CLINICAL STUDY PROTOCOL

Full Study Title: Sepsis Trials in Critical Care

Acronym: SepTiC

Product: Sargramostim (Trade name Leukine)

Development Phase: IV

Sponsor: Imperial College London

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This protocol has regard for the HRA guidance

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This protocol describes the SepTIC trial and provides information about procedures for enrolling participants to the trial. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres enrolling participants for the first time are advised to contact the Trial Coordination centre to confirm they have the most recent version. Problems relating to this trial should be referred, in the first instance, to the Trial Coordination centre.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act 2018 and other regulatory requirements as appropriate.

ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator or confidence interval (depending on context)
CRF	Case Report Form
CTA	Clinical Trial Authorisation
DKA	Diabetic Ketoacidosis
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GCP	Good Clinical Practice
GM-CSF	Granulocyte-macrophage colony stimulating factor
GMP	Good Manufacturing Practice
HHS	Hyperglycaemic Hyperosmolar State
HLA	Human Leukocyte Antigen
HRA	Health Research Authority
HTA	Health Technology Assessment
IB	Investigator's Brochure
ICHNT	Imperial College Healthcare NHS Trust
ICMJE	International Committee of Medical Journal Editors
ICTU	Imperial Clinical Trials Unit
ICU	Intensive Care Unit

IMP	Investigational Medicinal Product
INB	Incremental net monetary benefits
ITT	Intention to Treat
NIHR	National Institute for Health & Care Research
NOK	Next of Kin
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PerLR	Personal Legal Representative
PPI	Patient and Public Involvement
ProLR	Professional Legal Representative
QA	Quality Assurance
QALY	Quality-Adjusted Life Year
QC	Quality Control
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SSAR	Suspected Serious Adverse Reaction
STP	State Transition Probabilities
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group

TSC	Trial Steering Committee
UKRI	United Kingdom Research and Innovation
WBC	White Blood Cell

Table of Contents

TRIAL SUMMARY

TITLE: Sepsis Trials in Critical Care (SepTIC)

OBJECTIVES: To answer four primary research questions

1. Do rapid PCR-based microbiological diagnostics combined with procalcitonin improve outcomes and antibiotic stewardship compared to standard care in patients admitted to intensive care (ICU) with sepsis?
2. Does conservative fluid therapy with active removal of accumulated fluid (de-resuscitation) improve outcomes compared to standard care in patients admitted to ICU with sepsis?
3. Does GM-CSF compared to placebo improve outcomes in a high-risk subset of patients admitted to ICU with sepsis?
4. What is the relative cost-effectiveness of each of these interventions compared to current standard of care?

DESIGN: A multicentre pragmatic randomised, multi-factorial, open-label trial with an embedded randomised double-blind, placebo-controlled, parallel group trial.

SAMPLE SIZE: 3758 patients for the diagnostic and fluid trials. 1300 patients for the GM-CSF trial

PARTICIPANTS

Inclusion criteria

- Adults (≥16 years of age) admitted to ICU due to suspected sepsis and expected to stay for at least two calendar days (i.e. expected to still to be in ICU the day after tomorrow)
- Receiving intravenous antibiotics for suspected sepsis
- According to local clinical judgement, patient has received adequate initial early fluid resuscitation

Additional inclusion criteria for GM-CSF trial only (can be after initial trial entry):

- Intubated and mechanically ventilated and expected to continue for another 24 hours
- **Or** requiring two organ support (i.e. vasopressors or renal replacement therapy)
- An absolute lymphocyte count < 1.2 $x10^9$/L on two consecutive calendar days at least 12 hours apart, with no values >1.2 $x10^9$/L in between.

Exclusion criteria

- More than 24 hours since ICU admission (this does **NOT** apply for intervention 3, GM-CSF).
- Previously admitted to ICU due to sepsis on this hospital admission
- Not expected to survive 90 days, due to pre-existing chronic (end-stage) disease
- Not expected to survive initial resuscitation (24 hours)
- Neutropaenia (<0.5 neutrophils $x10^9$ /L) due to chemotherapy/malignancy (but not due to sepsis)
- A source of infection that will require a prolonged course of antibiotics, for greater than 21 days (e.g. infective endocarditis, osteomyelitis, hepatic or cerebral abscess, tuberculosis)
- Diabetic ketoacidosis (DKA) or hyperglycaemic hyperosmolar state (HHS)
- Within 21 days of a spontaneous subarachnoid haemorrhage
- Diabetes Insipidus

- Weight <40Kg

Additional exclusion criteria for GM-CSF trial only*:*

- More than 120 hours (5 days) since ICU admission
- Already receiving G-CSF or GM-CSF
- A total white blood cell count (WBC) >50 $x10^9$ /L
- Allergy, anaphylaxis or previous adverse reaction to GM-CSF or yeast-derived products
- Known to be pregnant or breastfeeding
- Known recent (required treatment within the last 5 years) haematological malignancy
- Solid organ or bone marrow transplantation
- Patient weight >125kg

TREATMENT/MAIN STUDY PROCEDURES

Diagnostic trial

PCR-based microbiological diagnostic combined with procalcitonin compared to standard microbiological testing

Fluid trial

A conservative fluid strategy with active removal of accumulated fluid (de-resuscitation) in ICU compared to standard care

GM-CSF trial

Subcutaneous GM-CSF for 8 days compared to matching placebo

OUTCOME MEASURES

PRIMARY ENDPOINT

- 90-day mortality combined with clinical state (in-hospital with organ support, in-hospital without organ support, discharged from hospital) over time

SECONDARY ENDPOINTS

- Duration of mechanical ventilation, vasopressor use, renal replacement therapy during index hospital admission up to 90 days
- Length of stay in ICU and hospital up to 90 days
- Antibiotic use (defined daily doses per 1000 occupied bed days and antibiotic-free days) during index hospital admission up to 28 days
- Infection relapse / recurrence or secondary infection requiring further antibiotic treatment during index hospital admission up to 28 days
- Adverse events and adverse drug reactions (including antibiotic related adverse events) during index hospital admission up to 28 days
- Health-related Quality of Life (EQ-5D-5L) and cognitive function (MoCA-Blind) at 6 months
- 1-year mortality
- Incremental costs per Quality-Adjusted Life Year (QALY), and Incremental Net monetary Benefits (INB)

2. BACKGROUND

Sepsis (life-threatening organ dysfunction caused by a dysregulated host response to infection) has significant patient burden and is a major healthcare problem. It is the most common cause of admission to intensive care with mortality rates of ~30%. For those that survive, it can be life-changing, with long term physical and psychological consequences. There remain many uncertainties about the early diagnosis and management of sepsis, particularly in high-risk populations.

This trial is designed in response to a commissioning brief from the NIHR (Ref 17/136) for trials to evaluate treatments to improve outcomes from sepsis. We have focused on important research questions that have recently been prioritised by the James Lind Alliance Emergency Medicine project. A recent article by international leaders in sepsis (including three of the co-applicants) highlighted the top ten priorities for clinical research in sepsis.(1) This included rapid microbiology diagnostics to guide therapy, restrictive or liberal fluid resuscitation, biomarker-guided immune stimulation therapy, and the use of multi-arm trials to simultaneously test multiple interventions. We have established a group of investigators to simultaneously and efficiently examine three important research topics in a single co-ordinated three-trial model. This provides both cost and time efficiency and, crucially, enables the establishment of a collaborative group that can provide a platform to address further questions beyond the life of this initial proposal.

Rapid antibiotic optimisation:

New diagnostic strategies based on modern technologies offer the opportunity to ensure that patients who have sepsis due to serious bacterial and fungal infection receive effective targeted treatment as early as possible. Conversely, in those without infection, who will not benefit from antibiotics, they can be safely stopped, avoiding side effects from unnecessary treatment.

Current clinical guidance states that when sepsis is suspected, and following urgent blood sampling for laboratory culture tests, immediate empirical broad-spectrum intravenous antibiotics should be delivered.(2) These are typically continued until more information about the infecting pathogen(s) becomes available. At this point, antibiotics can be rationalised as part of routine antimicrobial stewardship practice. Unfortunately, it typically takes around 72 hours for blood culture-based tests to identify pathogens and determine antibiotic sensitivities, allowing antibiotic treatment to be optimised. In sepsis, blood cultures provide important treatment information about pathogens in <30% of patients.(3) Usually, samples need to be processed in the laboratory for 5 days to be confident that they are negative. Opportunities for antibiotic optimisation within 48 hours of treatment are therefore currently limited. This is the point at which we propose that new rapid tests could affect an important change in practice.

Broad-spectrum antibiotic therapy is advocated for empirical treatment before laboratory results are available, as inadequate initial empirical antibiotic therapy is associated with increased mortality, with an odds ratio of 1.60 (95%CI 1.37-1.86) after adjusting for background conditions and sepsis severity.(4) However, broad-spectrum antibiotic use increases the risk of serious adverse events including secondary infection and antimicrobial resistance. Alterations to the host microbiome, with knock-on adverse effects on the patient are also increasingly appreciated.(5) Rapid identification of a pathogen(s) would allow earlier targeted treatment, resulting in either prompt escalation to ensure adequate cover from initial antibiotics, or switching to narrower-spectrum antibiotics. This more

individualised approach to antibiotic prescribing could bring improvements in patient outcomes and antimicrobial stewardship.

Rapid (within hours) non-culture, polymerase chain reaction (PCR) pathogen testing on whole blood samples has recently become available commercially and provide real opportunities to refine antimicrobial treatment decisions in sepsis.(6) For example, in the RADICAL multi-centre study,(7) changes in empirical antibiotics amongst patients admitted to intensive care was recommended in 178 (41%) of cases using a rapid pathogen test compared to standard microbiology tests. This increased to 57% for cases in which the rapid pathogen test results were positive and standard microbiology results were negative.

A NICE Diagnostic Guidance (DG 20 2016) reviewing the evidence for diagnostic accuracy of a variety of CE-marked rapid microbiology tests that are available to the NHS stated "*There is currently insufficient evidence to recommend the routine adoption in the NHS of [molecular assays] for rapidly identifying bloodstream bacteria and fungi. The tests show promise and further research to provide robust evidence is encouraged, particularly to demonstrate the value of using the test results in clinical decision-making… future studies should investigate using the rapid molecular tests in conjunction with other biomarkers, such as procalcitonin*". In an observational study the measurement of procalcitonin (PCT), a circulating host-response inflammatory marker associated with bacterial infection, was shown to provide additional diagnostic value in combination with rapid molecular pathogen tests aimed at clinical antibiotic treatment decisions.(8) We therefore plan to follow the NICE guidance and test PCR-based diagnostics combined with procalcitonin.

Such novel diagnostic tests have the potential to bring about a paradigm shift in how antibiotic therapy is prescribed in patients with sepsis. It is therefore crucial that we evaluate them in controlled trials to fully understand their effect on patient care and outcomes before they can be safely adopted into routine NHS practice. Without such trials there is a risk these expensive tests will be increasingly used in the NHS without any evidence of benefit.

Conservative fluid therapy

Fluid therapy is universally used as an integral part of the management of patients with sepsis. However, there is no clear consensus on how to best guide fluid administration. Early fluid resuscitation to correct significant hypovolaemia is essential to ensure adequate oxygen delivery to vital organs and tissues.(2) However, once initial hypovolaemia has been corrected (recommended to be within the first three hours of treatment), further intravenous fluid is often administered with the aim of improving global oxygen delivery. Large volumes of fluid may also be administered in the form of blood products, nutrition, 'maintenance fluid' and as a vehicle for drug delivery, and in the setting of systemic inflammation and capillary leak, as occurs in sepsis, this administered fluid is frequently sequestered within the extravascular compartment. The situation is compounded by endocrine influences and by acute kidney injury which predispose to ineffective excretion of accumulated fluid. The accumulation of a positive fluid balance in critically ill patients with sepsis is therefore very common.

Fluid overload is increasingly recognised as deleterious.(9) A strong and consistent association has been demonstrated between fluid accumulation in critical illness and poor outcomes, particularly mortality.(10, 11) Mechanisms by which this fluid overload may be harmful include:

- Elevated venous pressures and a reduced perfusion pressure gradient
- Direct injury to the endothelial glycocalyx layer, exacerbating capillary leak in sepsis and driving inflammation
- Haemodilution, leading to decreased oxygen delivery
- Tissue oedema and decreased capillary density leading to impaired oxygen diffusion.

As most of the available evidence comes from observational cohort studies, the potential for residual confounding remains, since more severely ill patients receive more fluid. Driven by evidence from these observational studies, and underpinned by mechanistic studies, three overlapping approaches to fluid overload are being tested in randomised trials.

1. Restrictive fluid resuscitation, typically involving earlier use of vasopressors. A small feasibility trial from Scandinavia found that patients treated with restrictive fluid therapy had reduced incidence of acute kidney injury (OR 0.46, 95%CI 0.23-0.92) and a trend to lower 90-day mortality (OR 0.71, 95%CI 0.36-1.40).(12) No difference in outcome was seen in the subsequent larger multicentre study in patients with septic shock, although there was some evidence of heterogeneity of treatment effect dependent on the use of respiratory support.(13) A similar US-based trial was terminated early due to futility.(14) Regardless of the efficacy or otherwise of restrictive approaches to fluid resuscitation, this accounts for only a small proportion of total administered fluid, and it therefore seems unlikely that this approach alone will prevent fluid overload. In the feasibility trial above, despite the administration of a lower volume of early resuscitation fluid, overall fluid balances did not differ between restrictive and standard care after 5 days in the ICU. Similar results have been reported in other studies.(15, 16) We do not plan to alter early resuscitation in SepTIC.
2. Minimisation of post-resuscitation fluid administration (conservative late fluid management). The majority of fluid intake in ICU patients is in the form of drug diluents, maintenance fluid and nutrition, rather than from resuscitation fluid, and ongoing accumulation of a positive fluid balance while in ICU is a common finding associated with adverse outcomes. (17-20) While avoidance of unnecessary 'maintenance' fluid and concentration of drugs and feeds in smaller volumes is sometimes possible, the majority of fluid intake is 'obligate'.
3. Active removal of fluid using diuretics or renal replacement therapy following the early resuscitation phase (de-resuscitation). Given multiple obligate sources of fluid intake, it is likely that active management of fluid accumulation is required to prevent or minimise accumulation of a positive fluid balance. In the above observational study,(19) a positive fluid balance at day 3 was independently associated with increased mortality, prolonged duration of ventilation and a longer ICU stay, even after correction for potential confounders such as illness severity and co-morbidities. Importantly, a lower mortality was seen in those patients who received de-resuscitation measures to achieve a negative balance by day 3 (adjusted odds ratio 0.29, 95%CI 0.12 – 0.69).

A systematic review and meta-analysis of randomised and quasi-randomised trials in critically ill patients compared a conservative approach to fluid administration or active de-resuscitation to either a liberal approach or standard care.(20) In the sepsis studies the risk ratio for mortality was 0.86 (95%CI 0.62-1.17) for a conservative or deresuscitative approach, while in the whole population there was a significant reduction in ICU length of stay (-1.88 days, 95%CI -3.64 to -0.12). In a subsequent feasibility trial, (The Role of Active De-resuscitation After Resuscitation-2 (RADAR-2), the investigators compared a multimodal conservative approach to fluids and de-resuscitation with

usual care with the aim of assessing the feasibility of achieving separation in fluid balance between the two treatment strategies. (21) Good separation between groups was achieved (397 +/- 4173mL vs 3692 +/- 4415mL after 5 days, P<0.01). Baseline imbalances between study arms confounded clinical outcomes, but serious adverse event rates were similar between study arms. In another small randomised controlled trial of deresuscitation versus usual care (IRIHS) good separation in fluid balance was achieved using diuretic therapy and was associated with a reduction in acute kidney injury progression in the diuretic arm. (22)

Fluid overload is therefore potentially an important iatrogenic contributary factor to adverse outcomes in sepsis, and thus there is an imperative to investigate the efficacy of strategies to reduce fluid overload in sepsis, particularly in view of current uncertainty as to optimal approaches and variability in current practice, which could be having an impact on patient outcomes.

Granulocyte-macrophage colony stimulating factor (GM-CSF)

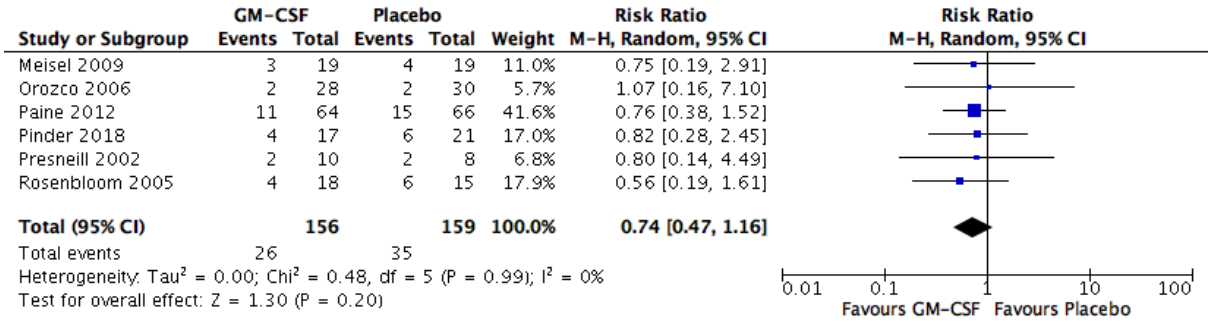
Although sepsis has classically been described as an inflammatory condition, the new sepsis definition recognises that it is a more diverse, dysregulated host response.(23) Many septic patients manifest impaired immune responsiveness and this is associated with an increased risk of mortality and secondary infection.(24) This sepsis-associated immune paresis is reflected in impaired function of circulating and tissue-migrated leukocytes, and in genomic signatures associated with profound immune dysfunction.(25-27) Arguably the best-characterised example of sepsis-associated immune cell dysfunction is the reduction in circulating monocyte HLA-DR, which has consistently been associated with adverse outcomes.(28) Accelerated lymphocyte apoptosis and impaired bacterial killing by neutrophils are also well described.(29, 30)

Clear evidence for leukocyte dysfunction in sepsis has stimulated interest in 18mmune-stimulatory therapies, with a view to reversing immune paresis. GM-CSF is particularly attractive in this regard, given its known effects on stimulating both neutrophil and monocyte production and function, and the considerable clinical experience of its use in the treatment of chemotherapy-induced myelo-ablation in acute myeloid leukaemia. GM-CSF has been studied in small RCTs in sepsis, where it has proved to be safe, and associated with a rapid and sustained improvement in monocyte HLA-DR.(28-31)

In the broader ICU context, GM-CSF has also been found to be 18mmune-stimulatory and well-tolerated in respiratory failure and in patients at highest risk of ICU-acquired infection.(31-36) Importantly, severe critical illness itself drives impairment of neutrophil function, lymphocyte apoptosis, reduced monocyte HLA-DR and an elevation in regulatory T cells that are collectively associated with a significant increase in ICU-acquired infection.(37-39) GM-CSF restores neutrophil function *ex vivo.(40)* GM-CSF has been demonstrated to improve monocyte HLA-DR and the proportion of patients with functional neutrophils in a recent RCT.(36)

While GM-CSF studies in ICU to date have not been designed to assess mortality, we have conducted a meta-analysis of the data from the studies described above and a trend is seen toward reduced mortality (Table 1). This supports the need for a larger trial powered for mortality of GM-CSF in sepsis.

Table 1. Meta-analysis of GM-CSF studies in critical care assessing mortality



3. OBJECTIVES AND ENDPOINTS

Primary Objective

To assess the clinical effectiveness and cost-effectiveness of:

1) a rapid PCR-based microbiological diagnostic combined with procalcitonin (in the Diagnostic trial)
2) conservative fluid therapy with an active fluid de-resuscitation strategy (in the Fluids trial)
3) GM-CSF, using an enrichment strategy to both identify patients at higher risk of mortality (prognostic enrichment) and who are more likely to respond to treatment (predictive enrichment) (in the GM-CSF trial).

Secondary Objective

Collect blood samples from recruited patients to be stored in the Imperial Tissue Bank for use in later ethically approved studies.

Primary Endpoint

- 90-day mortality combined with clinical state (in-hospital with organ support, in-hospital without organ support, discharged from hospital) over time

Secondary Endpoints

- Duration of mechanical ventilation, vasopressor use, renal replacement therapy during index hospital admission up to 90 days
- Length of stay in ICU and hospital up to 90 days
- Antibiotic use (defined daily doses per 1000 occupied bed days and antibiotic-free days) during index hospital admission up to 28 days
- Infection relapse / recurrence or secondary infection requiring further antibiotic treatment during index hospital admission up to 28 days
- Adverse events and adverse drug reactions (including antibiotic related adverse events) during index hospital admission up to 28 days
- Health-related Quality of Life (EQ-5D-5L) and cognitive function (MoCA-Blind) at 6 months
- 1-year mortality
- Incremental costs per Quality-Adjusted Life Year (QALY), and Incremental Net monetary Benefits (INB)

4. STUDY DESIGN

This study will be performed at approximately 60 investigational sites in the UK. It is a multicentre pragmatic randomised, multi-factorial, open-label trial with an embedded randomised double-blind, placebo-controlled, parallel group trial.

Participants will be randomised to two antibiotic strategies and two fluid strategies. In a subset of more severely ill patients (~35% of the total trial population) a third randomisation will allocate patients to the addition of GM-CSF or placebo, as shown in Table 1 and Figure 1.

Design

Multicentre pragmatic randomised, multi-factorial, open-label trial with an embedded randomised double-blind, placebo-controlled, parallel group trial with internal pilot.

There will be up to three randomisations for each participant for the three trials i.e Diagnostic, Fluids or GM-CSF trial. In the first two, eligible patients with sepsis will be randomised on inclusion to

(i) either PCR and procalcitonin guided antibiotic therapy or standard care in a 1:1 allocation ratio.
(ii) either a conservative fluid strategy with de-resuscitation or standard care in a 1:1 allocation ratio.

In the eligible subset of more severely ill patients (~35% of the total trial population) the third randomisation will allocate patients to the addition of GM-CSF or placebo in a 1:1 ratio.

Enrolment and allocation to treatment arms will be performed using an online system to ensure concealment. Treatment allocation will be by minimisation and will include a 20% random element.(41) The minimisation stratification variables will be:

- Diagnostic trial: Centre, vasopressor use (Y/N), source of infection (community vs hospital acquired)
- Fluid trial: Centre, respiratory support (Y/N), vasopressor use (Y/N)
- GM-CSF trial: Centre, allocation to Diagnostic and Fluid trials, source of infection (community vs hospital acquired)

We include an internal pilot study to assess adequate recruitment rates to each of the trials. The internal pilot will run for the initial 8 months of recruitment (~350 patients) and run seamlessly into the main trial, if the success criteria are met (see data analysis section for details). The internal pilot outcome and recruitment will be examined at 4 and 8 months. The 4-month look will allow for corrective action only.

The TSC will monitor recruitment and completion of follow-up to the primary outcome (day 90).

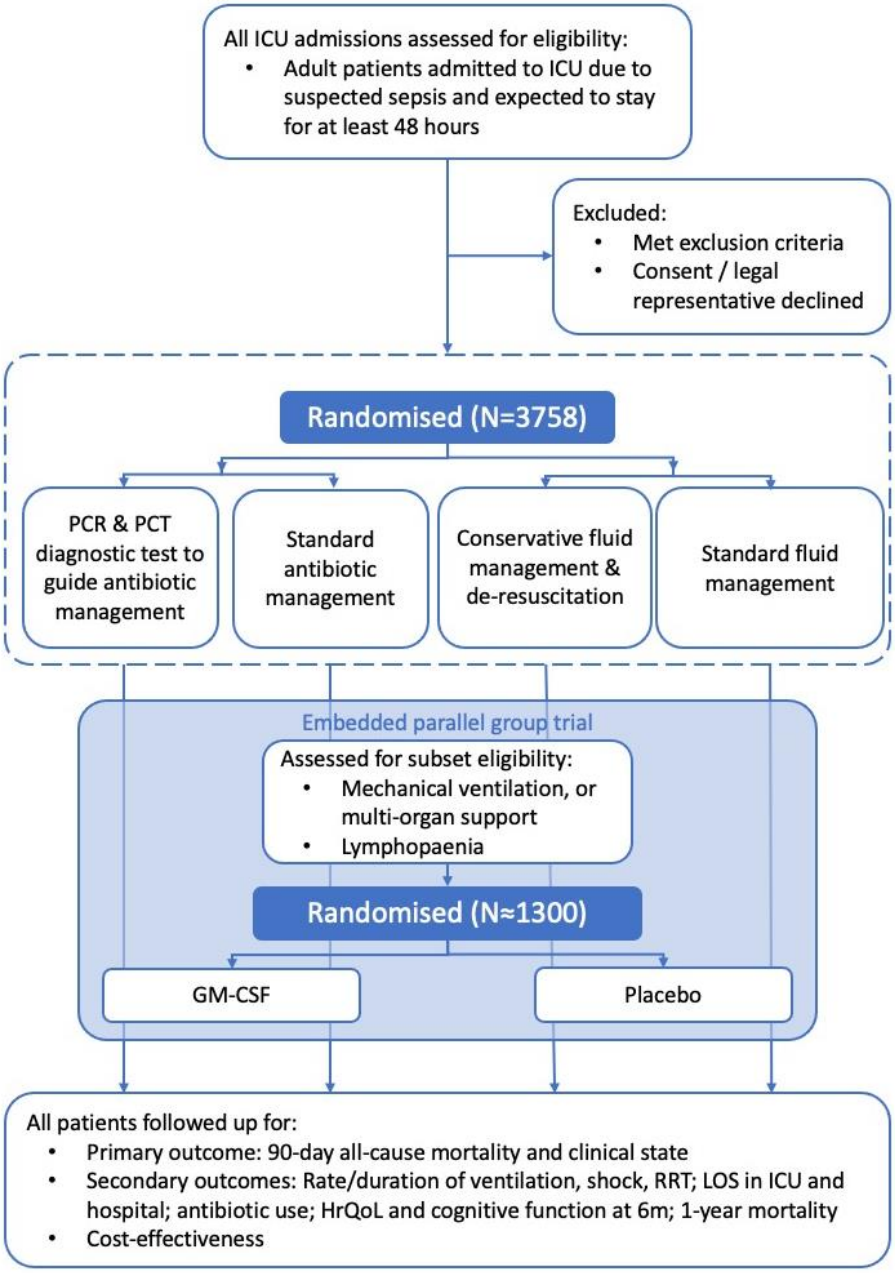
The TSC will also informally monitor measures of adherence:

- The median time to the PCR results being available in the intervention arm of the diagnostic trial
- The average separation in total fluid balance at day 3 in the fluid trial
- Adherence to IMP administration in the GM-CSF trial

Table 1: Summary of treatment groups

Trials	Number of participants	Intervention 1	Intervention 2
Diagnostic trial	3758	PCR and procalcitonin guided antibiotic therapy	Standard care
Fluid trial	3758	Conservative fluid therapy & de-resuscitation	Standard care
GM-CSF trial	1300	GM-CSF	Placebo

Figure 1: Study flow chart



5. PARTICIPANT ENTRY

Study setting and population

The target population is adult patients admitted to UK ICUs due to suspected sepsis.

Inclusion criteria

- Adults (≥16 years of age) admitted to ICU due to suspected sepsis and expected to stay for at least two calendar days (i.e. expected to still to be in ICU the day after tomorrow).
- Receiving intravenous antibiotics for suspected sepsis
- According to local clinical judgement, patient has received adequate initial early fluid resuscitation

Suspected sepsis definition: Within the context of this study, 'suspected sepsis' is defined as 'acute organ dysfunction associated with suspected infection'.(23) We do not mandate a specific definition for 'acute organ dysfunction' and will use local clinical decision. Patient characteristics underpinning local clinical decisions will be captured as part of the Case Report Form (CRF) which will include the Sequential Organ Failure Assessment (SOFA) score.

Additional inclusion criteria for GM-CSF trial only (can be after initial trial entry):

- Intubated and mechanically ventilated and expected to continue for another 24 hours
- **Or** requiring two organ support (i.e. vasopressors or renal replacement therapy)
- An absolute lymphocyte count < 1.2 $x10^9$ /L on two consecutive calendar days at least 12 hours apart, with no values >1.2 $x10^9$ /L in between.

Exclusion criteria

- More than 24 hours since ICU admission (this does **NOT** apply for intervention 3, GM-CSF). *Note:* As early intervention in sepsis is important, the aim should be to enrol eligible patients as soon after ICU admission as is practically possible.
- Previously admitted to ICU due to sepsis on this hospital admission
- Not expected to survive 90 days, due to pre-existing chronic (end-stage) disease
- Not expected to survive initial resuscitation (24 hours)
- Neutropaenia (<0.5 neutrophils $x10^9$ /L) due to chemotherapy/malignancy (but not due to sepsis)
- A source of infection that will require a prolonged course of antibiotics, for greater than 21 days (e.g. infective endocarditis, osteomyelitis, hepatic or cerebral abscess, tuberculosis)
- Diabetic ketoacidosis (DKA) or hyperglycaemic hyperosmolar state (HHS)
- Within 21 days of a spontaneous subarachnoid haemorrhage
- Diabetes Insipidus
- Weight <40Kg

Additional exclusion criteria for GM-CSF trial only*:*

- More than 120 hours (5 days) since ICU admission
- Already receiving G-CSF or GM-CSF
- A total white blood cell count (WBC) >50 $x10^9$ /L
- Allergy, anaphylaxis or previous adverse reaction to GM-CSF or yeast-derived products

- Known to be pregnant or breastfeeding
- Known recent (required treatment within the last 5 years) haematological malignancy
- Solid organ or bone marrow transplantation
  Patient weight >125kg

6. PROCEDURES AND MEASUREMENTS

Identification and recruitment of participants

Patients will be identified by local clinical and clinical research staff employed in the recruiting hospitals.

Screening and pre-randomisation evaluations

No additional tests or data are required for screening to assess eligibility for the trial. The screening will be conducted by local clinical and clinical research staff employed in the recruiting hospitals, using the routinely clinically collected data.

Randomisation and Blinding

Participants will be allocated to interventions using minimisation with a 20% random element.(41) Allocation to the first two interventions (diagnostic and fluid trials) will occur concurrently. Participants who are sicker on admission or deteriorate will be eligible to be allocated to the third intervention (GM-CSF trial). The following variables will be used for minimisation:

- Diagnostic trial: centre, vasopressor use (Y/N), source of infection (community vs hospital acquired)
- Fluid trial: centre, respiratory support (Y/N), vasopressor use (Y/N)
- GM-CSF trial: Centre, allocation in Diagnostic and Fluid trials, source of infection (community vs hospital acquired)

Concealment will be achieved through use of an online system; however, the diagnostic and fluid trials are open-label whereby participants, the clinical team and study team will not be masked to the interventions. No aggregated data by arm will be available to the study team throughout the trial.

The GM-CSF trial will be double-blind whereby the participants, clinical team, and study team will be masked to treatment allocation. This will be achieved by providing GM-CSF and matching placebo (supplied and imported from the USA by Partner Therapeutics) in similar vials and then masked by over-labelling the vials and packaging in individual numbered patient packs.

Code-breaking/ Unblinding

Each participant will be assigned a unique trial ID which is linked to the treatment allocation. The treatment code must not be broken except in medical emergencies when the appropriate management of the participant necessitates knowledge of the treatment, or in the event that expedited reporting to the Research Ethics Committee (REC) and MHRA of a Suspected Unexpected Serious Adverse Reaction (SUSAR) is required.

The trial EDC system (on OpenClinica) will include an automated unblinding facility, in case unblinding is required. In the event that emergency unblinding of an individual participant is required,

authorised staff (as documented on the delegation log) will follow trial procedures to unblind the participant in question and proceed with expedited reporting if required.

Investigators are encouraged to discuss the need for emergency unblinding with the Sponsor / Chief Investigator (or designee) / ICTU, if the circumstances permit such a discussion. If discussion prior to emergency unblinding cannot take place, the coordination centre (ICTU) should be informed afterwards.

Visit Schedule

Visit	**Day 0** (pre randomisation)	**Day 1** (post-randomisation)	**Day 2**	**Day 3-5**	**Day 6-12**	**Day 12-28**	**Day 90**	**Day 180**	**Day 365**
Screening	X								
Informed Consent (Patient consent/ PerLR/ ProLR / Retrospective Patient Information & consent)	Patient / PerLR / ProLR will be obtained initially. Retrospective patient consent will be obtained when the patient has recovered capacity to consent.								
Inclusion / Exclusion criteria	X								
Randomisation to diagnostic & fluid trials	X								
Randomisation to GM-CSF trial	Anytime up to 5 days (120 hours) after ICU admission once meets additional inclusion / exclusion criteria								
PCR & PCT test if in relevant arm of diagnostic trial.		X	PCT only on Day 2						
Conservative fluid management & de-resuscitation if in relevant arm of fluid trial		X	X	X					
Study drug (GM-CSF / placebo) administration		For 8 days after randomisation							

Research blood samples for all patients		X	Additional samples if in GM-CSF trial						
Data Collection / Follow up									
Baseline data	X	X							
Daily collection of clinical data up to Day 28 while in ICU		X	X	X	X	X			
Final hospital data collection on discharge from hospital (up to Day 90 max)							X		
Day-90 vital status							X		
EQ-5D-5L & MOCA-Blind. (Final contact with patient)								X	
1 year vital status (through data linkage only)									X
Final Visit									X

Treatment

Treatments within the trial will only be provided while the patients are in the ICU and will be provided by local clinical staff.

Diagnostic trial

Intervention – antibiotic optimisation using PCR-based pathogen testing and procalcitonin

A blood sample will be taken after randomisation for rapid PCR-based pathogen testing, to be sent to the designated molecular diagnostic laboratory.

Procalcitonin (PCT) will be measured on the day of inclusion and the next day (18 - 36 hours later). This will be measured in the local hospital laboratory or using a point of care device. If a sample has already been sent as part of clinical care on the day of inclusion that value can be used for trial purposes; it does not have to be repeated.

In addition, all standard microbiological sampling will continue as normal.

If the **PCR result is positive**, the antibiotic regimen will be optimised based on this result. The result from the PCR result will be sent to sites from the central coordination centre. The clinical team should seek advice from their local microbiology service as appropriate to discuss optimal antibiotic choice based on local sensitivity and resistance patterns of the identified organism(s).

If the **PCR result is negative AND the procalcitonin results are negative** (**both results <0.5 µg/L or the second procalcitonin result has dropped by >80% from the first result)** and there are no other positive microbiology results or clear objective evidence for infection, then the empiric antibiotics will be stopped. As well as standard microbiology culture techniques, other sources of evidence for infection, based on usual practice will be reviewed. These include pneumococcal and *Legionella* antigen tests, white cells on lumbar puncture, purpuric rash or evidence of faecal soiling seen at laparotomy. If any of these objective features of infection are present antibiotics will be continued according to local guidelines.

In all other patients (including if PCR is negative and the procalcitonin is positive, i.e. ≥0.5 µg/L without an 80% drop from first to second result), antibiotic prescriptions will be reviewed daily, in consultation with microbiology teams. Sites are advised to both de-escalate antibiotic therapy and prescribe short courses of antibiotics, as clinically appropriate, as recommended by Public Health England.(42)

Control – standard care

Patients will be prescribed antibiotics according to the local antibiotic prescribing guidelines and using standard microbiology sampling, culture and sensitivity testing. Best practice including daily review of antibiotics, in consultation with microbiology teams, is encouraged. Local teams are advised to both de-escalate antibiotic therapy and prescribe short courses of antibiotics, as clinically appropriate, as recommended by Public Health England.(42)

Fluid trial

Intervention – Conservative fluid therapy with de-resuscitation

A conservative fluid strategy will be followed as soon as possible after randomisation. Once initial early hypovolaemia has been corrected and in the absence of suspected or overt bleeding, or other fluid loss, a 250ml bolus of an isotonic crystalloid may be given if any of the following objective signs of possible hypovolaemia are present:

- Skin mottling beyond the area of the kneecap
- Blood pressure target cannot be maintained despite up-titration of noradrenaline or other vasoactive drugs
- Serum lactate ≥3 mmol/L
- Urine output < 0.25 ml/kg/h (on Day 1 only)

After administration of any fluid bolus, the patient should be re-assessed.

Further fluid boluses may be given if, on re-assessment, signs of possible hypovolaemia remain. No maximum volume of fluid boluses is specified. However, if there is no improvement after such fluid boluses, (for example after four boluses, 1000mL), the likelihood of benefit from further fluid boluses is very low and should not be given.

No routine (maintenance) intravenous fluid will be given other than to correct electrolyte abnormalities or to prevent ketosis, although replacement of bleeding or measured external fluid losses (e.g. vomiting, nasogastric losses, drain fluid) of more than 0.5 litre/day may be given in a 1:1 ratio. Normal feeding will continue as per local ICU protocol. Intravenous drugs will be given in the smallest acceptable volumes.

On a daily basis (days 2-5), patients will be assessed for cardiovascular stability. If stable (defined as a norepinephrine requirement <0.2 µg/kg/min or equivalent and not increasing, with no signs of possible hypovolaemia as described above) AND with signs of fluid overload (defined as >3000 ml cumulative positive fluid balance from ICU admission or oedema in more than one site [arms, legs, flanks, abdominal wall, chest x-ray]), then de-resuscitation will be given.

Deresuscitation will consist of combination diuretic therapy (oral indapamide* 5mg daily, and furosemide 0.25mg/kg IV bolus (to the nearest 10mg, maximum 40mg) followed by an infusion starting at 5mg/hr, titrated between 2-20mg/hr to aim for at least a negative fluid balance of ~1000 ml/day.
* Or any equivalent thiazide diuretic e.g. metolazone 5mg OD / bendroflumethiazide 5mg OD

Note, if there is excessive diuresis resulting in a larger negative balance or that the patient develops cardiovascularly instability the furosemide infusion should be reduced or stopped.

Patients requiring renal replacement therapy will not receive diuretics but the clinicians will target a similar negative balance through fluid removal.
There will be daily re-assessment according to previous criteria (oedema, fluid balance, cardiovascular stability). Once the patient is no longer assessed to be fluid overloaded (neutral fluid balance and/or no oedema), de-resuscitation will stop.

If eligibility criteria for deresuscitation are not met, diuretics should not be given and the patient should be reassessed the following day.

The conservative fluid therapy with de-resuscitation will continue until day 5 or the patient is discharged from ICU, whichever comes first.

See Appendix 1 for a treatment flowchart

Control – standard care

Patients will be prescribed fluids according to usual care, at the discretion of the treating clinicians. Intravenous drugs will be given in standard dilutions according to local ICU policy and maintenance fluid will be allowed if deemed required by the treating clinician. Fluid boluses will be given as deemed clinically indicated and sites will be encouraged to follow the Best Practice Statement in the Surviving Sepsis Campaign international guidelines that "fluid administration is continued as long as hemodynamic factors continue to improve… [applying] a fluid challenge technique".(2)

Fluid balance targets will be set by the treating clinicians and diuretic use will be allowed as clinically indicated.

5.6.3 GM-CSF

Intervention / control – GM-CSF (sargramostim) / placebo

In the subset of patients requiring mechanical ventilation, or with two or more organ failures and with persistent lymphopaenia (defined as an absolute lymphocyte count <1.2 $x10^9$ /L on two consecutive calendar days at least 12 hours apart, with no values >1.2 $x10^9$ /L in between) subcutaneous GM-CSF (sargramostim) or a matching placebo will be given.

The dose will be 500µg (two vials) for patients ≥50kg, and 250µg (one vial) for patients <50kg, once a day for 8 days in the ICU.

If the patient is discharged from the ICU before the 8th day then the study drug should be given on the ward until 8 doses in total have been, if logistics allow. It will not be a protocol deviation if this is not possible. If the patient is discharged hospital before the 8th day then the study drug will stop.

Follow-up

Patients will be followed up by the clinical research team daily whilst in ICU.

Once the patients have left ICU and been discharged to the general ward, they will be followed up prior to hospital discharge and at 90 days and 6 months. Follow-up at 1 year will be via medical records and data linkage with NHS records wherever possible.

Laboratory Evaluations

Blood tests

10ml of additional blood will be collected on inclusion into the trial and sent to a central lab for PCR-based pathogen testing.

Routinely collected clinical blood samples will be used to measure procalcitonin and absolute lymphocyte count measurement in local clinical laboratories at the recruiting hospital.

Exploratory / Research samples

In addition, 20ml of blood will be collected on inclusion and sent to a central lab for storage of DNA, RNA and serum in the Imperial Tissue Bank for analysis in other ethically approved studies. For patients in the GM-CSF trial three additional 7ml of blood samples for RNA storage will be collect on inclusion and days 3 and 5 after randomisation. If resources prevent collection of the exploratory / research samples this is not a protocol deviation.

Sample storage and analysis

The blood samples for PCR-based pathogen testing will be sent on the day of collection to the central laboratory for testing. Details of the processing, handling and shipping are provided in the separate sample handling manual. The results of these tests will be sent back to sites in two stages.

The initial report will state if pathogen DNA has been detected or not.

If pathogen (bacterial or fungal) DNA is detected this will be sequenced and pathogen identification will be reported to sites.

Details of the reporting process are provided in the separate diagnostic reporting manual.

If any sample material still remains after analysis this will be transferred to the Imperial Tissue Bank for analysis in other ethically approved studies

Incidental findings

There will be no clinical testing of samples beyond those described above for PCR-based pathogen testing that will be fed back to clinical care teams. Similarly, there are no additional clinical examinations other than routine clinical examination as part of standard care. Therefore, there will be no incidental finding reported to the patient, their clinical care team or their GP.

7. TREATMENTS IN GM-CSF TRIAL

Treatment arms

Eligible patients will be randomised to one of two treatment arms

GM-CSF (sargramostim) - 500µg (two vials) for patients ≥50kg, and 250µg (one vial) for patients <50kg, given subcutaneously once a day for 8 days.

or

Matching placebo - two vials for patients ≥50kg, and one vial for patients <50kg, given subcutaneously once a day for 8 days.

Investigational Medicinal Product Details

GM-CSF (sargramostim) for Injection drug product is a sterile, lyophilized white cake in a stoppered Type I, 5mL glass vial. Drug product comprises 250µg of active ingredient sargramostim (recombinant DNA human GM-CSF) with excipients Mannitol, Sucrose, and Tromethamine.

The matching placebo contains Mannitol, Sucrose, buffer and a hydrochloric acid solution in matching 5ml glass vials.

GM-CSF (sargramostim) is used to accelerate neutrophil recovery, decreasing infectious morbidity and mortality. The first marketing authorization was granted in 1991. The six approved indications for sargramostim are:

- To shorten time to neutrophil recovery and to reduce the incidence of severe, life-threatening, or fatal infections following induction chemotherapy in adult patients 55 years and older with acute myelogenous leukaemia.
- In adult patients with cancer undergoing autologous hematopoietic stem cell transplantation for the mobilization of hematopoietic progenitor cells into peripheral blood for collection by leukapheresis.
- For acceleration of myeloid reconstitution following autologous peripheral blood progenitor cell or bone marrow transplantation in adult and paediatric patients 2 years of age and older with non-Hodgkin's lymphoma, acute lymphoblastic leukaemia, and Hodgkin's lymphoma.
- For the acceleration of myeloid reconstitution following allogeneic BMT in adult and paediatric patients 2 years of age and older undergoing allogeneic BMT from human leukocyte antigen (HLA)-matched related donors.
- For treatment of adult and paediatric patients 2 years and older who have undergone allogeneic or autologous BMT in whom neutrophil recovery is delayed or failed.
- To increase survival in adult and paediatric patients from birth to 17 years of age acutely exposed to myelosuppressive doses of radiation (hematopoietic syndrome of acute radiation syndrome.

It is not currently licensed for use in any indication in the UK. The active drug and placebo have been provided by Partner Therapeutics

The trial is being carried out under a Clinical Trial Authorisation (CTA) from the MHRA. The drug is therefore only to be used by the named investigators, for the participants specified in this protocol, and within the trial.

The Investigator Brochure (IB) is used for drug safety and other reference in this trial.

Labelling and Packaging

Partner Therapeutics will be responsible for assuring that the quality of all IMPs are adequate for the duration of the trial and are in compliance with the Good Manufacturing Practice (GMP) standards.

Sargramostim (GM-CSF) and matched placebo will be imported from the USA. All drugs will be packaged, labelled and QP released according to the MHRA requirements and distributed to sites by Victoria Pharmaceuticals, Belfast. The Trial Co-ordination Centre will keep accurate records of supply to trial centres and destruction of unused IMP at the end of the trial.

Storage and Dispensing

The IMP will be delivered to the pharmacy at each participating site. Once received at site the IMP will be refrigerated and stored between 2 – 8°C. The pharmacy will then dispense the IMP to the ICU where this will be refrigerated until required. It is the responsibility of the site to ensure that accurate dispensing, prescriptions and returned records of the IMPs are maintained. The Study Coordination Centre will track supplies of IMPs via information from Victoria Pharmaceuticals and site IMP tracking documents. At the completion of the trial, the Trial Coordination Centre, via the monitor, will ensure the destruction of all returned dispensed IMPs (after close-out and before archiving). An IMP Management Plan will be generated to manage all aspects of IMP order, delivery, use and destruction during the course of the trial.

Accountability

Site pharmacies will be responsible for recording IMPs dispensed to the ICU. Preparation of all drug infusions will be recorded on the Drug Accountability Form and drug administration on the patient's prescription chart. The Trial Coordination Centre will provide sites with an Inventory Log to keep track of all IMP vials, whether infused, opened but not infused or unused. At the end of the study any remaining unused drug will be returned to the hospital pharmacy for recording.

Drug interactions / Precautions / Contraindications

Do not administer sargramostim (GM-CSF) to patients with a history of serious allergic reactions, including anaphylaxis, to human GM-CSF such as sargramostim, yeast-derived products, or any component of the product.

Sargramostim (GM-CSF) should be permanently discontinued if the total WBC count exceeds 50 $x10^9$ /L or if a serious allergic or anaphylactic reaction occurs.

Overdose of IMP

There is no clinical experience with sargramostim overdose. No specific antidote or detoxification measures can be recommended to date. If accidental overdose is suspected, the patient should be treated symptomatically.

Dose Modifications for Toxicity

In case of overdosage, discontinue sargramostim and monitor the patient for WBC increase and respiratory symptoms.

Study drug administration

Lyophilised drug product should be reconstituted with 1mL of Sterile or Bacteriostatic Water for Injection for a 250 µg sargramostim/mL solution for subcutaneous injection. Full details will be provided in a separate administration guide.

Permanent Discontinuation of Study Treatment and Withdrawal from Study

Permanent discontinuation of study treatment

Participants may discontinue study treatment for the following reasons:

- At the request of the participant or their personal/professional legal representative
- Adverse Event/ Serious Adverse Event
- Allergic reaction to IMP
- If the investigator considers that a participant's health will be compromised due to adverse events or concomitant illness that develop after entering the study.

Withdrawal from Study

Withdrawal from the study refers to discontinuation of study treatment and study procedures and can occur for the following reasons:

- Participant decision or their personal/professional legal representative
- Loss to follow-up

Procedures for Withdrawal from Study

Patients will be free to withdraw at any time. If the patient (or their personal/professional legal representative) wishes to withdraw from the study during the treatment period the treating physician will no longer follow the trial protocol and the study drug will be stopped. If the participant withdraws from the study this will be documented in the eCRF and medical records.

The patient will be able to either withdraw completely from the trial or from certain elements. Further follow-up visits as part of the clinical trial will cease. However, the participant will be asked if data collection through data linkage of routinely collected data, including long-term follow-up can continue.

Participants will be asked if previously collected, stored blood samples can be used for further analyses or if they would prefer their samples to be destroyed.

Already collected data will not be deleted as these will include important safety information which would be processed as part of a legitimate interest.

8. PHARMACOVIGILANCE

Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial participant administered a trial intervention / medicinal product and which does not necessarily have a causal relationship with this intervention / treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the trial medication, whether or not considered related to the IMP.

Adverse Reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions (ARs). The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected Adverse Reaction

An AR, the nature or severity of which is not consistent with the applicable product information as set out in the Reference Safety Information (RSI) (in the investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product).

When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the RSI section of the SmPC/IB which occur in a more severe form than anticipated are also considered to be unexpected.

Expectedness assessment will be performed by the Sponsor or person delegated by the Sponsor to assess expectedness. This is delegated to the local site PI.

Causality

The assignment of causality for adverse events should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigator. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Unrelated: No evidence of any causal relationship

Unlikely: There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).

Possible: There is some evidence to suggest a causal relationship (e.g. 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 (e.g. the patient's clinical condition, other concomitant treatments).

Probable: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Definite: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Severity of Adverse Events

Mild: Awareness of event but easily tolerated

Moderate: Discomfort enough to cause some interference with usual activity

Severe: Inability to carry out usual activity

Adverse Event recording

As this is a trial is conducted in critically ill patients with life-threatening sepsis then adverse events are expected to occur regularly in most, if not all, patients. Therefore, unless an adverse event is assessed to meet Serious Adverse Event criteria, these adverse events will not be reported in the case report form and simply noted in the patient's local medical record.

Abnormal Laboratory Test Results

Similarly due to the nature of the underlying critical illness, abnormal laboratory test results will be expected to occur daily for most, if not all, patients whilst in the ICU and therefore do not need to be reported as an AE/AR in the CRF. They will be recorded in the patients' medical record. Any clinically important abnormal laboratory tests will be repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the local investigator.

Serious Adverse Events (SAE)

Definition of SAE

An SAE is defined as any event that

- Results in death;
- Is life-threatening*;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**;
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;

* "Life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** "Hospitalisation" means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to the emergency department (without admission).

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a subject, or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Reporting of SAEs

As the primary outcome of the trial includes mortality, then death does not require reporting as an SAE unless, in the opinion of the local PI, the death was attributable to a study intervention / IMP or the trial protocol.

Similarly, the secondary outcomes have been selected to capture the most commonly occurring safety events in critically ill patients (e.g. organ failure and support, and new infection, including

Clostridium Difficile infection). Therefore, any events that are captured as an outcome in the eCRF do not require reporting as an SAE unless in the opinion of the local PI the event was attributable to a study intervention / IMP or the trial protocol.

Rapid reporting of all SAEs i.e. within 24 hours, occurring during the patient's ICU stay up to a maximum of 28 days must be performed as detailed in the study-specific safety reporting instructions.

Active monitoring of participants after discharge from ICU or after 28 days is not required, but if the investigator becomes aware of safety information that appears to be drug or trial related, involving a participant who participated in the study, even after an individual participant has completed the study, this should be reported to the Sponsor.

All SAEs will be reviewed by the Chief Investigator or a designated medically qualified representative to confirm expectedness and causality.

Reporting of SAEs and review by the CI will be via the trial data collection system (CRF/eCRF)

Definition of a Serious Adverse Reaction (SAR)

A SAR is defined as a SAE that is judged to be (possibly, probably or definitely) related to any dose of study drug administered to the participant.

Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any SAR that is NOT consistent with the applicable product information as set out in the Reference Safety Information (RSI) section of the Investigator Brochure (IB) or Summary of Product Characteristics (SmPC).

Reporting of SUSARs

SUSARs should be notified to the appropriate regulatory authority, the relevant REC and the Sponsor in accordance with regulatory requirements. SUSARs which are fatal or life-threatening will be reported not later than seven days after alerting the sponsor to the reaction. Any additional relevant information will be sent within eight days of the report.

A SUSAR which is not fatal or life-threatening will be reported within 15 days of first knowledge by the sponsor. The sponsor will inform all investigators about SUSARs occurring in the study.

Follow up of participants who have experienced a SUSAR should continue until recovery is complete or the condition has stabilised.

SUSAR reports will be unblinded prior to submission if required by national regulatory requirements.

Developmental Safety Update Reports / Annual Safety Reports

Developmental Safety Update Reports (DSUR) / Annual Safety reports will be submitted to the Sponsor, the Ethics Committee and Regulatory Authority in accordance with local / national regulatory requirements.

Pregnancy

GM-CSF is not approved in pregnancy and is an exclusion criterion for this intervention. Due to the life-threatening illness at the time of recruitment, pregnancy is not expected to occur. Should it occur pregnancy is not considered an SAE but should be recorded and followed up to ensure a congenital abnormality does not occur.

Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than three days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures.

9. STATISTICAL ANALYSES

Sample Size and power considerations

The sample size has been calculated based on 90-day mortality. To increase the power of the trial the primary outcome will include both 90-day mortality and clinical state (in-hospital without organ support, in-ICU with organ support, discharged) over 90 days. Simulations to further examine the power of the study under a range of conservative outcomes on the primary outcome with clinical state included have been performed on the planned trial sample size based on mortality alone.

The initial calculation used data from the ICNARC Case Mix Programme which reported a mortality rate of 31.8% for all sepsis admissions to ICU.(43) Allowing for the trial exclusion criteria we anticipate a 30% mortality rate in the standard of care arm.

In the diagnostic and fluid trials we aim to recruit 3758 patients to both trials. This is based on a clinically important target difference in mortality of 5%. Assuming 30% mortality, 5% reduction, 90% power, 5% two-sided type I error we will require 1674 per arm. We then inflate by 10% for a potential interaction effect between the intervention arms, and 2% to account for loss to follow-up.

The high-risk subset (35% eligibility for GM-CSF) will have a higher control mortality rate.(43-45) We assume a 45% mortality rate in the standard of care arm. Our meta-analysis for GM-CSF in sepsis trials demonstrated a relative risk for mortality of 0.74 (95%CI 0.49-1.14). We aim to recruit 1300 participants which will provide 90% power to detect 9% difference in mortality with type I two-sided error or 5%, and factoring in approximately 2% loss to follow up.

No adjustments for multiple comparisons have been made for these three trials as the comparisons are three distinct treatments where each result will provide a separate conclusion.(46)

In addition, we have undertaken simulations to further examine the power of the primary analysis of 90-day mortality and clinical states (in-hospital without organ support, in-hospital with organ support, discharged) for the antibiotic and fluid trials, assuming a more conservative reduction in mortality. Treating clinical state as an ordinal outcome which is repeatably observed over time increases statistical efficacy because it makes the most of all the information available. We define clinical status as an ordinal outcome with three passing states and one absorbing state.

Clinical status is categorised as:

1. Discharged from hospital

2. In-hospital without Organ Support
3. In-ICU with Organ Support
4. Death (absorbing state – i.e. cannot transition out of this state)

Using data from the VANISH and LeoPARDS trials (44, 47) we estimated the proportion in each clinical state on days 0, 7, 14, 28 and 90 days in the standard of care arm (Table 2).

Table 2: The anticipated probability of each clinical state over the first 90 days in the standard of care arm

State	0	7	14	28	90
1 = Discharge	0.00	0.06	0.21	0.42	0.60
2 = Hospital without support	0.15	0.48	0.32	0.17	0.05
3 = ICU with support	0.85	0.41	0.29	0.14	0.05
4 = Death	0.00	0.05	0.18	0.27	0.30

Simulations then estimated power for the new treatment under five scenarios, under which mortality reduction in the treatment arm was always kept at 3%, whereas the proportion transitioning to a better (lower) clinical state changed in each scenario, as shown in Table 3. Data for each simulation was generated using a Markov transition model. First, at least two sets of State Transition Probabilities (STP) corresponding to each timepoint and each scenario were estimated, using iterative methods to minimise the difference between the target clinical state probabilities and those derived from the STPs. Then, for each set of STPs, random draws of a standard (0,1) uniform distribution for each individual and timepoint were mapped onto the discrete clinical states by partitioning the interval [0,1] into sub-intervals with widths corresponding to the STPs. These simulations were analysed using a mixed effects proportional odds model which included the discrete timepoints, binary treatment variable and interactions between them as fixed effects, and participant as a random effect. In total 300 simulations were analysed for each scenario. The power was calculated based on the proportion of simulations from a two-sided hypothesis where the 95% confidence interval excluded the value of no difference (i.e. OR = 1).

Since the STPs used only imprecisely replicated the target probabilities, a slightly different power estimate was obtained for each scenario depending on which STPs were used. The final power estimate for each scenario was derived by fitting an approximate linear relationship between the 90-day change in mortality and the power as the STPs varied, and identifying the level of power corresponding to a 3% mortality reduction.

Table 3: Scenarios examining differing percentage change in clinical state achieved by day 90 in the intervention arm relative to the control arm

Scenario	Discharged	Hospital without support	Hospital with support	Mortality	Power
	Change (%)	Change (%)	Change (%)	Change (%)	
1	+ 1	+ 1	+ 1	-3	0.60
2	+ 1	+ 2	+ 0	-3	0.57
3	+ 1	+ 3	-1	-3	0.75
4	+ 3	+ 1	-1	-3	0.83
5	+ 4	+ 1	-2	-3	0.88

The power of the trial based on the simulation scenarios can be seen in final column of Table 3. The simulations have demonstrated that the trial will have good power under scenarios 3, 4 and 5 with power of 75%, 83% and 88% respectively. In summary the trial will have good power to detect a treatment difference when a minimal clinically important change in mortality of 3% by 90 days is combined with a reduction of 1% or more in participants with organ support in ICU by 90 days, and an increase of 3% or more participants to either in-hospital without support or discharge by 90 days.

Planned recruitment rate

It is expected that sites will recruit 2 patients per month on average.

Statistical analysis

Overview

We will undertake both a frequentist and Bayesian analysis on the primary outcome and a frequentist analysis on all secondary outcomes. For the primary outcome no multiple imputation will be used for missing outcome data as it is anticipated missing data will be extremely low due to the ability to retrieve information from routinely collected clinical data within medical records. The Bayesian analysis on the primary outcome will be used to facilitate interpretation and we will use vague prior distributions on the model parameters including the treatment effect parameter. In the frequentist approach we will report the Summary statistic (e.g. Odds Ratio) with 95% confidence intervals and the interpretation will focus on the magnitude of the intervention effect and precision of this estimate. In the Bayesian analysis we will report the probability of superiority, and the probability of superiority by a meaningful margin which will be pre-specified in the statistical analysis plan after consultation with Patient and Public Involvement and Engagement (PPIE) members and clinical teams.

For secondary outcomes where missing outcome data is greater than approximately 5% we will undertake multiple imputation and the model will be specified in the statistical analysis plan and will include the outcome as well as stratification variables.

Internal Pilot & success criteria.

We will undertake an internal pilot after 8 months from the start of recruitment when approximately 350 participants should have been recruited. We will also examine the internal pilot outcomes at 4 months to allow early corrective action if required but the trial will not be stopped based on this review.

We will assess recruitment to the trial for each of the three trials. These will be reviewed by the CI, the Trial Steering Committee and the funder. The criteria in Table 4 will be used to assess the pilot trial. If all three intervention arms meet the red criteria stopping the trial will be considered by the CI, the Sponsor and the Funder.

Table 4: The progression criteria to be assessed by the study team,TSC and funder

Progression criteria	Recruitment Criteria at 8 months	Proportion stopping antibiotics when PCT & PCR tests are both negative in diagnostic trial	Average separation in total fluid balance at day 3 between arms in the fluid trial
RED: Consider stopping the affected trial	Recruitment rate is less than 40% of planned	<40%	<250ml
AMBER: Explore methods to increase recruitment to the affected trial	Recruitment rate is between 40% and 95% of planned	40-80%	≥250ml and <750ml
GREEN: Continue without changes	Recruitment rate is ≥95%	>80%	≥750ml

The TSC will also informally monitor measures of adherence and data quality:

- The median time (and IQR) to the PCR results being available in the intervention arm of the diagnostic trial
- The proportion of participants who receive at least 50% and 75% of the drug or placebo in the GM-CSF trial
- Completeness of clinical state data over time

Estimand Definitions and Analysis on Primary Outcome

In randomised trials the occurrence of important post randomisation events that impact the outcome can create ambiguity as to what intervention effect has been estimated if they are not defined and well described as to how they have been handled in the analysis. In order to address a precisely defined research question as recommended by ICH-E9 guidelines, (48) we define the five attributes of the primary estimand so that there is clarity for which intervention effect is the primary one to be estimated. We will perform a sensitivity analysis on the primary estimand to examine the impact of any statistical assumptions. We will also undertake supplementary analysis on the primary outcome.

The estimands for the diagnostics, fluids and GM-CSF trials are set out in Supplementary Tables A, B and C respectively. Potential additional exploratory estimands are set out in Supplementary Table D. The key features of the estimands are summarised below.

<u>Primary estimand</u>

In the primary estimand for all three trials we aim to answer a 'treatment policy strategy' question - how effective the intervention is compared to routine clinical practice regardless of intercurrent events such as intervention non-adherence, standard diagnostic results and co-enrolment to other trials.

Supplementary estimands for intercurrent events

For the diagnostics trial, a key post randomisation event in routine clinical practice is the routine blood culture result that informs subsequent treatment. Although the diagnostics intervention may benefit all patients due to its rapidity relative to the blood culture test, it is potentially of greatest benefit to those for whom the blood culture result does not identify a target pathogen. A supplementary estimand will therefore use a 'principal stratum' strategy to assess the effect of the diagnostics intervention in only those patients who do not receive a positive blood culture result.

For the fluids trial, the main post randomisation factor that may affect the interpretation of results is the occurrence of fluid overload, which may confound or mediate the intervention effect. However, this is not a discrete event but may occur to a varying extent throughout the trial, and its influence on the results will be assessed not with a supplementary estimand but via a mediation analysis based on the number of days of fluid overload and component of the intervention given.

The diagnostics and fluids trials involve complex, pragmatically defined interventions, and thus it is not feasible to capture treatment adherence as a discrete post randomisation event. The treatment policy strategy is thus the only practical approach to adherence for these trials, but where possible, the interventions' impact on decision making and patient care will be captured via CRFs to enable monitoring of the various aspects of treatment adherence.

For the GM-CSF trial, supplementary estimands will assess the effect of the intervention in participants with varying levels of adherence, defined by the number of doses of treatment received. This will enable the relationship between dosage and treatment effect to be explored graphically.

Supplementary estimands for concomitant treatments

It may be of interest to consider supplementary estimands that assess the effect of each intervention in patients who simultaneously receive one or more of the other interventions. This may be relevant if future clinical practice incorporates one or more of the study interventions as standard and the additional effect of the remaining intervention(s) needs to be considered.

Exploratory estimands: treatment combinations

Further estimands as set out in Supplementary Table D will enable exploration of the combined effect of the interventions compared to standard of care in the event that each intervention in isolation shows no benefit.

Analysis population: We will use an Intention to Treat principle. Analyses will include all randomised participants in the arm they are allocated to regardless of the intervention received or intervention discontinuation but will exclude participants who are lost to follow-up with no data on the primary outcome at day 90, as no multiple imputation will be performed for the primary analysis. It is anticipated that >98% of all patients will have primary outcome data at day 90 and therefore there will be no substantial violation of the Intention to Treat principle. We expect that in this analysis population it will be possible to account for all participants' clinical status over time for the study

duration up to day 90, and reasonable to assume discharged status if no mortality or inpatient record can be identified via data linkage; therefore no missing primary outcome data is expected.

Analysis model: A single factorial model will be used to simultaneously estimate the estimands for the diagnostic and fluid interventions in the full study population, and will also include GM-CSF treatment status as a covariate, as this approach results in unbiased marginal odds ratios for the primary estimands.(49) A second factorial model, using data only for the subjects eligible for GM-CSF, will be used to estimate the estimands for the GM-CSF trial, and will include the other interventions as covariates.

In both cases, in order to estimate the difference due to the intervention effect over time we will fit a longitudinal Proportional Odds model. This will provide the between arm proportional Odds Ratio of moving from one clinical state to a better clinical state averaged over the 90 days follow-up period. We will also use the models to estimate the between-arm proportional Odds Ratio of moving to a better clinical state on Day 90, and the 90-day mortality Odds Ratio between intervention arms. Terms for interactions between timepoints and treatment will be included. The models will have a random intercept for each subject and will adjust for covariates including age and the variables used for minimisation.

Sensitivity analysis to Primary analysis

A sensitivity analysis will replace the factorial models with multiarm models, in which the assumption of no interaction between treatments is relaxed.(49)

Further sensitivity analyses may explore alternatives to the proportional odds model (such as the Generalized Ordered Logit/Partial Proportional Odds Model or multinomial logistic regression) for the primary outcome.

Analysis of secondary outcomes

Outcomes expressed as incidences or incidence rates will be examined using a suitable mixed effects regression model such as logistic, Poisson or negative binomial.

Outcomes where it is important to account for competing events such as death, or where the interest is in the timing or duration of the event, will be analysed as time-to-event or time-to-recovery data as appropriate, using sub-distribution hazard models(50) to account for the dependent competing risk of censoring when subjects transition to a less favourable clinical state (e.g. death).

All serious adverse events will be tabulated by arm and severity, displaying the number of participants with at least one adverse event and the number of adverse events. We will also calculate odds ratios and incidence rate ratios and their 95% CIs for binary and count SAE outcomes (including 1-year mortality) at SOC level using mixed effects logistic regression and Poisson, negative Binomial model or similar models for count data as appropriate.

Antibiotic use, quality of Life (EQ-5D-5L) and cognitive function (MoCA-Blind) will be summarised by arm (mean, standard deviation and 95% CI) and analysed using univariate linear mixed models.

All models used to analyse secondary outcomes will include the same adjusting variables as the corresponding primary analyses.

A detailed statistical analysis plan will be written prior to first data extraction from the database and will detail all analysis models and model checks to be performed.

10. ECONOMIC EVALUATION

Overview

We will undertake a full health economic evaluation for each of the three comparisons. Each evaluation will follow NICE methodological guidance in taking an NHS and personal social services perspective.(51) The interventions are: rapid PCR-based microbiological diagnostics combined with procalcitonin; conservative fluid therapy with de-resuscitation; and the use of GM-CSF in high-risk patients. We will estimate the incremental quality-adjusted life year (QALYs) and costs of each intervention compared to 'usual' care. We will report cost-effectiveness metrics, including the incremental costs per QALY and the incremental net monetary benefits (INB). We will calculate the INB by valuing any differences in mean quality-adjusted life year (QALY) between the comparison groups, at NICE recommended thresholds (e.g. £20,000 per QALY) and subtracting the incremental cost. The economic evaluation will proceed in two phases. In the first phase, we will undertake a 'trial-based' evaluation which directly uses information from each randomised comparison, but with the time horizon limited to the end of trial-follow-up (one year). In phase two we will use a model to extend the trial-based evaluation to report cost-effectiveness over the lifetime.(51) For both phases we will report the incremental costs, QALYs and INB of each intervention versus usual care

Trial-based evaluations

Each economic evaluation will extract patient-level information on resource use, in particular the length of stay in critical care, according to levels of organ support, and the overall length of hospital stay. We will collate this information from the ICNARC CMP database, for the index hospital admission and also any readmissions up to one year. We will combine information from hospital resource use including using levels of organ support to categorise, Healthcare Resource Group within critical care, with unit costs from the 'Payment by Results' database, to report total hospital costs up to one year.(52) The use of personal health services will be recorded by patient questionnaire at six months, and valued with unit costs from the Personal Social Services Research Unit (www.pssru.ac.uk).

We will combine responses to the EQ-5D-5L questionnaire at 90 days, with the NICE recommended approach to valuing EQ-5D-5L at the time of analysis, to report mean HRQoL for each randomised arm. We will calculate QALYs at one year by combining the HRQoL with survival time, and will previous research in sepsis in assuming that the individual's HRQoL reported 90 days is maintained at 12 months.(53)

For each of the three evaluations we will report the incremental QALYs, costs and INB over one year for each intervention versus usual care, overall, and according to the same pre-defined subgroups as for the evaluation of clinical effectiveness. We will extend the analytical approach taken in the main statistical analysis to allow for additional issues raised by the health economic evaluation, in particular those of multiple outcomes (costs and QALYs), and that these endpoints tend to have skewed distributions. The sensitivity analysis will test whether the results are robust to methodological assumptions, for example: the assumption that HRQoL at 90 days is maintained until 12 months, alternative assumptions about the unit costs (e.g. for sargramostim), and different cost-effectiveness thresholds (e.g £10,000, £30,000 per QALY).

Health economic modelling

We will develop mathematical models that use the trial-based estimates in capturing the long-term consequences of the interventions compared to usual care, according to avoided mortality or morbidity or cost differences. Decision-analysis modelling will be used to estimate the expected costs incurred and patient outcomes over the lifetime. The model will be constructed in line with latest NICE recommendations.(51) The above estimates from the study, together with measures of uncertainty will be used to populate the model. Key input parameters will include the underlying risk of mortality and morbidity associated with current care and the treatment effect sizes associated with combinations of interventions to estimate the health status of patients at defined time points, and the costs associated with care in hospital.

The model will extrapolate beyond the end of the study to estimate the QALYs conditional on the proportion of patients who are alive, and of these, the proportion that would have reduced health-related quality of life in the future. Estimates of long-term survival rates (which may be reduced due to prior sepsis) and of the disutility associated with observed adverse events in the study will be sourced from peer-reviewed literature. Similarly, any costs associated with long-term disabilities will be identified and included within the decision-analysis model.

The mathematical model will allow an explicit evaluation of the uncertainty in any conclusions drawn from the trial and allow value of information analyses to be performed to indicate if further research would be deemed as value for money. At present the precise modelling methodology to be implemented has not been determined. This decision will be made in conjunction with clinical experts and having assessed the available data.

11. REGULATORY, ETHICAL AND LEGAL ISSUES

Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the 2013 revision of the 1964 Declaration of Helsinki.

Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

Research Ethics Committee (REC) Approval

Initial Approval

Prior to the shipment of the IMP and the enrolment of participants, the REC must provide written approval of the conduct of the study at named sites, the protocol and any amendments, the Participant Information Sheet and Consent Form, any other written information that will be provided to the participants, any advertisements that will be used and details of any participant compensation.

Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the REC for approval. Amendments requiring REC approval may be implemented only after a copy of the REC's approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to participants may be implemented prior to receiving Sponsor or REC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Amendments to the protocol will be decided by the Chief Investigator and the Protocol Development Group and will be submitted to the Imperial College London Research Governance and Integrity Team for review prior to submission. Whether the changes in the protocol are substantial or non-substantial will be guided by the amendment tool as provided by the HRA/REC. An updated version and date of the protocol will be documented in the title and footer of the document, the approval pack (containing the updated protocol) will also be sent to all participating sites.

Annual Progress Reports

Annual Progress Reports will be submitted to the Research Ethics Committee (REC) and the Sponsor in accordance with local / national requirements. The Annual Progress Report will also detail all SAEs recorded.

End of Trial Notification

The REC will be informed about the end of the trial, within the required timelines.

The end of trial notification will be submitted within 90 days of the end of trial definition being met. In the event of a premature halt of the trial, the timeframe is 15 days, and the reasons should be clearly explained in the notification.

Regulatory Authority Approval

The study will be performed in compliance with UK regulatory requirements. Clinical Trial Authorisation from the Medicines and Healthcare products Regulatory Authority (MHRA) must be obtained prior to the start of the study. In addition, the REC/MHRA must approve amendments prior to their implementation (as instructed by the Sponsor), receive SUSAR reports and annual safety updates, and be notified of the end of the trial.

This study has Clinical Trials Authorisation from the UK Competent Authority; MHRA. Reference: IRAS 1005848

HRA approval

Health Research Authority (HRA) approval will be obtained prior to starting the study. Each participating site will confirm capacity and capability prior to commencing.

The HRA and all participating sites also need to be notified of all protocol amendments to assess whether the amendment affects the institutional approval for each site.

Non-Compliance and Serious Breaches

All protocol deviations and protocol violations will be reported via the eCRF/CRF and reviewed by the Chief Investigator and reported to the ICTU Head of QA on a monthly basis. Protocol violations will be reported to the Sponsor.

An assessment of whether the protocol deviation/violation constitutes a serious breach will be made.

A serious breach is defined as:

A breach of the conditions and principles of GCP in connection with a trial or the trial protocol, which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the UK trial participants; or
- The overall scientific value of the trial

The Sponsor will be notified within 24 hours of identifying a likely Serious Breach. If a decision is made that the incident constitutes a Serious Breach, this will be reported to the MHRA and REC within 7 days of becoming aware of the serious breach.

Insurance and Indemnity and Sponsor

The Sponsor has civil liability insurance, which covers this study in all participating countries. Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Imperial College London will act as the main Sponsor for this trial. Delegated responsibilities will be assigned to the NHS trusts taking part in the trial.

Trial Registration

The study will be registered on a trial database in accordance with requirements of the International Committee of Medical Journal Editors (ICMJE) regulations. The study will be registered on the ISRCTN registry.

Informed Consent

If the patient has capacity, they will always be approached to provide their informed consent. If the patient does not have capacity, a family member or independent doctor will be approached. Due to the emergency nature of the trial, if the patient lacks capacity and a family member or independent doctor is not available, a deferred consent model will be adopted to ensure the treatment for sepsis can start as soon as possible and consent will be sought soon after.

If the patient lacks capacity then a family member / next of kin (NOK) will be approached to provide their consent on behalf of the patient, (known as personal legal representative consent). This may be after treatment in the trial has already started due to the emergency nature of sepsis management. If this is the case, consent from the patient or their personal legal representative (PerLR) will be sought as soon as possible. If the PerLR is not able to attend in person, e-consent using the OpenClinica system will be used. The PerLR identity will be verified via a video link or other means, in line with the methods used by the clinical team to update family member / NOK about the clinical management of the participant. If a family member/NOK is not available a doctor who is not part of the study (i.e. not on the trial research delegation log) will be approached to give their professional legal representative (ProLR) consent. This would usually be a senior treating clinician of the patient. Once the patient regains capacity, they will be approached to provide their retrospective consent to remain in the study.

We will use a 2-hour window as established in other emergency UK ICU-based trials (ISRCTN18035454) to guide the emergency consent process if the patient lacks capacity.

- If the patient lacks capacity and a family member / next of kin is in attendance or will arrive within 2 hours, then seek PerLR consent.

- If the patient lacks capacity and a family member / next of kin is not available within 2 hours, then seek ProLR consent.
- If the patient lacks capacity and neither a PerLR nor a ProLR are available within 2 hours, then the patient can be included without prior consent.

Participants will be provided with a copy of the signed Participant Information Sheet/Informed Consent Form document. The original Informed Consent Form will be retained with the source documents.

Contact with General Practitioner

Patient GPs will be informed of the patient's enrolment in the study as part of the usual NHS discharge procedure at site. The study will not mandate that the site teams are required to send a separate, additional letter to the patient's GP.

Participant Confidentiality

The investigator must ensure that the participant's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to participants' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and RECs.

Data Protection and Participant Confidentiality

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

End of Trial

This study will end when the specified number of patients have been recruited, all patients have completed their 12-month follow-up and the database is hard locked.

Study Documentation and Data Storage

The investigator must retain essential documents until notified by the Sponsor, and for at least ten years after study completion. Participant files and other source data (including copies of protocols, CRFs, original reports of test results, IMP dispensing logs, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be retained. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

12. DATA MANAGEMENT

Source Data

Source documents include original documents related to the trial, to medical treatment and to the history of the participant, and adequate source documentation must be maintained to allow reliable verification and validation of the trial data. What constitutes the source data for this trial will be outlined in the Source Data agreement.

Language

CRFs will be in English. Generic names for concomitant medications should be recorded in the CRF wherever possible. All written material to be used by participants must use vocabulary that is clearly understood, and be in the language appropriate for the study site.

Database

Trial data will be collected on an electronic case report form (eCRF). Data will be entered via web-based database through electronic data capture (EDC). The database used to capture this information is the OpenClinica database. Data is entered into the database by the site team. The database will raise automatic queries and allow manual queries to also be raised which will be checked and validated by the Trial Manager and Monitor. All data, changes to data and query resolution will be included in an audit trail including dates. Specific instructions on how to enter data including drug naming and deal with queries are detailed in the eCRF completion guide. Automated Randomisation will be carried out using the OpenClinica system in accordance with ICTU specific SOPs.

Adverse events will be captured in the eCRF and all Serious Adverse Events will require sign off by the Principal Investigator at the site.

Data Collection

All data for the study will be entered into the cCRF via the OpenClinica database. These data will include demographics, previous medical history, blood results, vital signs, organ support and follow-up information. Details of procedures for eCRF/CRF completion will be provided in a study manual.

Archiving

All trial documentation, including that held at participating sites and the trial coordinating centre, will be archived for a minimum of 10 years following the end of the study.

13. STUDY MANAGEMENT STRUCTURE

The day-to-day management of the trial will be co-ordinated through the Imperial Clinical Trials Unit and the Chief Investigator.

The following groups and trial committees will be established

Trial Steering Committee

A Trial Steering Committee (TSC) will be convened including as a minimum an independent Chair, independent clinicians, independent statistician and health economist, lay members, the Chief

Investigator, Senior Statistician and Trial Manager. The role of the TSC is to provide overall supervision of trial conduct and progress. Details of membership, responsibilities and frequency of meetings will be defined in a separate Charter.

Trial Management Group

A Trial Management Group (TMG) will be convened including the Chief Investigator, co-investigators and key collaborators, trial statistician, a lay person and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Details of membership, responsibilities and frequency of meetings will be defined in separate terms of Reference.

Data Monitoring Committee

An independent Data Monitoring Committee (DMC) will be convened including at least an independent Chair and two other independent members. It will include suitable experienced clinicians / clinical trialists and statisticians. The role of the DMC is advisory to the TSC and Sponsor. It will monitor unblinded data emerging in the trial. Details of membership, responsibilities and frequency of meetings will be defined in a separate Charter

Early Discontinuation of the Study

The internal pilot study as detailed above provides the only formal stopping rules for the trial. The DMC may recommend early stopping of the trial or any intervention if there is a safety issue.

If these instances arise, guidance will be provided to local sites about continuation of interventions and follow-up visits.

Risk Assessment

A study-specific risk assessment will be performed prior to the start of the study to assign a risk category of ‘low’, ‘medium’ or ‘high’ to the trial. Risk assessment will be carried out by the ICTU Head of QA in collaboration with the Study Manager and the result will be used to guide the monitoring plan. The risk assessment will consider all aspects of the study and will be updated as required during the course of the study.

Monitoring

The study will be monitored periodically by trial monitors to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy and consistency of the data.

A monitoring plan will be devised based on risk analysis and described in detail in the monitoring manual by the project manager. Initiation visits will be conducted for all sites prior to the recruitment of participants. These visits will be conducted either remotely or on site depending on availability of the site and study team.

The trial will involve a combination of central, remote and on-site monitoring. On site visits will be conducted by trained monitors during the recruitment phase of the trial and after the trial as required by the protocol and trial procedures according to the monitoring manual to ensure patient safety, accurate data collection and reporting. Central monitoring will be conducted regularly where data queries and protocol deviations are reviewed and any required further site training is conducted.

Remote monitoring will also be utilised with sites in between on-site visits, to enable the study team to complete knowledge checks and follow up with training for new site members.

Quality Control and Quality Assurance

Quality Control will be performed according to ICTU internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

The study may be participant to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

Peer review

This trial was externally peer reviewed as part of the NIHR HTA funding process.

Public Involvement

Patient and Public Involvement (PPI) has been integral to the development of this proposal. The initial choice of interventions was based on the James Lind Alliance Emergency Medicine Priority Setting Partnership. We sought feedback on the trial design, interventions, outcome measures, consent process from patients and relatives through the ICUsteps and UK Sepsis Trust charities.

Three lay representatives will sit on the Trial Steering Committee and will provide input from a patient perspective at trial meetings. The representatives will provide valuable insight in trial management, study procedures, as well as any amendments we make to the study in the future. They will also contribute to result interpretation, reporting and dissemination.

Publication and Dissemination policy

We will publish the main results, as separate manuscripts for each of the three interventions, in major international peer-reviewed journals. We will ensure all publications meet UKRI open access policies. The heath economic analyses will either be included with these reports or in separate, more detailed evaluations. All of these publications will be in addition to the final NIHR HTA journal report.

We will set up a trial website that will provide information about the trial, including the easy read style and animated clip to provide patients and relatives with information about the trial. We will provide regular updates about the trial on the website and a trial-specific Twitter account. Final results will be available on this website. We won't notify individual patients of the results of the trial.

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information for the purposes of the study only.

It is understood by the investigator that the Sponsor will use information developed in this clinical study in connection with the development of the IMP/device and, therefore, may disclose it as required to other clinical investigators and to Regulatory Authorities. In order to allow the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide complete test results and all data developed during this study to the Sponsor.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

Therefore all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed.

A Clinical Study Report summarising the study results will be prepared and submitted to the REC within a year of the end of study. The results will also be submitted to the EudraCT results database in accordance with regulatory requirements.

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15. REVISION HISTORY

Version	Date	Summary of changes
1.0	21/FEB/2023	First version

SIGNATURE PAGE 1 (CHIEF INVESTIGATOR)

The signature below constitutes approval of this protocol by the signatory, on behalf of the Protocol Development Group, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Sepsis Trials in Critical Care (SepTiC)

Protocol Number: 22SM8039

Signed: _____

Anthony Gordon

Professor

Date: _____

SIGNATURE PAGE 2 (SPONSOR)

The signatures below constitute approval of this protocol by the signatory.

Study Title: Sepsis Trials in Critical Care (SepTiC)

Protocol Number: 22SM8039

Signed: _____

Rinat Ezra

Dr

Imperial College London

Date: _____

SIGNATURE PAGE 3 (STATISTICIAN)

The signatures below constitute approval of this protocol by the signatory.

Study Title: Sepsis Trials in Critical Care (SepTiC)

Protocol Number: 22SM8039

Signed: _____

Victoria Cornelius
Dr
Imperial College London

Date: _____

Signed: _____

Ed Waddingham
Dr
Imperial College London

Date: _____

SIGNATURE PAGE 4 (PRINCIPAL INVESTIGATOR)

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Sepsis Trials in Critical Care (SepTiC)

Protocol Number: 22SM8039

Address of Institution: _____

_____

_____

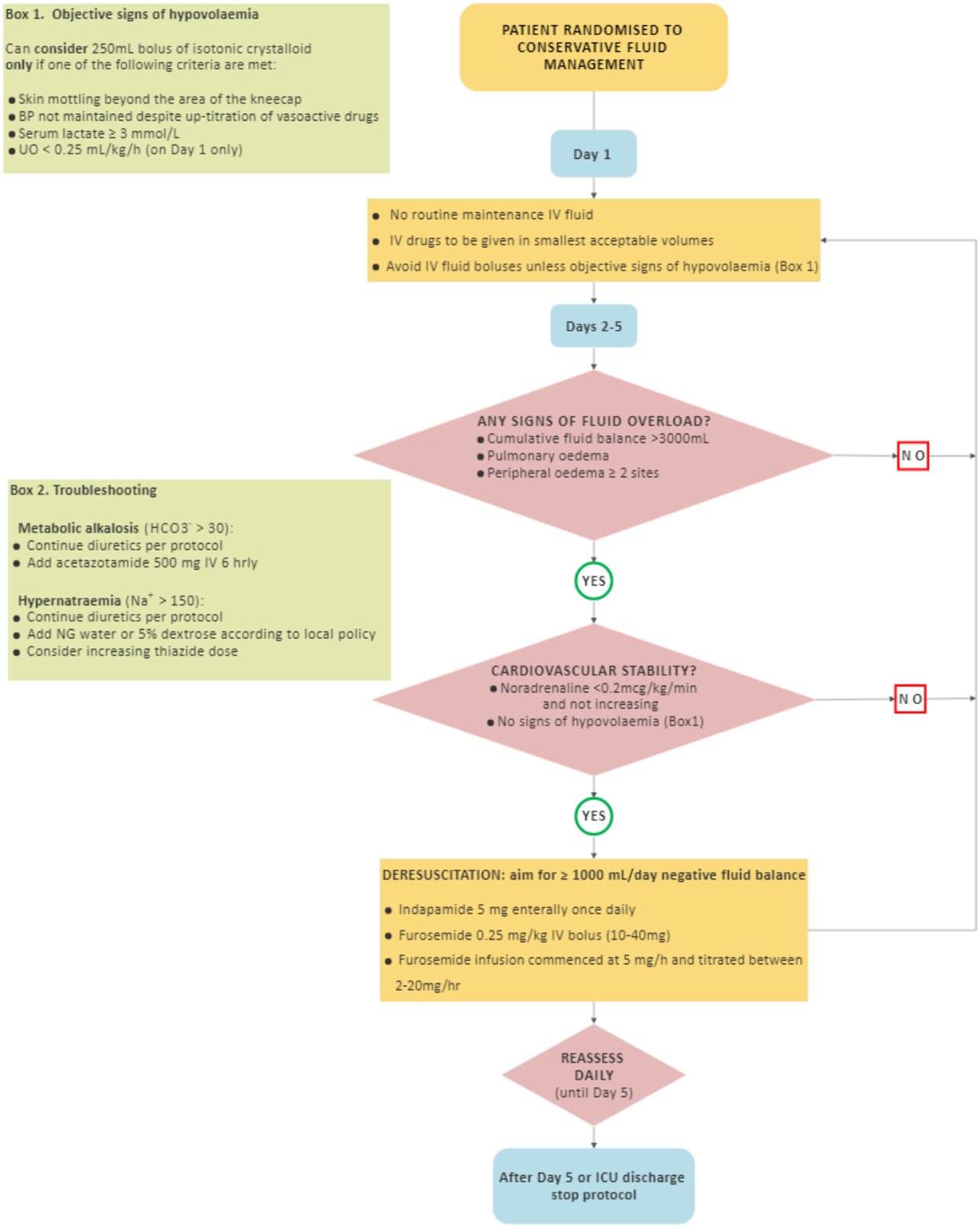
Signed: _____

Print Name and Title: _____

Date: _____

APPENDICES:

Appendix 1



Appendix 2: Estimand framework

SUPPLEMENTARY TABLE A: Primary and supplementary estimands for the diagnostics trial

Attribute	Primary estimand	Supplementary estimand for intercurrent events	Supplementary estimand for concomitant treatments
Population	Patients eligible for the diagnostics trial	Patients eligible for the diagnostics trial	Patients eligible for the diagnostics trial
Treatment condition(s)	Diagnostics intervention vs standard of care	Diagnostics intervention vs standard of care	**Diagnostics intervention plus fluids intervention vs fluids intervention alone**
Variable (outcome)	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days
Intercurrent events and the strategies used to handle them in the analysis	Intervention adherence and discontinuation – treatment policy[1]	Intervention adherence and discontinuation – treatment policy[1]	Intervention adherence and discontinuation – treatment policy[1]
	Blood culture results - treatment policy[1]	**Blood culture results - principal stratum[2] (participants with non-positive results)**	Blood culture results - treatment policy[1]
	Receiving other SEPTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[2]	Receiving other SEPTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[2]	Receiving other SEPTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[2]
	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]
	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]
Population-level summary measure	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.
	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.

Rationale for the estimand	**The aim of the trial is to assess whether the diagnostics intervention as given can improve on standard of care in the population of eligible patients.**	**If the trial does not demonstrate a positive result among the entire eligible population, it may still be of interest to assess the effect of the intervention in cases where current diagnostic practices are inconclusive.**	**If the fluids trial shows a positive result, then the fluids intervention may potentially be adopted as standard of care.**

[1] A treatment policy strategy considers the occurrence of the associated intercurrent event as irrelevant, and participant data are analysed regardless.

[2] A principal stratum strategy uses the intercurrent event as a means to target the population of interest in which to estimate the treatment effect eg the population that receive the intervention as intended

SUPPLEMENTARY TABLE B: Primary and supplementary estimands for the fluids trial

Attribute	Primary estimand	Supplementary estimand for concomitant treatments
Population	Patients eligible for the fluids trial	Patients eligible for the fluids trial
Treatment condition(s)	fluids intervention vs standard of care	**Fluids intervention plus diagnostics intervention vs diagnostics intervention alone**
Variable (outcome)	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days
Intercurrent events and the strategies used to handle them in the analysis	Intervention adherence and discontinuation – treatment policy[1]	Intervention adherence and discontinuation – treatment policy[1]
	Receiving other SEPTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[2]	Receiving other SEPTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[2]
	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]
	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]
Population-level summary measure	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.

	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.
Rationale for the estimand	**The aim of the trial is to assess whether the fluids intervention as given can improve on standard of care in the population of eligible patients.**	**If the diagnostics trial shows a positive result, then the diagnostics intervention may potentially be adopted as standard of care.**

[1] A treatment policy strategy considers the occurrence of the associated intercurrent event as irrelevant, and participant data are analysed regardless.

[2] A principal stratum strategy uses the intercurrent event as a means to target the population of interest in which to estimate the treatment effect eg the population that receive the intervention as intended

SUPPLEMENTARY TABLE C: Primary and supplementary estimands for the GM-CSF trial

Attribute	Primary estimand	Supplementary estimand for intercurrent events	Supplementary estimand for concomitant treatments
Population	Patients eligible for the GM-CSF trial	Patients eligible for the GM-CSF trial	Patients eligible for the GM-CSF trial
Treatment condition(s)	GM-CSF intervention vs standard of care	GM-CSF intervention vs standard of care	**Diagnostics intervention plus GM-CSF intervention vs diagnostics intervention alone;** **Fluids intervention plus GM-CSF intervention vs fluids intervention alone;** **All 3 interventions vs Diagnostics intervention plus fluids intervention**
Variable (outcome)	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days
Intercurrent events and the strategies used to handle them in the analysis	Intervention adherence and discontinuation – treatment policy[1]	**Intervention adherence and discontinuation – principal stratum[1] (participants receiving >=n doses – each value of n giving rise to a different estimand)**	Intervention adherence and discontinuation – treatment policy[1]
	Receiving other SepTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[1]	Receiving other SepTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[1]	Receiving other SepTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[1]
	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]

	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]
Population-level summary measure	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.
	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.
Rationale for the estimand	**The aim of the trial is to assess whether GM-CSF as given can improve on standard of care in the population of eligible patients.**	**If the trial does not demonstrate a positive result among the entire eligible population, it may still be of interest to assess the effect of the intervention in cases where treatment adherence is high.**	**If the diagnostic and/or fluids trials shows a positive result, then the fluids respective interventions may potentially be adopted as standard of care.**

[1] A treatment policy strategy considers the occurrence of the associated intercurrent event as irrelevant, and participant data are analysed regardless.

[2] A principal stratum strategy uses the intercurrent event as a means to target the population of interest in which to estimate the treatment effect eg the population that receive the intervention as intended

SUPPLEMENTARY TABLE D: Exploratory estimands

Attribute	Treatment combination estimand 1	Treatment combination estimand 2	Treatment combination estimand 3	Treatment combination estimand 4
Population	Patients eligible for the diagnostics and fluids trials	Patients eligible for the GM-CSF trial	Patients eligible for the GM-CSF trial	Patients eligible for the GM-CSF trial
Treatment condition(s)	**Diagnostics intervention plus fluids intervention vs standard of care**	**Diagnostics intervention plus GM-CSF intervention vs standard of care**	**Fluids intervention plus GM-CSF intervention vs standard of care**	**Diagnostics intervention plus fluids intervention plus GM-CSF intervention vs standard of care**
Variable (outcome)	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days	Mortality, in-ICU with organ support, in-hospital without organ support, discharged over 90 days
Intercurrent events and the strategies used to handle them in the analysis	Intervention adherence and discontinuation – treatment policy[1]	Intervention adherence and discontinuation – treatment policy[1]	Intervention adherence and discontinuation – treatment policy[1]	Intervention adherence and discontinuation – treatment policy[1]
	Blood culture results - treatment policy[1]	Blood culture results - treatment policy[1]	Blood culture results - treatment policy[1]	Blood culture results - treatment policy[1]
	Receiving other SEPTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[1]	Receiving other SepTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[1]	Receiving other SepTIC trial interventions – treatment policy[1], or (for sensitivity analysis) principal stratum[1]	Receiving other SepTIC trial interventions – treatment policy[1],

				or (for sensitivity analysis) principal stratum[1]
	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]	Use of other available treatments and medicines – treatment policy[1]
Population-level summary measure	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]	Co-enrolment in other clinical trials - treatment policy[1]
	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.	Odds Ratio from a proportional odds model across the 4 clinical states conditional on age and minimisation variables.
Rationale for the estimand	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.	Posterior Probability of being in a better clinical state using a Bayesian Proportional Odds model with vague priors.

[1] A treatment policy strategy considers the occurrence of the associated intercurrent event as irrelevant, and participant data are analysed regardless.

[2] A principal stratum strategy uses the intercurrent event as a means to target the population of interest in which to estimate the treatment effect eg the population that receive the intervention as intended