Does Extra-Corporeal Membrane Oxygenation alter Anti-infectives Therapy PharmacoKinetics in adult critically ill patients?

Short Study Title/Acronym: EAT-PK
IRAS number: 228196

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Signature Page

The Chief Investigator (CI) and the Research Office have discussed and agreed this study protocol. The investigators agree to perform the investigations outlined in this study protocol and to abide by this protocol except in the case of medical emergency that will be notified to the Research Office.

The Investigator agrees to conduct the trial in compliance with the study protocol and/or any subsequent amendments approved by the main REC and the Research Office, the Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the Research Governance Framework for Health & Social Care, 2nd Edition (2005), the Sponsor's SOPs, and any other applicable regulatory requirements.

This protocol has been written in accordance with the Sponsor's guidance for writing non-CTIMP protocols.

Chief Investigator (CI)		
Dr Anna Reed Consultant in Respiratory and Transplant Medicine		
Royal Brompton and Harefield NHS Foundation Trust (RB&HFT)	Signature	Date
Sponsor Representative		
Dr Jenny Rivers Associate Director of Research		
Royal Brompton and Harefield NHS Foundation Trust (RB&HFT)	Signature	Date

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1. LIST OF ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CI	Chief Investigator
CRF	Case Report Form
DMC	Data Monitoring Committee
EXACT	Exacerbations of Chronic Pulmonary Disease Tool
GAfREC	Governance Arrangements for NHS Research Ethics
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICF	Informed Consent Form
ISF	Investigator Site File
ISRCTN	International Standard Randomised
MUST	Malnutrition Universal Screening Tool
NHS R&D	National Health Service Research & Development
NIMP	Non- Investigational Medicinal Product
PerLR	Personal Legal Representative
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

2. STUDY PERSONNEL AND FACILITIES

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3. STUDY SYNOPSIS

Full study title:	Does Extra-Corporeal Membrane Oxygenation alter anti-infectives therapy pharmacokinetics in adult critically ill patients?
Short study title:	EAT-PK
Study drug (s) (non-IMPs):	Anti-infectives – including voriconazole, posaconazole and caspofungin
Chief Investigator:	Dr Anna Reed
Medical condition/disease under investigation:	Extracorporeal Membrane Oxygentation (ECMO)
Study duration:	3 years
Clinical phase:	Observational study
Primary Objective:	To study whether ECMO alters the PK of anti-infectives including voriconazole, posaconazole and caspofungin in critically ill patients on ECMO
Secondary Objectives:	▪ Develop Population PK models of anti-infectives, including voriconazole, posaconazole and caspofungin in critically ill patients on ECMO ▪ Develop Physiological-Based PK (PBPK) model of anti-infectives, including: voriconazole, posaconazole and caspofungin in critically ill patients on ECMO
Study population:	Critically ill patients on ECMO
Methodology:	Observational study to determine whether ECMO alters the PK of anti-infectives, by developing PK models.
Eligibility criteria:	***Inclusion criteria:*** 1. Age >18 years and <90 years 2. Currently undergoing ECMO for respiratory +/- cardiac dysfunction 3. Clinical indication for the anti-infectives, including voriconazole, posaconazole and caspofungin
	Exclusion criteria: 1. History of allergy to any of the study drug 2. Pregnant women
Study intervention: *(i.e. dose and mode of the study drug administration if applicable)***:**	
This is a non-interventional descriptive study in that the anti-infective drug selection and dosing will be at the discretion of the clinician, based on the clinical context and unit guidelines. Doses will be reconstituted and administered as per local hospital protocols in line with patient's routine care. Patients will be asked to provide additional blood samples over the course of the anti-infective dosing schedule, these samples will be taken from existing arterial lines to help guide treatment in future patients on ECMO receiving these anti-infectives.	

4. INTRODUCTION

4.1 BACKGROUND

Extracorporeal membrane oxygenation (ECMO) is an advanced life support system which allows for prolonged cardiopulmonary support in patients with life-threatening respiratory or cardiac failure (1, 2). It can be used as a bridge to either lung or heart transplantation, or to a long-term ventricular assist device. There are 2 types of ECMO – Venoarterial (VA) or Venovenous (VV) as shown in figure 1(3); in both the blood is drained from the venous system and oxygenated outside the body. In VV ECMO the blood is returned to the venous system; this is driven by the patients' own heart function, hence maintaining pulsatile blood flow, and provides respiratory support only. While in VA ECMO the blood is returned to the arterial system bypassing both the heart and the lungs so it can be used for supporting both respiratory and cardiac failure.

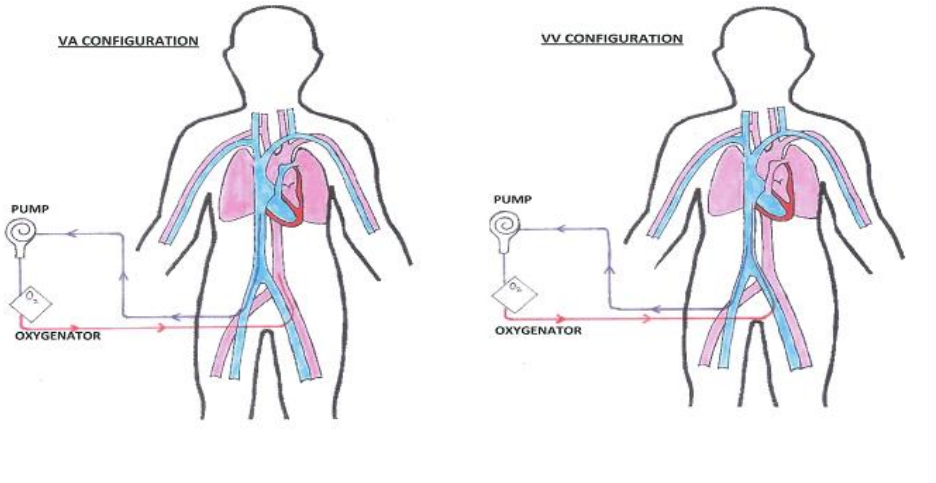


Figure 1. Schematic representation of ECMO.

In both modalities, blood is drained from the venous system (blue). In VA ECMO it is returned (red) to the arterial system, and in VVECMO it is returned to the venous system. The direction of blood flow in the ECMO circuit is indicated by arrows. (Submitted for publication Shekar *et al*).

Critically ill patients will often have a number of organ dysfunctions requiring support including mechanical ventilation and renal replacement therapy. These therapies together with the patients' underlying pathophysiological conditions are known to affect the pharmacokinetics (PK) of many drugs, such as the volume of distribution (Vd) and clearance (Cl) (3, 4). Where Vd refers to the volume of plasma in which the total amount of drug in the body would be required to be dissolved in order to reflect the drug concentration attained in the plasma; and Cl is the volume of plasma cleared of drug per unit time by the processes of metabolism and excretion.

Essentially, ECMO may alter the PK in a number of ways which include sequestration of the drug by the ECMO circuit (5, 6), increasing the Vd, alteration in renal and liver blood flow, altered plasma protein binding and decreased drug elimination. The impact of these PK changes can lead to either therapeutic failure or toxicity. The physicochemical properties of the drug will determine to what extent its PK is altered; in that those lipophilic drugs are

significantly sequestered in the circuit while hydrophilic drugs are significantly affected by hemodilution. Hence, the ECMO system itself may induce PK changes in a critically ill patient who already exhibits altered PK; the independent effects of ECMO add to the complexities in this group and are difficult to quantify.
Common mechanisms by which ECMO may alter PK include increased volume of distribution (Vd), drug adsorption in circuits and decreased drug elimination. Factors affecting PK on ECMO are summarised in Fig 2. Renal replacement whilst on ECMO adds increasing complexity to the PK of drugs because the presence of two extracorporeal circuits can make the estimation of PK parameters more difficult.

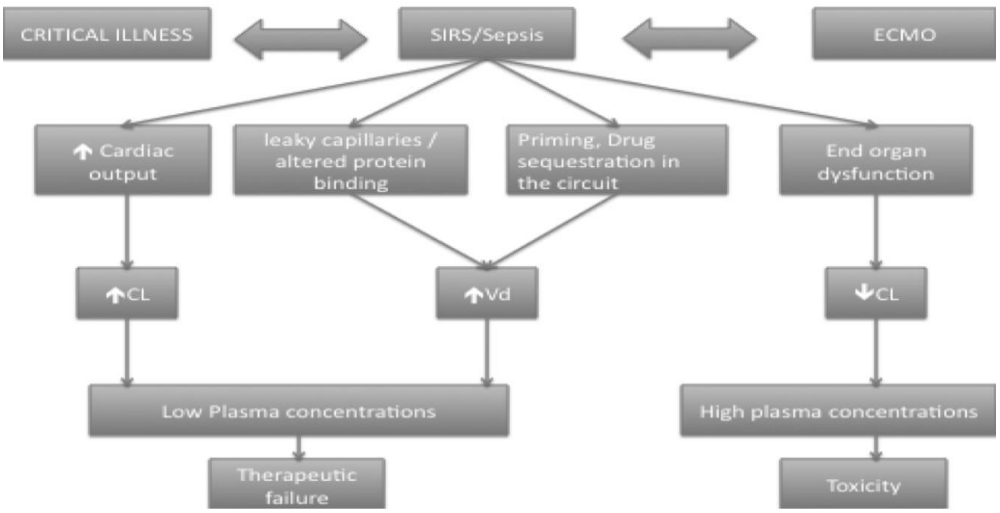


Figure 2. Impact of Critical illness, inflammation, ECMO on drug pharmacokinetics (submitted for publication Shekar *et al*). Vd volume of distribution), CL (clearance), SIRS (Systemic Inflammatory Response Syndrome).

4.2 PRE-CLINICAL DATA/CLINICAL DATA

There are a number of PK studies in patients with ECMO; the majority were performed in neonates and showed significant changes in the PK of antibiotic, sedative and analgesic drugs. These results cannot be extrapolated to adults as the physiologic processes that affect absorption, distribution, metabolism and excretion have not fully developed and are thus different to those in adults.
While the use of diuretics, anticoagulation, sedative, inotrope and vasopressor agents can be titrated to a measurable pharmacodynamic (PD) end-point, the use of anti-infectives cannot. Hence the application of PK principles in both selecting the appropriate anti-infective and its dosage regimen is important in optimizing outcome. Changes in antibiotic PK can lead to either therapeutic failure or toxicity in the patient; however, the potential emergence of resistant bacteria or fungi has wider implications.
There are a few case studies or small studies in adult patients with ECMO which have investigated vancomycin (7), oseltamivir (8-10), voriconazole (11, 12) and caspofungin(11, 12); the conclusions are preliminary and in the case of vancomycin and caspofungin they conclude with a recommendation of tailored dosing using therapeutic drug monitoring. However, not all of these drugs have their blood levels routinely measured *e.g.* caspofungin.
An ex-vivo study investigated the influence of plasma protein binding on sequestration in the

ECMO circuit and concluded that for drugs with similar lipophilicity, the extent of protein binding may determine the degree of circuit loss (13). It would be anticipated that both caspofungin and posaconazole would be prone to sequestration as they are both highly lipophilic and have a high degree of protein binding.
Currently there are no guidelines for dosing of drugs in patients with ECMO. The use of standard dosing in these critically ill patients can be sub-optimal, and ideally dosing should be individualized through the use of therapeutic drug monitoring.
There is a multi-centre, open label, descriptive PK study currently recruiting – ASAP ECMO: Antibiotic, Sedative and Analgesic Pharmacokinetics during Extracorporeal Membrane Oxygenation(14). This research will add to the evidence base for dosing in this group of patients.
Anti-infectives are commonly administered to critically ill patients on the intensive care unit, including those with ECMO. The antifungals: voriconazole, posaconazole and caspofungin are within the first- and second-line treatment guidelines at the Royal Brompton & Harefield NHS Trust (RB&HFT). Hence these drugs will be studied to determine whether the ECMO treatment alters their PK, which could lead to robust dosing guidelines in this group of patients.

Our proposed study will provide critical PK data to guide clinicians in administering "the right dose of the right drug, at the right time" during ECMO.

HYPOTHESES TO BE INVESTIGATED

ECMO significantly alters the PK of anti-infectives, which include posaconazole, voriconazole and caspofungin, thereby contributing to an elevated risk of therapeutic failure, drug toxicity and emergence of microbial resistance in critically ill adult patients on ECMO.

4.2.1 Results from our departmental ex-vivo experiments:

ECMO circuits were primed with whole human blood, sodium chloride 0.9% and Human Albumin Solution 20% with a final volume of approximately 700-750ml; then the investigated drug was added. In the case of caspofungin, 3.75mg was added to achieve 5mg/L concentration; posaconazole 3mg was added to achieve a concentration of approximately 4mg/L and for voriconazole 4mg was added to achieve 6mg/L concentration.

The ECMO circuits were maintained at a flow-rate of 4-5L/min, at physiological pH and temperature for 24 hours. Control samples (2ml) were withdrawn and stored in PET vacutainer tubes in a water bath at 37^0C. Serial samples (2ml) were collected at baseline, 30, 60, 120 and 360mins and at 12 and 24 hours; the concentrations of voriconazole and posaconazole were quantified using HPLC-mass spectrometry and caspofungin using a HPLC assay with fluorescence detection. The experiments were repeated four times for each caspofungin and posaconazole and twice for voriconazole and mean drug concentration vs time graph plots were drawn (figs 5.1.2a and 5.1.2b)

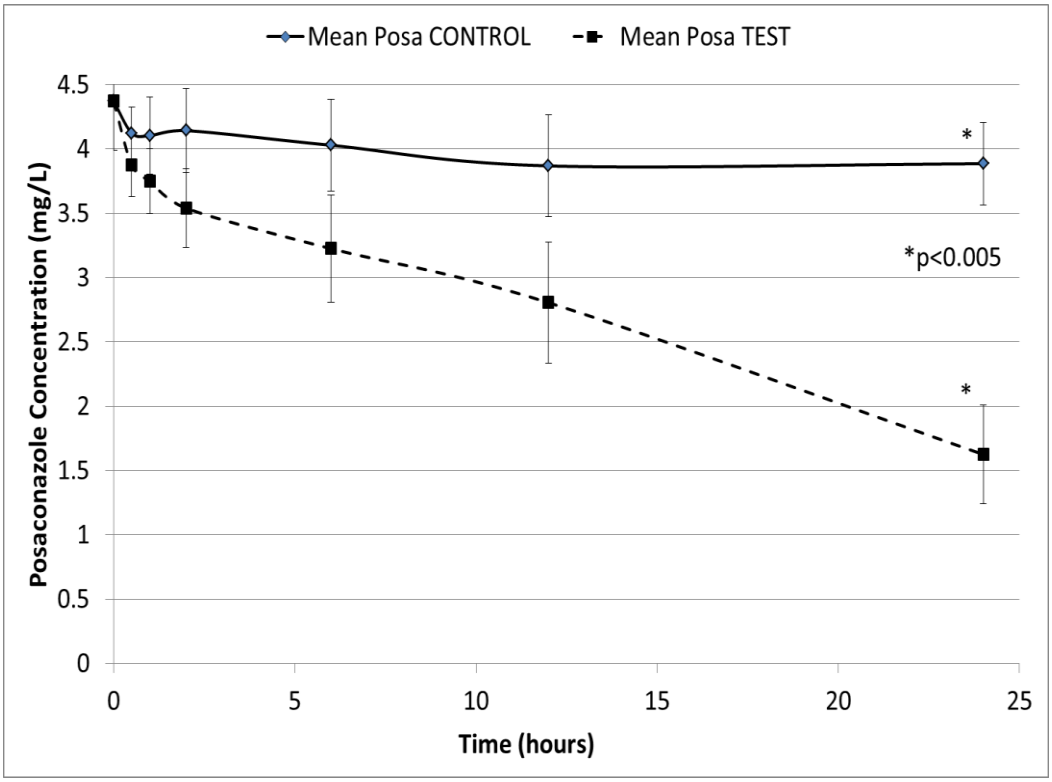


Figure 3. shows that there is a mean loss of 63% in posaconazole concentration in the ex-vivo ECMO model compared with 11% in the controlled samples over 24 hours ($p<0.005$).

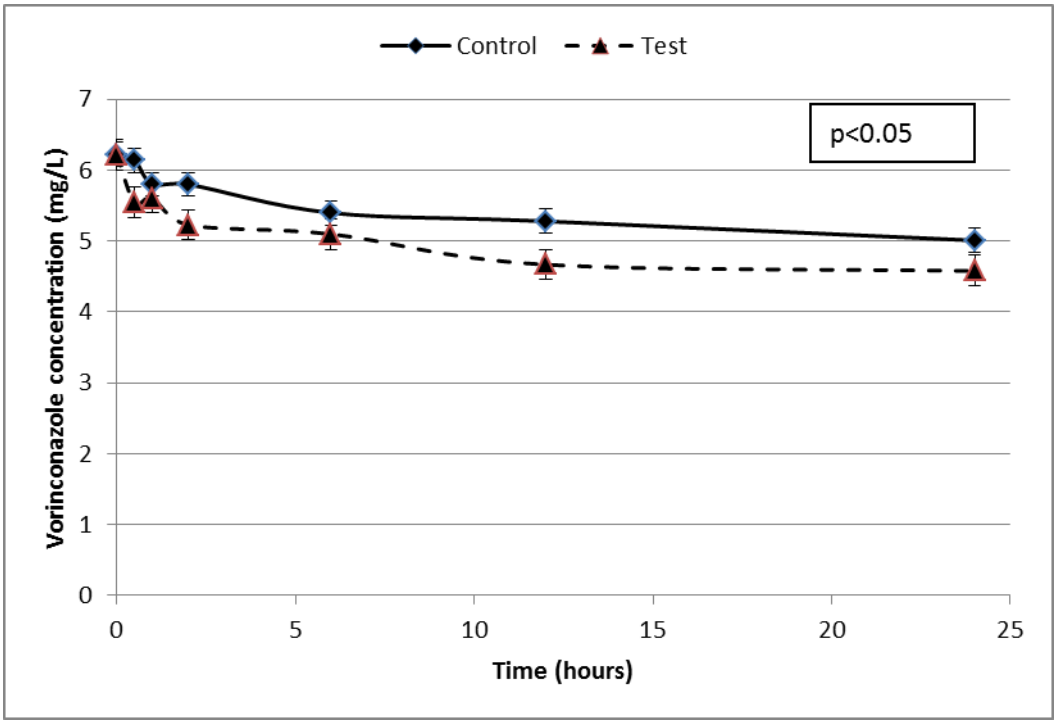


Figure 4. Mean voriconazole concentration vs time plot over 24 hours in blood-primed ECMO circuit showed a mean loss of 27% in the ex-vivo ECMO model compared with 19.2% in the controlled sample ($p<0.05$)

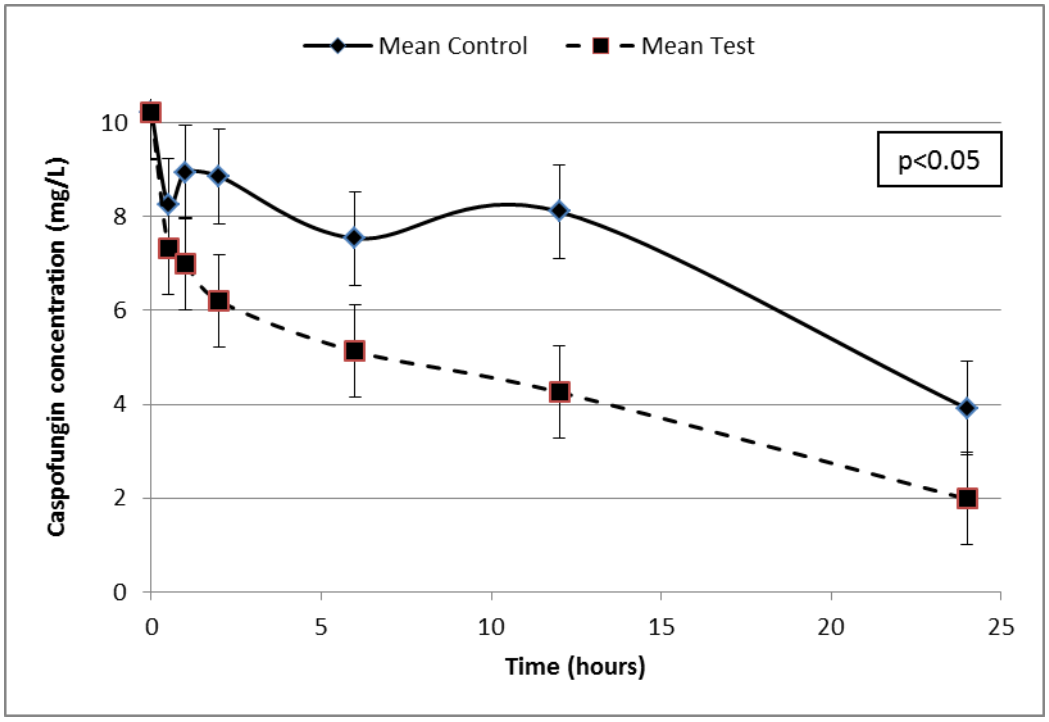


Figure 5. Mean caspofungin concentration vs time plot over 24 hours in blood-primed ECMO circuit shows a mean loss in concentration of 80% in the ex-vivo ECMO model compared with 61% in the controlled samples over 24 hours (p=ns). Mean AUC 40.9±11.9 in blood-primed ECMO circuit compared with 48.03±14.4 in the control (p=0.024)

4.3 STUDY RATIONALE AND RISK/BENEFIT ANALYSIS

This is a non-interventional descriptive study in that the anti-infective drug selection and dosing will be at the discretion of the clinician, based on the clinical context and unit guidelines. Doses will be reconstituted and administered as per local hospital protocols in line with patient's routine care. Patients will be asked to provide additional blood samples over the course of the anti-infective dosing schedule, these samples will be taken from existing arterial lines.

The main benefit of this study will be to help guide treatment in future patients on ECMO receiving these anti-infectives.

4.4 MANAGEMENT OF POTENTIAL STUDY RISKS

As this is an observational study, the burdens and risks to patients will be minimal. All samples will be collected from patients while they are recovering on a ventilator in the ICU. As such, it is routine clinical practice for most patients to remain sedated for a variable period of time.

Nearly all blood samples on the ICU are taken from in-dwelling vascular cannula (such as central venous catheters or arterial lines), so patients will not experience any additional pain or discomfort during blood sampling. Bloods will be taken by experienced staff members, and will probably go unnoticed by the patient. Patients on the ICU will routinely have a

urinary catheter inserted to monitor urine output. Urine samples will be collected from a bag that drains the bladder *via* the urinary catheter.

5. STUDY OBJECTIVES

5.1 PRIMARY OBJECTIVE

- Do adult patients with extracorporeal membrane oxygenation (ECMO) have altered pharmacokinetic profiles to commonly used anti-infectives, including caspofungin, posaconazole and voriconazole?

5.2 SECONDARY OBJECTIVES

a) Develop a physiologic based pharmacokinetic (PBPK) model for adult patients with ECMO
b) Develop a population PK model for voriconazole in adult patients with ECMO
c) Develop a population PK model for posaconazole in adult patients with ECMO
d) Develop a population PK model for caspofungin in adult patients with ECMO

6. TRIAL DESIGN

6.1 OVERALL DESIGN

Anti-infective drug selection and dosing will be at the discretion of the clinician, based on the clinical context and unit guidelines. Doses will be reconstituted and administered as per local hospital protocols and based on routine standard care.

Blood samples will be drawn from an existing arterial line and collected in 3 ml tubes with Lithium Heparin anticoagulant.

All patients will be sampled over a single dosing period on the first or second day of ECMO treatment, or of an antibiotic course where antibiotics are commenced whilst the patient is on ECMO. Where possible, sampling during one extra dosing interval will occur 4-8 later while on ECMO treatment and/or prior to the next tubing change. Where two or more anti-infectives of interest are prescribed for one patient, collect data on timing of administration for both drugs and sample according to the antibiotic with the longer dosing interval.

Blood samples (2ml) will be collected from an existing arterial line at the following time points 0 (pre-starting infusion, pre-NG/PO dose), 1 (end of infusion or 1hour post NG/PO dose), 2, 3, 4, 8 and either 12 (for twice daily regimen) or 24 (for once daily regimen) hours over the dosage interval.

Where a patient is receiving medications where a validated drug assay exists in addition to the study drug (such as other anti-infectives), analysis of the additional therapy will also be attempted where practical.

6.1.1 Data collection

For each patient various de-identified clinical and demographic data will be collected using the study Case Report Form (CRF):

- Patient demographics- Age, gender, weight, height
- Clinical details - Admission diagnosis, allergies
- S.Bilurubin, S.creatinine on day of study
- Disease Severity Scores – Sequential Organ Failure Assessment (SOFA) score on days 1, 3 and 5 & Acute Physiology and Chronic Health Evaluation II (APACHE II) on admission will be recorded
- Average daily flows on ECMO
- Type of oxygenator and pump head
- Use of Renal replacement therapy
- Doses and route of administered anti-infective agent
- Fluid balance, blood product requirements will be recorded on a daily basis.

6.1.2 Pharmacokinetic Sample Analysis

Plasma samples will be analysed using validated methodology at the Royal Brompton & Harefield NHS Foundation Trust (RB&HFT), Harefield Hospital Immunology Laboratory. Both total and unbound antibiotic concentrations will be determined in plasma. Where a patient is receiving antibiotic or antifungal therapy additional to the study drug, analysis of the additional therapy will also be attempted where practical.

6.1.3 Data analysis

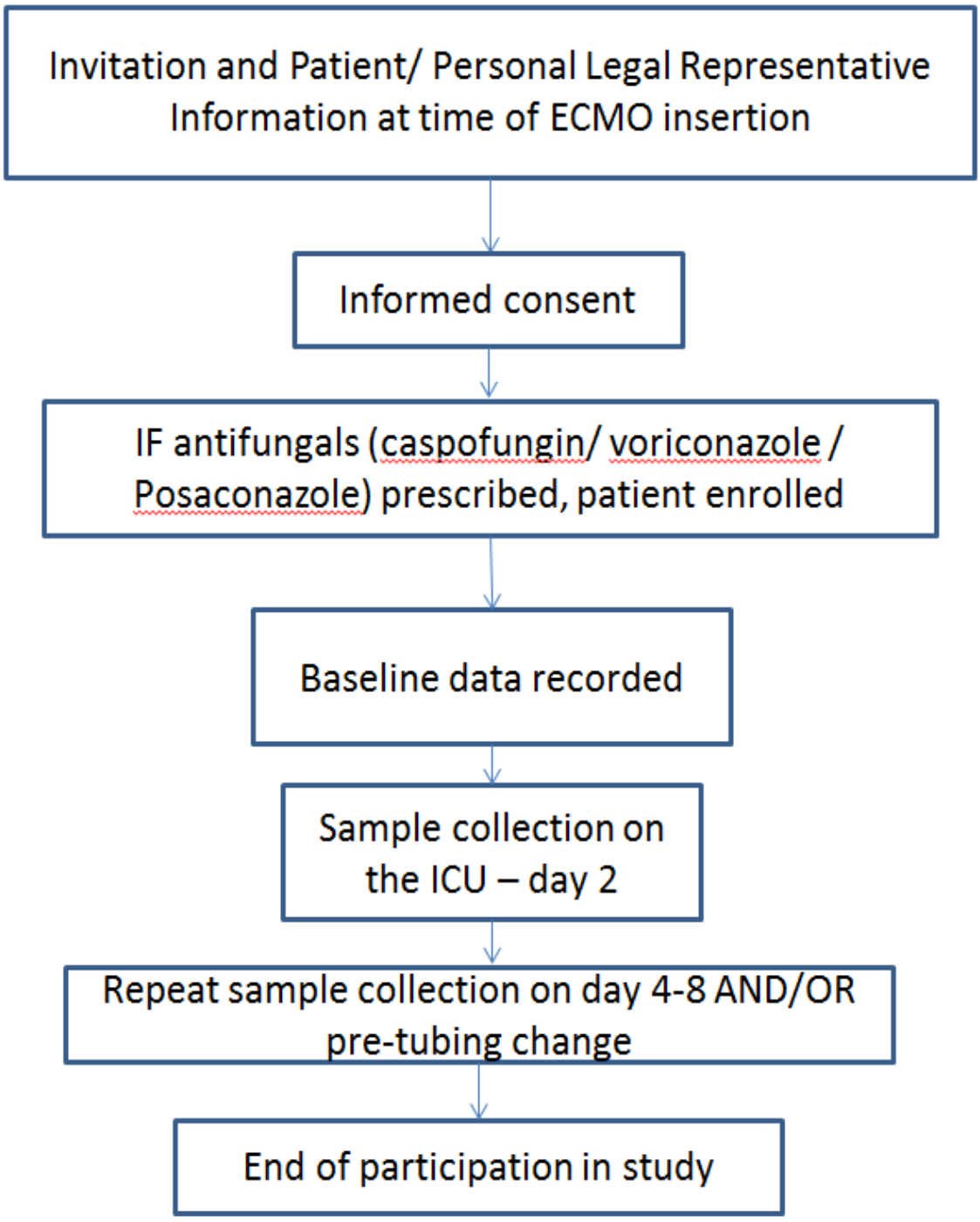
Population PK model: Sample analysis results will be interpreted with the pharmacokinetic computer software NONMEM to develop a population pharmacokinetic model. The model will aim to determine if correlations exist between clinical interventions (*e.g.* ECMO settings) demographic and clinical factors and drug pharmacokinetics. If one or more of the variables are found to have a significant effect on the pharmacokinetics of the drug, then it can be incorporated into the final pharmacokinetic model. The final model can then be used to undertake Monte Carlo simulations of different drug doses to determine optimal dosing regimens for critically ill patients receiving ECMO.

PBPK model: Sample analysis results will be interpreted with the pharmacokinetic computer software SimCyp (or PK-Sim or Berkley Madonna) to develop a PBPK model.

6.2 TREATMENT AND RATIONALE

- Patients will have a clinical indication for the anti-infectives: voriconazole, posaconazole and caspofungin. Anti-infective drug selection and dosing will be at the discretion of the clinician, based on clinical context and unit guidelines.
- Our objective is to define the interplay between critical illness and the extracorporeal circuits (ECMO) that result in altered drug PK during ECMO.

6.3 SCHEMATIC OF TRIAL DESIGN


Invitation and Patient/ Personal Legal Representative Information at time of ECMO insertion
Informed consent
IF antifungals (caspofungin/ voriconazole / Posaconazole) prescribed, patient enrolled
Baseline data recorded
Sample collection on the ICU – day 2
Repeat sample collection on day 4-8 AND/OR pre-tubing change
End of participation in study

7. ELIGIBILITY CRITERIA

7.1 INCLUSION CRITERIA

- Age >18 years and <90 years
- Currently undergoing ECMO for respiratory +/- cardiac dysfunction
- Clinical indication for the anti-infectives: voriconazole, posaconazole and caspofungin Anti-infective drug selection and dosing will be at the discretion of the clinician, based on clinical context and unit guidelines.

7.2 EXCLUSION CRITERIA

- History of allergy to any of the study drug
- Patients under the age of 18
- Pregnant women

7.3 DISCONTINUATION/WITHDRAWAL OF PARTICIPANTS AND STOPPING RULES

Should participants wish to withdraw from the trial, data collected to date will be kept and may be used for research purposes with the patient's agreement. All reasons for voluntary withdrawal from the study will be documented.

8. SUBJECT/PATIENT RECRUITMENT PROCESS

Patient recruitment at a site will only commence once the trial team has ensured that the following approval/essential documents are in place:

1. The main REC and the Health Research Authority (HRA) approval,
2. Final sponsorship
3. Confirmation of Capacity and Capability (C&C),
4. Local Site Delegation of Duties and Signature Log and the Investigator Site File (ISF) are set up by the Research Team.

All sites participating in the trial will also be asked to provide a copy of the following:

1. Signed Clinical Trial Site Agreement (CTSA) (if applicable),
2. Confirmation of Capacity and Capability.

9. STUDY PROCEDURES

9.1 INFORMED CONSENT

Critically ill patients admitted to the Intensive Care Units (ICU) at The Royal Brompton & Harefield NHS Foundation Trust who have a clinical indication for ECMO will be eligible for enrolment. Patients of interest will be receiving a study anti-infective for a clinical indication. A total of 12-15 patients will be enrolled for each study drug for this descriptive study.

Informed consent will be obtained by the Chief Investigator (CI), Principal Investigator (PI) and/or a nominated deputy as recorded on Sponsor's Delegation of Responsibilities Log. Only those members of the study team who have clinical responsibility for the care of patients under the care of the general medical service will be permitted to undertake informed consent. All individuals taking informed consent will have received training in Good Clinical Practice (GCP).

Consent to enter this study will be obtained after a full account has been provided of its nature, purpose, risks, burdens and potential benefits, and the patient has had the opportunity to deliberate. The patient will be allowed to specify the time they wish to spend deliberating, usually up to 24 hours.

Periods shorter than 24 hours will be permitted if the patient feels that further deliberation will not lead to a change in their decision, and provided the person seeking consent is satisfied that the patient has fully retained, understood and deliberated on the information given. This provision has been made with the support of our patient advisory group.
Likewise, periods longer than 24 hours will be permitted should the patient request this. The Investigator or designee will explain that the patients are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

A copy of the signed Informed Consent Form (ICF) along with a copy of the most recent approved Patient Information Sheet (PIS) will be given to the study participant. The original signed consent form will be retained at the study site (one filed in the medical notes and one field in the Investigator Site File (ISF). A copy of the consent form will also be given to the patient.

If new safety information results in significant changes to the risk–benefit assessment, the consent form will be reviewed and updated if necessary. All subjects, including those already being treated, will be informed of the new information, given a copy of the revised consent form and asked to re-consent if they choose to continue in the study.

Some patients will be sedated to facilitate mechanical ventilation and some patients will lack capacity due to the severity of their critical illness despite being awake on ECMO. It may not be possible to obtain prospective consent from the patient at the time of enrolment. As all the study drugs are already routinely used in the management of infection there is minimal extra risk from participation in this study.

9.1.1 Personal Legal Representative Consent

If the patient is unable to give consent, informed consent will be sought from the patient's 'Personal Legal Representative' (PerLR) who may be a relative, partner or close friend. The PerLR will be informed about the trial by the responsible clinician or a member of the research team and provided with a copy of the Personal Legal Representative Information Sheet and asked to give an opinion as to whether the patient would object to taking part in such medical research. The PerLR will be approached following 24 hours of screening, and will be given a further period of time to consider the patient's participation in the study. If

the PerLR decides that the patient would have no objection to participating in the trial they will be asked to sign the PerLR Consent Form which will then be counter signed by the responsible member of the research team. The PerLR will retain a copy of the signed Consent Form. A second copy will be placed in the patients' medical records whilst the original will be retained in the Investigator Site File (ISF).

9.1.2 Professional Legal Representative Consent

If the patient is unable to give informed consent, and attempts to meet and discuss with a PerLR have failed, then a doctor who is not connected with the conduct of the trial may act as a Professional Legal Representative (ProLR). The doctor will be informed about the trial by a member of the research team and given a copy of the Covering Statement for Professional Legal Representative. If the doctor decides that the patient is suitable for entry into the trial, then they will be asked to sign the ProLR Consent form. The ProLR will retain a copy of the signed Consent Form. A second copy will be placed in the patients' medical records whilst the original will be retained in the Investigator Site File (ISF).

Subsequently, if a relative, partner or close friend visits the patient before he or she has regained consciousness, then they should be informed about the patient's participation and also informed about the retrospective consent process.

9.1.3 Retrospective Patient Information

If and when the patient recovers and they regain the capacity to understand the details of the trial, a member of the research team will inform them of their participation in the trial. The patient will be given a copy of the Patient Information Sheet (PIS) to keep. The patient will be asked for consent to continue participation in the trial and to sign the Retrospective Consent Form. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient's medical records whilst the original will be retained in the Investigator Site File. If the patient does not want to continue participation in the study they will be given the choice of having the already collected data and samples excluded from the final analysis.

The right of the participant or their PerLR to refuse to participate without giving reasons must be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

9.1.4 General Practitioner (GP)

We will not be informing the patient's GP of their involvement in the trial because their involvement does not have any impact on the treatment that they will have received within the ICU or their future continued routine clinical treatment when participation in the trial has ended.

9.2 RANDOMISATION PROCEDURE

Not applicable

9.3 EMERGENCY UNBLINDING

Not applicable

10. STUDY ASSESSMENTS

10.1 SCREENING ASSESSMENTS

Critically ill patients admitted to the Intensive Care Units (ICU) at The Royal Brompton & Harefield NHS Foundation Trust, Royal Brompton Hospital and Harefield Hospital (2 sites), who have a clinical indication for ECMO will be screened for eligibility for enrolment. Patients of interest for enrolment will be receiving a study anti-infective for a clinical indication.

10.2 BASELINE ASSESSMENTS

For each patient various de-identified clinical and demographic data will be collected:

- Patient demographics- Age, gender, weight, height
- Clinical details- Admission diagnosis, allergies
- Serum Bilurubin, Serum Creatinine on day of study
- Disease Severity Scores – Sequential Organ Failure Assessment (SOFA) score on days 1, 3 and 5 & Acute Physiology and Chronic Health Evaluation II (APACHE II) on admission will be recorded
- Average daily flows on ECMO
- Type of oxygenator and pump head
- Use of Renal replacement therapy
- Doses and route of administered anti-infective agent
- Fluid balance, blood product requirements will be recorded on a daily basis.

10.3 STUDY PROCEDURES

There are no study drug treatments to be used as the patients of interest will be receiving a study anti-infective for a clinical indication.

Anti-infective drug selection and dosing will be at the discretion of the clinician, based on the clinical context and unit guidelines. Doses will be reconstituted and administered as per local hospital protocols and based on routine standard care.

Blood samples will be drawn from an existing arterial line and collected in 3 ml tubes with Lithium Heparin anticoagulant.

All patients will be sampled over a single dosing period on the first or second day of ECMO treatment, or of an antibiotic course where antibiotics are commenced whilst the patient is on ECMO. Where possible, sampling during one extra dosing interval will occur 4-8 days later while on ECMO treatment and/or prior to the next tubing change. Where two or more anti-infectives of interest are prescribed for one patient, collect data on timing of administration for both drugs and sample according to the antibiotic with the longer dosing interval.

Blood samples (2ml) will be collected from an existing arterial line at the following time points 0 (pre-starting infusion), 1 (end of infusion or 1 hour post-nasogastric administration), 2, 3, 4, 8 and either 12 (for twice daily regimen) or 24 (for once daily regimen) hours over the dosing interval.

10.4 SUBSEQUENT ASSESSMENTS

10.4.1 Pharmacokinetic Sample Analysis

Plasma samples will be analysed using validated methodology at the Royal Brompton & Harefield NHS Foundation Trust Immunology laboratory. Both total and unbound antibiotic concentrations will be determined in plasma. Where a patient is receiving medications where a validated drug assay exists in addition to the study drug (such as other anti-infectives), analysis of the additional therapy will also be attempted where practical.

10.6 SUMMARY CHART OF STUDY ASSESSMENTS

Please see the schematic table in Appendix 1.

11. METHODS

11.1 LABORATORY PROCEDURES

Blood will be taken for measurement of drug concentrations. The blood will be spun immediately and stored at -80°C for subsequent assays.

All samples will be stored in a pseudonymous form at The Heart Sciences Centre, Harefield Hospital. Biological samples will be pseudo-anonymised to allow sample identification from the same patient and for patient group purposes. We may choose to collaborate with academic or industrial partners by sharing the samples so that additional analyses can be performed, in order to derive maximum scientific value from the experimental effort. In case of samples being shipped elsewhere from the trial site a Material Transfer Agreement (MTA) will be in place. These partners may be within or outside of the European Union and the PIS will be drafted so that the patient is aware that s/he is consenting to this process also.

Drug concentrations will be measured using validated methodology using HLPC/MS.

After the completion of the study, relevant material may be retained in a research tissue bank for use in future research in other ethically approved projects. The patient information

sheet and consent form will clearly describe the intention to retain relevant material beyond the duration of this study, and retention of any relevant material will be subject to individual patient consent.

11.3 DEFINITION OF THE END OF TRIAL

The study will be completed following the last patient last visit (LPLV).

12. SAFETY REPORTING

12.1 ANTICIPATED COMPLICATIONS

As this is an observational study, the burdens and risks to patients will be minimal. All samples will be collected from patients while they are recovering on a ventilator in the ICU. As such, it is routine clinical practice for most patients to remain sedated for a variable period of time.
Nearly all blood samples on the ICU are taken from in-dwelling vascular cannulae (such as central venous catheters or arterial lines), so patients will not experience any additional pain or discomfort during blood sampling. Bloods will be taken by experienced staff members, and will probably go unnoticed by the patient. Patients on the ICU will routinely have a urinary catheter inserted to monitor urine output. Urine samples will be collected from a bag that drains the bladder via the urinary catheter

12.2 DEFINITIONS

Adverse Event (AE) — any untoward medical occurrence in a patient or clinical trial subject who is administered a treatment and which does not necessarily have a causal relationship with this treatment (*i.e.* any unfavourable or unintended change in the structure (signs), function (symptoms), or chemistry (lab data) in a subject to whom a treatment/study procedure has been administered, including occurrences unrelated to that product/procedure/device).

Serious Adverse Event (SAE) – is defined as an untoward occurrence that:

- Results in death; or
- Is life-threatening (places the subject, in the view of the Investigator, at immediate risk of death)
- Requires hospitalization or prolongation of existing hospitalization (hospitalisation is defined as an inpatient admission, regardless of length of stay; even if it is a precautionary measure for observation; including hospitalisation for an elective procedure, for a pre-existing condition)
- Results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)
- Consists of a congenital anomaly or birth defect (in offspring of subjects or their parents taking the study drug regardless of time of diagnosis)
- Is otherwise considered medically significant by the investigator.

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the outcomes listed in the definition of serious will also be considered serious.

12.2 Recording Adverse Events (AEs)

All Adverse Events will be recorded in the hospital notes and Case Report Form (CRF).

If the Investigator suspects that the disease has progressed faster due to the administration of the study treatment/procedure, then he/she will report this as an unexpected adverse event to the Sponsor and the main REC as detailed in Section 12.4.

12.3 Assessment of SAEs

Principal Investigator (PI) at all sites must report all SAEs to the Chief Investigator (CI) or a delegated individual in the research team. The CI and his research team at RBH are responsible for reporting events to the Research Office immediately and/or within 24 hours of becoming aware of the event using the Sponsor's SAE Reporting Form.

Classification and causality of Adverse Events (AEs) will be conducted by local PIs and reviewed by CI. The CI cannot downgrade the site PI's classification and if there is disagreement which cannot be resolved during formal discussion then the assessment of the site PI will be accepted. The CI, can however, upgrade the seriousness of an event without consultation with the site PI.

12.4 Reporting SAEs

All AEs that are to be reported to the Research Office must be recorded, signed and dated by the Investigator at site. Research Office accepts study specific SAE forms, HRA SAE Form or RB&HFT template SAE Reporting Form available here.

Information can be submitted in electronic format:

- E-mail: research.reporting@rbht.nhs.uk
- Fax: 0207 351 8829.

An SAE occurring to a research participant will be reported to the Research Ethics Committee (REC) that gave a favorable opinion of the study (the 'main REC'), the study Sponsor (RB&HFT Research Office) and the local R&D Office where in the opinion of the CI/PI the event was:

- **'related'**: that is, it resulted from administration of any of the research procedures; and
- **'Unexpected'**: that is, the type of event is not listed in the protocol as an expected occurrence.

Reports of related and unexpected SAEs will be submitted within 15 days of the CI/PI becoming aware of the event; using the SAE reporting form for non-CTIMPs published on the HRA website and entitled non-CTIMP safety report to REC. The form should be

completed in typescript and signed by the Chief Investigator (CI) prior to submission to the REC.

The coordinator of the main REC will acknowledge receipt of safety reports within 30 days. It is the responsibility of the CI and his/her research team to send a copy of the SAE notification and acknowledgement receipt to the Research Office.

The research team also has the responsibility to report SAEs occurring in a certain period (28 days) after a patient completes the study. Any SAEs reported to the Investigators during this phase must be documented in the patient's medical notes and submitted via an SAE form

12.6 ANNUAL PROGRESS REPORTS (APRS)

The Chief Investigator (CI) will prepare the APR for the study. It will be reviewed by the RO and sent to the main REC by the CI within 30 days of the anniversary date on which the favourable opinion was given by the main REC, and annually until the trial is declared ended.

12.7 REPORTING URGENT SAFETY MEASURES

The Sponsor and/or the Investigator may take appropriate urgent safety measures in order to protect the subjects of a clinical study against any immediate hazard to their health or safety. If safety measures are taken, the main REC approval is not required before the measure is taken.

The Investigator will immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the main REC and the study Sponsor of the measures taken and the circumstances giving rise to those measures.

In order to prevent any delays in the reporting timelines the Sponsor has delegated this responsibility to the CI/PI. Therefore the CI/PI must report any urgent safety measures to the main REC directly, and in parallel to the Sponsor. The REC coordinator will acknowledge receipt of urgent safety measures within 30 days.

13. DATA MANAGEMENT AND QUALITY ASSURANCE

13.1 CONFIDENTIALITY

All data will be handled in accordance with the Data Protection Act 1998, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care, 2nd Edition (2005), and the condition of the main REC approval.

The Case Report Forms (CRFs) will not bear the subject's name or other personal identifiable data. The subject's initials, Date of Birth (DOB) and trial Identification Number (ID), will be used for identification.

13.2 DATA COLLECTION TOOL

Case Report Forms (CRF) will be designed by the CI and the final version will be reviewed and discussed with the study Sponsor. All data will be entered legibly in black ink with a ball-point pen. If the Investigator makes an error, it will be crossed through with a single line in such a way to ensure that the original entry can still be read. The correct entry will then be clearly inserted. The amendment will be initialled and dated by the person making the correction immediately. Overwriting or use of correction fluid will not be permitted.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Delegation of Responsibilities Log will identify all trial personnel responsible for data collection, entry, handling and managing the database.

13.3 DATA HANDLING AND ANALYSIS

Patient participants will be allocated a study number. A database to collect the clinical data and drug concentrations will be designed. The database will be accessed via Trust computers with a log in for the research team.

The research will be conducted to ensure the confidentiality of personal data and to meet the requirements of the Data Protection Act 1998. The study will be followed according to the NHS Code of Confidentiality. All identifiable data will be treated in accordance with the Caldicott Principles.

We would also like to apply retrospectively to use any drug samples that we have collected from previous ECMO patients, as this will allow us to examine a larger cohort for improved statistical power.

13.4 ARCHIVING ARRANGEMENTS

The study documents (including the Trial Master File (TMF), Case Report Forms (CRFs), Informed Consent Forms along with the trial database) will be kept for a minimum of five years. They will be stored in locked offices within the Royal Brompton and Harefield NHS Foundation Trust (RB&HFT). The CI is responsible for the secure archiving of trial documents. The trial database will also be kept electronically on the RB&HFT computer network, for a minimum of five years.

The approved repository for longer retention of local materials for studies that involve RB&HFT patients is Box-It Storage UK. The study documentation will be prepared for archiving by the research team in line with the Research Office Archiving SOP and the transfer will be arranged by the Research Office.

14.STATISTICAL DESIGN

It is estimated that a minimum of 12 patients for each study drug will be sufficient for population PK analysis and is feasible to enrol. Although insufficient prior data in patients receiving ECMO exists, the minimum of 12 patients per antibiotic is based on data from previous non-interventional PK studies in critically ill patients. The power calculations supporting this sample size are based on previous work by Tam et al which suggest that 12

patients per drug obtains robust population PK parameter predictions with ~30% bias (and ~20% precision) which is considered acceptable given the high PK variability likely to exist in this patient population(15).

A total of 12-15 patients will be enrolled for each study drug for this descriptive study, there will be no formal statistical analysis plan.

The protocol has been reviewed and ongoing statistical support will be by the Trust's statistician, Mr Winston Banya.

The number of ECMO performed at any centre will vary, based on transplant activity from previous years; we would expect to recruit approximately 40 patients over the course of 24 months. and it has been decided that a formal sample size calculation is not required, as this is an observational pilot study. The sample size reflects our ECMO activity in previous years and internal consensus within the study team.

14.1 Statistical analysis plan

There will be no formal statistical analysis plan for this descriptive study.

Statistical support will be provided by the Trust statistician, Mr Winston Banya.

14.2 Pharmacokinetic modelling plan

Plots of drug doses over time for each patient will be used to visually identify trends and outliers. We will perform population PK modelling for each study drug of interest using a non-linear mixed effects modelling approach (NONMEM®) as previously described and also develop a Physiologically-based Pharmacokinetic (PBPK) model with the additional ECMO as a separate compartment.

This approach will be useful for the data to be generated in this study as we will be able to adequately describe sparse data (if required) and we will be able to detect the covariates that are associated with variability over time of volume of distribution (Vd) and clearance (Cl), which we believe are commonplace in the presence of ECMO. After developing and testing these PK models, we hope to perform dosing simulations (Monte Carlo Simulations) which can then form the basis for dosing guidelines for antibiotics use in patients on ECMO. This would be a significant advance for all centres globally that use ECMO, as no guidelines currently exist. The protocol will also allow us to study the influence of renal replacement therapy on PK during ECMO.

15. COMMITTEES IN INVOLVED IN THE STUDY

Trial Management Group (TMG) - normally includes those individuals responsible for the day-to-day management of the trial, such as the CI, statistician, trial manager, research nurse, pharmacist, and data manager. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself.

16. MONITORING AND AUDITING

The requirement for study monitoring or audit will be based on the internal Research Office risk assessment procedure and applicable Standard Operating Procedures (SOPs). It is the responsibility of the RO to determine the monitoring risk assessment and explain the rationale to the study research team.

Study monitoring and/or audit will be discussed with the CI before arrangements are made to conduct the visit.

16.1 DIRECT ACCESS TO SOURCE DATA

The Investigator(s)/institution(s) will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

17. ETHICS AND REGULATORY REQUIREMENTS

The Sponsor will ensure that the trial protocol, Patient Information Sheet (PIS), Informed Consent Form (ICF), GP letter and submitted supporting documents have been approved by the main Research Ethics Committee (REC), prior to any patient recruitment taking place. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical approval prior to implementation.

Before site(s) can enrol patients into the trial, the PI must apply for Site Specific Assessment from the Trust Research & Development (R&D) and be granted written NHS R&D approval. It is the responsibility of the PI at each site to ensure that all subsequent amendments gain the necessary approval. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

Within 90 days after the end of the trial, the CI and Sponsor will ensure that the main REC is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.

The CI will supply a final summary report of the clinical trial to the main REC and the Sponsor in parallel within one year after the end of the trial.

18. FINANCE

Funding has been granted internally as a studentship for Haifa Lyster.

19. INSURANCE AND INDEMNITY

NHS bodies are liable for clinical negligence and other negligent harm to individuals covered by their duty of care. NHS Institutions employing researchers are liable for negligent harm caused by the design of studies they initiate. The provision of such indemnity for negligent harm should be stated to the participant.

20. PUBLICATION POLICY

Data ownership rights will lie with the institution.

21. STATEMENT OF COMPLIANCE

The trial will be conducted in compliance with the protocol, Sponsor's Standard Operating Procedures (SOPs), GCP and the applicable regulatory requirement(s).

The study conduct shall comply with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws and statutes of the UK country in which the study site is located including but not limited to, the Human Rights Act 1998, the Data Protection Act 1998, the Medicines Act 1968, and with all relevant guidance relating to medicines and clinical studies from time to time in force including, but not limited to, the ICH GCP**,** the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version), the NHS Research Governance Framework for Health and Social Care (Version 2, April 2005).

This study will be conducted in compliance with the protocol approved by the main REC and according to RGF standards. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor and the main REC except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the Sponsor and the main REC as soon as possible.

22. LIST OF PROTOCOL APPENDICES

Appendix 1 *Summary Chart of Study Assessments*

23. REFERENCES

1. Bartlett RH. Extracorporeal life support: history and new directions. ASAIO J. 2005;51(5):487-9.
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Appendix 1 **Schedule of study assessments**

Study Procedures	ECMO insertion	Enrolment (commencement of antifungals)	Sample timing						
			T0 (pre-dose)	**T1** (end of infusion or 1 hour post-ng dose)	**T-2 hours**	**3 hours**	**4 hours**	**8 hours**	**12** (BD dosing) **or 24 hours** (OD dosing)
Informed Consent	X								
Inclusion/exclusion criteria	X	X							
Baseline Haematology		X							
Baseline Biochemistry		X							
Type of ECMO (VA / VV) Flow rate (L/min)		X							
CVVH (Y /N)		X							
APACHE II/III (on admission)		X							
Blood sampling (Day 2)			X	X	X	X	X	X	X
Repeat blood sampling (Day day 4-6 AND/OR pre-tube change)			X	X	X	X	X	X	X