NeoSep1

Statistical Analysis Plan for Part 1

An open-label randomised controlled trial comparing novel combination and currently used antibiotic regimens for the empiric treatment of neonatal sepsis with a run-in confirmatory pharmacokinetic phase

ISRCTN #: ISRCTN48721236

Version 1.0, 08-06-2023

In relation to protocol version: 1.0

Author	Position	Signature	Date
Wolfgang Stöhr	Trial Statistician, MRC Clinical Trials Unit at UCL, London	DocuSigned by: Wolfgang Stohr C02B7413DF134CE...	13-Jun-2023
Investigators for pharmacokinetic analysis			
Adrie Bekker	Stellenbosch University, Cape Town, South Africa	DocuSigned by: Adrie Bekker 088D93F6736143F...	13-Jun-2023
Tim Cressey	AMS-PHPT Research Unit, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand	DocuSigned by: Tim Cressey 1BBE17E54D2D44B...	13-Jun-2023
Joe Standing	UCL Institute of Child Health, London	DocuSigned by: Joe Standing 9E9D9201D011478...	13-Jun-2023
Reviewed by			
Ann Sarah Walker	Senior Statistician for NeoSep1, MRC Clinical Trials Unit at UCL, London	DocuSigned by: Ann Sarah Walker A21A8226533E49A...	13-Jun-2023
Michelle Clements	Independent Senior Statistician, MRC Clinical Trials Unit at UCL, London	DocuSigned by: Michelle Clements AD78954D2C79466...	13-Jun-2023
Approved by			
Mike Sharland	Chief Investigator, St George's, University of London	DocuSigned by: Michael Sharland 232789901B144D7...	21-Jun-2023
Seamus O'Brien	R&D Director, Global Antibiotic Research & Development Partnership (GARDP)	DocuSigned by: Seamus O'Brien D14B119D1B524F9...	13-Jun-2023
Shamim Qazi	TSC Chair, World Health Organization	DocuSigned by: Shamim Qazi 32DF8B1E2A284B7...	13-Jun-2023
Tim Peto	DMC Chair, University of Oxford	DocuSigned by: Tim Peto 99DD0A87112C477...	21-Jun-2023

Revision History

Version	Author	Date	Reason for Revision
Draft 0.01	Rebecca Turner Sarah Walker	04-Mar-2022	Protocol version 1.0
Draft 0.02	W Stöhr	31-Aug-2022	First draft
Draft 0.03	W Stöhr	16-Nov-2022	Incorporated Joseph Standing's comments on Draft 0.02
Draft 0.04	A Bekker TR Cressey J Standing	05-Dec-2022	Reviewed and added comments
Draft 0.05	Sarah Walker Francesca Schiavone W Stöhr	04-Jan-2023	Reviewed and responded to comments
Draft 0.06	W Stöhr	08-Feb-2023	Accepted changes made to draft 0.05, and responded to further comments by Subasree Srinivasan & Rashmi Mathur (GARDP)
Draft 0.07	W Stöhr	27-Feb-2023	Changes based on Michelle Clements' comments on Draft 0.06
Final 1.0	W Stöhr	08-Jun-2023	Accepted all changes in Draft 0.07

Notes: Rebecca Turner: Senior Statistician at MRC CTU at UCL; Francesca Schiavone: Clinical Project Manager for NeoSep1, MRC CTU at UCL.

TABLE OF CONTENTS

<u>Note</u>: This Statistical Analysis Plan deals only with Part 1 of the NeoSep1 trial. A separate Statistical Analysis Plan will be written for Part 2.

1 ABBREVIATIONS

Abbreviation	Expansion
AE	Adverse event
ALT	Alanine Aminotransferase
AMR	Antimicrobial resistance
AST	Aspartate transaminase
AUC	Area under the curve
BiPAP	Bilevel Positive Airway Pressure
BUN	Blood urea nitrogen
CI	Confidence interval
CL	Clearance
Cmax	Maximum concentration
CPAP	Continuous positive airways pressure
CRP	C-reactive protein
DMC	Data Monitoring Committee
EOT	End of treatment
FBC	Full blood count
Fm	Fraction of size and scaled clearance at birth
GARDP	Global Antibiotic Research and Development Partnership
HFNC	High flow nasal cannula
ISRCTN	International Standard Randomised Controlled Trial Number
IV	Intravenous
Km	Rate of postnatal maturation of clearance
MDR	Multi-Drug Resistant
MIC	Minimum Inhibitory Concentration
MRC CTU at UCL	Medical Research Council Clinical Trials Unit at University College London
NAESS	International Neonatal Consortium Neonatal Adverse Event Severity Scale
NeoOBS	Neonatal observational study
PK	Pharmacokinetics
PNA	Post-natal age
Q	Inter-compartmental clearance
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SGUL	St Georges University of London
StFU	Short term follow-up
$T_{½}$	Half-life (time to half the initial concentration)
Tmax	Time of the maximum concentration
TOC	Test of cure
TSC	Trial Steering Committee
UCL	University College London
V	Volume of distribution
Vp	Peripheral volume
WBC	White blood cells
WHO	World Health Organisation

2 BACKGROUND

While there is a high burden of neonatal sepsis globally, its impact is especially marked in low- and middle-income countries (LMICs), where there are an estimated 6.9 million annual episodes of possible serious bacterial infection and 680,000 related deaths. Increasing antimicrobial resistance (AMR), including a higher prevalence of AMR in isolates from septic neonates, threatens to undermine the effectiveness of World Health Organisation (WHO) recommended antibiotic treatments in these settings. The key threat is multi drug-resistant Gram-negative bacteria, where there are very few neonatal treatment options and increasing use of meropenem, driving carbapenem resistance. Current WHO guidelines, however, continue to recommend empiric first- and second-line regimens for neonatal sepsis that have remained unchanged for nearly 20 years.

Given increasing AMR, the coverage of current WHO-recommended regimens is expected to be low in many high-burden settings, and there is a clear need to re-evaluate the guidance for empiric treatment of neonatal sepsis in the hospital setting and to provide new options for treatment of MDR neonatal sepsis that have global relevance. Relevant regimens for comparison with WHO-recommended regimens should include antibiotics with a neonatal licence and provide good coverage for globally relevant extended-spectrum beta-lactamase producing organisms. Given the lack of evidence supporting much neonatal sepsis treatment and the severity of the condition, it also important to directly compare suitable novel regimens, including off-patent drugs with a neonatal licence but not currently widely used, to currently recommended and widely used regimens.

Therefore, in NeoSep1 three groups of empiric antibiotic regimens will be investigated:

- WHO-recommended regimens: ampicillin (or benzylpenicillin, amoxicillin or cloxacillin) + gentamicin, or the third generation cephalosporins, cefotaxime or ceftriaxone
- Broad spectrum antibiotics in common use in neonatal units with licenced and/or recommended neonatal doses: piperacillin/tazobactam, piperacillin/tazobactam + amikacin, ceftazidime, ceftazidime + amikacin, meropenem
- Older off patent antibiotics which have a licenced neonatal dose but are not currently widely used globally in neonatal units: fosfomycin, flomoxef and amikacin

These older off patent antibiotics will be tested as novel dual combinations, which is fosfomycin + amikacin, flomoxef + amikacin and fosfomycin + flomoxef. Since they have been infrequently used in neonatal populations, a run-in non-randomised pharmacokinetic study of these three combinations of fosfomycin, flomoxef and amikacin will be performed to confirm plasma drug levels at the proposed doses based on dosing recommendations and other studies, as well as collect safety data (NeoSep1 Part 1) before the start of the main randomised trial (NeoSep1 Part 2).

3 STUDY METHODS

3.1 TRIAL DESIGN

NeoSep1 Part 1 is an open label, pharmacokinetic and safety study among neonates admitted to hospital with clinical signs of sepsis in two countries. Participants will be enrolled into one of the following three sequential treatment cohorts (non-randomised):

- Fosfomycin and amikacin (cohort 1)
- Flomoxef and amikacin (cohort 2)
- Flomoxef and fosfomycin (cohort 3)

Intravenous doses used are those proposed by previous studies and current international dosing recommendations. If an included neonate clinically deteriorates, or fails to respond, then second-line treatment will be based on local clinician choice.

3.2 STUDY OBJECTIVES

The primary objective is to confirm that the recommended doses of fosfomycin and flomoxef, when used in combination with each other or with amikacin, to be studied in NeoSep1 Part 2 will provide adequate drug exposure in neonates with sepsis.

3.3 STUDY DURATION

The study duration for each participating neonate is 28 days from enrolment.

3.4 STUDY POPULATION

3.4.1 INCLUSION CRITERIA

1. Currently admitted to hospital
2. Aged ≤28 days (post-natal age)
3. Weight ≥1000g
4. Clinical diagnosis of a new episode of sepsis together with planned treatment with IV antibiotics
5. At moderate to high risk of death from this episode of sepsis, based on a neonatal sepsis severity score (NeoSep Severity Score; see 3.10); specifically, a baseline assessment NeoSep Severity Score of 5 or higher
6. IV antibiotics about to be started OR not received more than 24 hours of IV antibiotics for this episode of neonatal sepsis at the point of randomisation
7. Parent/guardian willing and able to provide written informed consent.

3.4.2 EXCLUSION CRITERIA

1. A known serious, non-infective co-morbidity including major congenital abnormalities (other than prematurity), anticipated to cause death within this admission
2. Previously enrolled in this trial
3. Current participation in any other clinical study of an Investigational Medicinal Product that is a systemic drug, unless it has received prior approval by the NeoSep1 Trial Management Group
4. Known contraindication to any of the trial antibiotics on the randomisation list for the relevant neonatal sub-population in that site

3.5 RANDOMISATION

Allocation to the three treatment cohorts will be done sequentially, without randomisation. For each treatment cohort, allocation to sampling time-points (see 3.7) will be done using pre-prepared randomisation lists with blocks of eight each comprising all unique combinations of time-points in random order.

3.6 OUTCOME MEASURES

3.6.1 PRIMARY ENDPOINTS (FOSFOMYCIN AND FLOMOXEF)

The following primary PK parameters will be derived from the population PK model:

- Clearance (CL)
- Central volume of distribution (V)
- Postnatal maturation function parameters: fraction of size and scaled clearance at birth (Fm) and the rate of postnatal maturation of clearance (Km)

3.6.2 SECONDARY ENDPOINTS (FOSFOMYCIN AND FLOMOXEF)

The following secondary PK parameters will be derived for fosfomycin and flomoxef from the population PK model:

- Maximum plasma concentration (Cmax)
- Time to Cmax (Tmax)
- Apparent terminal elimination half-life (t1/2)
- Area under the plasma concentration-time curve from 0 to last observed time point (AUC(0–last))
- Area Under the Curve to infinity (AUC(0–∞))
- Volume of distribution at steady state (Vss)

3.6.3 EXPLORATORY PK/PD ENDPOINTS

- Free drug AUC ratio to Minimum Inhibitory Concentration (MIC) (fosfomycin)
- Fraction of time for free concentration above MIC (flomoxef)
- Pharmacokinetic analysis of amikacin (if there is sufficient sample volume)

3.6.4 SAFETY ENDPOINTS

- Adverse events (AEs) based on the International Neonatal Consortium Neonatal Adverse Event Severity Scale (NAESS) through Day 28
- Modification (including discontinuation) of antibiotics for adverse reactions

3.7 TIMING OF OUTCOME ASSESSMENTS

PK samples will be taken on the following time-points, counting time from the calendar date of enrolment:

- Day 1 (baseline): date of enrolment
- Day 5 (± 1 day)

Each neonate enrolled in Part 1 will have 3 blood samples taken on Day 1 of dosing and will be allocated to one each from the a) early, b) middle and c) late time points, to determine plasma concentrations of flomoxef and fosfomycin and model the pharmacokinetic profiles. The allocated time points are:

a) early: 5 mins or 15 mins after end of dosing (window: ±5 mins)
b) middle: 30 mins or 60 mins after end of dosing (±10 mins)
c) late: 4h or 6h after end of dosing (±30 mins)

An additional sample will be collected prior to the first dose on Day 5 if baby is still on trial antibiotics.

Participants will also be followed up daily while on IV antibiotic treatment in hospital to collect clinical and safety information.

Trial Assessment Schedule for NeoSep1 Part 1

Visit type	Screening	Enrolment	Follow-up Treatment & Monitoring					TOC	StFU*
Timing (window)	Day 0	Day 1	Daily while on IV antibiotics	Day 3 (±1 day)	Day 5 (±1 day)	Day 7 (±2 day)	EOT [8] (if not Day 7 or 14)	14 (± 4 days)	28 (± 5 days)
Informed assent/consent	x[1]								
Verification of eligibility	x	x							
Enrolment to Part 1		x[2]							
Medical history	x	x							
Clinical review	x	x	x	x	x	x	x	x	x
C-reactive Protein	x[3]				x	x			
Full Blood Count (FBC)	x[3]				x	x[6]	x[6]	x[6]	x[6]
Urea & Electrolytes (U&Es)	x[3]				x	x[6]	x[6]	x[6]	x[6]
Liver function test (LFT)	x[3]				x	x[6]	x[6]	x[6]	x[6]
Creatinine	x[3]				x	x[6]	x[6]	x[6]	x[6]
Blood culture	x[4]			x[5]					
Administration of antibiotics		x	X	x	x	x	x		
Pharmacokinetic sample[7]		x			x				
Adverse event assessment		x	X	x	x	x	x	x	x
Concomitant medication		x	X	x	x	x	x	x	x

EOT= end of treatment, TOC = test of cure, StFU = short term follow-up visit (* by telephone / if clinically indicated, then hospital visit).
1 Written informed consent to be obtained from parent/guardian.
2 Treatment allocation in Part 1 and treatment initiation may be on the same day as the screening visit.

3 Lab results required within 48h before enrolment, but test can be done either at screening or randomisation or values from blood taken pre-screening.
FBC: Red blood count (RBC), white blood count (WBC) and differential, platelets. U&Es: including blood urea nitrate (BUN), sodium, potassium. LFTs: ALT, AST.
4 Blood must be taken for culture within 48h before enrolment but may precede screening visit by up to 48 hours if already taken for clinical management.
5 Repeat blood culture only if neonate switches treatment (at the time of switch) due to clinical deterioration or lack of response. Blood for culture should be taken before switch of antibiotics except in exceptional circumstances outside the responsible clinician's control.
6 Repeat blood tests only if abnormal at previous visit or baby's condition not stable.
7 Pharmacokinetic samples for Part 1. PK sample from CSF may also be collected if lumbar puncture is clinically indicated and baby receiving fosfomycin.
8 Planned duration of treatment at enrolment for blood culture-negative sepsis is to Day 7±2 days, for blood culture-positive sepsis is to Day 10 [-3,+4 days] if there is no switch to second-line. If antibiotics are switched to second-line, the total planned duration of antibiotic treatment including first and second line treatment is 14 ±7 days depending on the baby's condition.

3.8 SAMPLE SIZE

Approximately 60 neonates will be enrolled in total and sequentially allocated to each of the three treatment cohorts. 20 neonates with all 3 PK samples on Day 1 will be enrolled in each of the 3 sequential treatment cohorts. In addition, across all 3 cohorts, 10 neonates on fosfomycin and 10 neonates on flomoxef with a post-natal age under 7 days at the time of enrolment with complete Day 1 samples and Day 5 sample are required. The final sequential cohort will continue recruiting until this target is achieved. Any neonate without complete Day 1 PK samples will be replaced.

The sample size was calculated to ensure that there is at least 80% power to estimate the clearance (CL) and central volume of distribution (V) with 20% precision. The simulation-estimation analysis was carried out for flomoxef by scaling a published adult population pharmacokinetic model to a neonatal reference population. Scaling was performed through applying the concept of weight-based allometry to clearance and volume terms and using previously established maturation functions to further define clearance maturation based on post-natal and post-menstrual age. Prior to the simulation-estimation analysis, the scaled model was inspected against published neonatal flomoxef pharmacokinetic profiles to verify applicability of the scaling. For fosfomycin the model developed from NeoFos-001 was used (Kane, Gastine et al. 2021).

Six sampling time points were chosen to cover the dose interval on Day 1 (two early, two middle and two late time points), corresponding to three blood draws for each neonate, and the simulated population was randomly assigned postnatal age, postmenstrual age and weight combinations across the range expected for neonates.

3.9 SAFETY

Clinical examination will be performed daily while on IV antibiotic treatment in hospital and will explicitly record signs and symptoms relating to possible drug toxicities. If a baby has stopped IV antibiotics, a follow up (by telephone if discharged) on Days 14 and 28, respectively, will be performed to collect any AEs (NAESS or other) since last assessment.

The following laboratory tests will be carried out at screening and Day 5:

- Full Blood Count: red blood count, haemoglobin, white blood count and differential, neutrophils, platelets
- Blood urea nitrate, CRP, creatinine, ALT, AST, bilirubin
- Sodium, potassium

All adverse events, clinical and laboratory, will be graded using the neonatal adverse event severity scale (NAESS) (Salaets, Turner et al. 2019): for 35 AEs (e.g. neonatal convulsion, neonatal bradycardia), specific severity criteria are defined. The investigator must assess the causality of all events, serious and non-serious, in relation to the trial therapy. In Part 1 of NeoSep1, all adverse events will be summarised and graded. The adverse events reporting period begins upon subject enrolment in the trial (after the earliest of verbal assent or signed informed consent) and ends at the last visit of the patient (Day 28).
Of note, the limited number of neonates included in this PK study limits the power to conduct comparisons of adverse event, either within Part 1 or with other studies.

Serious adverse events (SAEs) are not an outcome measure in NeoSep1 because the neonates will be very sick when admitted, however, will be reported for pharmacovigilance purposes.

3.10 NEOSEP SEVERITY SCORE

The NeoSep Severity Score was developed for predicting 28-day mortality based on clinical information at the start of a new episode of sepsis. It was adapted from the WHO PSBI based scores for the hospital setting and developed from the NeoOBS study as described in the table below.

Factor (clinical signs in the 24h preceding start of clinical sepsis episode)	Score value if present
Time in hospital: ≤ 10 days	1
Gestational age: <37 weeks	1
Birth Weight: • >2 kg • 1-2 kg • <1 kg	 0 1 2
Congenital anomalies	2
Temperature • <35.5 °C • 35.5 to 37.9 °C • 38 to 38.9 °C • ≥39 °C	 1 0 1 2
Maximum respiratory support: • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 2 3 3
Abdominal distension	1
Difficulty in feeding	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement: • Lethargy only • No movement or movement only on stimulation +/- lethargy	 1 2

CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula.

3.11 NEOSEP RECOVERY SCORE

The NeoSep Recovery Score was developed from daily updated assessments of neonates' status in the NeoOBS study to predict mortality and guide clinical decision making.

Factor (clinical signs in the preceding 24h)	Score value if present
Temperature • <35.5°C • 35.5 to 37.9°C • 38 to 38.9 °C • ≥39 °C	 1 0 1 2
Maximum respiratory support: • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 2 3 3
Abdominal distension	1
Difficulty in feeding	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement • Lethargy only • No or movement only on stimulation +/- lethargy	 1 2
Cyanosis	1

4 STATISTICAL PRINCIPLES

4.1 ANALYSIS POPULATIONS

4.1.1 PK ANALYSIS

An as-treated approach will be taken whereby only patients who have received at least one dose of fosfomycin or flomoxef and who have at least one PK sample will be included.

4.1.2 SAFETY ANALYSIS

All participants with at least one dose of either fosfomycin, flomoxef or amikacin will be included in the safety analysis, whether or not they had complete PK samples.

4.2 STATISTICAL INTERIM ANALYSES AND STOPPING GUIDANCE

PK and safety data will be reviewed by an independent DMC. The NeoSep1 protocol states that there will be a review of safety data and preliminary analysis of pharmacokinetic samples (descriptive) when 20-40 neonates have been enrolled.

After further discussions among trial team and pharmacometricians, and reflecting the sequential nature of recruitment into different treatment cohorts and the relative amount of information available on fosfomycin and flomoxef, the PK review was expanded to the following review points (either descriptive or model based). As PK assays and modelling will take additional time, the safety and PK review may not happen at the same meeting.

1) after enrolment of the first 8-10 participants with complete Day 1 PK samples in Cohort 1 to assess fosfomycin plasma concentrations (descriptive) to check that concentrations are at least detectable
2) after enrolment of 20 participants with complete Day 1 PK samples in Cohort 1 for development of fosfomycin population PK model
3) after enrolment of the first 8-10 participants with complete Day 1 PK samples in cohort 2 to assess flomoxef plasma concentrations (descriptive) to check that concentrations are at least detectable
4) after enrolment of 20 participants with complete Day 1 PK samples in Cohort 2 for development of flomoxef population PK model

No formal stopping guidelines will be applied.

4.3 TIMING OF FINAL ANALYSIS

The final analysis of PK parameters for fosfomycin and flomoxef will be performed once at least 20 participants with complete Day 1 PK samples in Cohort 3 have been enrolled including a) at least 10 participants with a post-natal age of <7 days and complete Day 1 and Day 5 PK samples across cohorts 1 & 3 and b) at least 10 participants with a post-natal age of <7 days and complete Day 1 and Day 5 PK samples across cohorts 2 & 3.

4.4 MISSING DATA

Every effort will be made to collect all data as per the schedule of assessments, and we expect the number of missing data to be relatively minimal. Patients who withdraw consent will be excluded from trial analyses from the time they withdraw. When missing data occurs it is assumed that it will be at random, and all analyses will be based on observed data only.

4.5 CONFIDENCE INTERVALS AND P VALUES

This Part 1 study is not set up to perform formal hypothesis testing of group differences. If performed, all statistical tests, except where otherwise specified, will be two-sided, and no formal adjustment for multiple testing will be made (all significance tests will be interpreted in the context of the total number of comparisons performed). Final estimated PK outcomes will be presented with their associated relative standard errors (%RSE) and 95% confidence intervals.

4.6 STATISTICAL SOFTWARE

PK analyses will be performed using the software packages NONMEM (Version 7.4; ICONDevelopment Solutions, Ellicott City, MD, USA) and/or nlmixr (Version 1.0 or above) in R (Version 4.2 or above). The remainder will be carried out using Stata version 17 (or above).

5 ANALYSIS

5.1 RECRUITMENT

The following will be reported overall and by treatment cohort:

- Number screened and enrolled by calendar time (by calendar month and cumulative)
- Total screened and enrolled by centre, with dates of first and latest enrolment
- Eligibility: number and reasons for any participants found to be ineligible after enrolment

Reasons for not enrolling screened subjects will also be described.

5.2 BASELINE CHARACTERISTICS

Demography and other baseline characteristics will be summarised descriptively, overall and by treatment cohort. Categorical data will be summarised using the number and percentages of subjects. Continuous data will be presented using the number of subjects, mean, standard deviation, median, first quartile, third quartile, minimum and maximum.

- Sex: n (%) male, female
- Age (in days from birth)
- Time in hospital at presentation (days)
- Gestational age at birth (weeks)
- Birth weight (g): summarised as continuous variable, and n (%) in categories <1 kg, 1-2 kg, >2 kg
- Presence of congenital abnormalities: n (%)
- Temperature at presentation (°C): summarised as continuous variable, and n (%) in categories <35.5 °C, 35.5 to 37.9 °C, 38 to 38.9 °C, ≥39 °C
- Respiratory support at presentation: n (%) oxygen supplementation, CPAP/BiPAP/HFNC, invasive ventilation
- Abdominal distension at presentation: n (%)
- Difficulty in feeding at presentation: n (%)
- Evidence of shock at presentation: n (%)
- Lethargy or reduced/no movement at presentation: n (%) lethargy only, movement only on stimulation, no movement
- NeoSep Severity Score at presentation: median, first quartile, third quartile, minimum and maximum; may also be grouped, for example into low (score 0-4), medium (5-8) and high (9-16) risk groups, and presented categorically
- Mode of delivery: n (%) elective (planned) caesarean section, emergency caesarean section, vaginal delivery (spontaneous), vaginal delivery (assisted)
- HIV status: n (%) infected, exposed/uninfected, uninfected
- Any lines used in the past 24 hours: n (%) umbilical venous line, umbilical arterial line, peripheral arterial line, central venous line, PICC line
- Weight (g)
- Length (cm)
- Heart rate (beats per minute)
- Respiratory rate (breaths per minute)
- Oxygen saturation (%)

- Convulsions: n (%)
- Fast breathing (respiratory rate >60 breaths per minute): n (%)
- Chest indrawing: n (%)
- Start of IV antibiotics for this episode of sepsis before enrolment: n (%) overall; n (%) per antibiotic, total daily dose (mg or IU) per antibiotic, duration of treatment (hours)
- Systemic antibiotics taken for other reasons in preceding 7 days: n (%), n (%) per antibiotic, duration of treatment (hours)
- Sodium (mEq/L)
- Potassium (mmol/L)
- Blood Urea Nitrate (mg/dL)
- Creatinine (mg/dL)
- CRP (mg/L)
- ALT (U/L)
- AST (U/L)
- Total bilirubin (mg/dL)
- RBC (million cells/µL)
- Haemoglobin (g/L)
- WBC (10^9/L)
- Neutrophils (10^9/L)
- Platelets (10^9/L)

5.3 WITHDRAWAL / FOLLOW-UP

The following will be reported overall and by treatment cohort:

- Time between enrolment and last day of follow-up (days): median (IQR), range
- Short term follow-up visit (Day 28, final visit in Part 1): number (%) happened/missed; number (%) attended in clinic/assessed by telephone
- Time from enrolment to discharge (days)
- Withdrawal from trial participation before 28 days: number (%); description of reasons
- Lost to follow-up before 28 days: number (%)
- PK samples Day 1: number (%) participants with 3, 2, 1 or none of the scheduled samples
- PK sample Day 5: number (%) participants with sample, without sample, already stopped allocated antibiotics before Day 5.

5.4 PROTOCOL DEVIATIONS

Protocol deviations will be defined in the NeoSep1 Quality Management and Monitoring Plan and classified as critical, major or minor. The number of major and critical protocol deviations will be summarised for all participants, overall and by site.

5.5 PK ANALYSIS

Population PK modelling and dosing simulations will be undertaken with non-linear mixed-effects modelling to concentration-time data. The PK model will estimate the primary PK parameters clearance (CL), volume of distribution (V), intercompartmental clearance and peripheral volume. A covariate model will be used to quantify the effect of postnatal age (over and above weight and postmenstrual age) and renal function. The method of PK scaling (fixed allometric weight and postmenstrual age with estimated postnatal age and creatinine effects) is given in Kane et al (Kane, Gastine et al. 2021). PK outcomes (3.6.1, 3.6.2, 3.6.3) will be derived from the model. Model-based estimation of PK parameters will be undertaken using the first order conditional estimation method with interaction ('FOCEI') in NONMEM.

One- and two-compartment structural models will be compared. Inter individual variability (IIV) will be assumed to follow a log-normal distribution for clearance, volume and absorption rate constants, and a logit distribution for bioavailability. Estimation of IIV will be evaluated for all parameters. An additive, a proportional and a combined error model will be tested. In line with the v2 distribution, a drop in the log likelihood ratio of >6.64 per degree of freedom is needed to be significant at a level of P < 0.01 and >3.84 at a level of P<0.05.

Allometric (weight) scaling will be included using a fixed exponent of 0.75 on clearance terms and linear scaling on volume terms. A standard weight of 70kg will be used to enable comparison of parameter estimates with other studies. A previously published neonatal renal maturation function (Rhodin et al.) will also be added to clearance. Due to the narrow postmenstrual age (PMA) range of babies included in this study the Hill coefficient and time to 50% maturation will be fixed as with previous similar neonatal studies (Germovsek & Kent et al.; Germovsek & Lutsar et al.).

$$CL_i = CL_{std} \times \left(\frac{WT_i}{70}\right)^{0.75} \quad \text{Eq 1.}$$

$$V_i = V_{std} \times \left(\frac{WT_i}{70}\right) \quad \text{Eq 2.}$$

$$maturation = \frac{{PMA_i}^{3.4}}{47.7^{3.4} + {PMA_i}^{3.4}} \quad \text{Eq 3.}$$

While the Rhodin maturation function (Rhodin et al.) accounts for development of renal maturation in early life, there may also be a further effect on clearance maturation after birth regardless of gestational age that occurs over the first few days/weeks of life (Anderson et al.). This covariate may be best related to post-natal age (PNA) as has been observed by others (Germovsek & Kent at al.; Savic et al.) Therefore Equation 4 will also be evaluated (hM; fraction of clearance on the first day of life, set to day = 0; and hN, postnatal maturation rate constant).

$$PNA_{function} = \theta_M + (1 - \theta_M) \times \left(1 - e^{-PNA_i \times \theta_N}\right) \quad \text{Eq 4.}$$

The ability of serum creatinine concentration (SCR) to explain and reduce IIV on clearance will be tested according to Equation 6, where the measured SCR was standardized using typical serum concentration (TSCR) for age calculated based on the function published by Ceriotti et al. (Equation 5). The SCR levels utilized by Ceriotti et al. in defining Equation 5 were quantified using an enzymatic method, while a Jaffe method will be used in this study.

$$TSCR(\mu mol) = -2.37330 - 12.91367 \times ln_{(PNA_{years})} + 23.93581 \times \left(PNA_{years}\right)^{0.5} \quad \text{Eq 5.}$$

$$SCR_{function} = \frac{{SCR_i}^{\theta_{SCr}}}{TSCR} \quad \text{Eq 6.}$$

5.6 SAFETY

Safety data will be reported overall and by treatment cohort.

- 28-day mortality: Kaplan-Meier estimate
- Number (%) of participants experiencing an adverse event (any grade)
- Number (%) of participants experiencing an adverse reaction (possible, probable, or definitely related to study treatment)
- Number (%) of participants with modification (including discontinuation) of antibiotics for adverse reactions
- Maximum adverse event grade per participant
- Tabulation of adverse events, by body system, severity grade, and relationship to study treatment

Although not an outcome, a line listing of all SAEs will be generated.

5.7 ANTIBIOTIC TREATMENT

Exposure to allocated treatment will be summarised descriptively overall and by treatment cohort:

- N (%) receiving allocated treatment
- Change of allocated treatment: cumulative incidence of stopping all IV antibiotics, switching to second-line treatment, other change.
- N (%) receiving second-line treatment; description of second-line antibiotics
- Time to stop of all IV antibiotics: cumulative incidence
- Description of all non-allocated antibiotics used per participant: total number, type and class of antibiotics, reason started

5.8 CLINICAL FOLLOW-UP

Clinical status will be assessed at Day 3, 7, 14 and 28 based on the NeoSep Recovery Score (see 3.11).

The following parameters will be recorded daily whilst on IV antibiotics at each visit and will be described overall and by treatment cohort. Categorical data will be summarised using the number and percentages of subjects. Continuous data will be presented using the number of subjects, mean, standard deviation, median, first quartile, third quartile, minimum and maximum.

- Temperature (°C): summarised as continuous variable, and n (%) in categories <35.5 °C, 35.5 to 37.9 °C, 38 to 38.9 °C, ≥39 °C
- Respiratory support: n (%) oxygen supplementation, CPAP/BiPAP/HFNC, invasive ventilation
- Abdominal distension: n (%)
- Difficulty in feeding: n (%)
- Evidence of shock: n (%)
- Lethargy or reduced/no movement: n (%) lethargy only, movement only on stimulation, no movement
- Cyanosis: n (%)
- NeoSep Recovery Score: median, first quartile, third quartile, minimum and maximum; may also be grouped and presented categorically (for example <4, ≥4)
- Heart rate (beats per minute)
- Respiratory rate (breaths per minute)

- Oxygen saturation (%)
- Convulsions: n (%)
- Fast breathing (respiratory rate >60 breaths per minute): n (%)
- Chest indrawing: n (%)

Further assessments:

- CRP at Day 5 and Day 7; change from baseline
- Other laboratory values (see 5.2) at Day 5; including change in sodium and potassium in participants allocated to fosfomycin

5.9 MICROBIOLOGY

- Description of results of blood culture and CRF samples obtained before the start of antibiotics, isolation of any pathogen, including type of pathogen and antimicrobial susceptibility as determined locally by phenotypic methods
- Any new culture results (not present at baseline) will be described similarly.

5.10 CONCOMITANT MEDICATION

- Description of all concomitant medication used, by drug and class.

6 REFERENCES

Anderson BJ, Holford NHG. Negligible impact of birth on renal function and drug metabolism. Paediatr Anaesth 2018; 28: 1015–21.

Ceriotti F, Boyd JC, Klein G et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. Clin Chem 2008; 54: 559–66.

Germovsek E, Kent A, Metsvaht T et al. Development and evaluation of a gentamicin pharmacokinetic model that facilitates opportunistic gentamicin therapeutic drug monitoring in neonates and infants. Antimicrob Agents Chemother 2016; 60: 4869–77.

Germovsek E, Lutsar I, Kipper K et al. Plasma and CSF pharmacokinetics of meropenem in neonates and young infants: results from the NeoMero studies. J Antimicrob Chemother 2018; 73: 1908–16.

Kane Z, Gastine S, Obiero C, Williams P, Murunga S, Thitiri J, Ellis S, Correia E, Nyaoke B, Kipper K, van den Anker J, Sharland M, Berkley JA and Standing JF (2021). “IV and oral fosfomycin pharmacokinetics in neonates with suspected clinical sepsis.” J Antimicrob Chemother 76(7): 1855-1864.

Rhodin MM, Anderson BJ, Peters AM et al. Human renal function maturation: a quantitative description using weight and postmenstrual age. Pediatr Nephrol 2009; 24: 67–76.

Salaets T, Turner MA, Short M, Ward RM, Hokuto I, Ariagno RL, Klein A, Beauman S, Wade K, Thomson M, Roberts E, Harrison J, Quinn T, Baer G, Davis J, Allegaert K, International Neonatal Consortium (2019). “Development of a neonatal adverse event severity scale through a Delphi consensus approach.” Arch Dis Child 104(12): 1167-1173.

Savic RM, Cowan MJ, Dvorak CC et al. Effect of weight and maturation on busulfan clearance in infants and small children undergoing hematopoietic cell transplantation. Biol Blood Marrow Transplant 2013; 19: 1608–14.

DocuSign

Certificate Of Completion

Envelope Id: A3931E67AABA48E2B2C660F7F8C7C42C
Status: Completed
Subject: Please sign the NeoSep1_PART_1_Statistical Analysis Plan_v1.0_2023_06_08.docx
Source Envelope:
Document Pages: 19
Signatures: 10
Certificate Pages: 6
Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Enabled
Time Zone: (UTC) Dublin, Edinburgh, Lisbon, London

Envelope Originator:
Francesca Schiavone
90 High Holborn 2nd Floor London
London, London WC1V 6LJ
F.schiavone@ucl.ac.uk
IP Address: 144.82.114.195

Record Tracking

Status: Original
13 June 2023 | 09:19
Holder: Francesca Schiavone
F.schiavone@ucl.ac.uk
Location: DocuSign

Signer Events	Signature	Timestamp
Adrie Bekker adrie@sun.ac.za Security Level: Email, Account Authentication (Optional)	DocuSigned by: Adrie Bekker 088D93F6736143F... Signature Adoption: Pre-selected Style Using IP Address: 105.224.253.106	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 10:31 Signed: 13 June 2023 | 14:38
Electronic Record and Signature Disclosure: Accepted: 14 April 2022 | 13:17 ID: ed68e61d-a3a6-4fc3-81a0-7c8e2b8caea8		
Ann Sarah Walker rmjlasw@ucl.ac.uk Security Level: Email, Account Authentication (Optional)	DocuSigned by: Ann Sarah Walker A21A8226533E49A... Signature Adoption: Pre-selected Style Using IP Address: 86.140.253.239	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 09:39 Signed: 13 June 2023 | 09:39
Electronic Record and Signature Disclosure: Accepted: 18 May 2023 | 14:17 ID: 9ad929cc-a8ee-47b3-9d62-0b65b30a1dc0		
Joe Standing j.standing@ucl.ac.uk Professor of Pharmacometrics Security Level: Email, Account Authentication (Optional)	DocuSigned by: Joe Standing 9E9D9201D011478... Signature Adoption: Pre-selected Style Using IP Address: 194.82.240.136	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 09:26 Signed: 13 June 2023 | 09:27
Electronic Record and Signature Disclosure: Accepted: 13 June 2023 | 09:26 ID: 6568b32c-5d49-48d6-95a9-83b622023aea		
Michael Sharland msharland@sgul.ac.uk Michael Sharland Security Level: Email, Account Authentication (Optional)	DocuSigned by: Michael Sharland 232789901B144D7... Signature Adoption: Pre-selected Style Using IP Address: 86.169.193.180 Signed using mobile	Sent: 13 June 2023 | 09:24 Resent: 21 June 2023 | 08:16 Viewed: 21 June 2023 | 08:19 Signed: 21 June 2023 | 08:20
Electronic Record and Signature Disclosure: Accepted: 05 November 2020 | 13:36 ID: 66862256-a905-4c5d-ba30-bb75242d0362		

Signer Events	Signature	Timestamp
Michelle Clements michelle.clements@ucl.ac.uk Senior Statistician Security Level: Email, Account Authentication (Optional)	DocuSigned by: Michelle Clements AD78954D2C79466... Signature Adoption: Pre-selected Style Using IP Address: 144.82.114.199	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 10:09 Signed: 13 June 2023 | 10:09
Electronic Record and Signature Disclosure: Accepted: 13 June 2023 | 10:09 ID: 10475ff1-7a7b-4c2b-8297-24cedfce92e5		
SEAMUS O'BRIEN sobrien@gardp.org R&D Director GARDP Security Level: Email, Account Authentication (Optional)	DocuSigned by: SEAMUS O'BRIEN D14B119D1B524F9... Signature Adoption: Pre-selected Style Using IP Address: 81.140.178.187	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 20:38 Signed: 13 June 2023 | 20:38
Electronic Record and Signature Disclosure: Accepted: 12 June 2023 | 21:08 ID: 30ab8e0e-57a5-4312-b88a-c458a3ee256b		
Shamim Qazi qazis550@gmail.com Security Level: Email, Account Authentication (Optional)	DocuSigned by: Shamim Qazi 32DF8B1E2A284B7... Signature Adoption: Pre-selected Style Using IP Address: 213.55.244.218	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 12:17 Signed: 13 June 2023 | 12:18
Electronic Record and Signature Disclosure: Accepted: 03 July 2021 | 12:06 ID: b809a216-483b-4e32-ad61-5dbc1afcb28f		
Tim Cressey tim.cressey@cmu.ac.th Security Level: Email, Account Authentication (Optional)	DocuSigned by: Tim Cressey 1BBE17E54D2D44B... Signature Adoption: Pre-selected Style Using IP Address: 183.88.217.65	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 11:49 Signed: 13 June 2023 | 11:49
Electronic Record and Signature Disclosure: Accepted: 13 June 2023 | 11:49 ID: 0a0ad2ef-3d05-469b-bf6d-e579f40b9a46		
Tim Peto tim.peto@ndm.ox.ac.uk Security Level: Email, Account Authentication (Optional)	DocuSigned by: Tim Peto 99DD0A87112C477... Signature Adoption: Pre-selected Style Using IP Address: 2.97.9.123	Sent: 13 June 2023 | 09:24 Resent: 21 June 2023 | 08:16 Resent: 21 June 2023 | 18:03 Viewed: 21 June 2023 | 18:17 Signed: 21 June 2023 | 18:18
Electronic Record and Signature Disclosure: Accepted: 21 June 2023 | 18:17 ID: 3a12c975-ad86-4f9b-b805-1a681ae4700e		
Wolfgang Stohr w.stohr@ucl.ac.uk Security Level: Email, Account Authentication (Optional)	DocuSigned by: Wolfgang Stohr C02B7413DF134CE... Signature Adoption: Pre-selected Style Using IP Address: 144.82.114.253	Sent: 13 June 2023 | 09:24 Viewed: 13 June 2023 | 15:33 Signed: 13 June 2023 | 15:34
Electronic Record and Signature Disclosure:		

Signer Events	Signature	Timestamp
Accepted: 13 June 2023 | 15:33 ID: 9176078b-b622-4105-811a-6a2f3535debe		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	13 June 2023 | 09:24
Certified Delivered	Security Checked	13 June 2023 | 15:33
Signing Complete	Security Checked	13 June 2023 | 15:34
Completed	Security Checked	21 June 2023 | 18:18

Payment Events	Status	Timestamps

Electronic Record and Signature Disclosure

ELECTRONIC RECORD AND SIGNATURE DISCLOSURE

From time to time, MRC Clinical Trials Unit at UCL (we, us or Company) may be required by law to provide to you certain written notices or disclosures. Described below are the terms and conditions for providing to you such notices and disclosures electronically through the DocuSign system. Please read the information below carefully and thoroughly, and if you can access this information electronically to your satisfaction and agree to this Electronic Record and Signature Disclosure (ERSD), please confirm your agreement by selecting the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

Getting paper copies

At any time, you may request from us a paper copy of any record provided or made available electronically to you by us. You will have the ability to download and print documents we send to you through the DocuSign system during and immediately after the signing session and, if you elect to create a DocuSign account, you may access the documents for a limited period of time (usually 30 days) after such documents are first sent to you. After such time, if you wish for us to send you paper copies of any such documents from our office to you, you will be charged a $0.00 per-page fee. You may request delivery of such paper copies from us by following the procedure described below.

Withdrawing your consent

If you decide to receive notices and disclosures from us electronically, you may at any time change your mind and tell us that thereafter you want to receive required notices and disclosures only in paper format. How you must inform us of your decision to receive future notices and disclosure in paper format and withdraw your consent to receive notices and disclosures electronically is described below.

Consequences of changing your mind

If you elect to receive required notices and disclosures only in paper format, it will slow the speed at which we can complete certain steps in transactions with you and delivering services to you because we will need first to send the required notices or disclosures to you in paper format, and then wait until we receive back from you your acknowledgment of your receipt of such paper notices or disclosures. Further, you will no longer be able to use the DocuSign system to receive required notices and consents electronically from us or to sign electronically documents from us.

All notices and disclosures will be sent to you electronically

Unless you tell us otherwise in accordance with the procedures described herein, we will provide electronically to you through the DocuSign system all required notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you during the course of our relationship with you. To reduce the chance of you inadvertently not receiving any notice or disclosure, we prefer to provide all of the required notices and disclosures to you by the same method and to the same address that you have given us. Thus, you can receive all the disclosures and notices electronically or in paper format through the paper mail delivery system. If you do not agree with this process, please let us know as described below. Please also see the paragraph immediately above that describes the consequences of your electing not to receive delivery of the notices and disclosures electronically from us.

How to contact MRC Clinical Trials Unit at UCL:

You may contact us to let us know of your changes as to how we may contact you electronically, to request paper copies of certain information from us, and to withdraw your prior consent to receive notices and disclosures electronically as follows:
To contact us by email send messages to: s.assam@ucl.ac.uk

To advise MRC Clinical Trials Unit at UCL of your new email address

To let us know of a change in your email address where we should send notices and disclosures electronically to you, you must send an email message to us at s.assam@ucl.ac.uk and in the body of such request you must state: your previous email address, your new email address. We do not require any other information from you to change your email address.

If you created a DocuSign account, you may update it with your new email address through your account preferences.

To request paper copies from MRC Clinical Trials Unit at UCL

To request delivery from us of paper copies of the notices and disclosures previously provided by us to you electronically, you must send us an email to s.assam@ucl.ac.uk and in the body of such request you must state your email address, full name, mailing address, and telephone number. We will bill you for any fees at that time, if any.

To withdraw your consent with MRC Clinical Trials Unit at UCL

To inform us that you no longer wish to receive future notices and disclosures in electronic format you may:

i. decline to sign a document from within your signing session, and on the subsequent page, select the check-box indicating you wish to withdraw your consent, or you may;

ii. send us an email to s.assam@ucl.ac.uk and in the body of such request you must state your email, full name, mailing address, and telephone number. We do not need any other information from you to withdraw consent.. The consequences of your withdrawing consent for online documents will be that transactions may take a longer time to process..

Required hardware and software

The minimum system requirements for using the DocuSign system may change over time. The current system requirements are found here: https://support.docusign.com/guides/signer-guide-signing-system-requirements.

Acknowledging your access and consent to receive and sign documents electronically

To confirm to us that you can access this information electronically, which will be similar to other electronic notices and disclosures that we will provide to you, please confirm that you have read this ERSD, and (i) that you are able to print on paper or electronically save this ERSD for your future reference and access; or (ii) that you are able to email this ERSD to an email address where you will be able to print on paper or save it for your future reference and access. Further, if you consent to receiving notices and disclosures exclusively in electronic format as described herein, then select the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

By selecting the check-box next to 'I agree to use electronic records and signatures', you confirm that:

- You can access and read this Electronic Record and Signature Disclosure; and
- You can print on paper this Electronic Record and Signature Disclosure, or save or send this Electronic Record and Disclosure to a location where you can print it, for future reference and access; and
- Until or unless you notify MRC Clinical Trials Unit at UCL as described above, you consent to receive exclusively through electronic means all notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you by MRC Clinical Trials Unit at UCL during the course of your relationship with MRC Clinical Trials Unit at UCL.