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: NeoSep1

Version: 1.0
Date: 04-Mar-2022

ISRCTN #: ISRCTN48721236

Authorised by:

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Role: Neonatal Sepsis Project Leader
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Date: 07-MAR-2022

Name: Subasree Srinivasan
Role: GARDP Medical Director
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Date: 07 - MAR - 2022

Name: Professor Mike Sharland
Role: Chief Investigator
Institution: St George's, University of London

Signature:

Date: 07 - MAR - 2022

Name: Professor Ann Sarah Walker
Role: Senior Statistician
Institution: MRC Clinical Trials Unit at University College London, Institute of Clinical Trials and Methodology (MRC CTU at UCL, ICTM)

Signature:

Date: 07 - MAR - 2022

GENERAL INFORMATION

This document was constructed using the MRC CTU at UCL Protocol Template Version 9.0. The CTU endorses the Standard Protocol Items: Recommendations For Interventional Trials (SPIRIT) initiative. It describes the NeoSep1 trial, sponsored by the Global Antibiotics Research & Development Partnership (GARDP) and conducted collaboratively with the Medical Research Council (MRC) Clinical Trials Unit (CTU) at University College London (UCL), St George's, University of London (SGUL) and the Penta ID network.

This protocol provides information about procedures for entering participants into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but sites entering patients for the first time are advised to contact the trial team to confirm they have the most up-to-date version.

COMPLIANCE

International sites will comply with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (R2) and other applicable national regulations for each country where the trial will be conducted.

SPONSOR

GARDP is the trial Sponsor and has delegated responsibility for aspects of the management and conduct of the NeoSep1 trial to its collaborators, namely the MRC CTU at UCL, SGUL, the University of Antwerp and the Penta ID network. The delegation of responsibility is defined in separate agreements, but GARDP will remain responsible for the oversight of the trial according to the principles of GCP.

Queries relating to GARDP sponsorship of this trial should be addressed to Dr Subasree Srinivasan, Medical Director, and Sally Ellis, Children's antibiotics Project leader (see **Contact Details** section below).

FUNDING

GARDP, as the Sponsor, will be responsible for ensuring appropriate funding is in place to support this trial.

The MRC CTU at UCL is supported via core funding by the Medical Research Council, via UK Research and Innovation (UKRI) (grant number MC_UU_00004/05).

AUTHORISATIONS AND APPROVALS

This trial will be submitted for approval by Research Ethics Committees (REC), Institutional Review Boards (IRB) and Regulatory Authorities in each of the participating countries.

TRIAL REGISTRATION

This trial has been registered with the International Standard Randomised Controlled Trial Number (ISRCTN) Clinical Trials Register, where it is identified as ISRCTN48721236.

RANDOMISATIONS
Participating sites that have met all activation criteria are able to randomise via the electronic Data Capture system (eDC)

Serious Adverse Event (SAE) REPORTING
Please report all SAEs via the eDC system **within 24 hours of becoming aware** of an SAE

If you have any issues with reporting an SAE or have any questions please email mrcctu.neosep@ucl.ac.uk

SAEs that occur after the neonate has discontinued Investigational medicinal product (IMP) do not need to be reported in an expedited fashion within 24 hours but must be reported within 7 days of the site becoming aware

TRIAL ADMINISTRATION

Please direct all queries to the Trial Manager at the MRC CTU at UCL the first instance (mrcctu.neosep@ucl.ac.uk). Clinical queries will be passed to the Chief Investigator and/or Trial Physician and/or Sponsor as required.

GARDP

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PENTA

Prof Mike Sharland
Board Member of the Penta Foundation
MBBS BSc MRCP MD DTM&H FRCPCH

PARTICIPATING CENTRES AND CO-INVESTIGATORS

Please see **Section 3.2** for more information on participating centres

BIOANALYTICAL LABORATORY: PHARMACOKINETIC

Analytical Services International Ltd
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Hua Xu – Head of Clinical

BIOANALYTICAL LABORATORY: MICROBIOLOGY

University of Antwerp
Laboratory of Medical Microbiology
Universiteitsplein
Antwerp, B-2610, Belgium 1,

Study contact: to be identified

INDUSTRY COLLABORATORS (SUPPLYING TRIAL DRUGS ONLY)

INFECTOPHARM Arzneimittel und Consilium GmbH (Supply of fosfomycin)
Von-Humboldt-Str. 1
64646 Heppenheim
Germany

SHIONOGI (Supply of flomoxef)
Shionogi & Co., Ltd.
1-8, Doshomachi 3-chome, Chuo-ku,
Osaka 541-0045, Japan

For full details of all trial committees, please see **Section 16.**
NB: throughout this document, "MRC CTU at UCL" may be abbreviated to "CTU".

SUMMARY OF TRIAL

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Short Title of Trial	NeoSep1
Long Title of Trial	An open-label randomised controlled trial comparing novel combination and currently used antibiotic regimens for the empiric treatment of clinically diagnosed neonatal sepsis with a run-in confirmatory pharmacokinetic phase
Version	1.0
Date	04 MAR 2022
GARDP Protocol Number	Neo-Sep-001
ISRCTN #	ISRCTN48721236
Study Design	Part 1: Run-in sequential treatment cohort confirmatory pharmacokinetic and safety study Part 2: **P**ersonalised **RA**ndomised **C**ontrolled **T**rial (PRACTical) (Walker, White et al. 2021) comparing multiple different novel combination and currently used antibiotic regimens, including a Sequential Multiple Assignment Randomised Trial (SMART) design to allow randomisation to second-line antibiotic treatment where indicated.
Setting	Hospital
Trial phase	Phase III/IV/pragmatic public health
Type of Participants to be Studied	Hospitalised neonates aged ≤28 days and weighing >1000g with clinical signs of sepsis
Sponsor	GARDP
Chief Investigator	Prof Mike Sharland
PART 1 RUN-IN CONFIRMATORY PHARMACOKINETIC PHASE	
Interventions	Neonates hospitalised with clinical signs of sepsis will be enrolled into each of the following three sequential treatment cohorts (non-randomised)**:** • Fosfomycin and amikacin • Flomoxef and amikacin • Flomoxef and fosfomycin
Study Hypothesis	Recommended doses of fosfomycin and floxomef, in combination regimens to be studied in Part 2, will provide adequate drug levels in neonates with sepsis.
Primary Outcome Measure(s)	For fosfomycin and flomoxef, the following primary pharmacokinetic (PK) parameters will be estimated 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)
Secondary Outcome Measure(s)	For fosfomycin and flomoxef, the following secondary PK parameters will be derived from the population PK model: • Maximum plasma concentration (Cmax) • Time to Cmax (Tmax) • Apparent terminal elimination half-life (t1/2)

<table>
<tr><th>SUMMARY INFORMATION TYPE</th><th>SUMMARY DETAILS</th></tr>
<tr><td></td><td>• Area under the plasma concentration-time curve from 0 to last observed time point (AUC0–last)
• Area Under the Curve to infinity (AUC(0–∞))
• Volume of distribution at steady state (Vss)

Potential PK/PD relationships:
• Free drug AUC ratio to Minimum Inibitory Concentration (MIC) (fosfomycin)
• Fraction of time for free concentration above MIC (flomoxef)

Safety
• 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</td></tr>
<tr><td>Randomisation</td><td>None</td></tr>
<tr><td>Number of Participants to be Studied</td><td>Approximately 60 neonates will be enrolled and sequentially allocated to each of the three treatment cohorts. 20 evaluable neonates with all 3 PK samples on Day 1 will be required in each of 3 treatment cohorts.

In addition, across both fosfomycin containing cohorts, 10 neonates with a post-natal age under 7 days with complete Day 1 samples and Day 5 samples are required. The final sequential cohort will continue recruiting until both targets are achieved.</td></tr>
<tr><td>Duration</td><td>Approximately 9 months
Estimated date of First Participant First Visit (FPFV): Aug 2022
Estimated date of Last Participant Last Visit (LPLV): May 2023
Each neonate’s planned participation will be 28 days from enrolment.</td></tr>
<tr><td colspan="2">PART 2 – OPEN-LABEL RANDOMISED CONTROLLED TRIAL</td></tr>
<tr><td>Interventions</td><td>Participating sites will define locally relevant first and second-line treatment randomisation options for specific neonatal sub-populations in their site from the list below (second-line treatments will depend on first-line treatment received, and reflect an escalation of antibiotic therapy). A maximum of 8 first-line treatment options will be selected which will be informed by the feasibility assessment.

<table>
<tr><th>First-line treatment options</th></tr>
<tr><td>Ampicillin° and gentamicin</td></tr>
<tr><td>Cefotaxime or ceftriaxone</td></tr>
<tr><td>Fosfomycin and amikacin</td></tr>
<tr><td>Flomoxef and amikacin</td></tr>
<tr><td>Fosfomycin and flomoxef</td></tr>
<tr><td>Ceftazidime</td></tr>
<tr><td>Ceftazidime and amikacin</td></tr>
<tr><td>Piperacillin/tazobactam</td></tr>
<tr><td>Piperacillin/tazobactam and amikacin</td></tr>
<tr><td>Meropenem</td></tr>
</table>
<table>
<tr><th>Second-line treatment options</th></tr>
<tr><td>Cefotaxime or ceftriaxone</td></tr>
<tr><td>Fosfomycin and amikacin</td></tr>
<tr><td>Fosfomycin and flomoxef</td></tr>
<tr><td>Flomoxef and amikacin</td></tr>
<tr><td>Piperacillin/tazobactam ± amikacin*</td></tr>
<tr><td>Ceftazidime ± amikacin*</td></tr>
<tr><td>Meropenem</td></tr>
<tr><td>Locally selected therapy</td></tr>
<tr><td></td></tr>
<tr><td></td></tr>
</table>
°or benzylpenicillin or cloxacillin or amoxicillin
* use amikacin if not used first-line and susceptibility supported for this site

See Section 7 for details of how specific regimens will be chosen in each site site and details on use of locally selected therapy.</td></tr>
</table>

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Study Hypothesis	Mortality in hospitalised neonates with sepsis can be reduced by choosing a top-ranked antibiotic regimen compared with the World Health Organisation (WHO) recommended empiric antibiotic regimens for neonatal sepsis and other currently used regimens.
Primary Outcome Measure(s)	28-day mortality
Secondary Outcome Measure(s)	**Efficacy:** ▪ Clinical status, assessed at Days 3, 7, 14 and 28 after randomisation using a clinical recovery score based on data from the NeoOBS observational study (NeoSep Recovery Score) ▪ Clinically appropriate need for additional antibiotics beyond the first randomised treatment ▪ Clinically appropriate need for additional antibiotics beyond the first randomised and second (for failure) treatment ▪ Cure, defined as clinical improvement and no need for further antibiotic treatment for the original sepsis episode, at test of cure (TOC) visit (Day 14 ±3 days after randomisation) ▪ Length of stay during the index hospitalisation ▪ Systemic antibiotic exposure (days on antibiotics) during the index hospitalisation ▪ 90-day mortality ▪ Change in C-reactive protein to Day 3 and 7 from baseline (selected sites based on availability) **Safety**: ▪ Grade 3/4 adverse events (AEs) based on the International Neonatal Consortium Neonatal Adverse Event Severity Scale (NAESS) through Day 28 ▪ Adverse events of any grade related to antibiotics ▪ Modification (including discontinuation) of antibiotics for adverse reactions Note: Serious Adverse Events will be collected for pharmacovigilance and analysed descriptively but they are not trial outcome measures because the severity of illness of the population means that they will commonly be related to the underlying condition.
Randomisation	The main empiric treatment trial (Part 2) will use a novel **P**ersonalised **RA**ndomised **C**ontrolled **T**rial (PRACTical) design, in which each neonate is randomised only to pre-defined first-line regimens that are agreed with and clinically acceptable to each specific site for neonatal sub-population (ie early or late onset sepsis). The design will also include a Sequential Multiple Assignment Randomised Trial (SMART) design to allow randomisation to second-line treatment where indicated. For the second randomisation, randomisation lists of antibiotic options will be determined by the neonate's first randomised regimen and what is clinically appropriate for that specific site.
Number of Participants to be Studied	Approximately 3,000 neonates hospitalised with clinical signs of neonatal sepsis will be randomised.
Duration	36 – 42 months Estimated date of First Participant First Visit (FPFV): Q2 2023 Estimated date of Last Participant Last Visit (LPLV): Q3 2026 Each neonate's planned participation will be 90 days from randomisation.
Ancillary Studies/ Sub-studies	• Colonisation of key body sites by resistant bacteria to evaluate the selection of resistance during and after receipt of the novel regimens in individual neonates (selected sites) • Antimicrobial susceptibility testing of baseline blood culture isolates • Resource utilisation

TRIAL SCHEMA

Figure 1: Trial entry, treatment allocation and PK assessment (NeoSep1 Part 1)

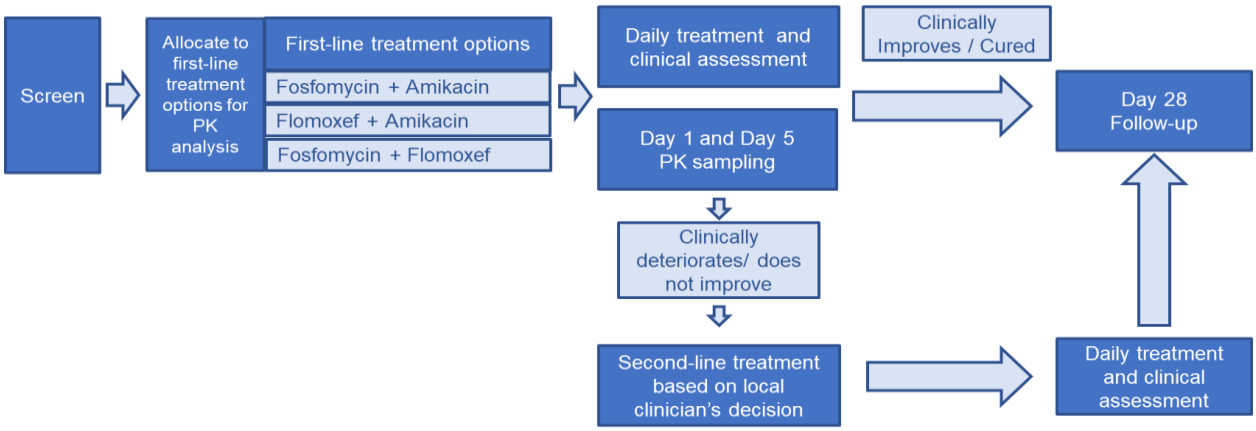
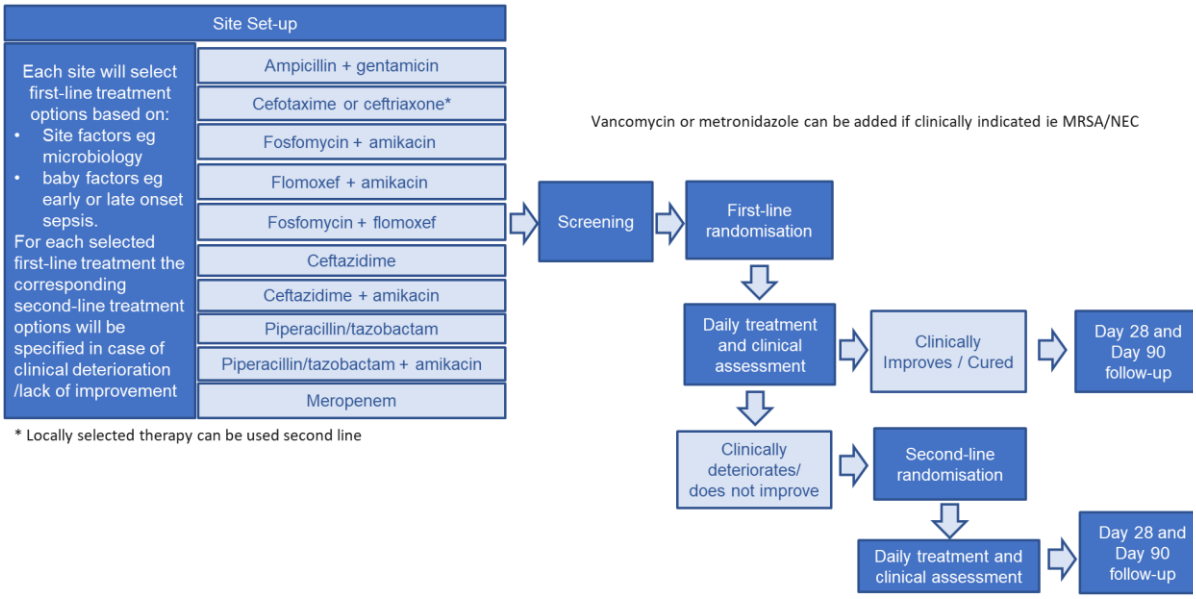


Figure 2: Trial Entry, Randomisation and first and second-line treatment (NeoSep1 Part 2)



Note: A maximum of 8 first line treatment options will be included in Part 2, which will be decided at a site level prior to the trial opening. See **Section 7** for details of management, including extending treatment duration depending on clinical judgement of baby's condition.

TRIAL ASSESSMENT SCHEDULE

Table 1: Trial Assessment Schedule (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.
Last FU visit for Part 1 participants will be on Day 28.

* 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. Trial related total blood sampling volumes should not exceed 3% of the total blood volume during a period of 4 weeks and not exceed 1% at any single time (TBV estimated to be 90 ml/kg body weight).
[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. See **Section 7** for further details of management, including extending treatment duration depending on clinical judgement of baby's condition.

Table 2: Trial Assessment Schedule (Part 2)

Trial Assessment Schedule									
Visit type	**Screening**	**Randomisation**	**Follow-up Treatment & Monitoring**				**TOC**	**StFU***	**LtFU***
Timing (window)	**Day 0**	**Day 1**	**Daily whilst on IV antibiotics**	**Day 3[7] (±1 day)**	**Day 7 (±2 day)**	**EOT [8] (Only if not Day 7 or 14)**	**14 (± 4 days)**	**28 (± 5 days)**	**90 (± 14 days)**
Informed assent/consent	x[1]								
Verification of eligibility	x	x							
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]	x[6]
Urea & Electrolytes (U&Es)	x[3]			x	x[6]	x[6]	x[6]	x[6]	x[6]
Liver function test (LFT)	x[3]			x	x[6]	x[6]	x[6]	x[6]	x[6]
Creatinine	x[3]			x	x[6]	x[6]	x[6]	x[6]	x[6]
Blood culture	x[4]			x[5]					
Administration of antibiotics		x	x	x	x	x			
Microbiology swab (peri-rectal) (substudy)		x				x	x		
Adverse events 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, LtFU = longer term follow-up visit. Last visit for Part 2 participants will be on Day 90.

* by telephone / if clinically indicated, then hospital visit.

[1] Written informed consent to be obtained from parent/guardian; at minimum, verbal consent must be obtained before randomisation.

[2] Randomisation and treatment initiation may be on the same day as the screening visit.

[3] Lab results required required within 48h before randomisation, but test can be done either at screening or randomisation or values from blood taken pre-screening.

All sites: FBC: Red blood count (RBC), white blood count (WBC) and differential, platelets
Selected sites: CRP, U&Es: including blood urea nitrate (BUN), sodium, potassium. LFTs: ALT, AST
[4] Blood must be taken for culture within 48h before randomisation, but may precede screening visit by up to 48 hours if already taken for clinical management.
Trial related total blood sampling volumes should not exceed 3% of the total blood volume during a period of 4 weeks and not exceed 1% at any single time (TBV estimated to be 90 ml/kg body weight).
[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] Randomisation to second-line treatment if the neonate fails to respond or clinically deteriorates (see **Section 7**).
[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 duration of antibiotic treatment including first and second line treatment is 14 ±7 days depending on the baby's condition. See **Section 7** for further details of management, including extending treatment duration depending on clinical judgement of baby's condition.
Note: Microbiology sub-study assessing colonisation will be conducted in selected sites only.

LAY SUMMARY

Babies admitted to hospital with sepsis are treated with medicines called “antibiotics”. In many countries, the antibiotics used are those recommended by the World Health Organisation (WHO). Other countries use different antibiotics based on local policies but unfortunately these are not always easily available. The use of different antibiotics also varies from baby to baby and between countries and hospitals.

More infections are now being caused by bacteria which are “resistant” to commonly used antibiotics. This means these antibiotics will not kill the bacteria and therefore will not cure the infection. These bacteria are often called multidrug resistant, as they are not killed by most of the antibiotics. We need to find new ways of treating these infections – using combinations of existing antibiotics is one possibility. Fosfomycin, flomoxef and amikacin are three antibiotics that could be combined into different two drug combinations. Another option is to give stronger antibiotics at the start of treatment. The problem with doing this is that not all babies will need these stronger antibiotics – and the more we use them, the more resistance will develop to these antibiotics. So using stronger antibiotics in lots of babies now, who don’t all need them, may mean that in future we will not be able to use them in any babies who might need them.

The NeoSep1 study will test how well giving fosfomycin and amikacin, OR flomoxef and amikacin OR fosfomycin and flomoxef works to treat babies 28 days old or younger who are in hospital with severe sepsis. It will also test how well these new combinations work compared to other antibiotics or combinations of antibiotics that are currently used globally. The study will be divided in two parts: Part 1 and Part 2.

Part 1 will measure the level of fosfomycin, amikacin and flomoxef in the baby’s blood; this is called a pharmacokinetic study or PK study. Each baby will get one of the three new combinations of antibiotics: fosfomycin and amikacin, OR flomoxef and amikacin OR fosfomycin and flomoxef. We will study 20 babies in each group, one after the other. We will use doses recommended in other studies. The information collected for Part 1 will confirm how much fosfomycin and/or flomoxef we should use in the next part of the study. We will also collect data on any side-effects. Babies in Part 1 will be followed up for 28 days.

In Part 2 of the study we will check how well these three combinations, as well as other antibiotics that are used routinely to treat sepsis in newborn babies, treat bacterial infections and stop babies dying.

A computer programme will assign antibiotic treatments at random (like the flip of a coin) and babies will get these antibiotics for approximately 7-10 days (their “first-line” treatment). If a baby’s condition gets worse during this time, or doesn’t get better as would be expected, doctors will be able to give them different antibiotics (also known as “second-line” treatment) to see if they do better with different antibiotics. Which specific second-line antibiotics each baby gets will also be chosen at random from a set of combinations that doctors would use after each first-line treatment. Babies in Part 2 will be followed up for 90 days, with a visit or telephone call 28 and 90 days after the baby entered the study to see if the baby is still doing well.

Over 3,000 babies will be included in Part 1 and 2 of the NeoSep1 study, from all over the world, and in particular from low and middle income countries such as South Africa, Kenya and other countries in Africa and South East Asia.

CONTENTS

ABBREVIATIONS

Abbreviation	Expansion
AE	Adverse event
ALT	Alanine Aminotransferase
AMR	Antimicrobial resistance
AR	Adverse reaction
AST	Aspartate transaminase
AUC	Area under the curve
BD	Bis in die (twice a day)
BiPAP	Bilevel Positive Airway Pressure
BSA	Body surface area
BUN	Blood urea nitrogen
CET	Cost-effectiveness threshold
CI	Chief Investigator
CI	Confidence interval
CL	Clearance
Cmax	Maximum concentration
CPAP	Continuous positive airways pressure
CPM	Clinical Project Manager
CSF	Cerebrospinal fluid
CTU	See MRC CTU at UCL
DMC	Data Monitoring Committee
DMP	Data Management Plan
DPA	(UK) Data Protection Act
DSUR	Developmental Safety Update Report
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
EMA	European Medicines Agency
EML	Essential Medicines List
EOT	End of treatment
ESBL	Extended Spectrum Beta-Lactamase (producing)
EU	European Union
FBC	Full blood count
FDA	(US) Food and Drug Administration
Fm	Fraction of size and scaled clearance at birth
FPFV	First participant first visit
g	Grams
GARDP	Global Antibiotic Research and Development Partnership
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
h	Hours
HFIM	Hollow Fibre Infection Model
HFNC	High flow nasal cannula
IB	Investigator Brochure

Abbreviation	Expansion
ICER	Incremental Cost Effectiveness Ratio
ICF	Informed consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IMP	Investigational medicinal product
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention-to-treat
IV	Intravenous
Km	Rate of postnatal maturation of clearance
LC-MS	Liquid chromatography–mass spectrometry
LFU	Longer term follow-up
LFT	Liver function test
LMIC	Low- and middle-income countries
LPLV	Last participant last visit
MDR	Multi-Drug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum Inhibitory Concentration
MOP	Manual of Operations
MRC	Medical Research Council
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
NIMP	Non-investigational-medicinal product
OD	Once daily
PD	Pharmacodynamics
PENTA	Paediatric European Network for Treatment of AIDS
PI	Principal Investigator
PIS	Patient Information Sheet
PK	Pharmacokinetics
PNA	Post-natal age
PRACTical	Personalised Randomised Controlled Trial (design)
pSBI	Possible serious bacterial infection
Q	Inter-compartmental clearance
Q(1,2,3, 4)	Quarter
QA	Quality Assurance
QC	Quality Control
QMAG	Quality Management Advisory Group
RBS	Red blood cells
RCT	Randomised controlled trial
REC	Research Ethics Committee
RGC	Research Governance Committee
SAE	Serious adverse event
SAP	Statistical Analysis Plan

Abbreviation	Expansion
SAR	Serious adverse reaction
SGUL	St Georges University of London
SMART	Sequential Multiple Assignment Randomised Trial
SOP	Standard operating procedure
SPC	Summary of Product Characteristics
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
SSA	Site-specific approval
SSG	Scientific Strategy Group
SSI	Site-specific information
StFU	Short term follow-up
SUSAR	Suspected unexpected serious adverse reaction
TM	Trial Manager
$T_{½}$	Half-life (time to half the initial concentration)
Tmax	Time of the maximum concentration
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
TOC	Test of cure
TSC	Trial Steering Committee
UAR	Unexpected adverse reaction
UCL	University College London
U&E	Urea and electrolytes
UKRI	UK Research and Innovation
V	Volume of distribution
Vp	Peripheral volume
WBC	White blood cells
WHO	World Health Organisation

1 BACKGROUND

1.1 NEONATAL SEPSIS: EXISTING EVIDENCE AND TREATMENT GUIDELINES

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 (pSBI) and 680,000 related deaths (Seale, Blencowe et al. 2013, Seale, Blencowe et al. 2014) (Thomson, Dyer et al. 2021). Increasing antimicrobial resistance (AMR), including a higher prevalence of Antimicrobial resistance (AMR) in isolates from septic neonates, threatens to undermine the effectiveness of WHO recommended antibiotic treatments in these settings (Downie, Armiento et al. 2013, Chaurasia, Sivanandan et al. 2019, Okomo, Akpalu et al. 2019, Bielicki, Sharland et al. 2020). 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. These multi drug resistant Gram-negative bacteria are now the dominant cause of neonatal sepsis in many high-burden settings (DeNIS 2016, Madhi, Pathirana et al. 2019).

Current World Health Organisation (WHO) guidelines, however, continue to recommend empiric first- and second-line regimens for neonatal sepsis that have remained unchanged for nearly 20 years, despite considerably higher observed rates of AMR (Fuchs, Bielicki et al. 2018) (**Table 3**).

Table 3: Current WHO recommendations for antibiotic therapy in infants aged 0-59 days with signs of possible serious bacterial infection or for prophylaxis (reproduced from Fuchs et al, 2018)

Reference	Conditions	Antibiotics	Dosing regimen
Pocket Book of Hospital Care for Children, 2013	Prophylaxis in neonates with documented risk factors	IM or IV ampicillin and gentamicin for at least 2 days	*Gentamicin* (IM/IV): First week of life Low-birthweight infants: 3 mg/kg once a day; normal birthweight: 5 mg/kg per dose once a day Weeks 2–4 of life: 7.5 mg/kg once a day *Ampicillin* (IM/IV): First week of life: 50 mg/kg every 12 h Weeks 2–4 of life: 50 mg/kg every 8 h *Benzylpenicillin (penicillin G)* (IM): First week of life: 50,000 U/kg every 12 h; weeks 2–4 and older: 50,000 U/kg every 6 h *Procaine benzylpenicillin* (IM): 50,000 U/kg once a day *Cloxacillin* (IV): First week of life: 25–50 mg/kg every 12 h Weeks 2–4 of life: 25–50 mg/kg every 8 h
	Case definition PSBI	IM or IV gentamicin and benzylpenicillin or ampicillin for at least 7–10 days	
	Greater risk of staphylococcus infection	IV cloxacillin and gentamicin for at least 7–10 days	
Managing possible serious bacterial infection in young infants when referral is not possible, 2015	Referral to hospital for young infants with PSBI is not possible	Option 1: IM gentamicin once daily for 7 days and oral amoxicillin twice daily for 7 days Option 2: IM gentamicin once daily for 2 days and oral amoxicillin twice daily for 7 days	*Gentamicin*: IM 5–7.5 mg/kg (for low-birthweight infants gentamicin 3–4 mg/kg) once daily *Amoxicillin*: Oral 50 mg/kg twice daily

A Cochrane review from 2004 for early-onset neonatal sepsis planned to meta-analyse data from trials to compare different regimens. But only 2 relevant studies including 127 neonates were identified, both published in the 1980s (Mtitimila and Cooke 2004). For late-onset neonatal sepsis, which includes sepsis episodes occurring while in hospital for management of other critical illness or conditions such as prematurity, the most recent Cochrane review is from 2021 (Korang, Safi et al. 2021). This included 5 trials and only 580 neonates. Each trial addressed a different 1:1 comparison and all the trials were deemed to be of low or very low quality. The authors concluded that current evidence was insufficient to support any antibiotic regimen being superior to another and called for further randomised controlled trials (RCTs) to be done.

However the key information needed to design a RCT, including what comparator(s) are being widely used, information on mortality to inform sample size calculation and clinical signs and

symptoms needed to define the population for the trial, was limited or not available, particularly in LMIC settings. A large neonatal observational study (NeoOBS) was therefore conducted in 19 sites in 11 countries including Bangladesh, Brazil, India, Kenya, South Africa, Uganda and Vietnam and enrolled 3204 infants (aged <60 days). Some key results from this study that informed the NeoSep1 clinical trial design include:

- There were 693 (21.7%) positive blood cultures that grew at least one organism. Gram-negative and Gram-positive pathogens were found in 355 and 196 infants, respectively (n=8 with both), and fungal pathogens in 21 (n=19 Candida spp). The most common pathogen isolated was *Klebsiella pneumoniae*
- The most common antibiotic regimens used were meropenem±vancomycin (n=438; 13.9%), ceftazidime±amikacin (n=435; 13.8%), piperacillin/tazobactam±amikacin (n=410; 13.0%), and ampicillin+gentamicin (n=403; 12.8%). WHO recommended second-line regimen cefotaxime or ceftriaxone were less common (<1/5 of regimens)
- Overall, 350 infants (11.3%; 95%CI 10.2-12.5%) died within 28 days of baseline blood culture
- A neonatal sepsis severity score (NeoSep Severity Score), based on infants' characteristics, supportive care and clinical signs at the time they presented with new signs of sepsis, was developed and validated using a random subset of Neo-Obs data from 15% of babies that were not included in the development of the score.

These findings were consistent with findings from another neonatal observational study (BARNARDS) which was undertaken to characterise the empiric antibiotic treatment in neonates with sepsis, in 12 sites located in 7 LMICs (Thomson, Dyer et al. 2021). In neonates diagnosed with clinical sepsis, blood culture was positive in 25% of the cases and of all the isolates, 87% were found to be Gram-negative bacteria (Thomson, Dyer et al. 2021). This study demonstrated increasing resistance to WHO standard of care antibiotics (ampicillin, gentamicin and third generation cephalosporins such as cefotaxime), and increased use of pipericillin/tazobactam, amikacin and ceftazidime particularly in Enterobacterales such as *Klebsiella pneumoniae*.

1.2 RATIONALE FOR EMPIRIC TREATMENT AND COMBINATION THERAPY FOR NEONATAL SEPSIS

Empiric treatment is considered necessary for any suspected infection which cannot be immediately confirmed microbiologically, and which can cause critical illness or death (Weiss, Peters et al. 2020). Neonates with sepsis frequently show non-specific signs and symptoms compatible with the early stages of an invasive bacterial infection and can rapidly develop multi-organ dysfunction with a high risk of mortality (Wynn, Kelly et al. 2017, Schmatz, Srinivasan et al. 2020), emphasising the importance of immediate treatment.

The aim of empiric treatment is to cover the typical spectrum of bacteria identified in neonates with positive cultures (Muller-Pebody, Johnson et al. 2011). However, due to the overlap between the signs of sepsis and other neonatal conditions such as prematurity or asphyxia, reliance on clinical signs and symptoms likely leads to more neonates being treated than are ultimately confirmed to have an invasive bacterial infection (Seale, Blencowe et al. 2014). This therefore creates a tension between the high use of broader spectrum antibiotics as empiric treatment to cover as many infecting pathogens with frequently high rates of resistance as possible, and the need to minimise the widespread use of broad spectrum antibiotics in neonates either without a bacterial infection or infection with a much less resistant pathogen to avoid the future development of AMR.

Given increasing and widespread AMR, the coverage of the current WHO-recommended regimens is expected to be low in many high-burden settings, although a risk-based approach, for example by timing of onset or underlying patient characteristics, are lacking (Williams, Isaacs et al. 2018, Bielicki, Sharland et al. 2020). The WHO recommends a single first-line and a single second-line regimen for all babies with sepsis globally, for both the community setting where rates of multi-drug resistant (MDR) pathogens are lower and the hospital setting, where rates of resistance are frequently much higher rates than in the community. While in many situations regimens based on WHO guidance may still be prescribed, alternative regimens, including empiric piperacillin/tazobactam and meropenem are also widely used in LMICs (Thomson, Dyer et al. 2021). Generally, a much more limited number of antibiotics are used in neonatal intensive care compared to pediatric or adult practice. Mostly, clinicians rely on established drugs with a neonatal licence or with a long history of use in the neonatal population (Hsia, Lee et al. 2019). New antibiotics targeting difficult to treat and resistant bacteria are often not accessible due to the lack of any neonatal dosing recommendations and may not be affordable in regions with the greatest need (Williams, Bradley et al. 2021). Combining different antibiotics into two drug regimens to improve coverage is common practice and provides an alternative to the empiric use of a broad-spectrum or other new antibiotics. For example, in one recent study, 6 of the top 10 regimens in use were combination regimens (Jackson, Hsia et al. 2019). In the community setting, efforts have been appropriately focussed on simplification of empriric treatment regimens. There is a clear need to re-evaluate the guidance for empiric treatment of neonatal sepsis in the hospital setting providing 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 (ESBL) producing organisms, alone or in combination. Given the lack of evidence supporting much neonatal sepsis treatment and the severity of the condition, it is critically 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. Developing novel options that are not carbapenem based is essential to avoid driving further resistance development (Elias, Moja et al. 2017). The only widely available treatment for carbapenem resistant infections in neonates is colistin, which has very challenging pharmcokinetics and toxicity concerns in this population.

1.3 RATIONALE FOR PROPOSED TRIAL DESIGN

While controlled trials are clearly needed, the standard approach of a large pragmatic randomised controlled trial with two arms comparing a single new treatment to the current standard of care, is unlikely to be feasible for the following reasons:

- There is no established single Standard of Care that would be acceptable in all settings of interest for all neonates. Many sites are now not using WHO-recommended regimens at all, or only for a specific types of neonates (N. Russell 2021)
- The specific novel regimens of interest also vary by site because of the varying burden of resistance to different drugs in different hospitals
- As a consequence, there is no clear set of regimens (including or excluding WHO-recommended regimens) that would be acceptable as standard of care across all settings of interest (N. Russell 2021)
- Such a standard two or multi-arm trial would make it difficult to include current within setting stratified approaches to decision-making (i.e. regimen selected based on a priori neonate-level or facility-level risk factors for resistant bacterial infection, including knowledge of local resistance patterns, early or late onset sepsis), which could support the continued use of some established narrow spectrum regimens in line with antimicrobial stewardship

- It is highly unlikely now that a single regimen will be the optimal option across all settings globally and patient subgroups

In addition, for antibiotics it may be argued that a single standard of care regimen potentially drives resistance to those recommended agents, and a paradigm shift is required to support diversification of prescribing. One mechanism of achieving this is a stratified risk-based regimen selection for different neonatal sub-populations, accompanied by prompt switching in the case of clinical deterioration or failure to respond, to avoid driving resistance development against single specific agents (Walker, White et al. 2021).

Theoretically, one can address these challenges by a network meta-analysis of multiple randomised controlled trials, each comparing different pairs of treatments, whose results can be combined to provide an overall picture of the evidence supporting different regimens. However, as described above, very few multi-centre, multi-country hospital-based neonatal sepsis antibiotic trials including relevant regimens have been done and none are underway (to our knowledge). Therefore, relevant data are unlikely to become available for many years.

This trial addresses these challenges by using a novel meta-analytic trial design which allows multiple parallel group comparisons from sites randomising different sub-populations of neonates across different sets of clinically relevant regimens for their site and specific sub-population known as Personalised RAndomised Controled Trial design (PRACTical) (Walker, White et al. 2021). This design has no specific single standard of care arm - each neonate is randomised between a set of regimens relevant to that neonate, and these sets differ from neonate to neonate and site to site. Importantly, the specific regimens and neonatal sub-populations most relevant to that site would be determined individually by each site **before** site initiation (see **Section 3**). **Table 4:** shows examples of how first-line treatment options might be tailored to different neonatal sub-populations, although sites are likely to differ based on many factors including local resistance patterns, local patterns of clinical care (eg on site maternity facilities), established routine clincial practice and local guidelines. Second-line options will depend on first-line regimen received.

Information about the relative performance of the different regimens in the trial would then be combined across the network of regimens being compared, using direct randomised evidence and indirect evidence across the network, to answer questions including “Of these different regimens, which is the best treatment to recommend?” “What is the ranking of these different treatments?”. Ranking can be done for multiple outcomes, including efficacy, safety, resistance, and cost.

Table 4: Examples of possible first-line randomisation lists for different sub-populations within a sites (based on NeoOBS study data) and examples of second-line options for a given first-line regimen

First-line treatment options	Early onset sepsis, preterm / term	Late onset sepsis, preterm, low amikacin resistance	Late onset sepsis, preterm, high amikacin resistence
Ampicillin + gentamicin	✓	×	×
Cefotaxime or ceftriaxone	✓	×	×
Fosfomycin and amikacin	✓	✓	×
Flomoxef and amikacin	✓	✓	×
Fosfomycin and flomoxef	✓	✓	✓
Ceftazidime	×	✓	✓
Ceftazidime+amikacin	×	✓	×
Piperacillin/tazobactam*	✓	✓	✓
Piperacillin/tazobactam+amikacin	×	✓	×
Meropenem	×	✓	✓

*If amikacin cannot be used

Second-line treatment options	First-line ampicillin + gentamicin	First-line Fosfomycin and amikacin	First-line ceftazidime
Cefotaxime or ceftriaxone	✓	×	×
Fosfomycin and amikacin	✓	×	×
Flomoxef and amikacin	✓	×	×
Fosfomycin and flomoxef	✓	×	×
Ceftazidime	×	✓	×
Ceftazidime+amikacin	×	×	×
Piperacillin/tazobactam*	✓	✓	✓
Piperacillin/tazobactam+amikacin	×	×	×
Meropenem	×	✓	✓
Locally selected therapy	×	✓	✓

One critically important aspect of empiric treatment is management of clinical deterioration or non-response, suggesting that either the neonate does not have a bacterial infection or that the antibiotic being used is insufficiently active against the infecting pathogen (which may not have been identified). Small blood volumes and other technical issues lead to low rates of positive blood cultures in neonatal sepsis. The potential benefits of using a novel combination of antibiotics to improve the early coverage of resistant pathogens for serious infections and clinical outcomes need to be balanced against the potential harm of higher selection of resistance associated with its routine first-line use in all neonates. A stewardship approach would suggest conserving the use of any new combination to second-line treatment in neonates with an inadequate clinical response. Arguably, it is prompt and effective management of non-response/deterioration that facilitates such a stewardship approach using narrower spectrum antibiotics empirically and reserving broader spectrum antibiotics for non-response/deterioration, rather than using broader spectrum antibiotics in all neonates empirically and hence driving resistance. However, this requires improved stratified first-line recommendations on the one hand and conservation of second-line agents for neonates with an inadequate response on the other hand – particularly as it is essentially similar antibiotic regimens that could be used for second-line and first-line.

This trial will directly address the question as to the potential advantages and disadvantages of using initial broader-spectrum empiric therapy versus narrower-spectrum empiric therapy with prompt switch to broader spectrum for clinical non-response/deterioration using a Sequential Multiple Assignment Randomised Trial (SMART) design (Almirall, Nahum-Shani et al. 2014). Specifically,

neonates randomised to an empiric regimen in the trial will be closely monitored for clinical non-response/deterioration, and if this occurs, they will be randomised to a second set of regimens, which will again depend on site appropriateness (particularly resistance patterns) as well as their first regimen (see **Table 4:)**. If there is only one or no trial regimen which the neonate can be randomised to second-line, then they may also receive local clinician-determined treatment as available in the site. This SMART randomisation will provide additional information about advantages and disadvantages of different approaches to sequencing first-line/empiric and second-line antibiotic therapy. There is a very limited evidence base to guide clinicians when it is safe to continue with the current first-line regimen and when to swtich to second-line therapy as the baby is deteriorating or not recovering appropriately. A novel neonatal sepsis recovery score that was adapted from WHO pSBI criteria for hospitalised neonates with sepsis and was developed from the NeoOBS study, using rates of recovery of simple clincial features and will be used to help inform clinical decision making (the NeoSep Recovery Score) (see **Section 8.3**).

1.4 EMPIRIC ANTIBIOTIC REGIMENS TO BE CONSIDERED IN NEOSEP1

Three groups of empiric antibiotic regimens will be included as options for selection into site-specific randomisation lists for the empiric treatment of specific neonatal sub-populations in NeoSep1:

- 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

For the third group, several candidate antibiotics were initially assessed using the following criteria:

- Established neonatal dose approved by a regulatory authority
- Antimicrobial activity against the most common current neonatal sepsis clinical isolates (Gram-negative and Gram-positive)
- Acceptable safety profile

Following this evaluation three antibiotics were selected: fosfomycin, flomoxef and amikacin.

Fosfomycin is a phosphonoic acid derivative discovered in 1969 as a natural product of *Streptomycetes* and *Pseudomonas syringae*. It acts as a bactericidal antibiotic by interfering with enzymes involved in the formation of bacterial cell walls (Li, Standing et al. 2017). Fosfomycin shows good activity against multi-drug resistant bacteria, including ESBL-producing Gram-negatives. The European Medicines Agency (EMA) label includes neonatal dosing recommendation as well as reccommendations that fosfomycin should be used in combination with other antibacterial agents to avoid selection of antibiotic-resistant mutants. As the parenteral formulation is a disodium salt, the recent single-centre NeoFosfo study investigated the effects of using intravenous fosfomycin on neonatal sodium levels in 60 neonates, to assess the potential risk of hypernatraemia. The study found no evidence of impact of fosfomycin on serum sodium levels and provided data to inform optimised dosing to achieve relevant pharmacodynamic targets. No other relevant safety signals were detected when comparing neonates receiving IV fosfomycin in addition to standard of care treatment for neonatal sepsis with those on standard of care treatment alone (Kane, Gastine et al. 2021).

Flomoxef was first produced in the 1980s and is an oxacephem antibiotic for parenteral administration similar to latamoxef (Simon, Simon et al. 1988). It is registered and widely used for

the treatment of neonatal sepsis across Japan, Taiwan and Korea with an approved neonatal dose. It has good activity against Gram-positive and Gram-negative bacteria, except *Pseudomonas aeruginosa,* and against anaerobes. Like all beta-lactams, the oxacephems achieve their bactericidal effects by binding to the transpeptidases (pencillin binding proteins) responsible for cross-linking peptidoglycan layers into the bacterial cell wall. Unusually, flomoxef is stable against certain beta-lactamases, in particular ESBLs except AmpC, and can therefore be used to target many multi-drug resistant Gram-negative infections. As a cephalosporin, it has a favourable toxicity profile with hypersensitivity reported as the only major side effect (Ito and Ishigami 1991). A recent systemic literature search evaluated available clinical and pharmacokinetic data on flomoxef use in neonates, constructed a population pharmacokinetic model and used this to simulate drug exposures of different flomoxef regimens. Individual-level clinical and pharmacokinetic data were extracted for 313 and 146 neonates, respectively, with population clinical data extracted for a further 199 neonates. The final population PK model incorporated body weight and postnatal age as covariates. Probability of target attainment analyses predicted that IV regimens of 20 mg/kg q12h, 20 mg/kg q6–8h and 40 mg/kg q6–8h are adequate for neonates aged 0–7, 7–14 and 14–28 days, respectively (Darlow and Hope 2021).

Amikacin is an aminoglycoside patented in 1971, and like other aminoglycosides achieves its bactericidal effects through interfering with bacterial protein production by binding to the ribosome. Amikacin is already used, usually in combination with a beta-lactam antibiotic, for the treatment of neonates in regions with a high prevalence of gentamicin resistance. It is the third and fifth most prescribed antibiotic in neonatal care in South-East Asia and Europe/the Eastern Mediterranean, respectively (Hsia, Lee et al. 2019). Considerable work has been done to define optimal dosing of amikacin for neonates, including in low-resource African settings (Amponsah, Adjei et al. 2017, Hughes, Johnson et al. 2017). While both oto- and nephrotoxicity are concerns when aminoglycosides are used in other populations, these adverse events occur at a very low rate in neonates. Long term renal impairment is very rare as there is a marked growth in renal size and function through infancy.

These three antibiotics have been assessed in detail using Hollow Fibre Infection Models (HFIM) to determine their pharmacokinetic and pharmacodynamic properties when used as part of a combination against relevant Gram-negative organisms. HFIM is an *in vitro* pre-clinical model that can assess the ability of antibiotics to kill and prevent emergence of resistance in bacteria when exposed to dynamic pharmacokinetic drug,replicating human time-concentration profiles of each drug in humans. Therefore, HFIM reflects the drug concentration modulations that are close to that seen in humans, in contrast to simplier static models (e.g. time-kill assays) that use drug concentrations that may not reflect *in vivo* activity.

The HFIM experiments consisted of a 16-arm 4 x 4 dose ranging experiment (that included doses that produces 0%, 20%, 50% and 80% of maximal bacterial killing for the test strain) to quantify pharmacodynamic interaction (i.e., the presence of synergy). This was followed by an assessment of the same combinations at clinically relevant doses against resistant Enterobacterales strains with different minimal inhibitory concentrations (MIC) for each of the antibiotics included e.g. high MIC to fosfomycin and low MIC to amikacin or vice versa (Kent, Turner et al. 2014, Darlow, Docobo-Perez et al. 2021). Each of the three combinations, fosfomycin and amikacin, flomoxef and amikacin, and fosfomycin and flomoxef were studied for determination of spectrum coverage (using minimum inhibition concentration assays), and potential synergistic antibacterial activity (using checkerboard assays), against a panel of 40 strains of bacterial species representative of those that cause neonatal sepsis in LMIC settings. This panel included 10 isolates for each of the following species: *E. coli* and *K. pneumoniae* ESBL producers, MRSA and group B streptococci (GBS). The Gram-negative bacterial

strains were selected to ensure that they were ESBLs producers from classes A, C and D, that are the target of the empiric therapy in the Neonatal Sepsis program.

The combination of fosfomycin and amikacin demonstrated synergy upon modelling of the experimental outputs (Darlow, Docobo-Perez et al. 2021). The combination regimen achieved prompt and sustained reduction in bacterial growth when tested at clinically relevant doses against resistant Enterobacterales strains with high fosfomycin and amikacin MICs. Similar synergy was seen with fosfomycin and flomoxef using similar protocols (Darlow and Hope 2021). The same assessment was completed on flomoxef and amikacin which demonstrated at least an additive effect using similar protocols (data on file) confirming all three combination regimens as clear options to be evaluated for the empiric treatment of neonatal sepsis caused by MDR pathogens.

In NeoSep1, we therefore propose to test the three novel dual combinations of these three existing, off-patent drugs, namely fosfomycin+amikacin, flomoxef+amikacin and fosfomycin+flomoxef. Reflecting that they have been infrequently used in neonatal populations, we will perform a run-in non-randomised pharmacokinetic study of these three combinations of fosfomycin, flomoxef and amikacin to confirm plasma drug levels at the proposed doses based on dosing recommendations and other studies, as well as collect safety data (Part 1) before the start of the main randomised trial (Part 2).

Additional antibiotics may be added during the main trial (Part 2) as first or second line treatment options following ethical and regulatory approval. This could be due to emerging data on effectiveness, resistance, toxicity, or availability of new antibiotics.

1.5 TRIAL OBJECTIVES

1.5.1 PRIMARY OBJECTIVES

In Part 1, the primary objective is to confirm that the recommended doses of fosfomycin and flomoxef, when used in combination with each other or amikacin to be studied in Part 2 will provide adequate drug exposure in neonates with sepsis.

In Part 2, the primary objective is to provide a ranking of eight different clinically relevant antibiotic regimens for first-line empiric and second-line (after lack of response/deterioration) treatment in terms of 28-day mortality as the primary outcome measure. It will flexibly compare these multiple different relevant treatment regimens to enable the trial to be run in sites worldwide with very different background rates of resistance and patterns of routine clinical care by randomising each participant to locally relevant antibiotic regimens agreed prior to site initiation. The trial will ensure generalisability by focusing inclusion based on clinical symptoms associated with high mortality risk in the NeoOBS study, which have been developed into a novel neonatal sepsis severity score – the NeoSep Severity Score.

1.5.2 SECONDARY OBJECTIVES

A secondary objective of Part 1 is to collect safety data.

In Part 2, a secondary objective is to also provide a ranking of clinically relevant antibiotic regimens based on other efficacy and safety secondary outcomes, as well as on health economic measures and the potential selection of resistance at both the individual baby and neonatal unit level.

The trial data will provide data to inform the balance between efficacy, safety, cost and propensity for resistance selection that will influence facility-level and national decision-making about adoption of studied regimens, and potential future inclusion in WHO guidelines.

2 PROTOCOL IMPLEMENTATION PLAN

The NeoSep1 protocol will be implemented in two parts to:

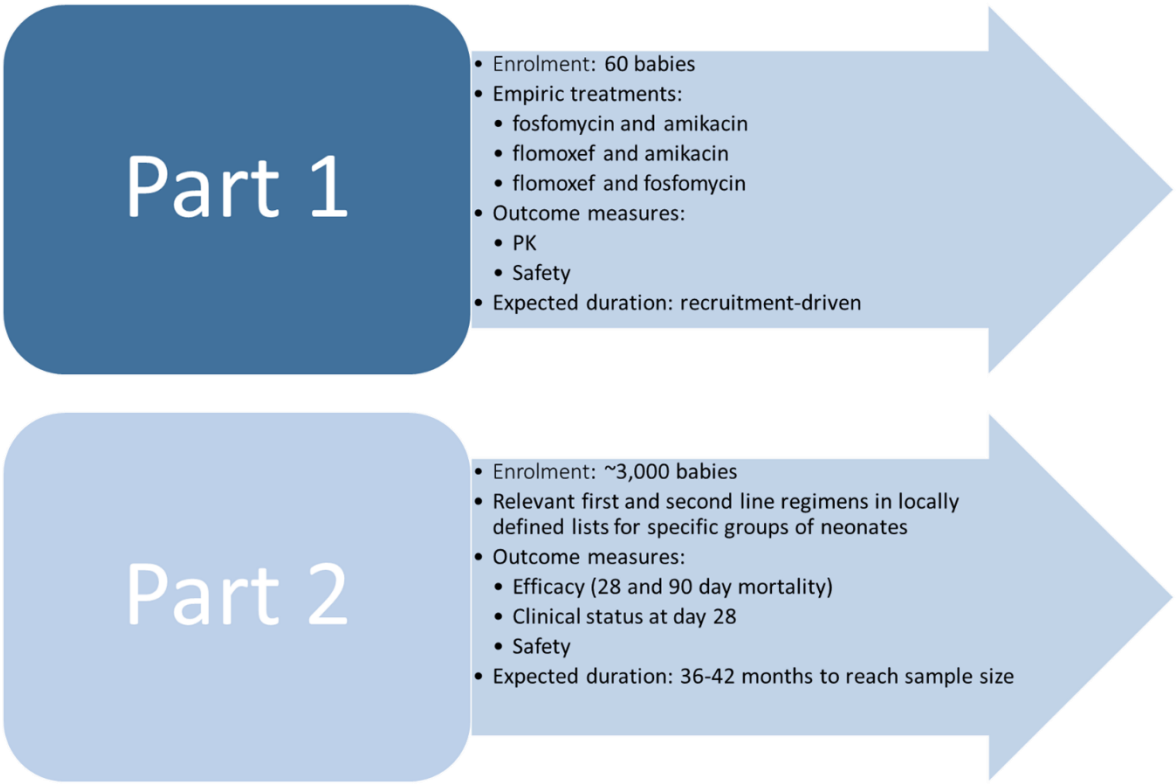
- Enable a staged approach to the protocol roll-out
- Allow the independent Data Monitoring Committee (DMC) to carefully monitor the accumulating data without posing unnecessary safety risks to participants
- Ensure the feasibility of the trial is established in a controlled manner across participating centres

Protocol implementation will be **milestone-driven**; in particular:

- Part 1: To confirm pharmacokinetics of combinations including two off patent drugs: fosfomycin and flomoxef, together and each in combination with amikacin
- Part 2: To determine the ranking of these three novel regimens, currently used WHO-recommended regimens and other common broad-spectrum regimens when used routinely as either first-line empiric or second-line therapy for neonatal sepsis, in terms of 28-day mortality

PK and safety data will be reviewed by an independent DMC, composed of members internationally recognised in their field of expertise eg antibiotic research, neonatology; for more information see **Section 16**.

Once the PK data confirms the planned doses of fosfomycin and flomoxef in Part 1, the trial protocol will proceed to Part 2.



2.1 TRIAL PART 1

Two participating countries will be involved in Part 1. Sites have been selected through a feasibility process conducted by the Sponsor based on the following criteria:

- Disease prevalence
- Clinical and research infrastructure
- Existing successful recruitment and collaboration in other relevant and GARDP-sponsored trials and studies in AMR

No specific recruitment target will be set for participating centres. There is not a fixed number that need to be enrolled in each cohort by centre in order to ensure that Part 1 completes as quickly as possible overall, but a minimum and maximum range will be set for each site. Recruitment will be allocated across 3 sites and sequentially across the three combination treatments.

Sixty evaluable neonates will be included in Part 1 (see **Section 11.3.1**). Hospitalised neonates will be sequentially allocated to receive:

- Fosfomycin and amikacin (first 20 neonates providing complete Day 1 PK samples)
- Flomoxef and amikacin (second 20 neonates providing complete Day 1 PK samples)
- Flomoxef and fosfomycin (third 20 neonates providing complete Day 1 PK samples)

If an included neonate clinically deteriorates, or fails to respond, then second-line treatment will be based on local clinician choice.

Doses used are those proposed by previous studies and current international dosing recommendations. There will be a review of safety data and preliminary analysis of pharmacokinetic samples when 20-40 neonates have been enrolled.

The Ethics Committees and Regulatory Authorities of the countries participating in Part 1 will be informed of the outcome of the results from Part 1 and any recommendations from the DMC following endorsement from the Trial Steering Committee (TSC). The final decision on proceeding to Part 2, including confirmation of dose, will be approved by the Sponsor.

2.2 TRIAL PART 2

Part 2 of the NeoSep1 protocol involves a PRACTical and SMART randomised trial design (see **Section 3.2** for more information on participating sites). No specific fixed recruitment targets will be set for participating centres in order to ensure that Part 2 completes as quickly as possible overall, but a minimum and maximum range will be set for each site. The trial aims to recruit approximately 3,000 neonates in total.

As part of set-up activities for Part 2, each site will define which first-line treatment regimens in **Table 4** are clinically appropriate for each participating neonatal unit. Each neonatal unit may define different sub-populations of neonates and a range of antibiotic regimens that it is appropriate to randomise that specific population in that neonatal unit between. For example, this may depend on local resistance patterns, whether the neonate is inborn (born in the recruiting hospital and not left since birth) or outborn (admitted from the community), birth weight, and postnatal age (early or late onset sepsis). This will follow local protocols and practice and so will not be centrally determined, rather reflecting the regimens that would be **locally relevant** for use outside of the trial in that setting. For each first-line regimen in a site's randomisation list, the site will also determine relevant second-line options. The responsible clinician at each site will also be able to select site-specific alternative regimens to use second-line that would not be available or acceptable to other sites. The first-line and second-line treatment options agreed with each site will be included in a country

specific protocol appendix that will be submitted to and approved by relevant ethics committees and regulatory authorities prior to the start of Part 2 of the trial (see country-specific appendix).

Part 2 of the protocol implementation plan will embed:

- A feasibility phase (10% of total sample size; 300 neonates)
- A main recruitment phase

Analysis of the feasibility phase of Part 2 of the protocol implementation plan will focus on the:

- Assessment of recruitment feasibility, which will be formally reviewed by the Trial Management Group (TMG), TSC and Sponsor and will address questions such as
    - Is the recruitment process, including consenting process, acceptable?
    - Are sites able to randomise patients appropriately to second-line treatments?
- Assessment of compliance with randomised first and second-line treatment strategies

Depending on findings, a protocol amendment may be submitted. The trial will then continue to the the main recruitment phase which will determine the efficacy, safety, antibiotic resistance development and health economic outcomes (main phase).

3 SELECTION OF SITES AND CLINICIANS

3.1 SITE ENROLMENT PLAN

A minimum and a maximum recruitment goal will be agreed at each site as relevant for each of Part 1 and Part 2. Within this range recruitment is competitive and the goal of both parts of the trial is rapid overall recruitment. For Part 1, the minimum is 10 and the maximum is 40 participants per site. For Part 2 the minimum is 50 and the maximum is 400 participants per site.

3.1.1 FEASIBILITY SITE ASSESSMENT

A feasibility site questionnaire will be sent to all prospective sites for Part 2 which will include information on local antibiotic prescribing and resistance patterns, routine patterns of care and clinical practice, resource availability, capacity and research experience.

3.2 PARTICIPATING SITES AND INVESTIGATOR SELECTION CRITERIA

Once a site has been identified, the trial team will provide the site with a copy of the current protocol.

3.2.1 PI'S QUALIFICATIONS & AGREEMENTS

The following criteria must be met by the local PI in order to fulfil their role and responsibility as clinical lead at the site:

1. The investigators should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial at their site and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, ethics committees, and/or regulatory authority(ies) as required by the country.

2. The investigator should be thoroughly familiar with the appropriate use of the investigational products as described in the protocol, in the Reference Safety Information (RSI) provided by the Sponsor, as appropriate.

3. The investigator should be aware of, and should comply with, the principles of GCP and the applicable regulatory requirements. A record of GCP training should be accessible for all investigators.

4. The investigator/site should permit monitoring and auditing by the Sponsor, and inspection by the appropriate regulatory authority(ies).

5. The investigator is responsible for supervising any individual or party to whom the investigator delegates trial-related duties and functions conducted at the trial site.

6. If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution should ensure this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.

7. The investigator should maintain a delegation log of appropriately-qualified persons to whom the investigator has delegated significant trial-related duties.

8. The investigator should sign an investigator statement, which verifies that the site is willing and able to comply with the requirements of the trial.

3.2.2 ADEQUATE RESOURCES

Adequate resources for the conduct of the trial are expected and in particular:

1. The investigator should be able to demonstrate a potential for recruiting a sufficient number of suitable participants within the agreed recruitment period (that is, the investigator regularly treats the target population).

2. The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

3. The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

4. The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

5. The site should have sufficient data management resources to allow prompt data return to the MRC CTU. Sites that have previously participated in GARDP or MRC CTU-coordinated trials should have a proven track record of good data return.

3.2.3 SITE ASSESSMENT

Each selected clinical trial site must complete the NeoSep1 Accreditation Form, which includes the Investigator Statement, Signature and Delegation of Responsibilities Log, and staff contact details. The Investigator Statement verifies that the site is willing, and able to comply with the requirements of the trial. This will be signed by the Principal Investigator (PI) at the site. In addition, and in compliance with the principles of GCP, all site staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to the MRC CTU at UCL. The CTU must be notified of any changes to trial personnel and/or their responsibilities. An up-to-date copy of this log must be stored in the Trial Master File (TMF) at the site and also at the MRC CTU.

3.3 PARTICIPATING SITES AND INVESTIGATOR SELECTION CRITERIA

All participating centres and investigators must meet the selection criteria detailed in **Section 3.2** in order to participate in the trial. There are no additional exclusion criteria.

3.4 APPROVAL AND ACTIVATION

A Clinical Trial Authorisation (CTA) will be obtained prior to the trial opening to recruitment according to the national regulatory authorities' requirements.

Site training will be performed prior to the activation of the site and will include all processes for the trial including but not limited to protocol training, data management procedures, procedures for handling of investigational medicinal product, adverse event reporting procedures, procedures for

laboratory samples, and frequency and expectations for any monitoring visits. A log of attendees will be kept in the TMF as a record of participants present at all types of training events.

Before a site can open to recruitment, formal Sponsor Site Green light/Accreditation will be completed to document that the site has met all the requirements to participate in the trial. Written confirmation of site activation will be sent to the PI. A Randomisation Pack will be provided to the site. The site's pharmacist will also be informed of the site's activation and (if applicable) an initial drug order will be dispatched to the named pharmacist (see the trial's Manual Of Operations (MOPs) for more detail).

1. The site should conduct the trial in compliance with the protocol as agreed by the Sponsor and, if required, by the regulatory authority(ies), and which was given favourable opinion by the relevant ethics committee.
2. The PI or delegate should document and explain any deviation from the approved protocol and communicate this with the trial team at the CTU.

4 SELECTION OF PATIENTS

There will be **no exceptions** to eligibility requirements at the time of randomisation. Questions about eligibility criteria should be addressed prior to attempting to randomise the participant.

The eligibility criteria are the standards used to ensure that only those for whom the trial is medically appropriate are considered for inclusion. Neonates not meeting the criteria should not be enrolled in the trial. For the safety of the neonates, as well as to ensure that the results of this trial can be useful for making treatment decisions regarding other neonates in similar situations, it is important that no exceptions be made to these criteria for admission to the trial.

Neonates will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

4.1 PATIENT 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), adapted from the WHO PSBI based scores for the hospital setting and developed using baseline clinical information and subsequent mortality from the NeoOBS study as described in **Table 5:5**; specifically, a baseline assessment NeoSep Severity Score of 5 or higher
6. Can receive at least 2 of the potential treatment options, ensuring randomisation is possible (Part 2 only)
7. 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
8. Parent/guardian willing and able to provide consent (written or, if their baby is severely ill, verbal consent confirmed by written consent as soon as possible). Verbal consent allows for administration of first-line antibiotics at no or minimal delay (see **Section 4.5** for details).

4.2 PATIENT 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 (IMP) that is a systemic drug, unless it has received prior approval by the NeoSep1 Trial Management Group (TMG)
4. Known contraindication to any of the trial antibiotics on the randomisation list for the relevant neonatal sub-population in that site (see **Section 6**; these will vary according to the antibiotics on the specific randomisation list)

Table 5: NeoSep Severity Score for predicting 28-day mortality based on clinical information at the start of a new episode of sepsis.

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 – 38.9 °C • ≥ 39 °C	 1 0 1 2
Maximum respiratory support: • None • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 0 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

Note: CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula. Table 5 indicates the criteria that define the NeoSEP Severity Score that was adapted from WHO pSBI criteria for hospitalised neonates with sepsis and based on the data generated from the NeoOBS study. See Manual of Operations (MOP) for details of assessment for each factor, based on the NeoOBS MOP. Babies weighing less than 1kg (1000 grams) at the point of enrolment are not eligible for this trial, but heavier babies may be included even if they weighed less than 1kg at birth.

The NeoSEP score allocates two points to any congential abnormality, indicating these babies are at higher risk of mortality. The exclusion criteria of major congenital abnormality anticipated to cause death **within this admission** aims to exclude neonates who are very unlikely to be able to benefit from any antibiotic regimen due to the severity of their condition; including these babies would dilute the differences between randomised groups.

4.3 NUMBER OF PATIENTS

4.3.1 PART 1

Enrolment will continue until twenty evaluable neonates have complete Day 1 PK samples for each of the three sequential treatment cohorts included in Part 1 (see **Section 2.1**) (approximately 60 neonates in total, with 20 evaluable babies per treatment cohort). Across both fosfomycin cohorts, at least 10 neonates with a post-natal age over 7 days with complete Day 1 samples and the Day 5 sample are also required; the final sequential cohort will continue recruiting until both targets are achieved. Recruitment is expected to be completed in 9-12 months.

4.3.2 PART 2

Approximately 3,000 neonates will be randomised in Part 2 across all participating sites. Recruitment is expected to be completed between 36-42 months.

4.4 CO-ENROLMENT GUIDELINES

Concurrent participation in any other clinical trial of a drug will not be permitted for the duration of the initial follow up period, i.e. within 28 days after randomisation. Participation in other studies that do not involve a systemic drug (ie topical intervention) may be acceptable but should be discussed with the TMG. The TMG will consider co-enrolment of NeoSep1 participants into other trials where the interventions do not conflict with the NeoSep1 objectives on a case-by-case basis (for both Part 1 and Part 2 of this protocol).

4.5 SCREENING PROCEDURES & PRE-RANDOMISATION INVESTIGATIONS

Most neonates with sepsis present as clinical emergencies, where delay in enrolment and therefore prompt antibiotic treatment, due to requiring a written consent procedure would be unacceptable.

In **Part 1**, because the objective requires trial specific procedures on Day 1 including blood samples that are not required for care, written informed consent must be obtained prior to enrolment.

In **Part 2**, because the objective is clinical and neonates will only be randomised to antibiotic regimens that the site judges would be appropriate to use in routine clinical practice and additional investigations are minimal, a two stage consent process will take place, with verbal consent obtained from the parent/guardian of the neonate first, to be confirmed by written informed consent as soon as possible. If the neonate's condition is not an emergency, for example if the neonate is admitted for other conditions and starts to deteriorate with early signs of sepsis, then written consent should be obtained where possible before randomisation.

In emergency situations in Part 2, verbal consent will be sought from parents or guardians by the admitting medical team, if it is considered that the full consent process would significantly delay treatment initiation, and consequently could be detrimental to the neonate's health. Written informed consent will be sought once the neonate's clinical condition has been stabilised, ideally within 48 hours from verbal consent. Caregivers will be provided with a verbal description in the local language of the trial and will be given the opportunity to "opt out" of the clinical research. The clinician will record the fact that verbal consent has been given on the relevant randomisation eCRF; written informed consent will be sought as soon as feasible; whether, and when, this was provided documented on a subsequent eCRF.

All parents or guardians will be given an information sheet in their usual language containing details of the NeoSep1 trial. The sheet will be read aloud to those who are unable to read, and the written

consent form signed by an independent witness where parents are not able to sign their name but provide only a thumbprint. Parents and guardians will be encouraged to ask questions about the trial prior to signing the consent form. The right of the parent/guardian to refuse to participate without giving reasons must be respected.

If written consent is not provided following verbal consent, the neonate will be excluded from analyses from the time consent is refused. If the neonate dies before written consent is obtained, written consent will not be sought to avoid distress to the parents/guardians, but the neonate will be included in the analysis to ensure this primary outcome is recorded.

It must be made completely and unambiguously clear that the parent or guardian of a neonate is free to refuse the participation of their neonate, in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment (or that of their neonate). Consent must be sought again if a neonate's legal guardian changes. Signed consent forms must be kept by the investigator and documented in the eCRF and a copy given to the parent/guardian.

5 REGISTRATION & RANDOMISATION

The neonate's eligibility for enrolment will be confirmed by the electronic Data Capture system (eDC) prior to enrolment (Part 1) or randomisation (Part 2) (see **Sections 4**, **5.1** and **5.2**).

Enrolment assessments will be performed as summarised in the Trial Assessment Schedule (see **Table 1: and Table 2:**), including taking blood for culture before initiating IV antibiotics or as soon as possible after initiation (unless a blood culture has already been performed in the 48h preceding enrolment). The clinician should complete the relevant screening and enrolment electronic Case Report Form (eCRF) which should be data entered directly onto the secure web-based trial database. Neither Part 1 or Part 2 are blinded so there are no unblinding procedures for this trial.

A trial register will be kept at the clinical site and will record all neonates who are eligible and invited to join the trial. Those accepting will have initials, date of admission, age (in days), randomisation date and unique trial identifier recorded. Those who refuse will have initials, date of admission, age (in days) and reason for refusal (if provided) recorded. The register will be kept in a secure place in each clinical site; must be available for monitoring, audit and inspection; and will be the responsibility of the Principal Investigator at that site.

Neonates in the microbiological substudy in Part 2 (conducted in specific sites only) should have peri-rectal swabs taken as soon as possible after consent, and wherever possible before initiation of antibiotics (with date and time the swab was taken recorded). This is in order to assay baseline commensal flora, including presence of any resistance genes, as early as possible in the antibiotic treatment course. A sample of faecal material may be taken instead of a peri-rectal swab if available from a nappy.

5.1 ENROLMENT PRACTICALITIES: PART 1

Approximately 60 participants in Part 1 will be enrolled sequentially across the 3 treatment cohorts:

- Fosfomycin and amikacin (first 20 evaluable neonates)
- Flomoxef and amikacin (second 20 evaluable neonates)
- Flomoxef and fosfomycin (third 20 evaluable neonates)

Any neonate without complete Day 1 PK samples will be replaced. Enrolment will be carried out via the electronic Data Capture (eDC) system following correct completion of the eCRF. Participants will be allocated a unique identifier. More information on enrolment procedures can be found in the trial MOP.

ENROLMENT
Participating sites that have met all activation criteria are able to enrol eligible participants via the electronic Data Capture system (eDC)

5.2 RANDOMISATION PRACTICALITIES: PART 2

Randomisation to first-line treatment regimens and second-line regimens will be carried out via the eDC system; this system will be tailored to each participating centre's agreed treatments for different sub-populations of neonates, confirmed during the feasibility site assessment phase.

Eligible participants will be allocated a unique trial identifier; this identifier will **not** change if the patient is further randomised to second-line treatment following lack of response or clinical deterioration. Not all participants will be randomised to second-line treatment. Randomisation to second-line treatment will only occur if the neonate does not improve clinically or clinically deteriorates (see **Section 7**), and there are two or more options in the relevant randomisation list for that neonate (i.e., randomisation is possible).

More information on enrolment procedures can be found in the trial MOP. Further details on the process of randomisation can be found in **Section 11**.

RANDOMISATIONS
Participating sites that have met all activation criteria are able to randomise eligible participants via the electronic Data Capture system (eDC)

A manual randomisation process will not be in place when the main electronic system is not working. Sites are advised to contact the CTU if there are any issues with the enrolment and randomisation process.

6 TRIAL TREATMENTS

6.1 INTRODUCTION

Neonates will be allocated to a first-line regimen and potentially, following lack of response or clinical deterioration, to a second-line regimen. More details on allocation to different treatment regimens in Part 1 and Part 2 of the protocol are provided in **Section 5**. Details on first-line and second-line treatment options are provided in **Section 7**. All antibiotics that are part of any first-line or second-line regimen in the Part 1 study or Part 2 trial are IMPs, specifically ampicillin, amoxicillin, amikacin, benzylpenicillin, cloxacillin, cefotaxime, ceftazidime, ceftriaxone, flomoxef, fosfomycin, gentamicin, meropenem, piperacillin/tazobactam.

For each antibiotic, treatment should be commenced as soon as possible and ideally within 3 hours after enrolment, in line with the Survival Sepsis Campaign Children's Guidelines. Treatment is expected to continue for 7±2 days based on clinician's judgment of the baby's condition but may be extended depending on wheter a pathogen is isolated from the baseline blood culture, the babies condition etc. The responsible clinician will be responsible for determining when discontinuation, a change of treatment or continuation of treatment is required (see also **Section 7** for details on treatment modification for clinical non-response or deterioration). Antibiotic-specific contra-indications should be considered when assessing participants against exclusion criteria 4 (see **Section 4.2**) and relevant blood tests performed if neccesary as per local standard of care.

Information specific to each antibiotic including dosing and dose modification, interruption and discontinuation is provided below and summarised in **Table 6**. For all antibiotics except aminoglycosides (gentamicin and amikacin) and fosfomycin, a variation in dosing of ±20% from the minimum and maximum given in **Table 6** below is allowed following standard practice and is not considered an overdose or underdose. For fosfomycin, the permitted variation is ±10%. All infusions should be via a vein. Information on storage and dispensing for all antibiotics is given in **Sections 6.11** and **6.12,** respectively. Treatment should not be shared with any other trial participants or neonates outside the trial. More information on pharmacy procedures for all antibiotics can be found in the Manual of Operations (MOP).

Table 6: Summary of dosing for trial IMP

Treatment	Total daily dose for sepsis¥	Total daily dose for suspected / confirmed meningitis¥	Number of divided doses per day	Minimum IV infusion duration
Amikacin	15 mg/kg*	15 mg/kg**		30 minutes
Ampicillin / amoxicillin	100-150 mg/kg**	200-400mg/kg**	2-3	10 minutes
Benzylpenicillin	100,000-200,000 IU/kg**	100,000-200,000 IU/kg**	2-4	10 minutes
Cefotaxime	100-150 mg/kg**	150-200mg/kg**	2-3	20 minutes
Ceftazidime	100-150 mg/kg**	100-150 mg/kg**	3	15 minutes
Ceftriaxone	80-100 mg/kg**	80-100 mg/kg**	1-2	60 minutes
Cloxacillin	100-150 mg/kg**	100-150 mg/kg**	2-3	30 minutes
Flomoxef	120-150 mg/kg **	120-150 mg/kg **	2-3	30 minutes
Fosfomycin	200-300 mg/kg *	200-300 mg/kg *	2	15 minutes
Gentamicin	5-7 mg/kg*	5-7 mg/kg**	1	20 minutes
Meropenem	60 mg/kg **	80-120 mg/kg**	3	15 minutes
Piperacillin/tazobactam	240-300 mg/kg** (piperacillin)	N/A	3-4	30 minutes

* ±10%
** ±20%
¥ Dosing is weight-based and adjusted by gestational age and postnatal age, as necessary.
Note: Bolus administration possible for all IMPs.

Recommendations for IV administration include IV infusion with a recommended infusion time and by slow IV bolus, if possible. Administration as a slow IV bolus should never be more rapid than over 3 minutes, and ideally over 5 minutes in all cases. If slower IV bolus administration is necessary, this is noted below. No IMP should ever be administered through an arterial line.

The acceptable solvents for reconstitution and dilution are listed for each IMP. No other solvents should be used. When selecting the most appropriate solvent from several options, the final target volume and general state of the neonate should be taken into account, including the possible impact of sodium and glucose administration when using normal saline and glucose solutions, respectively, see details below for specific drugs. Reconstituted solutions should not be used if any particulate matter or clouding is visible. Reconstituted IMP should be administered as soon as possible. After opening, unused portions must not be stored or shared and should be discarded immediately.

Further details for all IMP will be provided in the MOP, including additional instructions for reconstitution, compatibilities and storage once reconstituted.

Where reference is made to severe renal impairment this is defined as a serum creatinine >150 µmol/L or a urine output of <0.7 ml/kg per hour.

6.2 AMIKACIN

6.2.1 ADMINISTRATION

IV infusion over 30-60 minutes or by slow IV bolus.

6.2.2 STANDARD DOSING

15 mg/kg every 24 hours.

6.2.3 Reconstitution and Dilution

Reconstitution is not necessary. Suitable solvents for dilution include, Glucose Infusion 50 mg/ml (5%), 0.9% NaCl solution for infusion or Ringer's Lactate Solution.

6.2.4 Dose modifications or interruptions

Dose adjustments in neonates with severe renal impairment may be necessary in line with local practice.

6.2.5 Precautions for Use

Amikacin should not be initiated in neonates with known maternal myasthenia gravis.

6.2.6 Additional Safety Information

Avoid concurrent use with furosemide where possible. Indomethacin may increase neonatal amikacin plasma concentrations. Consider monitoring serum creatinine, if routinely available at site, when co-administering with other nephrotoxic agents, including polymixin B, colistin, vancomycin and other aminoglycosides, where possible.

6.2.7 Overdose

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. If the total daily dose exceeds 20 mg/kg, monitor serum creatinine immediately and after 12 hours. If values are abnormal or urine output is declining, repeat after 24 hours. Consider follow-up audiology, if available.

6.2.8 Supply and Labelling

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.3 AMPICILLIN / AMOXICILLIN

6.3.1 Administration

IV infusion over 10 minutes or by slow IV bolus.

6.3.2 Standard Dosing

Suspected or confirmed sepsis

- 0 to 7 days post-natal age (PNA): 50 mg/kg every 12 hours
- ≥ 8 days PNA: 50 mg/kg every 8 hours

For suspected meningitis total daily dose of up to 200-400 mg/kg in 2-3 divided doses may be administered (see **Section 7.3**).

6.3.3 Reconstitution and Dilution

Reconstitute prior to administration. Reconstitute with Glucose Infusion 50 mg/ml (5%), 0.9% NaCl solution for infusion or Ringer solution.

6.3.4 Dose modifications or Interruptions

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.3.5 PRECAUTIONS FOR USE

Ampicillin should not be initiated in neonates with a history of jaundice or hepatic impairment associated with the prior use of ampicillin.

6.3.6 ADDITIONAL SAFETY INFORMATION

Anticoagulant use is extrmely uncommon in neonatal care. Avoid concurrent use with anticoagulants due to the risk of increased bleeding tendency wherever possible, but if necessary, consider monitoring coagulation.

6.3.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown, but doses up to 300-400 mg/kg per day have been used. There are no specific management recommendations for overdose other than those listed in **Section 6.19.**

6.3.8 SUPPLY AND LABELLING

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.4 BENZYLPENICILLIN SODIUM

6.4.1 ADMINISTRATION

IV infusion over 10 minutes or by slow IV bolus.

6.4.2 STANDARD DOSING

- 0 to 7 days PNA: 50,000 IU/kg (equivalent to 30mg/kg) every 12 hours
- ≥ 8 days PNA: 50,000 IU/kg (equivalent to 30mg/kg) every 6 or 8 hours

6.4.3 RECONSTITUTION AND DILUTION

Reconstitute prior to use with Water for Injections or 0.9% NaCl solution for infusion. When administering as IV infusion, 0.9% NaCl solution for infusion is a suitable solvent for dilution.

6.4.4 DOSE MODIFICATIONS OR INTERRUPTIONS

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.4.5 PRECAUTIONS FOR USE

None specific.

6.4.6 ADDITIONAL SAFETY INFORMATION

Avoid concurrent administration with methylprednisolone.

6.4.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. There are no specific management recommendations for overdose other than those listed in **Section 6.19.**

6.4.8 SUPPLY AND LABELLING

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.5 CEFOTAXIME

6.5.1 ADMINISTRATION

IV infusion over 20 minutes or by slow IV bolus.

6.5.2 STANDARD DOSING

- 0 to 7 days PNA: 50 mg/kg every 12 hours
- ≥ 8 days PNA: 50 mg/kg every 8 hours

For suspected meningitis, a total daily dose of up to 150-200 mg/kg in 2-3 divided doses may be administered (see **Section 7.3**).

6.5.3 RECONSTITUTION AND DILUTION

Reconstitute prior to administration. Reconstitute with Water for Injection. Dissolve reconstituted product in Glucose Infusion 50 mg/ml (5%), 0.9% NaCl solution for infusion or Ringer Lactate Solution.

6.5.4 DOSE MODIFICATIONS AND INTERRUPTIONS

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.5.5 PRECAUTIONS FOR USE

None specific.

6.5.6 ADDITIONAL SAFETY INFORMATION

Cefotaxime should be injected over 5 minutes or longer when given as a slow IV bolus.

6.5.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown, but doses up to 200 mg/kg per day have been used. If the total daily dose exceeds 200 mg/kg, monitor for signs of encephalopathy (reversible on discontinuation), especially seizures.

6.5.8 SUPPLY AND LABELLING

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.6 CEFTAZIDIME

6.6.1 ADMINISTRATION

IV infusion over 15 minutes or slow IV bolus.

6.6.2 STANDARD DOSING

- 0 to 7 days PNA: 50 mg/kg every 12 hours

- ≥8 days PNA: 50 mg/kg every 8 hours

6.6.3 Reconstitution and Dilution

Reconstitute prior to administration with 0.9% NaCl solution for infusion, Glucose Infusion 50 mg/ml (5%) or Glucose Infusion 100 mg/ml (10%).

6.6.4 Dose modifications or Interruptions

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.6.5 Precautions for Use

Ceftriaxone should not be given to neonates in the following situations:

- Requiring treatment with calcium-containing intravenous solutions including total parenteral nutrition
- Known hyperbilirubinaemia requiring phototherapy

6.6.6 Additional Safety Information

Avoid concurrent use with furosemide. Avoid concurrent administration with amikacin and other aminoglycosides. Flush IV lines and giving sets in between ceftazidime and vancomycin administration to avoid precipitation.

6.6.7 Overdose

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. Transient encephalopathy has been described, monitor for seizures. There are no other specific management recommendations for overdose other than those listed in **Section 6.19**.

6.6.8 Supply and labelling

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.7 CEFTRIAXONE

6.7.1 Administration

IV infusion over 60 minutes or slow IV bolus.

6.7.2 Standard Dosing

- 80 mg/kg every 24 hours OR 50mg/kg every 12 hours if administered by slow IV bolus
- 100 mg/kg every 24 hours can be used if administered as IV infusion

6.7.3 Reconstitution and Dilution

Reconstitute prior to administration with Water for Injection, 0.9% NaCl solution for infusion or Glucose Infusion 50 mg/ml (5%).

6.7.4 Dose modifications or Interruptions

None specific.

6.7.5 PRECAUTIONS FOR USE

Ceftriaxone should not be given to neonates in the following situations:

- Requiring treatment with calcium-containing intravenous solutions including total parenteral nutrition
- Known hyperbilirubinaemia requiring phototherapy

6.7.6 ADDITIONAL SAFETY INFORMATION

Ceftriaxone should not be mixed with any solutions containing calcium because of the risk of a precipitate forming. Avoid concurrent use with anticoagulants. If unavoidable, monitor coagulation if possible.

6.7.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. There are no specific management recommendations for overdose other than those listed in **Section 6.19**.

6.7.8 SUPPLY AND LABELLING

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.8 CLOXACILLIN

6.8.1 ADMINISTRATION

IV infusion over 30 minutes or by slow IV bolus.

6.8.2 STANDARD DOSING

- 0 to 7 days PNA: 50 mg/kg every 12 hours
- ≥ 8 days PNA: 50 mg/kg every 8 hours

6.8.3 RECONSTITUTION AND DILUTION

Reconstitute prior to use with Water for Injections. Then dissolve in 0.9% NaCl solution for infusion, Glucose Infusion 50 mg/ml (5%) or Glucose Infusion 100 mg/ml (10%).

6.8.4 DOSE MODIFICATIONS OR INTERRUPTIONS

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.8.5 PRECAUTIONS FOR USE

None specific.

6.8.6 ADDITIONAL SAFETY INFORMATION

None specific.

6.8.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. Transient encephalopathy has been described for penicillins, monitor for seizures. There are no

other specific management recommendations for overdose other than those listed below in **Section 6.19.**

6.8.8 SUPPLY AND LABELLING

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.9 FLOMOXEF

6.9.1 ADMINISTRATION

IV infusion over 30 minutes or by slow IV bolus.

6.9.2 STANDARD DOSING

- 0 to 7 days PNA: 40 mg/kg injection/infusion 3 times per day (i.e., every 8 hours) - a total daily dose of 120 mg/kg/day
- ≥ 8 days: 50 mg/kg injection/infusion 3 times per day (i.e., every 8 hours) - a total daily dose of 150 mg/kg/day

6.9.3 RECONSTITUTION AND DILUTION

Reconstitute prior to administration. Reconstitute with Glucose Infusion 50 mg/ml (5%) or 0.9% NaCl solution for infusion. Do not use Water for Injection for reconstitution for IV infusion or injection.

6.9.4 DOSE MODIFICATIONS AND INTERRUPTIONS

Dose adjustments in neonates with severe renal impairment may be necessary taking in to consideration the impact of sepsis on kidney function. Changes will be based on the judgement of the local clinician.

6.9.5 PRECAUTIONS FOR USE

Flomoxef should not be initiated in neonates with active severe bleeding due to possible vitamin K deficiency.

6.9.6 ADDITIONAL SAFETY INFORMATION

Flomoxef can exacerbate vitamin K deficiency and its manifestations, primarily bleeding. Avoid concurrent use with furosemide if possible.

6.9.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. There are no specific management recommendations for overdose other than those listed below in **Section 6.19**.

6.9.8 SUPPLY AND LABELLING

Flomoxef manufactured by Shionogi, will be supplied to participating centres as arranged by the Sponsor. Flomoxef powder for solution for infusion will be supplied in 0.5, 5 or 1 g each in a 10ml glass vial. It will be labelled for clinical trials use only and according to local regulatory requirements.

6.10 FOSFOMYCIN

6.10.1 ADMINISTRATION

IV infusion over at least 15 minutes or by slow IV bolus.

6.10.2 STANDARD DOSING

Preterm neonates

- 0 to 7 days PNA or <1.5 kg: 100mg/kg every 12 hours
- ≥ 8 days PNA and ≥1.5 kg: 150 mg/kg every 12 hours

Term neonates

- Any PNA and ≥1.5 kg: 150 mg/kg every 12 hours

6.10.3 RECONSTITUTION AND DILUTION

Reconstitute AND dilute prior to administration. Reconstitute with Water for Injections and Glucose Infusion 50 mg/ml (5%) or Glucose Infusion 100 mg/ml (10%). Dilute with Water for Injections and Glucose Infusion 50 mg/ml (5%) or Glucose Infusion 100 mg/ml (10%) according to MOPs. Do not use sodium chloride containing solvents for reconstitution or dilution.

6.10.4 DOSE MODIFICATIONS OR INTERRUPTIONS

Dose adjustments in neonates with severe renal impairment may be necessary taking in to consideration the impact of sepsis on kidney function. Changes will be based on the judgement of the local clinician.

6.10.5 PRECAUTIONS

Fosfomycin should not be initiated in neonates with known serum sodium ≥150 mmol/L.

6.10.6 ADDITIONAL SAFETY INFORMATION

None specific.

6.10.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. If the total daily dose exceeds 350 mg/kg, monitor electrolytes immediately if possible and after 4 hours. If values are abnormal repeat after 8 hours.

6.10.8 SUPPLY AND LABELLING

For NeoSep1, fosfomycin manufactured by Infectopharm will be supplied to participating centres as arranged by the Sponsor. Fosfomycin 40 mg/ml powder for solution for infusion will be supplied in clear type-II glass bottles with a rubber stopper and pull-off cap containing 2g or 4g fosfomycin (in 30 ml bottle). It will be labelled for clinical trials use only according to local regulations.

6.11 GENTAMICIN

6.11.1 ADMINISTRATION

IV infusion over 20 minutes or by slow IV bolus.

6.11.2 STANDARD DOSING

- 0 to 7 days PNA: 5 mg/kg every 24 hours

- ≥ 8 days PNA: 7 mg/kg every 24 hours

6.11.3 Reconstitution and Dilution

Reconstitution is not necessary. When used as an IV infusion, dissolve in Glucose Infusion 50 mg/ml (5%), 0.9% NaCl solution for infusion.

6.11.4 Dose modifications and interruptions

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.11.5 Precautions for Use

Gentamicin should not be initiated in neonates with known maternal myasthenia gravis.

6.11.6 Additional Safety Information

Avoid concurrent use with furosemide, where possible. Consider monitoring serum creatinine where possible, if routinely available at site, when co-administering with other nephrotoxic agents, including polymixin B, colistin, vancomycin and other aminoglycosides.

6.11.7 Overdose

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown. If the total daily dose exceeds 9 mg/kg, monitor serum creatinine immediately if possible and after 12 hours. If values are abnormal or urine output is declining, repeat after 24 hours. Consider follow-up audiology, if available.

6.11.8 Supply and labelling

Local hospital stock will be used for the trial; local hospital stock will be appropriately labelled before dispensing.

6.12 MEROPENEM

6.12.1 Administration

IV infusion over 15 minutes or by slow IV bolus.

6.12.2 Standard Dosing

20 mg/kg every 8 hours.
For suspected meningitis total daily dose of up to 120mg/kg in 3 divided doses may be administered (see **Section 7.3**).

6.12.3 Reconstitution and Dilution

Reconstitute prior to administration. Reconstitute with Water for Injection. For administration as an IV infusion, may be directly reconstituted with Glucose Infusion 50 mg/ml (5%) or 0.9% NaCl solution for infusion.

6.12.4 Dose modifications or Interruptions

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.12.5 PRECAUTIONS FOR USE

None specific.

6.12.6 ADDITIONAL SAFETY INFORMATION

Monitor liver function in neonates with known pre-existing liver disorders. Avoid concurrent use of valproic acid or valproate (antiepileptic) due to an increased risk of seizures.

6.12.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown, but doses up to 120 mg/kg per day have been used. There are no specific management recommendations for overdose other than those listed in **Section 6.19**.

6.12.8 SUPPLY AND LABELLING

It is anticipated that local hospital stock will be used for the trial. If meropenem cannot be sourced locally it will be provided from a central stock provided by the Sponsor; stock will be appropriately labelled before dispensing.

6.13 PIPERACILLIN/TAZOBACTAM

6.13.1 ADMINISTRATION

IV infusion over 30 minutes or by slow IV bolus.

6.13.2 STANDARD DOSING

Based on piperacillin component:

- 0 to 7 days PNA: 80 mg/kg every 8 hours
- ≥ 8 days PNA: 100 mg/kg every 8 hours

6.13.3 RECONSTITUTION AND DILUTION

Reconstitute prior to administration. Reconstitute with Water for Injection, Glucose Infusion 50 mg/ml (5%) or 0.9% NaCl solution for infusion. Dissolve reconstituted product in Glucose Infusion 50 mg/ml (5%), 0.9% NaCl solution for infusion or Ringer Solution.

6.13.4 DOSE MODIFICATIONS OR INTERRUPTIONS

Dose adjustments in neonates with severe renal impairment may be necessary and should be done according to local practice.

6.13.5 PRECAUTIONS FOR USE

None specific.

6.13.6 ADDITIONAL SAFETY INFORMATION

Avoid concurrent use of piperacillin/tazobactam and vancomycin. If used together, monitor serum creatinine, if available.

6.13.7 OVERDOSE

The maximum daily dose above which dose-dependent toxicities may occur in neonates is unknown, but doses up to 400 mg/kg per day (piperacillin) have been used. If the total daily dose exceeds 400 mg/kg (piperacillin) monitor for seizures.

6.13.8 SUPPLY AND LABELLING

It is anticipated that local hospital stock will be used for the. If piperacillin/ tazobactam cannot be sourced locally it will be provided from a central stock; local hospital stock will be appropriately labelled before dispensing.

6.14 TREATMENT COMBINATIONS

Fosfomycin and flomoxef must only be administerd with each other or with amikacin. Neither fosfomycin nor flomoxef should be administered concurrently with other antibacterial agents

Where multiple agents are given as trial treatment combinations, each agent should be administered consecutively. When given as IV infusion, the drug with the shorter infusion time should generally be administered first, followed by the drug with the longer infusion time. Therefore, the treatment sequence should be as shown in **Table 6**. The IV line and giving set should be flushed before administering the second drug by IV infusion.

When given as slow IV bolus, no particular order needs to be respected. IV lines and and giving sets should be flushed before administering the second drug.

When one drug is given as a slow IV bolus and the other as an IV infusion, the drug given as a slow IV bolus should be administered first. The IV line and giving set should be flushed before administering the second drug by IV infusion.

When Beta lactams (penicillins, cephalosporins, piperacillin/tazobactam, flomoxef and meropenem) and aminoglycosides (amikacin, gentamicin) are being given through the same IV line, they should be given consecutively, with the beta-lactam administered first.

Table 7: Infusion durations and ordering for combination trial treatments

Treatment combination	First infusion	Treatment gap	Second infusion*
Fosfomycin+ amikacin	Fosfomycin	5 min**	Amikacin
Flomoxef+ amikacin	Flomoxef		Amikacin
Fosfomyin+ flomoxef	Flomoxef		Fosfomycin
Ampicillin or other penicillin + gentamicin	Ampicillin		Gentamicin
Ampicillin or other penicillin + amikacin	Ampicillin		Amikacin
Piperacillin/tazobactam + amikacin	Piperacillin/tazobactam		Amikacin
Any cephalosporin other than ceftriaxone + amikacin	Cephalosporin		Amikacin

* Slow push administration possible for second infusion
**Treatment gap can be up to 10 minutes

6.15 STORAGE

Fosfomycin and flomoxef must be kept segregated from clinical stock throughout the course of the trial to ensure appropriate accountability. For more information on storage conditions the MOP should be consulted to ensure products are used within the required conditions.

6.16 DISPENSING

Infusions should be prepared following the specified dilution requirements; once prepared the product should be used as soon as possible.

Information on dispensing procedures is specified in the MOP. Dispensing will be recorded in the relevant pharmacy documentation, as required.

6.17 ACCOUNTABILITY & UNUSED DRUGS

Accountability must be maintained for all IMPs dispensed to participants. Any diluted product that has been dispensed from pharmacy must be destroyed if not used for the treatment of a trial participant. Vials containing undiluted product should be stored for drug accountability. Expired products should be quarantined prior to destruction according to local procedures and recorded in the appropriate trial-specific log. Logs may be collected centrally for monitoring purposes. More information on accountability and pharmacy procedures, including destruction procedures, can be found in the trial MOP.

6.18 COMPLIANCE & ADHERENCE

Data on trial treatment administration, including frequency, doses, start time of infusion/injection and duration, will be collected via the eDC system as part of the trial data collection. As treatment is IV and will be administered by nurses, no other compliance and adherence data will be collected; reasons for missed IV doses will be collected.

6.19 TRIAL MEDICATION OVERDOSE

Whilst all care should be paid to ensure the correct treatment dose is administered to participants, overdoses are possible (defined as >20% of the maximum dose provided in **Table 6,** >10% for fosfomycin, or total daily doses exceeding maximum doses in use as specified above). There is no specific antidote for any of the trial IMP. These should be handled by the responsible clinician as follows:

- Review treatment administration immediately when the overdose is identified
- Use symptomatic and supportive therapy as clinically required, including measures to accelerate elimination (e.g. ensuring adequate hydration), symptomatic treatment of any adverse reactions (e.g. convulsions).

All instances of overdoses must be reported to the CTU as soon as possible after occurring and within a maximum of 24 hours. The CTU will triage with the clinical team at SGUL and provide additional advice as needed. Information on overdosing will also be captured on the trial's eDC system (please refer to the MOPs for more information).

6.20 PROTOCOL TREATMENT DISCONTINUATION

In consenting to the trial, parent/guardians are consenting to trial treatment, trial follow-up and data collection for their neonate. However, a parent/guardian may decide to stop trial treatment early or trial treatment may be stopped early for any of the following reasons:

- Blood culture positive for a pathogen that is not susceptible to trial treatment (note: if the neonate is clinically responding well the clinician should make a judgement about the relevance of local susceptibility results, particularly where two drug combination therapy is being used
- Any change in the neonate's condition that justifies the discontinuation of treatment in the clinician's opinion
- Withdrawal of consent for trial treatment by the neonate's parent/guardian

As the neonate's participation in the trial is entirely voluntary, their parent or legal guardian may choose to discontinue trial treatment at any time without penalty or loss of benefits to which they are otherwise entitled. Although the parent or legal guardian is not required to give a reason for discontinuing trial treatment, a reasonable effort should be made to establish this reason while fully respecting their rights.

It should be clear to the parent or legal guardian and recorded in the patient notes what aspect(s) of the trial the participant is discontinuing their participation. These could include:

- Withdrawal from further treatment
- Withdrawal from sample collections
- Withdrawal from further trial follow-up
- Withdrawal from use of routine health records

Information on any level of patient discontinuation should be recorded on the relevant electronic Case Report Forms (eCRFs).

Participants should remain in the trial for the purpose of follow-up, regardless of treatment received, unless consent is specifically withdrawn for this. Data on patients who stop trial treatment or follow-up early will be kept and included in analysis. Consent to use pseudonymised data in analysis cannot be withdrawn retrospectively; only consent for future data collection can be withdrawn. If participant has ongoing SAEs we will collect follow-up safety data, until resolution or there is no further change. If a patient ceases follow-up early, refer to **Section 8.7**.

6.21 NON-TRIAL TREATMENT (CONCOMITANT MEDICATIONS)

All oral, intravenous, intramuscular and topical treatments for any condition are considered a concomitant medication, including blood transfusion. All clinically indicated medications are permitted in trial participants; information on the medication (but not the dose or frequency) will be collected on the eCRF. Medicines to be used with caution are indicated in **Section 6.2 to 6.13** for each antibiotic (under "**ADDITIONAL SAFETY INFORMATION**").

Patients who go on to receive non-trial antibiotics following early cessation of trial antibiotics will remain on trial, following the same visit schedule where possible, for the purposes of follow-up and data collection (unless they withdraw their consent from all stages of the trial).

In particular, the routine use of specific antibiotics for particular suspected infections is permitted. These include vancomycin for the treatment of MRSA and other Gram-positive infections, metronidazole, for anaerobic infections, including necrotising enterocolitis (NEC) and colistin for carbapenem-resistant infections.

6.22 CO-ENROLMENT GUIDELINES

Co-enrolment in previous or future trials is considered in **Section 4.4**.

7 FIRST-LINE AND SECOND-LINE REGIMENS

7.1 FIRST-LINE TREATMENT REGIMENS

Neonates will be allocated to a first-line treatment option, sequentially across treatment cohorts in Part 1 and by randomisation in Part 2.

As part of set-up activities for Part 2, each site will define which first-line treatment regimens in **Table 8** (**Part 2**) are clinically appropriate for specific sub-populations of babies in each participating neonatal unit. Each neonatal unit will define the list of antibiotics that they decide is appropriate to randomise that specific population in that neonatal unit to, as outlined in **Section 2.2**. For each site, these are listed in the country-specific appendices.

Ten regimens are under consideration for inclusion in the PRACTical design, but only eight regimens will be included in the trial. The sample size calculation is therefore based on 8 first-line regimens (see **Section 11.3.2**). WHO-recommended regimens (ampicillin (amoxicillin or benzylpenicillin or cloxacillin) + gentamicin, cefotaxime or ceftriaxone) and the three novel two-drug combinations of existing off-patent antibiotics (fosfomycin, flomoxef and amikacin) will be included in the trial. However, we expect that during site set-up activities, two of the five broad-spectrum regimens (piperacillin/tazobactam, piperacillin/tazobactam + amikacin, ceftazidime, ceftazidime + amikacin or meropenem) will be excluded from Part 2 of the trial, based on feedback from the site feasibility (ie no or very few sites select it for any sub-population of neonates). Any changes to the agreed local treatment regimens will only be implemented following approval by ethics committee and regulatory body, except for any changes in antibiotic treatments required in response to local outbreaks.

Table 8: First-line treatment options

Part 1

First-line treatment options
Fosfomycin and amikacin
Flomoxef and amikacin
Fosfomycin and flomoxef

Part 2

First-line treatment options
Ampicillin (amoxicillin or benzylpenicillin or cloxacillin) + gentamicin
Cefotaxime or ceftriaxone
Fosfomycin and amikacin
Flomoxef and amikacin
Fosfomycin and flomoxef
Piperacillin/tazobactam
Piperacillin/tazobactam + amikacin
Ceftazidime
Ceftazidime + amikacin
Meropenem

Note: see **Section 6** for details of drug administration, dosing etc. Regimens in *grey* will be included in the final randomisation lists for Part 2; two regimens in white will be dropped based on site relevance.

First-line treatment is expected to continue for 7±2 days for babies with culture-negative sepsis and 10 [-3,+4] days for culture-positive sepsis. If antibiotics are switched to second-line treatment, the

total expected duration of antibiotic treatment including first and second line is 14 ±7 days depending on the baby's condition.

Dose modification may occur due to toxicity; please refer to **Section 6.2 to 6.13** for more information on each recommended antibiotic dose modification.

7.2 LACK OF RESPONSE TO OR CLINICAL DETERIORATION ON FIRST-LINE TREATMENT

Initial response to first-line treatment should be formally assessed on Day 3 (window ±1 day) after initiation of first-line treatment for all patients (Part 1 and Part 2). However, in the situation where the neonate's clinical condition deteriorates rapidly between 24 and 48 hours, first-line treatment should be switched to second-line immediately (see **Section 7.3** for details of second-line treatment). **A blood culture must be taken whenever possible before switching to second-line treatment**.

Based on the NeoOBS study, the majority of neonates with successful treatment outcomes have improved their clinical status at 48-96 hours. The time-updated NeoSep Recovery Score, including 6 clinical signs and level of respiratory support required was developed from daily updated assessments of neonates' status in the NeoOBS study and was strongly associated with mortality (see Table 9:). At Day 3, a score of 4 or higher was the most predictive of babies who died in the following 4 days, whether this was an increase from baseline or lack of initial response or an improvement but not to levels below this. Therefore, a NeoSep Recovery Score of 4 or higher on Day 3 should be considered as a prompt to switch to second-line treatment. Neonates whose NeoSep Recovery Score is 3 or lower on Day 3 should remain on first-line treatment and continue to be monitored daily throughout the planned duration of first-line treatment (7±2 days) for babies with culture-negative sepsis and 10 [-3,+ 4] days for babies with culture-positive sepsis. If their score increases to 4 or higher they should switch to second-line treatment. These scores have been designed to assist with clinical decision making and are not binding, still allowing local clinical judgement as to whether switch to second-line treatment is in the best interest of the child.

Table 9: NeoSep Recovery Score using time-updated clinical information 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 – 38.9 °C • ≥ 39 °C	 1 0 1 2
Maximum respiratory support: • None • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 0 2 3 3
Abdominal distension	1
Difficulty in feeding	1
Evidence of shock including cold peripheries	1

Factor (clinical signs in the preceding 24h)	Score value if present
Lethargy / no or reduced movement • Lethargy only • No or movement only on stimulation +/- lethargy	 1 2
Cyanosis	1

Note: CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula. Table 9 indicates the criteria that define the NeoSEP Recovery Score that was developed based on the data generated from the NeoOBS study. See Manual of Operations (MOP) for details of assessment for each factor, based on the NeoOBS MOP.

In 10-20% of neonates, a pathogen will be isolated from a baseline blood culture, and susceptibility results will usually become available between Day 2-4. These results should be taken into account in the decision as to whether to switch to second-line, together with the neonate's clinical status. For example, if a susceptibility test result suggests that the pathogen isolated from a baseline blood culture was resistant to a drug the neonate is receiving, but the neonate has significantly improved clinically on that regimen, switching is not mandated regardless of the NeoSep Recovery Score. If antibiotics are switched the total duration of antibiotic treatment including first and second line treatment should be 14 ±7 days.

Switch to non-trial antibiotics, and continuation of antibiotics for longer than planned above, is permitted in the case of rapid deterioration and/or long-term non-response and/or identification of other complications of sepsis (e.g. meningitis), but all neonates will continue to be followed up "on-study, off-study-treatment" for the complete duration of follow-up.

7.3 MENINGITIS

Symptoms and signs of meningitis in neonates are often subtle and difficult to determine clinically. Neonatal meningitis is rare and is most commonly diagnosed when a range of screening bacteriological specimens including a lumbar puncture are taken from a neonate as a "septic work up" for suspected clinical sepsis. Investigations for meningitis, including a lumbar puncture, should be performed as guided by local policies and decided by the neonate's local doctor and clinical team.

Neonates with suspected clinical meningitis, where no lumbar puncture is performed, should either remain on allocated first-line regimen or switch to second line (including randomisation) the same as for babies with clinical sepsis and no suspicion of meningitis. This also includes neonates where the lumbar puncture results are suggestive of meningitis (eg high white cell count), but there is no positive bacterial culture, apart from babies who are receiving piperacillin-tazobactam based regimens, where consideration should be given to switching to alternative regimens, such as the WHO recommended ceftriaxone/cefotaxime and gentamicin depending on the baby's condition.

Neonates with a positive culture from the cerebro-spinal fluid (CSF) result from the lumbar puncture should be considered in a similar way to neonates with a positive blood culture. If the pathogen is sensitive to the antibiotic the baby is receiving, then these should be continued. Dose adjustment will be necessary for certain antibiotics and details will be given in the MOP. If the pathogen isolated is resistant to the antibiotics the neonate is receiving or there is any clinical concern, then at the local clinician's discretion the antibiotic may be changed to locally used regimens for neonatal performing a lumbar puncture to identify undiagnosed meningitis.

The WHO Pocket Book 2013 (WHO 2013) recommends a combination of ampicillin and gentamicin for three weeks for the treatment of neonatal meningitis, or alternatively ceftriaxone/cefotaxime and gentamicin for three weeks (WHO 2013). Neonates with a diagnosis of meningitis who are making a good clinical recovery and are on an appropriate regimen, including all of the novel trial combinations, can continue on the same regimen for three weeks.

7.4 SECOND-LINE TREATMENT REGIMENS

In Part 1, second-line treatment is based on clinician choice.

In Part 2, for each first-line regimen in a site's randomisation list, the site will also determine relevant second-line regimens as part of site feasibility (listed in the country-specific appendices). This list may include specific antibiotics or regimens used in particular sites that are not available in other sites as a "locally selected therapy" option. Any such drugs will be used according to local clinical practice and guidelines. Randomisation to second-line therapy will enable direct comparison of the general strategic approach of using broad-spectrum antibiotics empirically as first-line in all neonates compared to using narrower-spectrum antibiotics first-line empirically and only escalating to broad-spectrum antibiotics in the group of babies who do not respond or deteriorate clinically.

If the responsible clinician has decided to switch treatment, and providing that there are two or more clinically appropriate second-line regimens available, taking into account the neonates first-line regimen, resistance patterns at the site and any susceptibility testing results from a pathogen isolated from the individual neonate, then second-line treatment will be randomised.

Eight regimens are under consideration for inclusion in the PRACTical design for second-line treatment (**Table 9**). The three novel two-drug combinations of existing off-patent antibiotics will be included in the implemented second-line randomisation lists and "locally selected therapy" which will be site specific and determined prior to site initation. Changes to the local treatment regimens can be implemented following approval by ethics committee and regulatory bodies, except for changes in antibiotic treatments required in response to local outbreaks.

Table 10: Second-line treatment options in Part 2

Second-line treatment options
Cefotaxime or ceftriaxone
Fosfomycin and amikacin
Fosfomycin and flomoxef
Flomoxef and amikacin
Ceftazidime ± amikacin
Piperacillin/tazobactam ± amikacin
Meropenem
"Locally selected therapy"

Note: With any regimen (other than the fixed three novel combination regimens of fosfomycin+amikacin; fosfomycin+flomoxef; flomoxef+amikacin), amikacin can be continued in the second line treatment including if it was given first line, at the discretion of the local treating physician.

Where two or more clinically appropriate regimens are not available, neonates should be switched to "locally selected therapy" without randomisation.

Regardless of specific second-line regimen, all infants will continue to be followed up until Day 28 (Part 1) or Day 90 (Part 2) after enrolment/randomisation.

In the rare situation where the neonate's clinical condition deteriorates rapidly while on second-line treatment, further treatment will be at the discretion of the local physician.

Additional antibiotics may be added during the trial as first or second line treatment options as part of a protocol amendment following ethical and regulatory approval.

8 ASSESSMENTS & FOLLOW-UP

8.1 TRIAL ASSESSMENT SCHEDULE

The complete trial assessment schedule is presented in **Table 1: and Table 2:**. These assessments should be conducted on all patients participating in Part 1 (**Table 1:**) and Part 2 (**Table 2:**).

All screening assessments must be performed after informed consent has been obtained (for Part 2 this may be verbal, followed by written as soon as possible given the neonate's condition). Neonates will be followed up at the following time points, counting time from the calendar date of enrolment (Part 1) or randomisation (Part 2) as Day 1. Trial visit schedules will be prepared for each neonate at randomisation, and neonates should be followed on that same schedule, until the final follow up, even if their trial medication is discontinued prematurely. The target dates for trial contacts are determined by the date of randomisation and are not affected by subsequent events. Sites may choose to re-schedule contacts to allow for public holidays or other unavoidable circumstances that affect the scheduled visit date, but the re-scheduled visit or contact should preferably be in the window period, as follows:

- Baseline (laboratory tests should be within 48 hours before randomisation)
- Daily while on IV antibiotic treatment in hospital
- Day 3 (± 1 day)
- Day 5 (± 1 day) (Part 1 only)
- Day 7 (± 2 days)
- End of treatment (EOT) (Day 5 to 21): if EOT is on Day 7 or 14 visits can be combined.
- Day 14 (± 4 days)
- Day 28 (± 5 days)
- Day 90 (± 14 days) (Part 2 only)

Treatment duration will be 7±2 days for neonates that are culture-negative and 10 [-3,+ 4] days for neonates that are culture-positive with no switch to second-line. If antibiotics are switched the total duration of antibiotic treatment including first and second line is 14 ±7 days depending on the baby's condition.

All visits should ideally be face to face: those from day 14 onwards may be via telephone if necessary. Enrolment (Part 1) or Randomisation (Part 2), treatment initiation, and PK sampling (Part 1) may be on the same day as screening.

Medical history should be taken as part of enrolment procedures to ensure patient's eligibility and document baseline status. Locator information, including physical address and contact phone numbers and relevant social history will also be collected at baseline. Baseline laboratory test results and blood culture are required within 48h before randomisation, but can be done either at screening or randomisation or values from blood taken pre-screening within the time window may be used. Clinical examination (including vital signs and temperature) and treatment administration data will be collected at all follow-up assessments, including an evaluation of AEs and SAEs. Resource utilisation will also be collected at these follow-up assessments. After Day 3 (Part 2) or Day 5 (Part 1), routine laboratory assessments will only be repeated if abnormal at the previous visit or baby's condition is not stable, to avoid additional blood tests in neonates. Data on all safety assessments will be collected via the eDC system and monitored centrally.

Parents/guardians will be given a card with the contact details for the trial research team at their site, and encouraged to return to the site if the infant becomes acutely unwell during the follow up period.

8.2 PROCEDURES FOR ASSESSING PK (PART 1)

The PK study aims to confirm dosing of fosfomycin and flomoxef, with a particular focus on understanding early postnatal age maturation to guide dosing.

Each neonate enrolled in Part 1 will have 3 blood samples and will be allocated to one each from the a) early, b) middle and c) late time points, taken on Day 1 of dosing 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
b) middle: 30 mins **or** 60 mins after end of dosing
c) late: 4h **or** 6h after end of dosing

An additional sample will be collected prior to the first dose on Day 5. PK sampling time points may be subject to change based on emerging data. In total, four PK blood samples of 0.5ml each will be drawn from each participant. More information on PK sampling can be found in the PK MOP.

If cerebrospinal fluid (CSF) is taken during trial treatment administration (e.g. to investigate possible meningitis), then any leftover material not required for diagnostic purposes will be retained for PK analysis. No pre-dose samples are required as neither fosfomycin nor flomoxef are currently used routinely in the participating sites.

Samples will be shipped at regular intervals to a central laboratory in the UK for processing. The MOP contains full details of PK procedures and sample handling, including storing plasma aliquots separately for shipping. Plasma concentrations will be determined with highly sensitive LC-MS/MS methods.

8.3 PROCEDURES FOR ASSESSING EFFICACY

The primary measure of efficacy in NeoSep1 is death within 28 days of randomisation; death within 90 days of randomisation is a secondary outcome. Vital status will be ascertained after discharge through contact with the parent/guardian, either by a scheduled hospital visit or telephone call. Parents/guardians will be encouraged to return to the trial site if the infant becomes acutely unwell during trial follow-up.

Clinical status will be assessed at Day 3, 7, 14 and 28 after randomisation based on a neonatal clinical recovery score (NeoSep Recovery Score) and incorporating information on clinical signs (whose presence/absence will be recorded at each visit) and vital signs over time following randomisation. The NeoSep Recovery Score is based on updated information every day a neonate is on antibiotics for treatment of sepsis, and is designed to align with but distinct from the baseline NeoSep Severity Score which relates clinical parameters recorded at the the start of a new episode of sepsis to overall mortality. In contrast, the NeoSep Recovery Score specifically includes time-updated assessments to monitor how an individual neonate's risk changes over time on antibiotics. The details of the NeoSep Recovery Score and its use are included in **Section 7.2** and **Table 9:**.

Cure at the test of cure (TOC) visit will similarly be defined based on this score and whether additional antibiotics have been given.

Other efficacy measures include clinically appropriate need for non-allocated antibiotics beyond the original first or second randomised treatment, length-of-stay and duration of systemic antibiotic exposure in the index hospitalisation, which will be determined from regular in-hospital follow-up and medical notes, including recording of all antibiotics received. Clinical appropriateness of non-allocated antibiotics will initially be defined according to whether a neonate meets the criteria for switch to second-line treatment (see **Section 7.2**). All non-allocated antibiotics in Part 1 will be reviewed by a group containing independent members to determine algorithms to define this prospectively in Part 2.

C-reactive protein (CRP) will be assessed in all neonates at baseline, Day 5 and Day 7 in Part 1 and at at baseline, Day 3 and Day 7 in selected sites in Part 2. The reason for moving the first post-baseline timepoint to Day 5 in Part 1 is to align with the blood draw for the fourth PK sample.

8.4 PROCEDURES FOR ASSESSING SAFETY

The clinical examination will explicitly record signs and symptoms relating to possible drug toxicities. Adverse events (clinical and laboratory) will be graded using the clinically based 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.

Part 1: All adverse events of any grade will be reported on eCRFs.

Part 2: All adverse events of any grade that lead to modification (including discontinuation) of antibiotics or are considered related to antibiotics will be reported on eCRFs, as will any Grade 3 or 4 adverse events.

Assessing safety is important, however all patients eligible for the NeoSep trial are critically ill, with complex pre-existing co-morbidities such as prematurity and due to the complexity of their condition are at increased risk of experiencing AEs as defined in **Section 9**. This has been demonstrated in other neonatal sepsis trials (Gilbert, Brown et al. 2019, Juul, Comstock et al. 2020, Ruel, Capparelli et al. 2021). Many of these events are expected as a result of the patient's medical condition and standard treatment received in hospital but are very likely not to be related to trial interventions. Consequently, Grade 1 and 2 AEs (according to NAESS) occurring as a result of the patient's medical condition or standard hospital treatment will not be reported on eCRF (Part 2 only). Pre-existing conditions identified before trial drug administration do not qualify as AEs unless they worsen (**Section 9.1.2**), but should be documented in the patient's medical notes. In Part 1, all AEs of any grade will be reported on eCRFs in order to investigate associations with PK.

See **Section 9** for pharmacovigilance reporting of Serious Adverse Events (SAEs) and other notable events. SAEs are not an outcome measure in NeoSep1 because the neonates will be very sick when admitted. SAEs are therefore not likely to be informative as trial endpoints as they will commonly reflect the underlying disease process rather than any impact of the trial treatment, but will be collected for pharmacovigilance purposes.

The following laboratory tests should be carried out in all enrolled neonates at screening and Day 5 for Part 1 (to minimise blood sampling) and Day 3 (Part 2):

- Full Blood Count (FBC): red blood count (RBC), white blood count (WBC) and differential, platelets (Part 1 and 2)
- Blood urea nitrate (BUN), creatinine (Part 1 and 2)
- Liver function tests: ALT, AST, bilirubin (Part 1 and 2)

- Sodium, potassium: (Part 1 all babies and Part 2 in selected sites as part of a sub-study)

Date and time of sample collection will be recorded on the relevant eCRF, targeting the time windows in **Table 1: and Table 2:**. Any abnormal test on Day 3 should be repeated at subsequent scheduled assessement until normal or stable.

Additional safety blood tests or investigations may be performed to investigate symptoms or monitor emergent laboratory test abnormalities as clinically indicated following routine clinical practice. Management should follow local standard of care. Results of any laboratory tests not listed above that are conducted as part of routine clinical practice will be recorded on eCRFs.

8.5 MICROBIOLOGY PROCEDURES

8.5.1 BLOOD/CEREBROSPINAL FLUID (CSF) CULTURE

If a neonate does not respond to first-line treatment, a repeat blood culture should be taken before switch to second-line treatment and consideration should be given to conducting a lumbar puncture. Results from this blood culture, and any other cultures of blood, urine or cerebrospinal fluid (CSF) conducted as part of routine clinical practice, will be recorded on the relevant eCRF.

For clinical management, each site will use a local microbiology laboratory, to report pathogen identification and antimicrobial susceptibility following local practice. All significant microbiology isolates cultured from baseline or follow-up blood samples will be centralised for retrospective standardised assessment of susceptibility to trial antibiotics. Central analysis of the clinical isolates could include confirmation of the identity of the clinical isolate. On selected isolates, antibiotic susceptibility testing including MIC determination to standard antibiotics and fosfomycin and flomoxef. In addition, whole genome sequencing may be conducted on clinical microbiology isolates including to characterise genotypic mechanisms of resistance.

8.5.2 MICROBIOLOGY SUB-STUDY (SELECTED SITES) (PART 2 ONLY)

In addition, selected sites will seek informed consent to recruit neonates into a microbiology sub-study. Neonates will have peri-rectal swabs taken at baseline to assay carriage of commensal organisms and of antimicrobial resistance genes. Collection should be as soon as possible,before initiation of intravenous antibiotics, wherever possible. A sample of faecal material may be takenfrom a nappy instead of a peri-rectal swab. Peri-rectal swabs are minimally invasive, can be taken in a standardised manner independent of passing stools using a soft swab and very soon after enrolment, minimizing antibiotic pre-exposure.

In this sub-study, sample collection will be repeated at the end of IV treatment, and at Day 14 where this assessment is either done in hospital or face to face, to test for acquisition of antibiotic resistant genes during treatment and hospitalisation.

Further details on sample collection are provided in the MOP. Samples will be shipped to University of Antwerp, Belgium, for processing. All processing of samples from this sub-study will be done in batches and not in real-time, so there will be no impact on clinical management of the neonates.

8.6 HEALTH ECONOMICS

Policymakers require information on the costs, health effects and equity implications of alternative interventions when considering how to allocate limited resources to meet the population's health needs. We will estimate costs and cost-effectiveness of the trial's treatment strategies evaluated using generic health measures (e.g. disability-adjusted life-years (DALYs)-averted) to allow for

comparison with other interventions. Resource use and total costs will be estimated using trial data and other sources (e.g. unit costs/prices) to be representative of LMIC countries in general. The cost-effectiveness of more effective but more expensive alternatives will depend upon whether the health gains offered are large enough compared to other calls on limited country budgets to be deemed "value-for-money", so we will outline criteria upon which this assessment can be made.

The trial will measure healthcare-related costs in trial neonates, starting at randomisation and continuing for the duration of follow up. Costs incurred by the parents/guardians (transport, indirect and companion person's costs) will be obtained by parent/guardian reports and be recorded in eCRFs. Family information (e.g. parental age, educational level and broad measures of socio-economic status) will be recorded on eCRFs at baseline. Household level cost data will be collected through a brief follow-up survey. Reported transport costs will be confirmed using local information on distance and cost of transport. Information on hospitalisations (number, reason, and duration of stay) and other healthcare resource utilisation (eg NICU, ward days, outpatient visits, medications, and procedures) will be recorded on eCRFs.

For the cost-effectiveness analysis, we will calculate an average cost per infant treated. From the provider's perspective, this cost will include patient-specific resources (medications, investigations, products, supplemental oxygen, intravenous fluids, procedures etc) in addition to overhead and staff costs incurred during each admission. The price/cost/charge for each of these resources will be obtained from trial sites and from trial financial data.

Overhead and staff costs will be collected from routine hospital expenditure data. Overheads include running costs such as electricity, water, cleaning, laundry, security, administration and maintenance costs. In addition, routine hospital expenditure data will provide an estimate of the hospital expenditure on staff (anonymised). Overhead and staff costs will be allocated to an inpatient day using the patient day equivalent method in order to estimate an overhead and staff cost per inpatient day or per admission. The patient day equivalent for each site will be estimated from routine hospital statistics such as number of inpatient days and number of outpatient or emergency department visits.

8.7 EARLY STOPPING OF FOLLOW-UP

See **Section 6.20** for discontinuation of protocol treatment. If a parent/guardian chooses to discontinue their neonate's trial treatment, the neonate should always be followed up providing they are willing, that is, they should be encouraged to continue follow-up; if they do not wish them to remain on trial follow-up, however, their decision must be respected and the neonate will be withdrawn from the trial completely and no additional follow up data will be collected for the participant. If the participant has ongoing related AEs (Part 1) or SAEs at the time of consent withdrawal, the outcome of the AR or SAE will be collected. The CTU should be informed of this using the appropriate documentation.

If follow-up is stopped early, the pseudoanonymised medical data collected during their participation in the trial will be kept and used in the analysis; consent cannot be withdrawn for the use of historical pseudonymised data already collected. This will be included in the participant information sheet. Consent for future use of stored samples already collected can be refused when leaving the trial early (but this should be discouraged and should follow a discussion).

Patients may change their minds about stopping trial follow-up at any time and rejoin the trial.

In Part 1, patients who stop trial follow-up early (ie prior to Day 28 follow-up) will not be replaced, unless they stopped participation before all three Day 1 PK samples were taken.

8.8 PATIENT TRANSFERS

As each site may be the only trial site in a relatively large area, it will not be possible to transfer a participant from one investigational site to another investigational site. However, some sites do transfer neonates to more local hospitals when their condition has stabilised. Wherever possible, neonates should remain at the site until end of IV treatment, and ideally until 14 days (unless discharged before this time). If it is necessary to transfer a participant before Day 14, then subsequent visits may take place over the telephone with the hospital to which the neonate was transferred. This routine transfer does not count as a new hospitalisation for the purposes of SAE reporting.

8.9 LOSS TO FOLLOW-UP

All attempts should be made to reach at least the Day 28 follow up assessment (when the primary endpoint is assessed), in person or via telephone. The Day 90 assessment may also be conducted via telephone if the neonate is not expected to be seen at the hospital to assess the baby's vital status.

For operational management at sites, an infant will be classified as "lost-to-follow-up" (meaning no further attempts at contact are made) only when three unsuccessful attempts have been made to contact the parent/guardian following non-attendance at a face-to-face follow-up, including telephone calls in the first instance, and if feasible then attempts to visit the home (based on the location provided at baseline). If an infant is contacted after being classified as "lost-to-follow-up", the relevant follow-up form should be completed, regardless of the length of time it takes to re-establish contact with the family, in order to record the infant's vital status at the time of the missed visits.

8.10 COMPLETION OF PROTOCOL FOLLOW UP

The protocol follow up will end after the last scheduled follow-up visit of the last randomised participant in Part 2. Sites will be closed once data cleaning is completed and the database undergone its final lock; the regulatory authorities and ethics committee will be informed of the trial closure as required by local regulations.

9 SAFETY REPORTING

The principles of GCP require that both investigators and Sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials. These procedures are described in this section of the protocol. **Section 9.1** lists definitions, **Section 9.3** gives details of the investigator responsibilities and **Section 9.4** provides information on CTU responsibilities.

9.1 DEFINITIONS

The definitions of adverse drug reactions, events or suspected unexpected serious adverse reactions are given in **Table 10**. Serious adverse events are collected for pharmacovigilance purposes but given the severity of illness of the eligible population and the fact that the majority of SAEs will not be related to the trial medications, they are not outcome measures in the trial.

Table 11: Definitions

TERM	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial participant to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: ▪ Results in death ▪ Is life-threatening* ▪ Requires hospitalisation or prolongation of existing hospitalisation** ▪ Results in persistent or significant disability or incapacity ▪ Consists of a congenital anomaly or birth defect ▪ Is another important medical condition***

*The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation.

*** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation or development of drug dependency.

9.1.1 MEDICINAL PRODUCTS

An investigational medicinal product (IMP) is defined as the tested investigational medicinal product and the comparators used in the study.

Adverse reactions include any untoward or unintended response to drugs. Reactions to an IMP, including comparators, should be reported appropriately.

9.1.2 ADVERSE EVENTS

Adverse Events, as defined in **Table 10**, **include**:

- An exacerbation of a pre-existing illness
- Laboratory abnormalities which are judged clinically significant (hence meet the definition of untoward medical occurrence)
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the study treatment

Adverse Events do **not include**:

- Medical or surgical procedures; however, the condition that leads to the procedure is the adverse event (and that should be captured in the eDC)
- Laboratory abnormalities which are not judged clinically significant (hence do not meet the definition of untoward medical occurrence, e.g. isolated abnormal measurement without clinical signs or consequence)
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisations where no untoward or unintended response has occurred, e.g. social admissions
- Overdose of medication without signs or symptoms. Overdoses with clinical symptoms will be reported as AEs (and SAEs if 'seriousness' criteria are met).

9.1.3 ADVERSE EVENTS REPORTING PERIOD

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 for Part 1 and Day 90 for Part 2.

In addition, any SAE that occurs after the adverse event reporting period, that the investigator assesses as related to the IMP, should also be reported to the Sponsor.

9.1.4 OTHER STUDY-SPECIFIC REQUIREMENTS

None.

9.2 OVERDOSES

Overdoses of >20% of the maximum recommended dose in **Table 6** (>10% for fosfomycin), without signs or symptoms are not entered as adverse events but should be reported to CTU as in **Section 6.19**.

Any clinical symptoms emerging as a result of an overdose should be reported as an AE in the relevant eCRF. The seriousness of such an AE should also be considered and reporting of an overdose-related SAE done as required.

9.3 INVESTIGATOR RESPONSIBILITIES

All AEs should be recorded in the patient's medical notes. AEs which are trial outcomes (grade 3 or 4, or causing a modification (including discontinuation) of or judged related to an antibiotic) should be reported in the AE eCRF and sent to the CTU within **the agreed timescale**, as specified in the MOP.

SAEs should be notified to the CTU within 24 hours of the investigator becoming aware of the event.

9.3.1 INVESTIGATOR ASSESSMENT

9.3.1.A Seriousness

When an AE or AR occurs, the investigator responsible for the care of the patient must first assess whether or not the event is serious using the definition given in **Table 10 (Section 9.1)**. If the event is serious, then an SAE Form must be completed and the CTU notified within 24 hours.

9.3.1.B Severity or Grading of Adverse Events

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded using the gradings in the neonatal adverse event severity scale (Salaets, Turner et al. 2019) [Grade 1 (mild) to Grade 5 (death)]. This scale is directly relevant to neonates, whereas standard toxicity grading scales are not applicable to this specific population. For 35 AEs (e.g. neonatal convulsion, neonatal bradycardia) specific severity criteria are defined. Whilst some of the criteria require highly specialised staff and/or equipment (eg neonatal opthalmology), all criteria have a clinical component, and can be graded according to generic severity criteria described in **Appendix 2**.

9.3.1.C Causality

The investigator must assess the causality of all events, serious and non-serious, in relation to the trial therapy using the definitions in **Table 11**.

There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is:

- unrelated or unlikely to be related, the event is classified as unrelated, and this is recorded on the relevant eCRF.
- possible, probable or definitely related, then the event is classified as related, and this is recorded on the relevant eCRF.

Table 12: Assigning Type of AE Through Causality

RELATIONSHIP	DESCRIPTION	AE TYPE
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	AR or SAR*
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	AR or SAR*
Possible	There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (for example, the patient's clinical condition, other concomitant treatments).	AR or SAR*

RELATIONSHIP	DESCRIPTION	AE TYPE
Unlikely	There is little evidence to suggest that there is a causal relationship (for example, the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (for example, the patient's clinical condition, other concomitant treatment).	Unrelated AE or SAE
Unrelated	There is no evidence of any causal relationship	Unrelated AE or SAE

*related

9.3.1.D Notification

Whilst the neonate is taking IMP, the CTU should be notified of all SAEs within 24 hours of the investigator becoming aware of the event regardless of causality. After IMP is discontinued, the CTU should be notified of all SAEs within 7 working days of the investigator becoming aware of the event.

9.3.2 NOTIFICATION PROCEDURE OF SAES FROM INVESTIGATOR TO CTU/SPONSOR

1. The SAE Form must be completed by an investigator (named on the Signature List and Delegation of Responsibilities Log, who is responsible for the patient's care; this will be either the Principal Investigator or another medically qualified person with delegated authority for SAE reporting). Due care should be paid to the grading and causality of the event, as outlined above. In the absence of the responsible investigator, the form should be completed and signed by a member of the site trial team and entered onto the eDC system. The responsible investigator should subsequently check the SAE Form, confirm causality, make changes as needed, sign and then re-send to the CTU as soon as possible. The initial report must be followed by detailed, written reports.

   The minimum criteria required for reporting an SAE are:
   - the participant's unique trial identifier and date of birth
   - name of investigator reporting the adverse event
   - the IMP
   - and why it is considered serious.

2. The SAE Form must be entered onto the eDC or sent by email to mrcctu.neosep@ucl.ac.uk

3. Follow-up: patients must be followed up (see **Section 9.3.3**). Additional annotated information and/or copies of test results may be provided separately. The patient must be identified by participant's unique trial identifier, date of birth and initials only. The patient's name should not be used on any correspondence and should be deleted from any test results.

4. Staff should follow their institution's procedure for local notification requirements.

Serious Adverse Event (SAE) REPORTING
Please report all SAEs via the eDC system **within 24 hours of becoming aware** of an SAE

If you have any issues with reporting an SAE or have any questions please email mrcctu.neosep@ucl.ac.uk

SAEs that occur after the neonate has discontinued IMP do not need to be reported in an expedited fashion within 24 hours but must be reported within 7 days of the site becoming aware

* Procedures will be in place to ensure reporting of SAEs in the event of power outage or interruption of access to clinical database. Details on how to report events in such cases will be provided at site training. SAEs will be reported via secure email in case of issues with internet connection (mrcctu.neosep@ucl.ac.uk).

9.3.3 AE FOLLOW-UP

AEs must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised or until the Day 28 (Part 1) or 90 (Part 2) follow-up assessment.

For SAEs, follow-up should continue after completion of protocol treatment if necessary. A further SAE Form, indicated as 'Follow-up' should be completed and sent to the CTU as information becomes available.

9.4 SPONSOR RESPONSIBILITIES

9.4.1.A Expectedness

The definition of an unexpected adverse reaction (UAR) is given in **Table 10**. An unexpected adverse reaction is one not previously reported in the current representative Reference Safety Information (RSI) or one that is more frequent or more severe than previously reported. The RSI will be a Summary of Product Characteristic, depending on the IMP, as defined elsewhere.

The Sponsor (or designee) has ultimate responsibility for determination of expectedness.

9.5 CTU RESPONSIBILITIES

Designated Sponsor's medical representatives (or a medically-qualified delegate) will review all SAE reports received. The causality assessment given by the local investigator at the hospital cannot be overruled; in the case of disagreement, both opinions will be provided in any subsequent reports. The CTU will also keep all investigators informed of any safety issues that arise during the course of the trial.

The CTU will provide data to be incorporated into Annual Safety Reports as required by Regulatory Authorities and Ethics Committees.

9.6 REPORTING TO ETHICS COMMITTEES AND REGULATORY AUTHORITIES

The Sponsor's designee will be responsible for the reporting of SUSARs and other SARs to the regulatory authorities and the research ethics committees in the countries in which the trial is taking place, according to local requirements. The safety management plan willprovide further details.

10 QUALITY ASSURANCE & CONTROL

10.1 RISK ASSESSMENT

The Quality Assurance (QA) and Quality Control (QC) considerations have been based on a formal Risk Assessment, which acknowledges the risks associated with the conduct of the trial and how to address them with QA and QC processes. QA includes all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled.

This Risk Assessment has been reviewed by the Sponsor and CTU's Research Governance Committee (RGC) and has led to the development of all quality management documents (see also **Section 10.3.3**) which will be separately reviewed by the Sponsor and Quality Management Advisory Group (QMAG).

The Sponsor will also document a separate risk assessment as per required GARDP's procedures.

10.2 CENTRAL MONITORING OF DATA AT CTU

CTU staff will review electronic Case Report Form (eCRF) data for errors and missing data points.

Other essential trial issues, events and outputs will be detailed in the Monitoring Plan that is based on the trial-specific Risk Assessment.

10.3 ON-SITE MONITORING

The frequency, type and intensity for routine monitoring and the requirements for triggered monitoring will be detailed in the Monitoring Plan. This plan will also detail the procedures for review and sign-off.

10.3.1 DIRECT ACCESS TO PATIENT RECORDS

Participating investigators should agree to allow trial-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required. Patients' consent for this must be obtained.

10.3.2 CONFIDENTIALITY

The trial will follow the principles of the United Kingdom (UK) Data Protection Act (DPA), regardless of the countries where the trial is being conducted. All applicable national laws will be followed to ensure compliance with data handling requirements. In particular, the investigators must ensure that neonate's anonymity will be maintained and that their identities are protected from unauthorised parties. Participants will be assigned a trial identification number and this will be used on eCRFs; they will not be identified by name. The investigator will keep securely a patient trial register showing participants'unique trial identifiers, names, initials and dates of birth, held only at the local site. The participants' unique trial identifier and date of birth or a laboratory tracking number will identify all laboratory specimens, eCRFs, and other records and no names will be used on forms or samples, in order to maintain confidentiality. All paper records will be kept in locked locations. Clinical information will not be released without written permission, except as necessary for monitoring by the trial monitors as well as for audits and regulatory inspections.

10.3.3 MONITORING

Prior to trial start, a monitoring plan will be developed. The site principal investigator will allow the monitors to visit the site and facilities where the trial will take place in order to verify compliance with the trial protocol, principles of ICH Good Clinical Practice (GCP) and WHO Good Clinical Laboratory Practice (GCLP) for laboratories. Training sessions on GCP, GCLP and on protocol implementation will be organised for the investigators and all trial staff, as appropriate to their role, prior to recruitment start. A MOP will be distributed to all the trial centres and the trial's MOPs will be distributed to all laboratories.

Trial monitoring will be carried out by dedicated monitors according to the agreed monitoring plan, depending on the recruitment rate, to verify data quality and trial integrity. The site Principal Investigator must allow the monitor to:

- monitor the site, the laboratories, the facilities, the equipment and the material used for the trial
- meet all members of his/her team involved in the trial
- consult all of the documents relevant to the trial, including those filled by the trial nurse and trial pharmacist
- check that the eCRF has been correctly completed
- review the completion and accuracy of pharmacovigilance documentation and consistency with the eCRF
- directly access source documents for comparison of data therein with the data in the eCRF/data or forms sent for pharmacovigilance
- verify that randomisation has been conducted in accordance with the corresponding MOP and that no breach occurred in allocation concealment
- verify the collection, transport, storage and shipment of biological samples
- verify that the trial is carried out in compliance with the protocol and national regulatory requirements
- verify the proper handling and management of trial treatments

At the end of each monitoring visit, and based on monitoring visit reports, the Sponsor will be responsible for working with the PIs on the management of:

- recruitment rates, ineligibility, non-compliance, protocol violations and dropouts overall and in each trial centre
- completeness and timeliness of data entry
- compliance with GCP, GCLP and applicable regulations
- any protocol deviations as defined in the monitoring plan

A final close out visit will be conducted at the end of the trial, after the last participant last visit (LPLV), and once the database is locked.

In addition to the monitoring activities foreseen above, the trial may be evaluated by external auditors appointed by the Sponsor and by representatives from national regulatory authorities or ethics committees who must be allowed access to eCRFs, source documents, trial files, and trial facilities.

10.4 SOURCE DATA

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial participants. Source data are

contained in source documents and are defined by EU guidelines as all information in original records that are used for the reconstruction and evaluation of the clinical trial. Source documents are the first place where the source data are recorded. These can include hospital records, clinical and office charts, laboratory notes, X-rays, and pharmacy dispensing records.

Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g. via an audit trail). Each data element should only have one source.

A source data plan will be agreed with each site as part of the green light process. This plan will define the source documents and the data therein. The document will also specify for each site where the eCRF will be the source data. **For this trial, the eCRFs may be the source document for data elements including but not limited to:**

- clinical signs and symptoms
- all adverse events (Part 1 only)
- patient level health economic data
- sub-study samples (participating sites)

The following data should all be verifiable from source documents, which may include paper notes and electronic health records:

- signed consent forms
- dates of visits including dates any trial samples were taken and processed in the laboratory
- eligibility and baseline values
- dates IMP dispensed and (if necessary) drugs returned
- pharmacy or clinic IMP accountability and prescription logs
- adverse events of any grade that lead to modification (including discontinuation) of antibiotics and adverse events judged related to antibiotics
- severe (grade 3/4) adverse events
- serious adverse events

10.5 AUDIT AND REGULATORY INSPECTIONS

An auditor appointed by the Sponsor may carry out independent audit on all collaborating partners and participating centres. GARDP SOPs will be followed.

Representatives from national regulatory authorities or ethics committees may perform inspections. All external auditors / inspectors must be allowed access to eCRFs, source documents, trial files, and trial facilities. The informed consent form includes details on the possibility of source notes to be inspected and audited.

11 STATISTICAL CONSIDERATIONS

11.1 METHOD OF RANDOMISATION

11.1.1 PART 1

Neonates in Part 1 will be sequentially assigned to three treatment cohorts: there is no randomisation.

11.1.2 PART 2

The trial will use a personalised randomised controlled trial (PRACTical) design (Walker, White et al. 2021), in which each neonate is randomised only to regimens that are considered clinically acceptable for that specific site and sub-population. The design will also incorporate the use of a Sequential Multiple Assignment Randomised Trial design (SMART) to allow randomisation to second-line treatment where required.

As each sub-population in each site will have a separate randomisation list, simple 1:1 randomisation between all trial treatments in each randomisation list will be used for both first-line and second-line randomisations.

11.2 OUTCOME MEASURES

11.2.1 PART 1

Primary endpoints (fosfomycin and flomoxef)
The following primary PK parameters will be derived for fosfomycin and flomoxef 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)

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)

Potential PK/PD relationships:

- Free drug AUC ratio to Minimum Inhibitory Concentration (MIC) (fosfomycin)
- Fraction of time for free concentration above MIC (flomoxef)

The following safety data will be summarised

- All adverse events in Part 1 will be summarised and graded using the clinical Neonatal Adverse Event Severity Scale) (Salaets, Turner et al. 2019)

Other endpoints

- Pharmacokinetic analysis of amikacin (if there is sufficient sample volume)

11.2.2 PART 2

Primary Endpoint

- 28-day mortality

Secondary Endpoints

Efficacy

- Clinical status at Days 3, 7, 14 and 28 after randomisation
- Clinically appropriate need for additional antibiotics beyond the first randomised treatment, as assessed by an algorithm validated by an independent group
- Clinically appropriate need for additional antibiotics beyond the first randomised and second (for failure) treatment, as assessed by an algorithm validated by an independent group
- Cure, defined as clinical improvement and no need for further antibiotic treatment for the original sepsis episode, at test of cure (TOC) visit (Day 14 ± 3 days after randomisation)
- Length of stay during the index hospitalisation
- Systemic antibiotic exposure (days on antibiotics) during the index hospitalisation
- 90-day mortality
- Change in C-reactive protein from baseline to Day 3 and Day 7

Safety (all graded using the neonatal adverse event severity scale) (Salaets, Turner et al. 2019):

- Grade 3/4 adverse events (AEs) through Day 28 after randomisation
- Adverse events of any grade related to antibiotics
- Modification (including discontinuation) of antibiotics for adverse reactions

Note: serious adverse events will be collected for pharmacovigilance but are not trial outcome measures given the severity of illness of the population.

11.3 SAMPLE SIZE.

11.3.1 PART 1

20 neonates with all 3 PK samples on Day 1 will be enrolled in each of the 3 sequential treatment cohorts. In addition, across both fosfomycin cohorts, 10 neonates with a post-natal age under 7 days with complete Day 1 samples and Day 5 samples are required. The final sequential cohort will continue recruiting until both targets are achieved.

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 (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.

11.3.2 PART 2

Sample size is calculated based on simulations, given the number of different regimens involved.

At the first randomisation, we have assumed that personalised randomisation lists (Walker, White et al. 2021) will be drawn from a list of 8 regimens according to three different patterns, reflecting their acceptability in different sites. At the second randomisation, we have assumed that personalised randomisation lists will be determined by the neonate's first randomised regimen, and include all regimens that are broader spectrum and do not contain any regimen in the first-line treatment.

Sample size calculations are informed by preliminary analyses from the neonatal observational study (NeoOBS). Following treatment under the first-line/second-line strategies available, 28 day mortality is expected to vary from 10-20%. Fixed values for first-line and second-line regimen effects have been selected to achieve this variation. We have assumed an equal split between the three assumed patterns of randomisation, 5% early mortality before second randomisation and 25% of neonates switching to a randomised second-line treatment. Simulations were performed to investigate how much information would be provided by the planned trial design under varying sample sizes.

It is estimated that using "top-ranked" strategies based on information gained from a trial including 3000 neonates would achieve 80% of the maximum possible reduction in mortality, for each neonate compared with selecting a random regimen for each neonate, with 90% chance of mortality being within 2% of the best strategy or 80% chance of being within 1% of the best strategy. In sensitivity analyses, we varied three assumptions to allow unequal patterns of randomisation, 50% switching to randomised second-line treatment, and reversed ordering of mortality across strategies and obtained similar results.

Neonates for whom verbal assent is confirmed by written consent, and neonates that die before verbal consent will contribute to the total sample size. This is in order to ensure that these children contribute to the primary outcome (mortality at Day 28) and to SAE pharmacovigilence. If verbal consent is not confirmed by written consent then no further data will be collected and the neonate will not count towards the sample size.

A sample size review will be conducted when 50% of participants have completed the Day 28 follow-up visit, as part of an interim analysis. This will update the sample size calculations in the light of accumulating evidence about the frequency of use of each personalised randomisation list and the overall mortality rate and hence consider whether recruitment should continue to the original target or be modified, or whether for example, the randomised allocation ratio should be varied from 1:1 to randomise more neonates to less represented regimens. Any decision to increase the sample size is a Sponsor decision in collaboration with the TSC.

11.4 INTERIM MONITORING & ANALYSES IN PART 2

In Part 2, a feasibility phase will enrol approximately 10% of the trial cohort (300 patients) to assess the feasibility of implementing the study at the participating sites. This will focus on:

- Assessing recruitment compliance with first-line treatment options
- Assessing implementation of second randomisation and compliance to second-line treatment options

A Data Monitoring Committee (DMC) Charter will be drawn up that describes the membership of the DMC, relationships with other committees, terms of reference, decision-making processes. The

Charter will also contain a description of stopping guidelines. See **Section 16** for details on DMC membership.

For Part 2, the DMC will meet within 6 months after the trial opens; although the DMC will in general meet every 6-9 months, the frequency of subsequent meetings will be determined by the DMC and could be more frequent if deemed necessary. The DMC will also review safety parameters (sodium and potassium) for fosfomycin containing treatment regimens as part of a sub-study in selected sites. The DMC can recommend premature closure or reporting of the trial, or that recruitment to any randomised group be discontinued or modified. Such recommendations would be made if, in the view of the DMC, there is proof beyond reasonable doubt that one of the allocated strategies is better than any other in terms of a difference of clinically significant magnitude in a primary outcome. The guiding statistical criteria for "proof beyond reasonable doubt" is a Haybittle-Peto type rule based on the 99.9% credible interval.

11.5 ANALYSIS PLAN (BRIEF)

The analyses are described in detail in a full Statistical Analysis Plan. This section summarises the main issues.

11.5.1 PART 1

Population PK modelling and dosing simulations will be undertaken with non-linear mixed-effects modelling. The PK model will estimate the primary PK parameters clearance, volume of distribution, 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 will be derived from the model.

Safety outcomes will be summarised in each group, but the limited number of neonates included in this PK confirmatory study limits power to conduct comparisons either within Part 1 or with other studies.

11.5.2 PART 2

The primary analysis population is intention-to-treat, including all randomised neonates, regardless of treatment received. This corresponds to estimating the impact of the effectiveness of the strategies.

Analysis of the trial data will be carried out by using network meta-analytic methods to compare the first-line/second-line strategies and to rank strategies with respect to each outcome exploiting both the direct randomised comparisons and the indirect information across the network. Although we are focusing on comparing strategies in our primary analysis using intention to treat, we will examine the impact of re-randomisation (switch), in a secondary analysis using inverse probability of treatment weighting.

As follow-up for the primary endpoint is short (28 days post-randomisation) loss-to-follow-up should be low, and so the primary comparison between randomised groups will be conducted using binomial regression. The primary analysis will be conducted on observed data. Secondary analyses may use multiple imputation with chained estimating equations to impute outcomes if the number with incompletely ascertained outcomes exceeds 10% 28 days post-randomisation. Imputation will be done separately within each randomised group to allow for unknown interactions, and will be based on outcomes and main baseline characteristics as recommended.

In the primary analysis, we will present risk ratios comparing each strategy against the control strategy of WHO regimens (ampicillin + gentamicin followed by ceftriaxone/cefotaxime). We will determine which strategies perform best with respect to mortality, safety, cost and resistance over two steps. As a first step, we will examine rankings with respect to mortality and safety, to identify a set of antibotic regimens that dominate the others, i.e. are safer and more effective. Rankings of strategies from best to worst will be presented in a table and also illustrated in a plot showing performance in both dimensions. The rankings of this remaining set of regimens with respect to resistance either in infecting isolates on carriage in the microbiology substudy will then be examined as a second step to determine how this affects the ranking on mortality and safety. Costs (which may vary by region) will be examined independently in a health economic analysis overall and/or by region.

Particular sub-groups in which heterogeneity will be explored will be those factors which are used to define personalised randomisation lists tailored by each site (e.g. term vs pre-term, inborn vs outborn, age, culture positive vs culture negative including specific organisms eg *Klebsiella pneumoniae*, if there are sufficient numbers).

Secondary outcome analyses will use similar methods; binomial regression for binary outcomes, and t-tests and normal linear regression (potentially on transformed data depending on the observed data distribution, adjusted for baseline values) for continuous outcomes.

Adverse event, including SARs, will be summarised by body system.

All baseline characteristics, including any pathogens identified from baseline blood cultures, will be described.

A Statistical Analysis Plan will be written and approved by the Trial Management Group (TMG), Trial Steering Committee (TSC) and the DMC before the first interim analysis is reviewed by the DMC.

12 ANCILLARY STUDIES

12.1 MICROBIOLOGICAL SUBSTUDY IN SELECTED SITES

The primary objective of the microbiological substudy is to evaluate the impact of trial treatments on the carriage of multidrug-resistant Gram-negative bacteria in neonates. The goal is to answer in depth questions regarding the potential impact of antibiotic management strategies on antimicrobial resistance by using novel microbiological methods to determine how these strategies relate to resistance in colonizing bacteria. Recognising the serious threat of antibacterial resistance to future child health, this will enable outcomes beyond the immediate health benefits of narrower compared to broader-spectrum antibiotics to be taken into account in clinical decision-making about antibiotic treatment strategies in neonates with sepsis.

Parents of neonates randomised in Part 2 in approximately 5 sites will be approached as for the main trial to provide consent for microbiological characterisation of the commensal faecal flora through peri-rectal sampling (or faecal sampling from nappies) at baseline, end of IV treatment and Day 14 post-randomisation.

A MOP will guide participating laboratories in the processes of sample collection and management locally, in order to optimise and standardise local procedures. The manual will include detailed instructions on sampling, transport and storage of study samples and strains. Training will be provided for PIs and other key local personnel from participating clinical sites to ensure optimal and standardised sampling from all participating neonates including quality assurance exercises and quality control. The samples will be stored at -80°C for batched transfer to the University of Antwerp for analysis which will be done in batches and not in real-time (so there are no implications for clinical management). Strain types and resistance genes of isolated bacteria will be further investigated.

Briefly, peri-rectal swabs will be transferred frozen at -80°C, and then they will be thawed and processed at the University of Antwerp. Total bacterial DNA will be extracted from uncultured swab samples following standard procedures, and shotgun sequenced using Illumina technology. We will determine species and functional richness and diversity and determine phylogenetic and functional composition of the metagenome. As a complementary methodology to the functional screen mentioned above, we will also mine the gut microbiome for known antibiotic resistance genes, as well as further investigate those that have detected in the functional screen. Based upon a manually compiled list of target resistance genes, hidden Markov models will be built and used to screen the gastrointestinal metagenomes generated within this project. This will allow the quantification of different resistance pools in the neonatal population, and the impact of antibiotic management strategies and hospitalisation on amplification of these pools.

12.2 HEALTH-ECONOMIC ANALYSES

We will assess the full economic costs of each randomised group from time of admission to hospital until death or 28 days' follow-up. A full economic costing approach includes financial as well as opportunity costs and is necessitated by the reality of severely constrained capacity within LMIC health systems. Our approach to costing establishes the utilisation of health services (e.g. inpatient days, diagnostic tests, medication and oxygen) directly from trial data specific to each randomised group. Within a decision analytic modelling framework, these utilisation estimates are multiplied by the full economic or site-specific unit cost of each service, diagnostic test or medicine. Unit costs are computed using a combined bottom up and step down approach, as appropriate (Drummond,

Sculpher et al. 2015). When valuing resources within the cost analysis that are paid from the research budget, we will use routine public sector salaries for staff and will seek to cost antibiotics that are sourced specifically for the trial at a level commensurate with a potential public sector funding decision in LMIC. In addition, care will be taken to exclude any costs that are incurred only as part of the research.

The data collection at the health system level will be complemented by a comprehensive assessment of costs incurred by households. These costs do not only include out-of-pocket expenditures for medical services (which we expect to be small because the trial will cover related costs), but also costs for transport to facilities, local food and accommodation, and income losses due to absences from work.

Once we have estimated our unit costs and utilisation, we will build a decision analytic model in order to estimate the cost per neonate treated within each randomised group, from time of first admission until 28 days follow-up. Deterministic sensitivity analyses will assess the impact of key parameter uncertainty (e.g. the cost of antibiotics within a scale-up scenario) and probabilistic sensitivity analysis will assess uncertainty around the relevant utilisation and outcome estimates from the trial (Drummond, Sculpher et al. 2015). Then, using outcome data from the trial and secondary sources as necessary, we will estimate a range of incremental cost-effectiveness ratios, including the cost per additional neonate cured and cost per life-year gained.

One of the key intentions of economic evaluation is to promote health care decisions that maximize population health within the available budget. To achieve this, a generic measure of outcome is needed in order to compare (in theory) across the full spectrum of diseases and patient groups within the particular setting (Thokala, Ochalek et al. 2018). Following this logic, the key ratio to be used will be the incremental cost per death averted (the incremental cost-effectiveness ratio (ICER)). Determining cost-effectiveness then requires the comparison of this ICER to other claims on limited resources (represented by a cost-effectiveness threshold (CET)). If the ICER<CET the intervention may be deemed “cost-effective”. The choice of CET will be based upon knowledge of the ICERs in recent funding decisions within the country health systems as well as international estimates.

One of our main economic hypotheses is that more effective treatment of neonates will lead to earlier discharge and substantially lower the financial and time burden to families, and that these differences will be particularly important for the poorest strata in each country. To quantify these differences, we will collect data on household’s human capital and living conditions and then classify households into site-specific quintiles. We will then estimate interacted impact models in a first step to assess whether health impacts vary across socio-economic subgroups. In a second step, we will quantify the total financial burden for all households, and quantify the share of households experiencing catastrophic expenditures. Catastrophic expenditure will be defined as total cost exceeding specific fractions of total monthly household income, with thresholds ranging between 10 and 50% (O’Donnell, Van Doorslaer et al. 2008). Household incomes will be computed based on the observed asset holdings using national surveys as references points (Fink, Victora et al. 2017). The generated evidence will complement the standard cost-effectiveness analysis (which abstracts from equity aspects) by assessing the overall equity and impact of these treatment regimens in general, and by assessing the extent to which these regimens can reduce socioeconomic gaps in particular.

13 REGULATORY & ETHICAL ISSUES

The trial protocol, the participant information sheets and consent forms, up-to-date versions of the IB or SPC, as well as principal investigators qualifications will be submitted to appropriate ethics committees and regulatory authorities, together with any other documents required (e.g. insurance, contracts).

The trial will not start in any site before written approval by the appropriate ethics committee, regulatory authority (where applicable) has been received, and the trial protocol and clinical trial agreement have been signed. Extensions, amendments and renewals of the approval must be obtained as necessary throughout the trial and also forwarded to the CTU and Sponsor.

13.1 COMPLIANCE

13.1.1 REGULATORY COMPLIANCE

International sites will comply with the principles of GCP as laid down by the ICH topic E6 (R2) and other applicable national regulations.

13.1.2 SITE COMPLIANCE

An agreement will be in place between the site and GARDP as Sponsor, or between the site and Penta ID or other organisations designated by the Sponsors, setting out respective roles and responsibilities.

The site will inform the CTU (and CTU will inform the Sponsor as appropriate) as soon as they are aware of a possible serious breach of compliance, so that the CTU/designee can report this breach as necessary. For the purposes of this protocol, a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or integrity of the participant, or
- The scientific validity of the trial

13.1.3 DATA COLLECTION & RETENTION

Any paper copies of data worksheets, clinical notes and administrative documentation should be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for a minimum of 25 years after the end of the trial. During this period, all data should be accessible, with suitable notice, to the Regulatory or equivalent authorities, the Sponsor, and other relevant parties in accordance with the applicable regulations. The data may be subject to an audit by the Regulatory authorities. Medical files of trial participants should be retained in accordance with the maximum period of time permitted by the hospital, or institution.

13.2 ETHICAL CONDUCT

The trial population of NeoSep1 is neonates with suspected clinically diagnosed neonatal sepsis. There is appropriate pressure to treat these neonates as soon as possible and with the most effective antibiotic treatment to treat the infection. Kidney function very rarely could be compromised by the use of some of the antibiotics used in the trial (as with the antibiotics used in routine clincial care of neonates with sepsis) but this risk is considered to be outweighed by the potential benefits.

Parents and/or guardians will be informed fully of known risks and possible benefits in the Patient Information Sheet, and this will be reinforced by discussions with the trial research teams at the individual sites.

Confidentiality of the neonates and their parents/guardians will be maintained throughout the trial. Data submitted on eCRFs by trial sites will be identified only by the unique trial identifier and date of birth (including random check letters to improve accuracy of identification). It is essential to collect actual date of birth given all participants will be under 1 month of age.

13.2.1 Informed Consent Process

Many neonates will present as emergencies where delay in study enrolment, and thus treatment, through a written consent procedure would be unacceptable. For Part 2 of the NeoSep1 trial we will therefore implement a two stage consent process, as has been used in previous trials in acutely unwell children admitted to hospital for emergency treatment (Maitland, Kiguli et al. 2011). Verbal consent will be sought from parents or guardians by the admitting medical team, if it is considered that the full consent process would significantly delay treatment allocation, and consequently could be detrimental to the neonate's health. Full consent will be sought once the neonate's clinical condition has been stabilised. Parents/guardians will be provided with a brief verbal description of the trial and will be given the opportunity to "opt out" of clinical research.The clinician will mark that verbal consent was obtained on the randomisation eCRF. Both the parent/guardian and the person taking consent will later sign the consent form which may also be witnessed by an independent person if the parent/guardian is not able to sign (see below). If verbal consent is not confirmed by written consent no further data from the participant will be used after effective consent withdrawal. In the event that the baby dies prior to written consent being obtained, this primary outcome will be used in analysis.

The rights of the parent to refuse consent for their baby to participate in the trial without giving a reason must be respected. After the participant has entered into the trial, the clinician remains free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. The reason for doing so, however, should be recorded; the participant will remain within the trial for the purpose of follow-up and for data analysis by the treatment option to which they have been allocated. Similarly, the parents and guardians must remain free to change their mind at any time about the protocol treatment and trial follow-up without giving a reason and without prejudicing their baby's further treatment.

Two original written informed consent forms (ICFs) must be completed, dated and signed personally by the parent(s)/legal guardian(s) and by the investigator or designated trial staff. The parent(s)/legal guardian(s) should be given one signed original form; the second original should be kept by the investigator.

If the parent(s)/legal guardian(s) is unable to read, a relative or an impartial witness should be present during the informed consent discussion. The parent(s)/legal guardian(s) must give consent orally and, if capable of doing so, complete, sign (or thumbprint) and personally date the information and consent form. The witness must then complete, sign and date the form to testify of the participant's understanding of the trial information and his/her willingness to participate, together with the investigator.

The ICF will be provided to the site principal investigators by the CTU. Any changes to the ICF suggested by the site principal investigator must be agreed to by the Sponsor before submission to the relevant ethics committee, and a copy of the approved version must be provided to the Sponsor and the CTU after ethics committee approval and before any participants are enrolled. Any change

to the ICF after the initial approval constitutes an amendment and must be submitted for approval to the ethics committee.

13.2.2 SAMPLE COLLECTION AND STORAGE AND ANALYSIS

The risks of drawing blood include pain and thrombophlebitis. The number of blood draws will be minimised and where possible will be taken using existing lines. In addition, careful aseptic technique will be used to minimise possibility of thrombophlebitis. No more than 1ml/kg of blood will be drawn for research at any one time, and no more than a total of 2.5ml/kg will be drawn for research during the entire study. Blood/plasma for pharmacokinetic analysis (**Section 8.2**, Part 1 patients only) will be collected to validate the doses of fosfomycin and flomoxef. The blood volumes for the pharmacokinetic analysis in part 1 is expected to be approximately 0.5 ml per time point and 4 time points per patient.

The number of blood samples for haematology and biochemistry analysis has been minimised to 1 at baseline (which can be a sample that is collected as part of routine clinical care) and Day 3. All subsequent blood samples only need to be done if clinically indicated; therefore, **it will not be considered a protocol deviation** if blood samples are not collected after Day 3 because they are not clinically indicated. Furthermore, if it is not feasible to collect a blood sample due the condition of the neonate, this **will not be considered a protocol deviation**.

Clinical isolates will be collected from all positive cultures collected from sterile sites (blood, CSF urine). Blood cultures specified in the protocol (**Section 8.5.1**) are according to WHO treatment guidelines for neonatal sepsis. Bacterial isolates from culture positive blood cultures will be evaluated centrally. This could include phenotypic and genotypic assessment.

Microbiology sub-study will include peri-rectal swabs in selected sites (**Section 8.5.2**). Peri-rectal swabs are minimally invasive, can be taken in a standardised manner independent of passing stools using a soft swab and very soon after enrolment, minimizing antibiotic pre-exposure. They have been successfully used in other antibiotic trials in sub-Saharan Africa investigating impact of antibiotic exposure on the gastrointestinal commensal flora in young children (personal communication, e.g. FLACSAM trial, ClinicalTrials.gov Identifier: NCT03174236), and were found to be highly acceptable to families.

Selected bacterial isolates from blood cultures or rectal swabs may be used in *in vitro* experiments of new and existing antibiotics or combination of antibiotics to inform future treatments for multi- drug resistant bacteria.

13.2.3 FAVOURABLE ETHICAL OPINION

Approval from the relevant ethics committee(s) is required, including local departments if applicable. National requirements for further approvals may differ. A copy of all local approvals must be provided to the Sponsor and CTU.

Trial progress and safety updates will be reported to the ethics committees, in accordance with local requirements and practices in a timely manner.

13.3 REGULATORY AUTHORITY APPROVALS

This protocol will be reviewed by/submitted to the national regulatory or equivalent authority, as appropriate in each country where the trial will be run.

The progress of the trial and safety issues will be reported to the regulatory authority, or equivalent, in accordance with local requirements and practices in a timely manner.

Safety reports, including expedited reporting and SUSARS if required, will be submitted to the regulatory authority in accordance with each authority's requirements in a timely manner.

13.4 TRIAL CLOSURE

The NeoSep1 trial will be considered closed once the pre-planned statistical analysis has taken place and results have been published. Site closure will take place ahead of final trial closure and ethics committees and regulatory authorities will be informed.

13.4.1 EARLY TERMINATION OF THE TRIAL

Both the Sponsor and the investigators reserve the right to terminate the trial at any time prior to inclusion of the intended number of participants, but they should intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures after review and consultation. In terminating the trial, the Sponsor and the investigators will ensure that adequate consideration is given to the protection of the participants' interests.

Reasons for early termination of the trial, overall or at one site, by the Sponsor may include but are not limited to:

- enrolment rate too low
- protocol violations
- inaccurate or incomplete data
- unsafe or unethical practices
- questionable safety of a trial treatment
- following the recommendation of the DMC or ethics committee
- administrative decision

Reasons for early termination of the trial by the investigators may be:

- insufficient time or resource to conduct the trial
- lack of eligible participants

In the event that the trial is terminated early, the investigator has to:

- complete the eCRF to the greater extent possible
- answer all questions of the Sponsor or their representatives related to data of participants enrolled at the site prior to trial termination
- ensure that parents/guardians of neonates enrolled in the trial who had not yet reached a follow up time point are informed promptly and followed up with the necessary medical care
- provide in writing the reasons for their decision to the ethics committee and the Sponsor
- follow end of trial procedures as per the protocol and MOP

In the event that the trial is terminated early an abbreviated clinical study report will be prepared.

14 INDEMNITY

As Sponsor, GARDP is liable for the conduct of the trial. To this end GARDP has taken out insurance as required by local law covering the conduct of the trial, and in particular, in respect of any claim made by a parent/guardian for damages suffered by a study subject resulting from participation in the trial.

15 FINANCE

GARDP as the Sponsor is responsible for ensuring the trial has adequate funds to be carried out. GARDP is responsible for overseeing all financial activities in the trial and notify partners of any changes in funding in a timely manner. A written agreement with the site PI and GARDP will outline the funding arrangements to sites.

Core funding to support the trials is also provided by the MRC to the MRC CTU.

16 OVERSIGHT & TRIAL COMMITTEES

There are a number of committees involved with the oversight of the trial. These committees are detailed below.

16.1 TRIAL MANAGEMENT GROUP (TMG)

A Trial Management Group (TMG) will be formed comprising the Sponsor representative's, Chief Investigator, other lead investigators (clinical and non-clinical) and members of the MRC Clinical Trials Unit (CTU). The TMG will be responsible for the overall running and management of the trial. It will meet approximately three times a year. The full details can be found in the TMG Charter. On a day-to-day basis, operational management will be delegated from the TMG to a smaller team including the Sponsor's representative, Chief Investigator and other representatives of SGUL and MRC CTU.

16.2 TRIAL STEERING COMMITTEE (TSC)

The Trial Steering Committee (TSC) has membership from the TMG plus independent members, including the Chair. The role of the TSC is to provide independent advice and guidance on the trial conduct through its independent Chair to the Sponsor. The ultimate decision for the continuation of the trial lies with the Sponsor. Further details of TSC functioning are presented in the TSC Charter.

16.3 THE DATA MONITORING COMMITTEE (DMC)

An independent Data Monitoring Committee (DMC) will be formed. The DMC will be the only group who sees the confidential, accumulating data for the trial. Reports to the DMC will be produced by the CTU statisticians. The DMC will meet within 6 months of the trial opening; the frequency of meetings will be dictated in the DMC charter. The DMC will consider data using the statistical analysis plan (see **Section 11.5**) and will advise the Sponsor. The DMC can recommend premature closure or reporting of the trial, or that recruitment to any treatment be discontinued. The ultimate decision for the continuation of the trial lies with the Sponsor.

Further details of DMC functioning, and the procedures for interim analysis and monitoring are provided in the DMC Charter.

16.4 ROLE OF STUDY SPONSOR

GARDP is the study Sponsor. GARDP is overall responsible for all aspects of the set-up, conduct, management, finance and reporting of the NeoSep1 trial in accordance with the local laws, regulations and the Principles of ICH GCP (E6). This includes:

- Decision related to confirming the doses of fosfomycin and flomoxef before proceeding to Part 2
- Provision of fosfomycin and flomoxef from relevant partners

GARDP will delegate responsibilities for some of the activities required to implement the trial to Penta Foundation, MRC Clinical Trials Unit at UCL, and St George's, University of London.

GARDP's intent as Sponsor and supported by project partners is to use the results from the trial, where possible, to support broader objectives related to access to the new and existing antibiotic regimens evaluated as part of this trial.

17 COMMUNITY ENGAGEMENT

In consultation with study site teams, and with regard to local cultural practices, community engagement will be discussed, and a strategy will be agreed, as appropriate for that site/country. Information about this will be included in the country specific appendix. This could consider the following aspects:

- Engagement with local stakeholders as appropriate to the setting which could include parent/ guardian forums, local parents groups or organisations focussing on care of neonates, Community representatives, Patient representative organisations, Ministry of Health or other organisations identified by study site teams or other members of the local study team eg clinical monitor.
- Training in community engagement for the healthcare providers such as Clinical officers, nurses and other hospital staff who may be involved in patient care of trial participants, as appropriate to the setting.

The objective of this is to inform relevant members of the local community about the trial and its progress and ultimately the results. We will engage key stakeholders through meetings, supported by the Sponsor but led by local team representatives. At these meetings, information and feedback will be given and received.

17.1 TRAINING IN COMMUNITY ENGAGEMENT

As part of the site set-up process, we will determine training requirements for community engagement, both of the local trial team (see **Section 3.4**) but also of the broader hospital community as appropriate for the individual setting. This could include Clinical Officers, Physicians Assistants, Nurses and others as appropriate for their involvement in the trial in that unit. In addition, all investigators will complete relevant courses in Good Clinical Practice ethical training specifically addressing research involving human participants (see **Section 3.4**).

18 TRIAL REPORT, PUBLICATION AND DISSEMINATION OF RESULTS

18.1 FINAL TRIAL REPORTS

A clinical trial report will be prepared by the Sponsor with input from investigators as appropriate. One copy of the final trial report must be dated and signed by the Sponsor's medical monitor, principal investigators, trial statistician and the clinical trial manager before being transmitted to the regulatory authorities and local ethics committees if required.

18.2 PUBLICATION

A publication policy will be drafted reflecting the following principles:

- All parties including GARDP, MRC CTU, SGUL, Penta, and participating sites will contribute to preparation of publication
- Upon trial completion and finalisation of the trial report, the results of the trial will be submitted for publication to a peer-reviewed journal and posted in a publicly accessible database of clinical trial results
- Authorship of any publication will be based on the uniform requirements for manuscripts submitted to biomedical journals as defined by the International Committee of Medical Journal Editors (ICMJE)
- The PK and safety data from Part 1 will be published separately from Part 2, both in a peer-reviewed journal.

19 DATA AND/OR SAMPLE SHARING

Data will be shared according to a controlled access approach, based on the following principles:

- No data should be released that would compromise the ongoing trial
- There must be a strong scientific or other legitimate rationale for the data to be used for the requested purpose
- Investigators who have invested time and effort into developing the trial should have a period of exclusivity in which to pursue their aims with the data, before key trial data are made available to other researchers; details on data sharing will be covered in a separate site agreement
- The resources required to process requests should not be under-estimated, particularly successful requests which lead to preparing data for release. Therefore adequate resources must be available in order to comply in a timely manner or at all, and the scientific aims of the study must justify the use of such resources
- Data exchange complies with Information Governance and Data Security Policies in all of the relevant countries

Researchers wishing to access NeoSep1 trial data should contact the TMG in the first instance. Approval will be sought from the Sponsor and all requests will be reviewed and discussed by the TMG.

20 PROTOCOL AMENDMENTS

Version 1.0 DD-MMM-YYYY: first version

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22 APPENDICES

APPENDIX 1: COUNTRY SPECIFIC APPENDIX

INVESTIGATORS

Add details of country and site investigators

INSTITITION

Name
Address
Country

Country Lead Investigator: to be added
Qualification: to be added

Tel: to be added
Email: to be added

INSTITITION

Name
Address
Country

Site Principal Investigator: to be added
Qualification: to be added

Tel: to be added
Email: to be added

Sub-Investigator: to be added
Qualification: to be added

Tel: to be added
Email: to be added

Investigator: to be added
Qualification: to be added

Tel: to be added
Email: to be added

Investigator: to be added
Qualification: to be added

Tel: to be added
Email: to be added

LABORATORY (IF APPLICABLE)

INSTITITION

Name
Address
Country

Contact: to be added
Qualification: to be added

Tel: to be added
Email: to be added

FIRST LINE TREATMENT OPTIONS (TO BE DEFINED BY SITE / COUNTRY)

Part 2

Treatment option for X population- edit as required

First-line treatment options
Ampicillin (amoxicillin or benzylpenicillin or cloxacillin) + gentamicin
Cefotaxime or ceftriaxone
Fosfomycin and amikacin
Flomoxef and amikacin
Fosfomycin and flomoxef
Piperacillin/tazobactam
Piperacillin/tazobactam + amikacin
Ceftazidime
Ceftazidime + amikacin
Meropenem

Note: see **Section 6** for details of drug administration, dosing etc. Regimens in *grey* will be included in the final randomisation lists for Part 2; two regimens in white will be dropped based on site relevance.

Treatment option for Y population - edit as required

First-line treatment options
Ampicillin (amoxicillin or benzylpenicillin or cloxacillin) + gentamicin
Cefotaxime or ceftriaxone
Fosfomycin and amikacin
Flomoxef and amikacin
Fosfomycin and flomoxef
Piperacillin/tazobactam
Piperacillin/tazobactam + amikacin
Ceftazidime
Ceftazidime + amikacin
Meropenem

Note: see **Section 6** for details of drug administration, dosing etc. Regimens in *grey* will be included in the final randomisation lists for Part 2; two regimens in white will be dropped based on site relevance.

SECOND LINE TREATMENT OPTIONS

Provide details of locally selected therapy

OTHER LOCAL REQUIREMENTS

<<Edit as required>>

STUDY MONITOR

<<Edit as required>>

INVESTIGATOR SIGNATURE PAGE

PRINCIPAL INVESTIGATOR'S NAME	
TITLE	
INSTITUTION	
ADDRESS	

PRINCIPAL INVESTIGATOR STATEMENT
I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (R2) and other applicable national regulations.
I understand that as Principal Investigator I am responsible for the conduct of the trial at this site and will ensure that all colleagues and supporting staff assisting with the trial are adequately informed about the protocol, the investigational products and their trial related duties
I will use only the informed consent form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this trial if required by national law.
I agree that the sponsor or its representatives shall have access to any source documents from which electronic case report form information may have been generated as well as all relevant trial's essential documents

PRINCIPAL INVESTIGATOR'S SIGNATURE	
DATE **(DD-MMM-YYYY)**	

APPENDIX 2

INC NEONATAL ADVERSE EVENT SCALE V1.0