervention are sent for culture and susceptibility testing

AND

f. Infection is caused or presumed to be caused by mircroorganisms susceptible to the intravenous study medications (ceftazidime/NXL104 plus metronidazole or meropenem)

## 4.3 EXCLUSION CRITERIA

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

1. Patient diagnosed with traumatic bowel perforation with surgery within 12 hours; perforation of gastroduodenal ulcers with surgery within 24 hours. Other intra-abdominal processes in which the primary etiology is not likely to be infectious.
2. Patient with abdominal wall abscess or small bowel obstruction without perforation or ischemic bowel without perforation.
3. Patient with simple cholecystitis; or gangrenous cholecystitis without rupture; or simple appendicitis; or acute suppurative cholangitis; or infected necrotizing pancreatitis or pancreatic abscess
4. Patient whose surgery will include staged abdominal repair, or "open abdomen" technique, or marsupialization.
5. Patient known at study entry to have intra-abdominal infections that are caused by pathogens resistant to the study antimicrobial agents.
6. Patient with evidence of sepsis with shock not responding to intravenous fluid challenge or anticipated to require the administration of vasopressors for $\geq$ 12 hours.
7. Patient with perinephric infections.
8. Female patient with infection of the genital tract.
9. Patient with indwelling peritoneal catheter.
10. Patient with history of serious allergy, hypersensitivity (e.g., anaphylaxis), or any serious reaction to carbapenem or cephalosporin antibiotics or other beta lactam antibiotics.
11. Patient with APACHE II score > 25 (see appendix 6).
12. Patient who is considered unlikely to survive the 6- to 8-week study period.
13. Patient who is unlikely to respond to 5 to 14 days of antibiotic therapy.
14. Patient with rapidly progressive or terminal illness, including acute hepatic failure or respiratory failure.
15. Male patient who is not willing to abstain from sexual intercourse with a fertile woman without use of a condom/spermicide while taking the study drug and for at least 90 days after treatment with study drug.
16. Female patient who is pregnant or breastfeeding, or fertile woman not practicing adequate methods of contraception (as defined in inclusion criteria); or planning to become pregnant within 1 month of the study.
17. Patient who received systemic antibacterial agents within the 72-hour period prior to study entry, unless either of the following pertains:
    - Patient treated with nonstudy systemic antibiotic consisting of postoperative (or postdrainage) therapy of no more than 24 hours of an appropriate antimicrobial regimen for patients not considered to have failed a previous regimen;
    - Patient is considered to have failed the previous treatment regimen. In this case, preoperative treatment of any duration with nonstudy systemic antimicrobial therapy for peritonitis or abscess is permitted provided that;
        a) the treatment regimen has been administered for at least 72 hours and is thought to have been inadequate
        b) findings of infection were documented at surgery
        c) operative intervention is intended no more than 24 hours after study entry
        d) specimens for bacterial cultures and susceptibility testing are taken at operative intervention
        e) no further nonstudy antibacterials are administered after enrollment
18. Patient who needs effective concomitant systemic antibacterials (other than vancomycin for documented Methicillin Resistant *S. aureus* or Enteroccal infections) in addition to those designated in the 2 study groups.
19. Patient with concurrent infection that may interfere with the evaluation of response to the study antibiotic.
20. Patient with a BMI > 45 kg/m$^2$.
21. Patient with Hematocrit <30% or Hemoglobin <10 g/dL.

22. Patient with absolute neutrophil count (ANC) less than 1500/$mm^3$. Patient with ANC as low as 1000/$mm^3$ may be enrolled if this is directly related to the acute infection.
23. Patient with Platelet count <100,000/$mm^3$.
24. Patient with Coagulation (prothrombin time [PT] and partial thromboplastin time [PTT] and/or INR) tests >1.5 times the upper limit of the range of normal values (ULN) used by the laboratory performing the test. Patients who are on anticoagulant therapy with values >1.5 times ULN may be enrolled provided these values are stable within the therapeutic range.
25. Patient with an estimated creatinine clearance < 50mL/min by Cockcroft-Gault formula. If a patient is dehydrated he/she should be rehydrated and creatinine re-measured before calculating creatinine clearance.
26. Patient with abnormal liver function:
    a. Alanine transaminase (ALT), Aspartate transaminase (AST) > 3 times ULN values used by the laboratory performing the test. Patients with elevations of AST and/or ALT up to 5 times ULN are eligible if these elevations are acute and directly related to the infectious process being treated. This must be documented.
    b. Bilirubin >3.0 times ULN, unless isolated hyperbilirubinemia is directly related to the acute infection or known Gilbert's disease.
    c. Alkaline Phosphatase >3.0 times ULN. Patients with values >3.0 times ULN and <5.0 times ULN are eligible if this value is historically stable.
    d. Acute hepatitis, chronic hepatitis, cirrhosis, acute hepatic failure, or acute decompensation of chronic hepatic failure should be excluded.
27. Immunocompromised patient, such as:
    a. HIV infection, with either an AIDS-defining condition (e.g., Kaposi's sarcoma, *Pneumocystis carinii* pneumonia) or a CD4+ T-lymphocyte count < 200/$mm^3$
    b. metastatic or hematological malignancy requiring chemotherapeutic interventions
    c. splenectomized patient or patient with known hyposplenia or asplenia
    d. receiving maintenance corticosteroid therapy (> 20 mg/day equivalent prednisolone)
28. Patients who participated in any other clinical study that involves the administration of an investigational medication at the time of presentation, during the course of the study, or during the 30 days prior to study start.
29. Patient or legal representative unable to provide written informed consent for any reason.
30. Patient is in a situation or has a condition that, in the investigator's opinion, may interfere with optimal participation in the study.
31. Patient unlikely to comply with protocol, e.g., uncooperative attitude, inability to return for follow-up visits, and unlikelihood of completing the study.
32. Patient who has previously been treated with the investigational product (NXL104).
33. Patient with known inflammatory bowel disease.
34. Patient who has received more than one dose of ceftazidime for treatment of this infection.
35. Patient who had been previously enrolled in this study.

Any waiver of these inclusion and exclusion criteria must be approved by the sponsor on a case-by-case basis prior to enrolling the subject. This must be documented by both the sponsor and the investigator.

### 4.4 SUBJECTS OF REPRODUCTIVE POTENTIAL

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

- 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 (e.g., condoms or diaphragms together with spermicidal foam or gel) during treatment and for at least 28 days after dosing with study medication.

Men must be willing to abstain from sexual intercourse with a fertile woman without use of a condom/spermicide while taking the study drug and for at least 90 days after treatment with study drug.

# 5 STUDY TREATMENTS

## 5.1 DETAILS OF STUDY TREATMENTS

Patients will be randomized 1:1 to ceftazidime/NXL104 plus metronidazole OR meropenem. Study treatments will be prepared as outlined in Appendix 1.

**NXL104/ceftazidime plus metronidazole group**

**NXL104 powder for solution**

| | |
|---|---|
| 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 | 500mg NXL104 |
| Manufacturer | MP5 10, rue des boules 63200 Riom France |

**Ceftazidime powder for solution**

| | |
|---|---|
| Formulation | Vial containing 2000 mg of ceftazidime powder to be diluted with 9mL of water for injection to obtain a 200mg/mL solution for IV infusion |
| Doses | 2000 mg of ceftazidime |
| Manufacturer | Sandoz |

NXL104 and ceftazidime will be added to the same infusion bag according to the instructions outlined in Appendix 1 and administered together by intravenous infusion over 30 minutes in a constant volume of 100mL with an infusion rate of 3.33mL/min (or 200mL/hr).

**Metronidazole**

| | |
|---|---|
| Formulation | Metronidazole injection, USP RTU® in 100mL single dose plastic containers each containing an iso-osmotic, buffered solution of 500mg metronidazole |
| Doses | 500mg |
| Manufacturer | Baxter |

Metronidazole is provided in 100mL single use plastic containers each containing 500mg metronidazole. 500mg metronidazole should be administered IV over 60 minutes. Additives should not be introduced into metronidazole injection, USP RTU® (Appendix 5).

**Meropenem group**

**Meropenem**

| | |
|---|---|
| Formulation | 1 gram infusion vials |
| Doses | 1000mg in 100mL of compatible solution |
| Manufacturer | Astra Zeneca |

Meropenem is compatible with commonly used IV infusion fluids as outlined in the Merrem® package circular (Appendix 5) and is to be administered intravenously over 30 minutes. The placebo to metronidazole is 100mL of saline administered IV over 1 hour.

**Study drugs should not be physically mixed with or administered with any other drug other than as specified by protocol. Parenteral products should be visually inspected for particulate matter and discoloration prior to administration.**

### 5.2 DOSAGE SCHEDULE

Each patient will receive 6 intravenous infusions daily. Patients will be randomized to receive either 500mg NXL104/2000mg ceftazidime plus 500mg metronidazole IV q 8hr or meropenem 1000mg IV q 8hr. Patients randomized to meropenem group will receive placebo to metronidazole (100mL 0.9%saline) administered IV q 8hr as illustrated in the tables below.

**NXL104/ceftazidime plus metronidazole group**

| | | |
|---|---|---|
| 1st dose | 2 g Ceftazidime/ 500mg NXL104 in 100mL over 30 min. | 500mg metronidazole in 100mL over 1 hr |
| 2nd dose | 2 g Ceftazidime/ 500mg NXL104 in 100mL over 30 min. | 500mg metronidazole in 100mL over 1 hr |
| 3rd dose | 2 g Ceftazidime/ 500mg NXL104 in 100mL over 30 min. | 500mg metronidazole in 100mL over 1 hr |

**Meropenem group**

| | | |
|---|---|---|
| 1st dose | 1g Meropenem in 100mL over 30 min. | metronidazole placebo (100mL 0.9% saline over 1 hr) |
| 2nd dose | 1g Meropenem in 100mL over 30 min. | metronidazole placebo (100mL 0.9% saline over 1 hr) |
| 3rd dose | 1g Meropenem in 100mL over 30 min. | metronidazole placebo (100mL 0.9% saline over 1 hr) |

Details of the exact time of administration of medication (day/month/year, hr:min) will be documented in the drug accountability records and recorded on the case report form.

If *Enterococcus* spp. or methicillin resistant *S. aureus* (MRSA) is one of the pathogens isolated and specific therapy is indicated, in the opinion of the investigator, open-label vancomycin may be added to either of the study regimens according to the usual practice of the investigator. This will not affect the patient's evaluability. If vancomycin is started empirically it must be discontinued if MRSA or *Enterococcus spp.* are not isolated.

### 5.3 DURATION OF TREATMENT

Intravenous study antibiotic therapy will be continued for a period of time (minimum 5 full days; suggested maximum 14 full days) deemed appropriate by the investigator based upon fever and other signs and symptoms (described below) that demonstrate clear evidence of local and systemic improvement. These findings will be documented on the case report form for each patient.

The minimum duration of parenteral antibiotic therapy will be 5 days. After 5 days of study therapy and at the discretion of the investigator the study antimicrobial regimen may then be discontinued if the patient has shown clinical improvement by objective parameters such, including:

a) WBC is <12,500/µL.
b) Maximum temperature for the preceding 24 hours has been < 38°C orally without antipyretics or corticosteroids
c) Significant improvement of abdominal signs and symptoms
d) A return of bowel function and restoration of oral intake
e) No requirement for further antibiotic therapy

The need for continuation of therapy beyond 14 days should be discussed with the Sponsor.

### 5.4 TREATMENT ASSIGNMENT

Following a presumptive or definitive diagnosis of cIAI caused by microorganisms that are known or performing to be susceptible to the intravenous study antibiotics, written informed consent will be obtained prior to performing any study related procedures. Eligible patients will be randomized to 1 of the 2 study regimens in a 1:1 ratio according to the central allocation/randomization schedule.

Patients will be randomized by a central randomization process to ensure balance by severity of disease (Apache II score)

- Stratum 1: Apache II $\leq$ 10
- Stratum 2: Apache II > 10 and < 25

The pharmacist will obtain the patient's Apache II score from the clinical investigator's team,then will contact the IVRS and enter data. The IVRS will apply the central randomization algorithm to assign the patient to a treatment arm. The IVRS will select the drug vials to be dispensed from the site's inventory and report the unique vial identifiers. Study drug will be prepared by the study pharmacist as outlined in Appendix 1. Each patient must be given only the study medication matching the group to which he/she was randomized. The investigational products will be administered only to subjects included in this study following the procedures set out in the clinical study protocol.

Subjects withdrawn from the study retain their randomization number.

### 5.5 BLINDING, PACKAGING, AND LABELING

The investigational products will be packaged by
CRID Pharma
17, Parc des Vautes
39480, Saint-Gely-du-Fesc France

Vials will be labeled with the following information:

Protocol NXL104/2002
Vial number
Lot number
Patient number (to be filled in at site)
Vial of: (investigational drug specified as: NXL104 or ceftazidime or Merrem® or metronidazole)
Caution: New drug – limited by Federal law to investigational use
Keep out of reach of children
Sponsor: Novexel, Parc Biocitech, 102, Avenue Gaston Roussel, 93230 Romainville, France.

Each vial will be placed in a separate box that will be labeled with the following information:

Protocol NXL104/2002
Vial number
Lot number
Patient number (to be filled in at site)
Box containing 1 vial of (NXL104, ceftazidime metronidazole or meropenem)
Reconstitute according to protocol instructions
Store at 2 - 8°C
Caution: New drug – limited by Federal law to investigational use
Keep out of reach of children
Sponsor: Novexel, Parc Biocitech, 102 Avenue Gaston Roussel, 93230 Romainville, France.

NXL104 will be shipped to the investigator site at 2 - 8°C

Additional statements will be printed on the label(s) as required by local regulations.

The investigator must remain blinded to the I.V. study antibiotic regimen. In order to maintain this blind, assignment to treatment group and preparation of I.V. study antibiotics must be performed by someone other than the person who evaluates the patient for clinical response and determines if adverse experiences are related to study therapy. The study pharmacist or designee will receive open-labeled clinical supplies and following randomization of each patient, an unblinded confirmation listing the patient's assigned treatment. The confirmations are not to be furnished to anyone else involved with the study (i.e., investigator, patient). Treatments will be supplied to the investigator site in controlled quantities, on a schedule that reflects enrollment at the site.

### 5.6 SUPPLIES AND ACCOUNTABILITY

The study medication will be supplied by the sponsor (Novexel SA). The pharmacist will acknowledge receipt of all shipments of the investigational products through the IVRS, which will maintain the inventory. 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. IVRS will keep accurate records of the quantities of the investigational products dispensed, used, and returned for each subject. The study monitor will periodically 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

Administration of the investigational product will be supervised by the investigator or subinvestigator.
Any delegation of this responsibility must follow *Section 12.2.*

# 6 PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

## 6.1 PRIOR AND CONCOMITANT ILLNESSES

Additional illnesses present at the time informed consent is given are regarded as concomitant illnesses and must be documented in the case report form. Relevant past illnesses must also be documented in the case report form.

Illnesses first occurring or detected during the study, and worsening of a concomitant illness 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

All prescription and over-the-counter drug treatments taken by the subjects within 2 weeks prior to entry of the study or at any time during the study in addition to the investigational product are regarded as concomitant treatments and must be documented on the appropriate pages of the case report form.

### 6.2.1 Antimicrobial therapy

Prior Antimicrobial Therapy

a. Prior administration of systemic antimicrobial agents within 72 hours of study entry is not permitted, unless either of the following is true:

   1. treatment with nonstudy systemic antibacterial consisted of postoperative (or postdrainage) therapy of no more than 24 hours of an appropriate antimicrobial regimen for patients not considered to have failed a previous regimen

   OR

   2. Patient is considered to have failed the previous treatment regimen. In this case, preoperative treatment of any duration with nonstudy systemic antibacterial therapy for peritonitis or abscess is permitted provided that;

      (a) the treatment regimen was administered for at least 72 hours and is thought to have been inadequate.
      (b) findings of infection were documented at surgery.
      (c) operative intervention is intended no more than 24 to 48 hours after study entry.
      (d) specimens for bacterial cultures and susceptibility testing are taken at operative intervention.
      (e) no further nonstudy antibacterials are administered after enrollment.

b. All prior antimicrobial therapy for the 14 days prior to study entry is to be documented.

Concomitant Antimicrobial Therapy

1. Antibiotic peritoneal lavage is not permitted (peritoneal lavage with saline or other non-antibacterial containing solution is allowed).

2. The use of other systemic antimicrobials (other than vancomycin for documented MRSA or enterococcal infections) not specified by this protocol is not permitted during the study.

### 6.2.2 Non-antimicrobial therapy

1. Other medications that may be required for preexisting or concurrent conditions may be used provided they do not interfere with evaluations of the study antibiotic.
2. Glucocorticoid use, including duration and dosage, is to be documented
3. All medication administered within the 24 hour period prior to randomization and throughout study therapy should be recorded

NXL104

NXL104 is a novel, non β lactam, β lactamase inhibitor. No drug interaction studies have been performed yet with NXL104. However, NXL104 is primarily renally excreted and is not expected to cause or be affected by drug interactions involving the cytochrome P450 metabolizing enzymes.

Ceftazidime

Elevated levels of ceftazidime in patients with renal insufficiency can lead to seizures, encephalopathy, coma, asterixis, neuromuscular excitability, and myoclonia. Patients with estimated creatinine clearances of < 50 mL/min are excluded from this study.

Cephalosporins may be associated with a fall in prothrombin activity. Those at risk include patients with renal and hepatic impairment, or poor nutritional state, as well as patients receiving a protracted course of antimicrobial therapy. Prothrombin time should be monitored in patients at risk and exogenous vitamin K administered as indicated.

The ceftazidime package circular should be consulted for the full list of warnings and precautions associated with ceftazidime use.

Metronidazole

Two serious adverse reactions reported in patients treated with IV metronidazole have been seizures and peripheral neuropathy, the latter characterized mainly by numbness or paresthesias of an extremity. Metronidazole has been reported to potentiate the anticoagulant effect of warfarin and other oral coumarin anticoagulants, resulting in a prolongation of prothrombin time.

The simultaneous administration of drugs that induce microsomal liver enzyme activity, such as phenytoin or phenobarbital, may accelerate the elimination of metronidazole, resulting in reduced plasma levels; impaired clearance of phenytoin has also been reported. The simultaneous administration of drugs that decrease microsomal liver enzyme activity, such as cimetidine, may prolong the half-life and decrease plasma clearance of metronidazole.

Alcoholic beverages should not be consumed during metronidazole therapy because abdominal cramps, nausea, vomiting, headaches and flushing may occur. Psychotic reactions have been reported in alcoholic patients who are using metronidazole and disulfiram concurrently. Metronidazole should not be given to patients who have taken disulfiram within the last two weeks.

Metronidazole prescribing information should be consulted for the full list of warnings and precautions associated with metronidazole use.

Meropenem

Serious and occasionally fatal hypersensitivity (anaphylactic) reactions have been reported in patients receiving therapy with beta-lactams.

Seizures and other CNS adverse experiences have been reported during treatment with meropenem I.V. Close adherence to the recommended dosage and dosage schedules is urged, especially in patients with known factors that predispose to convulsive activity. Anticonvulsant therapy should be continued in patients with known seizure disorders. If focal tremors, myoclonus, or seizures occur, patients should be evaluated neurologically, placed on anticonvulsant therapy if not already instituted, and the dosage of meropenem I.V. re-examined to determine whether it should be decreased or the antibiotic discontinued.

In patients with renal dysfunction, thrombocytopenia has been observed but no clinical bleeding reported. The meropenem package circular should be consulted for the full list of warnings and precautions associated with meropenem use.

# 7 STUDY PROCEDURES AND SCHEDULE

## 7.1 OVERVIEW OF DATA COLLECTION

The primary efficacy variable will be the clinical outcome at the early follow-up Test of Cure (TOC) visit in the microbiologically evaluable population, which will be performed 2 weeks after the end of study therapy. Clinical outcome will be based on an evaluation by the investigator. Procedures and details of the initial and any subsequent operative intervention will be documented. In addition to antibiotic therapy, medical and surgical treatment of the infectious process will be carried out according to guidelines that may exist at the participating institution, and will be consistent with the investigator's usual practice.

The investigator will assess clinical outcome by comparing infection-related signs and symptoms, including physical examination findings specific to the abdominal process from the pre-therapy/entry visit to the post-therapy/TOC visit or the late post-therapy visit.

Details of interventional operative procedures will be documented, and the operative note with the initial and any subsequent intervention is to be submitted to the Sponsor after the 4 to 6 week follow-up. The initial intervention should be adequate as defined in the 1992 IDSA/FDA guidelines (37): a procedure in which all communications between the GI tract and peritoneal cavity are closed and no necrotic intestine is left and all infected collections are drained at the initial procedure. Anatomic site of infection, presence of abscesses (single or multiple), and/or peritonitis and etiologic mechanisms must be recorded.

The assessment of safety will be performed using the following criteria:

- Physical examination
- Vital signs
- Adverse events report (s)
- Laboratory variables
- ECGs

Microbiological outcome will be based on cultures and susceptibility testing. Cultures and susceptibility testing will be performed at the intervals specified in the table of study procedures and at any time that there is clinical or laboratory evidence of persistence or progression of the infectious process (including persistent fever, elevated WBC, or significant changes in the patient's clinical condition). When further surgical intervention is needed, or as clinically indicated, appropriate specimens from intra-abdominal, blood or other sites should be obtained for aerobic and anaerobic culture and susceptibility testing to NXL104/ceftazidime and meropenem of all organisms considered to the pathogens.

Additional assessments will include the following:

- Population pharmacokinetics (see Section 7.3.3).

The microbiological outcome will be evaluated according to computerized rules, utilizing the criteria outlined in Section 7.3 and the clinical outcome will be evaluated by the investigator according to the rules outlined in Section 7.3.

## 7.2 DESCRIPTION OF STUDY DAYS

The following observations will be made during the study as scheduled below:

### Visit 1 (eligibility screening) Day -1 to 0

- Informed consent will be signed.
- Inclusion/exclusion criteria will be reviewed.
- Demographic information (including height and weight) will be collected.
- Medical/surgical history will be taken
- Calculation of Apache II score (Appendix 6)
- Complete physical examination will be performed, including a detailed description of the history, nature, and extent of the infection, as well as documentation of body temperature
- Vital signs will be measured
- Infection-related signs and symptoms will be collected, including
    - Findings such as: abdominal pain, nausea, vomiting, tenderness to palpation, rebound tenderness, guarding, mass, ascites, as well as chills will be assessed by the investigator and graded as none, mild, moderate, or severe according to the following definitions:
        - None: no signs or symptoms
        - Mild: aware of sign or symptoms, but easily tolerated
        - Moderate: sign or symptom of enough intensity to cause interference with usual activity
        - Severe: sign or symptoms of enough intensity that incapacitate and interfere with usual activity.

  Other pertinent findings will be recorded, including the ability to take enteral feeding and passage of gas or solid stool.

- Microbiological samples (cultures and in vitro susceptibility testing to ceftazidime/NXL104, metronidazole, and meropenem) of all pathogens of specimens from the following sites will be collected, including:
    - The site of infection (cultures should not be taken from gallbladder bile, gallbladder wall, or bile from the common bile duct)
    - Blood culture (2 sets) is strongly recommended
    - Specimens from other clinically relevant intra-abdominal and other sites.

  NOTE: all organisms considered to be etiologic pathogens by the investigator will be retained by the clinical microbiology laboratory and sent to the central microbiology laboratory as described in the microbiology manual.

  All prestudy evaluations with the exception of cultures from the site of infection must be done within the 24 hour period prior to the start of study drug therapy.

  Cultures from the site of infection will be obtained within 24 hours prior to study entry for patients enrolled postoperatively and for those patients that are enrolled pre-operatively, cultures must be obtained within 24 hours following study entry.

- Laboratory studies of blood and urine for safety.
- Serum β-hCG for women of childbearing potential.
- Adverse events will be documented, if applicable.
- The schedule for collection of samples for population PK analysis as defined in Section 7.3.3 should be determined for each patient prior to the initiation of study therapy.

### Day 1 (Randomization)

- A 12 lead ECG will be performed (before any study medication is administered).

- After intraabdominal surgery has been performed, surgical wound examination will be performed by the investigator to assess symptoms of infection such as erythema, induration, tenderness, warmth, fluctuation, warmth, swelling, and superficial wound pain. Signs of infection should be recorded on worksheets and graded as mild, moderate or severe. The nature of discharge (purulent vs. serous) should be documented.
- Specific abdominal assessment findings such as abdominal pain, nausea, vomiting, tenderness to palpation, rebound tenderness, guarding, mass, ascites, as well as chills will be assessed by the investigator and graded as none, mild, moderate, or severe. Other pertinent findings including the ability to take enteral feeding and passage of gas or stool will be recorded.
- Eligible patients will be randomized. The investigator will determine the severity of disease by Apache II score and provide stratification to the study pharmacist or designee.
- Concomitant treatment will be assessed; previous antimicrobial treatment will be reviewed and documented.
- The first dose of study medication will be administered as outlined in Section 5.
- Adverse events and local tolerability at the site of study drug infusion will be assessed and documented.

**Daily During Intravenous Study Antibiotic Therapy**

- Study medication will be administered.
- Adverse events and local tolerability should be assessed daily while the patient is receiving IV study therapy.
- Vital signs, body temperature and a detailed description and evaluation of the intra-abdominal infection will be performed daily. Vital signs should be recorded at the same time each day. Body temperature should be measured at least every 8 hours and the daily maximum temperature recorded.
- Specific abdominal findings such as abdominal pain, nausea, vomiting, tenderness to palpation, rebound tenderness, guarding, mass, ascites, as well as chills will be assessed by the investigator and graded as none, mild, moderate, or severe. Other pertinent findings including the ability to take enteral feeding and passage of gas or stool will be recorded.
- Surgical wound examination will be performed by the investigator to assess symptoms of infection such as erythema, induration, tenderness, warmth, fluctuation, warmth, swelling, and superficial wound pain. Signs of infection should be recorded on worksheets and graded as mild, moderate or severe. The nature of discharge (purulent vs. serous) should be documented.

**Additional Procedures to be performed on Day 2 or Day 3**

- Blood cultures (at least two sets) within 48 to 72 hours of study entry if the baseline blood culture was positive.
- Laboratory safety studies must be performed **every 3 days** while on IV study therapy.

**Last Day of IV Study drug infusion (Day 5 to 14)**

- This is the last opportunity to collect blood samples for population PK evaluation.

**End of IV Therapy Visit (within 24 hours of the last dose of IV study therapy)**

- Targeted physical examination will be performed.
- Vital sign measurements and body temperature will be collected.
- Specific abdominal findings such as abdominal pain, nausea, vomiting, tenderness to palpation, rebound tenderness, guarding, mass, ascites, as well as chills will be assessed by the investigator and graded as none, mild, moderate, or severe. Other pertinent findings including the ability to take enteral feeding and passage of gas or stool will be recorded.
- Surgical wound examination will be performed by the investigator to assess symptoms of infection such as erythema, induration, tenderness, warmth, fluctuation, warmth, swelling, and superficial wound pain. Signs of infection should be recorded on worksheets and graded as mild, moderate or severe. The nature of discharge (purulent vs. serous) should be assessed.

inical status and response to IV therapy (see Section 11.1) and document the data on the CRF.

- When further surgical intervention is needed, or as clinically indicated, appropriate specimens from intra-abdominal, blood or other sites should be obtained for aerobic and anaerobic culture and susceptibility testing to NXL104/ceftazidime and metronidazole and meropenem of all organisms considered to be pathogens.
- Assessment of clinical outcome.
- Microbiological outcome will be assessed.
- Blood and urine samples will be collected for clinical laboratory safety tests and urinalysis.
- ECG will be performed.
- Adverse events will be documented.
- Concomitant treatment will be reviewed and documented.
- Study medication compliance will be documented.
- An appointment will be made for the early follow-up visit

**Follow-up Evaluations**

All patients who receive study antibiotic therapy will have two follow-up evaluations, at 2 weeks post-therapy (early follow-up) and at 4 to 6 weeks post-therapy (late follow-up). The early follow-up visit is the Test of Cure visit.

**Early follow-up Visit (2 weeks post-therapy)**

- Targeted physical examination, including vital signs (blood pressure, pulse rate, respiratory rate) and body temperature.
- Specific abdominal findings such as abdominal pain, nausea, vomiting, tenderness to palpation, rebound tenderness, guarding, mass, ascites, as well as chills will be assessed by the investigator and graded as none, mild, moderate, or severe. Other pertinent findings including the ability to take enteral feeding and passage of gas or stool will be recorded.
- Surgical wound examination will be performed by the investigator to assess symptoms of infection such as erythema, induration, tenderness, warmth, fluctuation, warmth, swelling, and superficial wound pain. Signs of infection should be recorded on worksheets and graded as mild, moderate or severe. The nature of discharge (purulent vs. serous) should be assessed.
- If further surgical intervention is needed, or as clinically indicated, appropriate specimens from intra-abdominal or other sites should be obtained for aerobic and anaerobic culture and susceptibility testing to NXL104/ceftazidime, metronidazole, and meropenem of all organisms considered to be pathogens.
- Assessment of clinical outcome.
- Assessment of microbiological outcome.
- ECG will be performed.
- Serum β-hCG for women of childbearing potential.
- Monitor for adverse experiences.

**Late Post-therapy Visit (4 to 6 weeks post-therapy)**

Follow-up evaluations should ideally be made by the investigator. However, this may not be practical for the late follow-up visit. If the late follow-up evaluation is conducted away from the investigator's site (e.g., at the patient's primary physician's office) the evaluation should be conducted by a physician or other healthcare provider, and written documentation of the assessment should be obtained by the investigator.

- Targeted physical examination, including vital signs and body temperature
- Specific abdominal findings such as abdominal pain, nausea, vomiting, tenderness to palpation, rebound tenderness, guarding, mass, ascites, as well as chills will be assessed by the investigator and graded as none, mild, moderate, or severe. Other pertinent findings including the ability to take enteral feeding and passage of gas or stool will be recorded.
- Surgical wound examination will be performed by the investigator to assess symptoms of infection such as erythema, induration, tenderness, warmth, fluctuation, warmth, swelling, and superficial wound pain. Signs

of infection should be recorded on worksheets and graded as mild, moderate or severe. The nature of discharge (purulent vs. serous) should be assessed.
- If further surgical intervention is needed, or as clinically indicated, appropriate specimens from intra-abdominal or other sites should be obtained for aerobic and anaerobic culture and susceptibility testing to NXL104/ceftazidime plus metronidazole and meropenem of all aerobic organisms considered to be pathogens.
- Assessment of clinical outcome
- Assessment of microbiological outcome
- Serum β-hCG for women of childbearing potential
- Concomitant treatment will be reviewed and documented.
- Investigator summary of case and operative note will be provided in narrative form.

## 7.3 METHODS OF DATA COLLECTION

### 7.3.1 Efficacy Variables

- Overall clinical assessment and detailed description and evaluation of the infectious process
- Microbiological cultures from intra-abdominal specimens and blood cultures

Timing of data collection:
- Baseline
- Within 24 hours of completion of parenteral therapy,
- At the early follow-up vist 2 weeks post-therapy, and
- At the 4- to 6-week late follow-up and as clinically indicated.

**Evaluation of Clinical Response**

The favorable final clinical response assessment is “cure”. The unfavorable final clinical response assessment is “failure”. Patients with a final clinical response assessment of “indeterminate” are considered to be clinically unevaluable. Patients with an appropriate clinical diagnosis, but from whom no etiologic bacteria are obtained will be identified as “no primary pathogen isolated” and will be considered nonevaluable for the primary analysis.

Clinical Response definitions are: cure, failure, and indeterminate. Reasons for failure will be indicated, according to the following clinical response definitions:

| Clinical Response | Definition |
|---|---|
| **Cure** | Complete resolution or significant improvement of signs and symptoms of the **index infection**. No further antimicrobial therapy or surgical or radiological intervention is necessary |
| **Failure** | a) Death related to intra-abdominal infection at any time point<br>b) Persisting or recurrent infection within the abdomen documented by the findings at re-intervention either percutaneously or operatively<br>c) postsurgical wound infections defined as an open wound with signs of local infection such as purulent exudates, erythema, or warmth that requires additional antibiotics and/or non-routine wound care or<br>d) patients who receive treatment with additional antibiotics for ongoing symptoms of intra-abdominal infection during the study antibiotic period |
| **Indeterminate** | Study data are not available for evaluation of efficacy for any reason, including:<br>(a) Death occurred during the study period and the index infection was clearly noncontributory<br>(b) Extenuating circumstances preclude classification as cure or failure |

## Microbiological Response

### 1) Assessment of Microbiological Response by Pathogen

The microbiology of each patient's infectious process will be determined by aerobic and anaerobic cultures of appropriate specimens, and by blood cultures in appropriate clinical situations. Results will be tabulated according to species, antibiotic susceptibility pattern, and microbiological response. Patients with an appropriate clinical diagnosis, but from whom no etiologic bacterial pathogens are obtained, will be identified as "no pathogen isolated" and will be considered microbiologically nonevaluable.

Microbiological response will be based on the results of the culture and susceptibility testing of specimens obtained at any time an operative intervention is performed during study therapy or posttreatment follow-up, if such a procedure is clinically indicated. It is expected that in situations of clinical cure, no further invasive procedures will be performed, and therefore the microbiologic response should be considered presumptive eradication. Evaluation at 4 to 6 weeks, post-therapy (test-of-cure) is the primary outcome assessment. Microbiological response will be assessed separately for each pathogen after completion of all follow-up using the definitions listed below. Microbiological responses other than "indeterminate" will be classified as "favorable" or "unfavorable."

Favorable microbiological response assessments include "eradication" and "presumptive eradication." Unfavorable microbiological response assessments include "persistence," "persistence acquiring resistance," "presumed persistence." Patients with a microbiological response assessment of "indeterminate" are considered to be microbiologically nonevaluable.

"Superinfection" and "new infection" will be considered separately.

### 2) Assessment of Overall Microbiological Response

Overall microbiological response will also be assessed as "favorable" or "unfavorable" for each patient. For patients from whom only one pathogen is isolated, the overall microbiological response assessment will be based on the microbiological response assessment for that pathogen.

For patients from whom more than one baseline pathogen is isolated, the overall microbiological response assessment will be "favorable" only if the microbiological response assessment for each of the baseline pathogens isolated is "favorable."

### 3) Definitions of Microbiological Response

Baseline pathogens will be categorized according to the following definitions:

| Microbiological Response | Definitions |
|---|---|
| Eradication | Absence of causative pathogens from appropriately obtained specimens at the site of infection |
| Presumptive Eradication | Absence of material to culture in a patient who had responded clinically to treatment. |
| Persistence | Any causative organism still present at or beyond the end of therapy from a culture of intra-abdominal abscess, peritonitis or surgical wound infection. |
| Persistence acquiring resistance | Continued presence of the original pathogen in cultures from the original site of infection obtained during or upon completion of therapy, and the pathogens that were susceptible to study drug pretreatment have become resistant to study drug therapy post-treatment. |
| Presumed persistence | Repeat cultures were not obtained because of the absence of material to culture in a patient who was assessed as clinical failure. |
| Indeterminate | a) Entry culture either not obtained or no growth<br>b) Assessment not possible because of protocol violation<br>c) Any other circumstance which makes it impossible to define the microbiological response. |

## Emergent Infections

Pathogens that are identified after baseline will be categorized according to the following definitions and summarized separately.

| Microbiological Response | Definitions |
|---|---|
| Superinfection | Emergency of new pathogen during therapy, either at the site of infection or at a distant site with emergence or worsening of signs and symptoms of infection. |
| New Infection | Eradication of the original pathogen followed by replacement (at the same site and after completion of therapy) by a new species or by a new serotype or biotype of the same organism in the presence of signs or symptoms of infection. If a pathogen is isolated from a site distant to the primary infection after study therapy is completed, then this is to be designated as a new infection. |

### 7.3.2 Safety data

Safety will be evaluated on the basis of:

- Adverse events based on spontaneous reporting, questioning by the study staff and review of the medical record
- Local tolerability
- Vital signs: blood pressure, heart rate.
- Physical examinations
- ECG parameters from 12 lead-ECGs
- Laboratory safety: biochemistry, hematology, and urinalysis

#### Physical examination

At screening, randomization/entry, daily while on IV study drug therapy, and at the early and late follow-up visits patients will undergo a physical examination as indicated above. If pathologic findings emerge or worsen from the baseline assessment, a nonserious adverse event page of the case report form should be completed for these findings. If a finding meets the criteria for a serious adverse event, the appropriate procedures for reporting such events should be followed (see Section 8. *Adverse events)*.

### Vital signs

Vital signs will be closely monitored and the subject's daily maximum temperature, maximum heart rate, respiratory rate, and lowest blood pressure reading will be recorded on the hospital source document. The data corresponding to the day of each visit will be reported in to the case report form.

### ECG

Twelve lead ECGs will be performed on pretherapy/entry visit before starting treatment, at the end of IV study therapy and at the early follow-up visit (2 weeks post-therapy). All ECGs will be collected and kept in the study file.

### Adverse events

Adverse events observed by the investigator or reported by the subject will be documented as described in Section 8. *Adverse events.*

## Laboratory variables (see Study Procedures)

**NOTE: laboratory studies of blood and urine for safety must be performed every 3 days while on parenteral antibiotic therapy**

The following variables will be determined as outlined in the table of Study Procedures:

| **Haematology** | **Biochemistry** | **Urinalysis** |
|---|---|---|
| • haemoglobin<br>• RBC<br>• haematocrit<br>• WBC (total and differential)<br>• platelet count<br>• INR (PT)<br>• PTT<br>• Coombs test | • SGOT/AST<br>• SGPT/ALT<br>• alkaline phosphatase<br>• creatinine<br>• total bilirubin<br>• total protein<br>• glucose<br>• albumin<br>• blood urea nitrogen<br>• inorganic phosphorous<br>• calcium<br>• sodium<br>• potassium<br>• chloride<br>• CO2<br>• β hCG | • pH<br>Semiquantitative<br>• glucose<br>• protein<br>• leukocytes<br>• erythrocytes<br>• sediment |

**Safety follow-up**

At completion of antibiotic therapy patients should be followed at least 14 days for safety. Patients who withdraw from study prior to completing a total of 5 days of therapy need to complete the follow-up evaluations, and must also be followed up for safety purposes for at least 14 days after the date that study antibiotic was discontinued.

### 7.3.3 Pharmacokinetic data

Blood samples for population pharmacokinetics will be collected on tubes containing potassium oxalate and sodium fluoride. As far as possible, five samples will taken from each patient during the course of therapy within the following pre-defined time windows:

- at any time within the 15 minutes prior to, or after, stopping any infusion
- at any time between 30min and 1h30 after stopping any infusion
- at any time between 2h30 and 3h30 after stopping any infusion
- at any time between 6h30 and 7h30 after stopping any infusion

Every attempt should be made to obtain at least one sample from each of the 4 time windows for each patient. Whenever two samples have to be taken within the same time window, this should be done preferably on two separate occasions (*i.e.* two distinct infusions). In the absence of prior knowledge of the PK model structure and associated PK parameters, the sampling time windows could not be optimized. Sampling times were selected with the objective to obtain, where feasible, multiple drug levels per patient at different times to capture PK data in the vicinity of Cmax, in the initial (distribution) phase, and in the terminal phase including one sample in the vicinity of Cmin.

To encourage diversity in the pharmacokinetic dataset, the sampling dates are deliberately left at the convenience of investigators and should ideally vary, both within and between patients. All reasonable measures will be taken to ensure accurate information on dosing history (doses, dates and times of dosing including times of infusion start and stop) and timing of samples relative to dosing.

Every effort should be made to centrifuge blood samples within 4 hours of their collection. Plasma will then be separated and stored at or below –20 C for no longer than 2 months, until transfer to a deep freezer at or below –70 C and further shipment to the bioanalytical site. Under these conditions of sample handling and storage, NXL104 and ceftazidime have demonstrated stability in human plasma for a total duration of 12 months.

# 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.

The investigator will evaluate all adverse experiences as to the intensity or severity of adverse events will be graded as follows:

- **Mild** – Awareness of sign or symptom, but easily tolerated. Not expected to have a clinically significant effect on the subject's overall health and well-being. Not likely to require medical attention.

- **Moderate** – discomfort enough to cause interference with usual activity or affects clinical status. May require medical intervention.

- **Severe** – incapacitating or significantly affecting clinical status. Likely requires medical intervention and/or close follow-up.

### 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 judgement 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.

**Determination of relationship of adverse events to study drug**

The investigator must make the determination of relationship to study drug for each adverse event. The Investigator should decide whether, in their medical judgment, there is a reasonable possibility that the event may have been caused by the trial therapy. The following guidance may be helpful:

- **Certain**: There is a reasonable causal relationship between the Study Medication and the AE. The event responds to withdrawal of Study Medication (dechallenge), and recurs with rechallenge when clinically feasible.

- **Probable:** There is a reasonable causal relationship between the Study Medication and the AE. The event responds to dechallenge. Rechallenge is not required.

- **Not likely**: There is a temporal relationship to Study Medication administration, but there is not a reasonable causal relationship between the Study Medication and the AE.

- **Unrelated**: There is not a temporal relationship to Study Medication administration (too early, too late, or Study Medication not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

### 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. A significant overdose in this study is defined as more than twice the prescribed dose of the investigational product in a single 24 hour period.

In addition, any pregnancy diagnosed in a female subject or in the female partner of a male subject during treatment with the investigational product must be reported to the sponsor immediately. Information related to the pregnancy must be given on a "Drug Exposure Via Parent – Data Collection" form that will be provided by the sponsor.

### 8.1.4 Tolerability

Daily assessment of local tolerability

The tolerability of IV NXL104/ceftazidime and IV meropenem at the local site of infusion will be evaluated by the blinded investigator and recorded daily. Evaluation should be based on investigator inspection and patient comments regarding the intensity of signs and symptoms at the site of study drug infusion, including: pain, erythema, swelling, tenderness, phlebitis, rash, and ulceration. The intensity of the finding should be assessed by the following severity scale:

**None:** no signs or symptoms of intolerance

**Mild:** aware of signs and symptoms, but easily tolerated

**Moderate:** signs and symptoms of such intensity as to interfere with usual activities.

**Severe:** signs and symptoms that are incapacitating

**Reactions at the site of infusion are adverse experiences if the abnormality represents a clinically significant adverse change from baseline**.

## 8.2 PERIOD OF OBSERVATION

For the purposes of this study, the period of observation for collection of adverse events extends from the time the subject gives informed consent until 14 days after the end of study therapy.

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 study monitor, who will forward it to the sponsor's Pharmacovigilance department.
- If the adverse event is listed as an alert term (see Section 8.1.3) even if the "alert term" is non-serious, 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 adverse event is detected. This form must be sent to the study monitor, 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. The **"**Serious Adverse Event/Expedited Report from a Clinical Trial" form and the instructions are provided in the investigator's study file.

The investigator must also inform the study monitor in all cases. The sponsor 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" form.

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 or at the request of their legally authorized representative*
- If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being
- Development of signs or symptoms of a rapidly progressive underlying disease that would preclude evaluation of therapy, or that would render continuation in the study inadvisable, in the judgment of the investigator.
- At the specific request of the sponsor

"Legally authorized representative" means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research.

Patients withdrawn from the study regimen for any reason will receive therapy deemed appropriate by the investigator.

All patients should be followed, whenever possible, until the 4 to 6 week posttreatment follow-up visit for the final outcome assessment with the exception of patients who are considered clinical failures. If withdrawal from study therapy is a consequence of clinical failure, these patients will be considered as such for analysis. However, patients must be followed for safety for at least 14 days after discontinuation of study therapy.

If an additional antibiotic is required for nosocomial infection outside the abdomen at least 5 days into the study, all discontinuation of IV procedures should be performed on the day this new therapy is initiated, whether or not study therapy is discontinued.

## 9.2 DISCONTINUATION OF STUDY MEDICATION

Subjects must be withdrawn from study medication under the following circumstances:

- Deterioration of the clinical condition or delayed response: If after at least 48 hours of treatment, it is the investigator's opinion that the subject needs to be discontinued because of failure to improve clinically, the subject should be discontinued and evaluated as a clinical failure.
- Presence of a resistant pathogen that could be considered as causative; (e.g. isolated from adequate sources) persistent pathogen or superinfection. However, in the case of a clear clinical improvement, the originally assigned regimen may be continued at the discretion of the investigator.
- The occurrence of alarming adverse events that may be related to study medication.
- AST or ALT $\geq$ 3 times the upper limit of normal (ULN) range for subjects with normal transaminase levels at baseline and AST or ALT $\geq$ 5 times the ULN for subjects with abnormal transaminase levels at baseline unless due to another clearly defined cause such as the intra-abdominal infection.
- Impaired renal function, as shown by creatinine clearance $\leq$ 50 mL/min.
- Lost to follow-up (The investigator must make every effort to contact subjects lost to follow-up)

In all cases, the reason for withdrawal from study or discontinuation of study medication 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 Adverse Events*.

As far as possible, all examinations scheduled for the EOT visit should be performed (if possible, within 72 hours) on all subjects (see *Section 7.2 Description of study days).* A urine culture and urinalysis should be performed.

Wherever possible, subjects should continue with the study schedule as planned, completing the post-therapy/TOC and late post-therapy visits as scheduled.

## 9.3 REPLACEMENT OF SUBJECTS

Subjects will not be replaced.

# 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 IDENTIFICATION OF INVESTIGATIONAL PRODUCTS

The unblinded pharmacist may provide a patient's randomization to an investigator if considered a medical emergency. If not an emergency, the investigator should contact the study monitor to discuss the rationale for unblinding. If it is considered medically necessary to unblind study therapy, the blinded investigator should complete clinical assessments prior to unblinding.

## 10.3 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

Continuous variables (e.g., age) will be summarized by the number of subjects, mean, standard deviation (SD), median, minimum, maximum, and number of missing values. Categorical variables (e.g., race) will be summarized by frequencies and percentages of subjects in each category.

## 11.1 ANALYSIS VARIABLES

The primary analysis variable for efficacy will be the clinical response at the early follow-up, Test of Cure (TOC) Visit, performed 2 weeks post-therapy in the microbiologically evaluable population.

The primary safety variable will be the incidence of adverse experiences.

The secondary analysis variables for efficacy will include:

- The clinical response in baseline microbiologically evaluable patients with cIAI at the end of IV therapy and at the late follow-up 4 to 6 weeks post-therapy.
- The clinical response in clinically evaluable patients with cIAI at the end of IV therapy, at the Test of Cure visit, and at the late follow-up 4 to 6 weeks post-therapy.
- The microbiological response in patients with cIAI at the end of IV therapy, at the Test of Cure visit and at the late follow-up 4 to 6 weeks post-therapy.

Additional analysis variables for efficacy and safety will be defined in a Statistical Analysis Plan (SAP) prior to finalization of the database.

## 11.2 STUDY POPULATIONS

The following study populations are to be used for statistical analyses:

- Microbiologically evaluable (ME) population
- Clinically evaluable (CE) population
- Microbiological modified intent to treat (mMITT) population
- Safety population
- PK population

### Evaluability criteria

CE at TOC: All randomized subjects who have an appropriate diagnosis of intraperitoneal infection confirmed by operative findings and received an adequate course of therapy and have sufficient information to determine clinical outcome at TOC.

CE at Late Follow Up Visits: All randomized subjects who have an appropriate diagnosis of cIAI, receive an adequate course of therapy and have adequate data to make an outcome assessment at the respective follow up visit (not indeterminate).

ME at TOC: A subset of CE subjects who also have at least one etiologic pathogen isolated from a clinically relevant specimen (peritoneal fluid, abscess fluid, peritoneal surface of infected organ prior to the incision of a

hollow viscus, or blood culture in appropriate clinical setting) in the initial/prestudy culture that is susceptible in both study agents. Patients with a polymicrobial infection where one or more pathogens are resistant in vitro to the study antibiotic may be kept on study therapy at the discretion of the investigator, and will be considered evaluable.

ME at Late Follow Up visits: A subset of CE subjects who also have at least one etiologic pathogen identified from a relevant specimen that this susceptible to study antibiotics.

Microbiological modified intent to treat population (mMITT): All randomized subjects who receive at least 1 dose of study drug and meet the disease definition of IAI and have at least one bacterial pathogen identified at study entry regardless of susceptibility.

Safety population: All subjects who receive at least one dose of study treatment.

PK Population: All randomized subjects who receive at least one dose of study treatment and have at least one measurable plasma concentration of NXL104.

## 11.3 STATISTICAL METHODS

**Efficacy**

The primary analysis variable for efficacy will be the clinical outcome at the early follow-up, Test of Cure (TOC) Visit, performed 2 weeks post-therapy in the microbiologically evaluable population. The population for the primary analysis will be "ME Population" as defined in Section 11.2. The stratified Mantel Haenszel test, accounting for baseline disease severity (Apache II score <=10 vs >10 and <25), will be used to determine treatment effect on clinical outcome (cured vs. failure). Significance will be set at 5% for all analyses. Primary analysis will also be carried out on the mMITT population. No adjustments for multiplicity will be made.

Appropriate statistical tests will be used to evaluate treatment effect on secondary analysis variables. Methods and populations to be used will be detailed further in a separate SAP.

**Safety**

The population for safety and tolerability data analysis will be the "Safety Population," defined in Section 11.2

All safety and tolerability variables will be tabulated and summarized according to treatment group.

The AE verbatim terms will be coded to system organ class and preferred term for summary purposes using the MedDRA dictionary. Treatment emergent adverse events (TEAEs) that are reported during the study will be summarized by system organ class, preferred term, and treatment group. Treatment-emergent AEs (TEAEs) are defined as those that occur or worsen after the first dose of the study medication. TEAEs will also be summarized by severity and relatedness to treatment. All AEs will be listed. Serious adverse events will also be summarized and listed as described above for AEs.

Local tolerability, assessment of abdominal signs and symptoms, vital signs, ECGs, laboratory tests and physical examinations will be summarized by treatment group and visit as appropriate.

Withdrawals from the study will be summarized by treatment group. Reasons for withdrawal will also be summarized.

Concomitant medications will be coded using WHODrug and summarized by treatment group according to preferred drug name and drug class.

**PK Analysis**

The population for pharmacokinetic analyses is the "PK Population," defined above in Section 11.2.

The derived PK parameters will be tabulated and presented with descriptive statistics and graphs.

Complete details of all 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 and the study being unblinded.

### 11.4 INTERIM ANALYSIS

No interim analysis is planned for this study.

### 11.5 SAMPLE SIZE JUSTIFICATION

There is no formal sample size calculation for this study as this is an early phase clinical trial. The sample size is therefore based on currently accepted standards for this type of investigation. Consequently, 200 subjects will be included in the study, comprising of approximately 100 in each treatment group. Previous studies suggest that approximately 65% of enrolled patients are considered microbiologically evaluable [42,43,44]. This will provide information on which to base the next phase III pivotal study.

# 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 subinvestigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

## 12.3 SUBJECT INFORMATION AND INFORMED CONSENT

Before being enrolled in the clinical study, 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.

### *For subjects able to give informed consent*

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.

If the subject is unable to read, oral presentation and explanation of the written informed consent form and information to be supplied to subjects must take place in the presence of an impartial witness. Consent must be confirmed at the time of consent orally and by the personally dated signature of the subject or by a local legally recognized alternative (e.g., the subject's thumbprint or mark). The witness and the person conducting the informed consent discussions must also sign and personally date the consent document.

A copy of the signed consent document must be given to the subject. The original signed consent document will be retained by the investigator.

If a subject is not in a position to give informed consent because of his or her physical or mental condition, the consent of a legally authorized representative* must be sought. The consent must be confirmed at the time of consent by the personally dated signature of the representative and by the personally dated signature of the person conducting the informed consent discussions. A copy of the signed consent document must be given to the representative. The original signed consent document will be retained by the investigator. Local legal requirements must be observed and informed consent must be sought from the subject as soon as possible afterwards, if feasible. This procedure must have prior agreement from the independent ethics committee (IEC)/institutional review board (IRB).

* "Legally authorized representative" means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research.

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 after documentation on **all** ethical and legal requirements for starting the study has been received by the sponsor. 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
- Expedited safety reports from the sponsor, as soon as possible
- Periodic reports on the progress of the study

## 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:

- 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 subinvestigators and other appropriately qualified persons to whom the investigator has delegated significant trial-related duties, together with their roles in the study and their signatures
- 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 15 years

## 12.10 LIABILITY AND INSURANCE

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

## 12.11 FINANCIAL DISCLOSURE

Before the start of the study, the investigator will disclose to the sponsor any proprietary or financial interests he or she might hold in the investigational products or the sponsor company as outlined in the financial disclosure form provided by the sponsor. The investigator agrees to update this information in case of significant changes during the study or within one year of its completion. The investigator also agrees that, where required by law or regulation, the sponsor may submit this financial information to domestic or foreign regulatory authorities in applications for marketing authorizations.

Similar information will be provided by each subinvestigator to whom the investigator delegates significant study related responsibilities.

# 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 close-out 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

This study will be performed using an electronic case report form (eCRF). The investigator and study site staff will receive system documentation, training and support for the use of the eCRF.

All protocol-required information collected during the study must be entered by the investigator, or designated representative in the eCRF. All data entry, modification or deletion will be recorded automatically in an electronic audit trail indicating the individual subject, original value, the new value, the reason for change, who made the change and when the change was made. All data changes will be clearly indicated with a means to locate prior values. The system will be secured to prevent unauthorized access to the data or the system. This will include the requirement for a user ID and password to enter or change data. The investigator will maintain a list of individuals who are authorized to enter or correct data and their system ID.

All electronic data entered by the site (including an electronic audit trail) as well as computer hardware and software (for accessing the data) will be maintained or made available at the site in compliance with applicable record retention regulations. The computerized system is able to generate accurate and complete copies of records in both human-readable and electronic form for inspection, review and copying by regulatory authorities, the IEC/IRB, and an auditor authorized by the sponsor. Site documentation will identify the software and hardware systems used to create, modify, maintain, archive, retrieve or transmit data.

The investigator or designated subinvestigator, following review of the data in the eCRF, will confirm the validity of each subject's data by electronic signature or by signing a paper printout of a listing of all patients enrolled in the study.

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

The sponsor will retain the original eCRF data and audit trail. A copy of all completed eCRFs will be provided to the investigator.

## 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.

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

The investigator (or coordinating 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.

It is agreed that, consistent with scientific standards, publication of the results of the study shall be made only as part of a publication of the results obtained by all sites performing the protocol.

# 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 E6)

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

Study Manager/Clinical Manager/Chief Medical Officer

Date:_____ Signature:_____

Name (block letters): _____

## 15.2 DECLARATION OF INVESTIGATOR

***For US and non-US studies conducted under a US IND***

I confirm that I have read the above protocol. I understand it, and I will work according to the principles of GCP as described in 21 CFR parts 50, 54, 56, and 312 and according to applicable local requirements.

**Investigator**

Date: _____ Signature: _____

Name (block letters): _____

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 †‡ ***(insert relevant country or organization)***.

I agree to obtain, in the manner described in this clinical study protocol, written informed consent or witnessed verbal 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 agree to disclose any proprietary or financial interests I may hold in the investigational product or sponsor company as specified in Section 12.11. ***(covered studies only)***

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 a 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

## Appendix 1: Preparation of Study Drug Infusion

Solutions of NXL104 for intravenous infusion will be prepared under aseptic conditions no later than 24h before administration. Two doses should be prepared at one time using 1 vial of NXL104 and 2 vials of ceftazidime. The solutions will be stored at +2°C to +8°C temperature in light protected condition.

The study medication includes both NXL104 and ceftazidime, which will be administered together in a single infusion bag. NXL104 vials include 1000mg lyophilised powder to be reconstituted with 10mL of water for injection to obtain the concentration of 100mg/1mL.

Ceftazidime 2g vials will be reconstituted using 9mL of water for injection to obtain 10mL solution containing 200mg/1mL. Attention must be paid during reconstitution and drawing of the required amount of reconstituted ceftazidime for further dilution in the infusion bags as $CO_2$ released with in the vials will increase the pressure. The pressure has to be released by inserting a needle with the vials in upright position (see the summary of product characteristics of ceftazidime given in appendix 5 for more details)

The final solutions to be administered will be prepared by diluting under aseptic conditions an appropriate amount of NXL104 and ceftazidime with 100 mL Glucose 5%. From this preparation, a volume of 100 mL will be infused in 30 min.

First, 10mL of NXL104 should be withdrawn from a single vial of reconstituted NXL104. 5mL, containing 500mg NXL104, should be added to each of 2 infusion bags containing 5% glucose solution.

Next, 10mL of ceftazidime should be withdrawn from one reconstituted ceftazidime vial and added to one infusion bag containing 5% glucose and NXL104. Repeat with a second ceftazidime vial for the 2nd infusion bag.

This process should be repeated each time study drug is prepared. Study drug prepared from any given vial of NXL104 may only be used for 1 patient. If study drug is stopped for a given patient before the second bag is administered, it should be discarded and noted on the drug accountability record. It should not be administered to another patient.

The appropriate amount of NXL104 + ceftazidime will be transferred into the infusion bag containing 100 mL with 5 % glucose according to the following table.

**Preparation of the final solution to be administered**

| **NXL104 +Ceftazidime** | | | | | |
|---|---|---|---|---|---|
| | Dose NXL104 (mg) | Number of NXL104 vials required/ subject | Concentration of NXL104 to be prepared (mg/mL) | Preparation of the solution | |
| NXL104 | 500 | 1 vial for 2 doses | Add 10mL water to vial (100mg/mL) | 5mL | Add to 100mL 5% Glucose |
| + ceftazidime | 2000 | 1vial per dose | Add 9mL water to vial (200mg/mL) | 10mL | |

# Appendix 2: Sample Collection Procedure for PK 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 ref 367922 containing sodium fluoride (10mg) and potassium oxalate (8mg)
- Polypropylene specimen stabilization/storage tubes (13 x 7.5 cm)
- Vacutainer collection needles, 21g or 22g x 1" and needle holders
- 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 –20 ° C or colder, with routine temperature monitoring and documentation
- The facility supplied specimen labels containing the following information: study number (NXL104/2002), subject number, treatment number, date, day #, time in relation to start of last infusion, and identification of analyte, "NXL104 assay".

## PLASMA COLLECTION FOR PK:

• Collect all blood samples (2x4 mL) for NXL104 assay in tubes containing sodium fluoride (10mg)/potassium oxalate (8mg) (Vacutainer FX reference number 367922).
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.
• Within 4 hours of blood collection, 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 –20°C.
• The label on each tube will state: NXL104/2002, Patient number, treatment number, Date, Day #, time elapsed since the start of last infusion.
• Storage of plasma tubes at -20°C for no longer than 2 months, then transfer to the bioanalytical lab for storage at -70◦C..

## SAMPLE LABELLING, STORAGE, PACKING AND SHIPMENT

The actual blood sample withdrawal date and time for plasma will be captured in the electronic source data capture system. The actual dosing date and times (start and end) of the study medication (NXL104) administration will also be captured in the electronic source data capture system.

## SPECIMEN STORAGE PRIOR TO SHIPMENT (for plasma 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. Plasma will be separated and stored at or below –20◦ C for no longer than 2 months, until transfer in a deep freezer at or below –70◦ C and further shipment to the bioanalytical site. Under these conditions of sample handling and storage, NXL104 and ceftazidime have demonstrated stability in human plasma for a total duration of 12 months. . 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

Instructions for shipment of PK samples to the analytic laboratory will be provided separately.

# Appendix 3: Microbiological Procedures

1. Specimen Samples

Specimens should be collected, transported, processed and interpreted according to the standard policies of the Local Microbiology Laboratory.

Specimen Collection
Specimens should be obtained from patients that meet the inclusion criteria. The microbiology laboratory should confirm that an appropriate sample has been submitted for processing as this is critical to successfully meeting the study objectives.

1.2 Specimen Processing/Susceptibility Testing

1.2.1. Specimens should be processed as soon as they are received in order to isolate and identify all pathogenic organisms. Specimen processing should be performed according to the Local Microbiology Laboratories' routine methods using appropriate aerobic and anaerobic culture media. Gram-staining of specimens and pure cultures of isolates are encouraged, the results of which need to be provided to study coordinator, as source documentation for transcribing onto the Case Report Form (CRF).

1.2.2 Isolated pathogens will be susceptibility tested per standard operating procedures in place at the local laboratory to assist in routine patient management.

1.2.3 Sites will be provided with three disk diffusion test reagents which will include NXL104/Ceftazidime , Meropenem and Ceftazidime.
Antimicrobial disks must be stored frozen at minus 20◦C or below until needed. Sites will perform disk susceptibility testing on all Gram-negative isolates according to the CLSI document M2-A9: Performance Standards for Antimicrobial Disk Susceptibility Tests; approved Standard-Ninth Addition (2006). All isolates will be tested using commercially-prepared Mueller-Hinton Agar obtained by the site. Plates should be read after 16-18 hours of incubation and zone diameters must be measured and interpreted according to the CLSI M100-S18 document or the document provided for NXL/Ceftazidime. Zone diameter results for each disk reagents will be recorded by the site on the CRF. A laminated card will be provided to the site for determining susceptibility to each of the three tested agents when using the disk diffusion method.

1.2.4 Quality control (QC) testing of the appropriate ATCC isolates is to be performed concurrently when testing isolates from study subjects. The QC recommendations can be found in the CLSI M100-S18 document as well as on the provided QC log and should be interpreted according to the ranges located in these documents. Susceptibility testing and QC testing results should be available for review by the Clinical Study Monitors. A QC log sheet for disk diffusion results has been provided and should be used to document all testing events and be available to the study monitor. These QC results do not need to be sent to the Central Microbiology Laboratory.

**1.3 Interpretation of Culture Results**

All organisms isolated by the Local Microbiology Laboratory and deemed to be contributory to the infectious process will be identified according to their standard policies, guidelines, and routine methods. The identifications of all isolated organisms (Gram-positive and –negative) need to be provided to the Study Coordinator as source documentation for transcribing onto the CRF and bacterial isolates will be referred to the Central Microbiology Laboratory. **Organisms not considered pathogens are presumed to be normal colonizers or contaminants and are not to be forwarded to the Central Microbiology Laboratory.**

### 1.4 Isolate Processing

- Each purified bacterial isolate will be identified throughout the study process using protocol labels, provided by the Central Microbiology Laboratory, as described in the Microbiology Manual for the study.

## 2. ISOLATE RETENTION AND REFERRAL

All isolates must be stored at the Local Microbiology Laboratory until the completion of the study and disposed of only upon consultant and approval of the sponsor. The Central Microbiology Laboratory will provide storage media and storage containers for isolate retention at **minus 20◦C or below**. Frozen isolates will be identified using the provided cryovial labels. Isolates will require identification and susceptibility confirmation by the Central Microbiology Laboratory which will provide all shipping materials, instructions and will cover the cost of isolate referrals.

All aerobic bacterial isolates obtained from clinical specimens, are to be provided to the Central Microbiology Laboratory accompanied with microbiology specimen information (i.e., Isolate Submission Form). The isolates will be identified by using the appropriate label for isolate submission which is to be placed onto the culture identification report section of the Isolate Submission Form. All isolates obtained from a single specimen must be purified and documented on the same Isolate Submission Form with different culture ID #'s.

The site should also attach and send the locally-generated antibiogram information obtained using the sites routine method (e.g. Vitek or other results) which must be identified using the local antibiogram report label.

Purified isolates will be sent to the Central Microbiology Laboratory as directed in the Microbiology Manual. As stated above, isolates are also to be stored at the Local Microbiology Laboratory as frozen samples in the cryovials and media supplied by the Central Microbiology Laboratory. Frozen isolates will be identified with pre-printed culture ID # labels that will match labels used for culturing for purity and referring isolates on the swabs.

Isolates will be sent via overnight courier to the Central Microbiology Laboratory upon completion of local laboratory processing . The Central Microbiology Laboratory will monitor study site compliance for all isolates submitted and serve to coordinate all aspects of laboratory processing.

The Central Microbiology Laboratory will re-identify all isolates received to the level of genus and species and will perform reference susceptibility testing on isolates using reference broth microdilution methods. Identification discrepancies will be resolved by consultation between the Central Microbiology Laboratory and the study site utilizing the following tracking method. The Central Microbiology Laboratory will check the box located at the bottom of the Isolate Submission Form with a yes or no response and FAX this form to the site for each submitted isolate(s). In the event of a discrepance (i.e a checked no response), The Central Microbiology Laboratory will generate an Isolate Submission Clarification form that will accompany the Isolate Submission Form to resolve discrepant results. The original archived isolate must be re-shipped to the Central Microbiology Laboratory for identification confirmation and/or re-testing antimicrobial susceptibility. Sites will be asked to re-test isolates under special circumstances only.

## 3. DATA COLLECTION

Site and patient information and bacterial isolate data will be collected by the Local Microbiology Laboratory and entered onto the Isolate Submission Form provided by the Central Microbiology Laboratory. Each specimen that is accessioned for the study with an aerobic isolate recovered will require an individual form. If more than one pathogen is identified in a sample, each bacterial species can be placed onto a single form which has space for up to three individual isolates. In the unlikely event that more than three isolates are considered to be causative pathogens, please contact the Central Microbiology Laboratory. Information will include the following:

- Subject Identification
    - Site/Subject #
- Specimen Information
    - Local Accession #
    - Visit
    - Specimen Source
    - Collection Date
    - Collection Time
- Culture Identification
    - Culture ID #
    - Species Identification (Genus and species if available)

## 4. STUDY MATERIALS

The Central Laboratory will provide the required materials as outlined in the Microbiology manual. Please check that each item has been received when the study commences. Note that the materials may not arrive at the same time as this protocol.

## Appendix 4: 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

### *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.

# Appendix 6: Apache II scores

## APACHE II Severity of Disease Classification System

**Glasgow Coma Score (GCS)**
(circle appropriate response)

| **Eyes open (E)** | **Motor response (M)** | **Verbal - Response (V)** |
|---|---|---|
| 4 - spontaneously | 6 - to verbal command | 5 - oriented and controversed |
| 3 - to verbal command | 5 - localizes to pain | 4 - confused and disoriented |
| 2 - to painful stimul | 4 - withdraws to pain | 3 - inappropriate words |
| 1 - no response | 3 - decorticate | 2 - incomprehensible sounds |
| | 2 - decerebrate | 1 - no response |
| | 1 - no response | |

**COMA SCORE† = E + M + V**
**GCS = 15 - Coma Score**

† Patients scoring 3 or 4 have an 85% chance of dying or remaining vegetative, while scores above 11 indicate 5 to 10% likelihood of death or vegetative state and 85% chance of moderate disability or good recovery. Intermediate scores correlate with proportional chances of patients recovering.

**B. Age Points**

| **Age** | **Points** |
|---|---|
| <44 | 0 |
| 45-54 | 2 |
| 55-64 | 3 |
| 65-74 | 5 |
| >75 | 6 |

**Age points = _____**

**C. Chronic Health Points**

If any of the 5 CHE categories is answered with yes give +5 points for nonoperative or emergency postoperative patients, or +2 points for elective postoperative patients

Liver - Cirrhosis with PHT or encephalopathy
Cardiovascular - Class IV angina or at rest or with minimal self care activities
Pulmonary - chronic hypoxemia or hypercapnia or polycythemia of PHT >40 mm Hg
Kidney - chronic peritoneal or hemodialysis
Immune - immune compromised host

**Chronic Health Points =_____**

**APACHE-II Score (sum of A+B+C)**

| | |
|---|---|
| APS points | A |
| Age points | +B |
| Chronic Health Points | +C |
| **Total APACHE-II** | |

APACHE II Severity of Disease Classification System

APACHE II Score Form

A. Acute Physiology Score:

| | PHYSIOLOGIC VARIABLE | HIGH ABNORMAL RANGE | | | | | LOW ABNORMAL RANGE | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | +4 | +3 | +2 | +1 | 0 | +1 | +2 | +3 | +4 |
| 1 | Temperature rectal (°C) | ≥41 | 39-40.9 | | 38.5-38.9 | 36.0-38.4 | 34-35.9 | 32-33.9 | 30-31.9 | ≤29.9 |
| 2 | Mean arterial pressure = (2 x diastolic+systolic)/3 | ≥160 | 130-159 | 110-129 | | 70-109 | | 50-69 | | ≤49 |
| 3 | Heart rate (ventricular response) | ≥180 | 140-179 | 110-139 | | 70-109 | | 55-69 | 40-54 | ≤39 |
| 4 | Respiratory rate (nonventilated or ventilated) | ≥50 | 35-49 | | 25-34 | 12-24 | 10-11 | 6-9 | | <5 |
| 5 | Oxygenation<br>A-$aDO_2$ or $PaO_2$ (mm Hg)<br>a)$FiO_2$>0.5:record A-$aDO_2$ | ≥500 | 350-499 | 200-349 | | <200 | | | | |
| | b)$FiO_2$<0.5:record only $PaO_2$ | | | | | >70 | 61-70 | | 55-60 | <55 |
| 6 | Arterial pH If no ABGs record Serum HCO3 below | ≥7.7 | 7.6-7.69 | | 7.5-7.59 | 7.33-7.49 | | 7.25-7.32 | 7.15-7.24 | <7.15 |
| 7 | Serum Sodium | ≥180 | 160-179 | 155-159 | 150-154 | 130-149 | | 120-129 | 111-119 | ≤110 |
| 8 | Serum Potassium | ≥7 | 6-6.9 | | 5.6-5.9 | 3.5-5.4 | 3-3.4 | 2.5-2.9 | | <2.5 |
| 9 | Serum Creatinine (mg/dL)<br>Double Point for acute renal failure | ≥3.5 | 2-3.4 | 1.5-1.9 | | 0.6-1.4 | | <0.6 | | |
| 10 | Hematocrit (%) | ≥60 | | 50-59.9 | 46-49.9 | 30-45.9 | | 20-29.9 | | <20 |
| 11 | White Blood Count | ≥40 | | 20-39.9 | 15-19.9 | 3-14.9 | | 1-2.9 | | <1 |
| 12 | Glasgow Coma Scale<br>(Score = 15 minus actual GCS) | 15-GCS= | | | | | | | | |
| A | Total Acute Physiology Score (APS) | Sum of the 12 individual variable points = | | | | | | | | |
| * | Serum HCO3(venous-mMol/L)<br>Not preferred, use if no ABGs | <52 | 41-51.9 | | 32-40.9 | 22-31.9 | | 18-21.9 | 15-17.9 | <15 |