1.0 Title Page

Full / Long Title

A randomised, blinded placebo-controlled Phase 2a study to evaluate the safety and efficacy of Artesunate treatment in severely injured trauma patients with traumatic haemorrhage

Short title and/or Acronym

TOP-ART

Sponsor: Queen Mary, University of London

Representative of the Sponsor:

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REC Number : TBC

Sponsor Reference: REDA 009531

2.0 Research Reference Numbers

IRAS Number:	169165
EudraCT Number:	2015-000301-40
ISRCTN Number / Clinical trials.gov Number:	N/A

3.0 Signature Pages

Chief Investigator Declaration

I confirm that the following protocol (Version 2.0, dated 19th May 2016), has been written by me and I, as the Chief Investigator, agree to conduct the trial in compliance with this version of the protocol.

I will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), and all subsequent amendments of the clinical trial regulations, current Research Governance Framework, the World Medical Association Declaration of Helsinki (1996), GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the sponsor.

I also confirm that I will make the findings of the study publically available through publication and/or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator: Professor Karim Brohi

Chief Investigator Site: The Royal London Hospital, Barts Health NHS Trust

Signature: .....

Date: ...../...../.....

Name (please print):

.....

Statistician Declaration

The clinical study as detailed within this research protocol (Version 2.0, dated 19th May 2016), involves the use of an investigational medicinal product and will be conducted in accordance with the current Research Governance Framework for Health & Social Care the World Medical Association Declaration of Helsinki (1996), Principles of ICH E6-GCP, ICH E9 - Statistical principles for Clinical Trials, ICH E10 - Choice of Control Groups and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Statistician: Dr Bernard North

Job title: Senior Statistician

Statistician Site/Organisation: Queen Mary University of London

Signature: .....

Date: ...../...../.....

Name (please print): .....

Principal Investigator

I, as Principal Investigator confirm that I have read and understood the following protocol (Version 2.0, dated 19th May 2016) and that I agree to conduct the trial in compliance with this version of the protocol. I will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), and any subsequent amendments of the clinical trial regulations, current Research Governance Framework, GCP guidelines, the World Medical Association Declaration of Helsinki (1996), the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the sponsor.

Principal Investigator Name: Professor Karim Brohi

Principal Investigator Site: The Royal London Hospital, Barts Health NHS Trust

Signature:.....

Date: ...../...../.....

Name (please print): .....

This page must be signed by each PI at every site, and kept in the ISF, a copy of this page must be sent to the lead site/coordinating centre as evidence.

4.0 Key Trial Contacts

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Laboratories	1. Department of Haematology and Department of Clinical Biochemistry, The Royal London Hospital, Barts Health NHS Trust 2. Trauma Research Laboratory, Ward 12D, The Royal London Hospital, Barts Health NHS Trust 3. Mahidol Oxford Tropical Medicine Research Unit, Bangkok
Funder(s)	The Wellcome Trust
Clinical Trials Unit	N/A
Statistician	Dr. Bernard North Phone: 020 7882 349 E-mail: b.v.north@qmul.ac.uk Fax: 020 7882 3890
Trials pharmacist	Barts Health Clinical Trials Pharmacist Phone: 020 3594 6680 Fax: 020 7277 7386
Committees (DMC, TSC, TMG)	DMC –Data Monitoring Committee Reviews trial safety data and advises the sponsor on future management of the trial. TSC – Trial Steering Committee Provides overall supervision of the trial and monitors trial progress and conduct, advising on scientific credibility. TMG – Trial Management Group Provides day to day management of the trial

5.0 Trial Summary

Full title	A randomised, blinded placebo-controlled phase 2a study to evaluate the safety and efficacy of Artesunate treatment in severely injured trauma patients with traumatic haemorrhage.
Short title and/or Acronym	TOP-ART
Trial Design Methodology	Randomised, blinded placebo-controlled, parallel group study.
Phase of the Trial	Phase 2a
Study Duration	27-months.
Study setting	Single-site NHS Major Trauma Centre
Investigational Medicinal Product(s) and Treatment Groups	IMP: • Artesunate provided as a white crystalline powder in a glass vial containing 110mg, supplied with a second glass vial of reconstitution diluent which is a sterile phosphate buffer comprised of a mixture of sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous and water for injection to achieve a 0.3 M solution with a pH of 8.0 (7.9-8.1). • Matching placebo will comprise of the same, buffered diluent drawn up to the volume that would be necessary to reconstitute active IMP according to the subjects' weight. The sterile phosphate buffer is provided in a glass vial and is comprised of a mixture of sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous and water for injection to achieve a 0.3 M solution with a pH of 8.0 (7.9-8.1). Treatment group 1 (low dose intervention group): Subjects will be dosed at 2.4 mg/kg. Treatment Group 2 (high dose intervention group): Subjects will be dosed at 4.8 mg/kg. Control Group: Subjects will be receiving placebo at the same dose as treatment groups 1 or 2 respectively
Medical condition or disease under investigation	Trauma haemorrhage
Planned Sample Size	105
(Maximum) Treatment duration	1-2 minutes (time taken to administer bolus injection of IMP or comparator)
Follow up duration	90 Days
End of Trial definition	Point at which all surviving Subjects have completed 90-day mortality check.

6.0 Protocol Contributors

Key Protocol Contributors	Full contact details including phone, email and fax numbers
Sponsor	Queen Mary, University of London Joint Research Management Office Phone: 020 7882 7260 E-mail: sponsorsrep@bartshealth.nhs.uk Fax: 020 7882 7276
Funder	Wellcome Trust Phone: +44 (0) 20 7611 8336 E-mail: s.nicholas@wellcome.ac.uk Fax: +44 (0) 20 7611 8857
IMP Provider	Sigma Tau Industrie Farmaceutiche Riunite S.p.A. Phone: +39 06 9139 3434 E-mail: fabrizio.giorgi@sigma-tau.it Fax: +39 06 9139 3638

The sponsor (Queen Mary, University of London) has responsibility for ensuring the relevant trial standards are met and to ensure arrangements are put and kept in place for trial management, monitoring and reporting. The funder (Wellcome Trust) has responsibility for awarding funding according to pre-agreed milestones, although the conduct of the trial itself will remain separate.

The final decision regarding the design, conduct, data analysis and interpretation, manuscript writing and dissemination of results lies with the Sponsor and Trial Steering Committee.

7.0 List of Contents

8.0 List of Abbreviations / Glossary of Terms

AE	Adverse Event
ACCU	Adult Critical Care Unit
ANOVA	Analysis of Variance
AR	Adverse Reaction
BHT	Barts Health NHS Trust
CI	Chief Investigator
CIOMS	Council for International Organisations of Medical Sciences
CONSORT	CONsolidated Standards of Reporting Trials
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CRO	Contract Research Organisation
CTCOFR	Composite Time to Complete Organ Failure Resolution
CTIMP	Clinical Trial of Investigational Medicinal Product
DHA	Dihydroartemesinin
DMC	Data Monitoring Committee
EC	European Commission
eGFR	Estimated Glomerular Filtration Rate
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Union Drug Regulating Authorities Clinical Trials
eNOS	Endothelial Nitric Oxide Synthase
FiO2	Fraction of inspired Oxygen
GCP	Good Clinical Practice
GCS	Glasgow Coma Score
GMP	Good Manufacturing Practice
GP	General Practitioner
GSK-3β	Glycogen Synthase Kinase-3β
hCG	Human Chorionic Gonadotrophin
HTA	Human Tissue Act
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements

	for Registration of Pharmaceuticals for Human Use
IgE	Immunoglobulin E
IMP	Investigational Medicinal Product
INR	**International Normalised Ratio**
ITT	Intention To Treat
ISRCTN	International Standard Randomised Controlled Trial Number
JRMO	Joint Research Management Office
LR	Legal Representative
LC-MS/MS	Liquid Chromatography-Mass Spectrometry
MA	Marketing Authorisation
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
Main REC	Main Research Ethics Committee
MOF	**Multiple Organ Failure**
MORU	Mahidol Oxford Research Unit
n	number
NF-□B	Nuclear Factor-κB
NHS R&D	National Health Service Research & Development
PaO2	Partial pressure of arterial Oxygenation
PEEP	Positive End Expiratory Pressure
PI	Principle Investigator
pKa	$-\log_{10}(Ka)$ Ka represents the acid dissociation constant
PK/PD	Pharmacokinetic/Pharmacodynamic
PP	Per Protocol
PT	Preferred Term
QMUL	Queen Mary University of London
RGF	Research Gorvernance Framework
RIFLE	Risk, Injury, Failure, Loss of function, Endstage renal failure
RRT	Renal Replacement Therapy
Subject	An individual who takes part in a clinical trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction

SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Management File
TMG	Trial Management Group
TSC	Trial Steering Committee
WHO	World Health Organisation
WWARN	World-Wide Antimalarial Resistance Network

9.0 Introduction

9.1 Background

Multiple organ failure is common (30%) in the severely injured population and, if not fatal, results in worse outcomes such as prolonged critical care and hospital stays, and poor long-term quality of life. Development of multiple organ failure occurs early (within two days of admission) and correlates with an increase in nosocomial infections and if it persists, mortality (Minei *et al.* 2012). This figure rises rapidly as the degree of blood loss and shock intensifies. Patients requiring at least four units of red cell transfusions have longer critical care (9 vs. 5 days) and hospital stays (25 vs. 15 days). They also have a higher mortality (35% vs. 6%). Even modest improvements in these figures would have a significant improvement on patient experience, quality of life and the costs of healthcare.

Currently there are no specific treatments for organ failure. A therapeutic agent that reduces the incidence and severity of multiple organ failure could have a major global impact on the patient outcomes and resource utilisation.

The World Health Organisation (WHO) recommends intravenous Artesunate (a semi-synthetic artemisinin derived from the plant *Artemisia annua*) as the treatment of choice for severe malaria (WHO, 2010). Artesunate has very few adverse effects and the recommended intravenous bolus dose in adults/children with malaria is 2.4 mg/kg. Artesunate has been used by thousands of patients with malaria without important adverse effects (Sinclair *et al.* 2012). In man, Artesunate has a half-life of less than 30 mins and its active metabolite dihydroartemesinin (DHA) of less than 3 hrs. According to the WHO, the dose of Artesunate does not have to be adjusted in patients with renal failure or liver dysfunction.

In the first phase of the study, the dose of Artesunate (single bolus intravenous injection) proposed for administration to patients with trauma-haemorrhage is identical to the dose currently recommended by the WHO for the treatment of malaria (2.4 mg/kg), although in malaria Artesunate is repeatedly administered (every 12hrs), whilst in this study only one bolus dose will be injected. Thus, no significant adverse effects with Artesunate in patients with trauma-haemorrhage are expected. Provided the interim analysis demonstrates no safety concerns, the trial will proceed with the higher dose of Artesunate (4.8 mg/kg)—see also Section 9.2 for further safety data.

Pre-Clinical Data

We have discovered that the intravenous injection of small doses of Artesunate (1-10 mg/kg) upon resuscitation after severe haemorrhage (rat) reduces the multiple organ failure after trauma-haemorrhage by enhancing the resistance of organs against injury by a) activating well-known cell-survival pathways, and b) reducing excessive inflammation.

We have discovered preclinical (rat) efficacy of low doses of Artesunate (up to 10 mg/kg) in trauma-haemorrhage i.e. removal of approximately 40% of blood volume for 90 mins followed by resuscitation for 4 hrs resulting in multiple organ (kidney, lung, liver) failure. Intravenous injection upon resuscitation (after 90 mins of haemorrhage) of low dose Artesunate (1-10 mg/kg) attenuates the multiple organ failure (renal dysfunction, liver injury, neuromuscular injury) caused by severe haemorrhage by 50-90% (Fig. 1) by activating well-known survival pathways or by inhibiting pro-inflammatory pathways (Fig 2).

The most effective dose was 3 mg/kg, and a higher dose (10 mg/kg) offered no further significant benefit. Using the same protocol, we have then evaluated the effects of 2.4 or 4.8 mg/kg

intravenous artesunate in rats with severe haemorrhagic shock. This study confirmed that Artesunate caused a dose-related reduction in multiple organ injury/dysfunction (Fig 3).

When given after a prolonged (90 min) period of severe haemorrhage (leading to a fall in mean arterial blood pressure to 35 mmHg), Artesunate (2.4 or 4.8 mg/kg i.v.) attenuates the renal (glomerular) dysfunction, liver injury and neuromuscular injury by 50 to 90%. In the liver of rats with haemorrhagic shock, Artesunate also activates a well-known survival pathway resulting in activation of Akt (pro-survival), activation of eNOS (vasodilator and pro-survival), inhibition of GSK-3 and NF-B (anti-inflammatory), which contribute to or account for the observed beneficial effects of Artesunate.

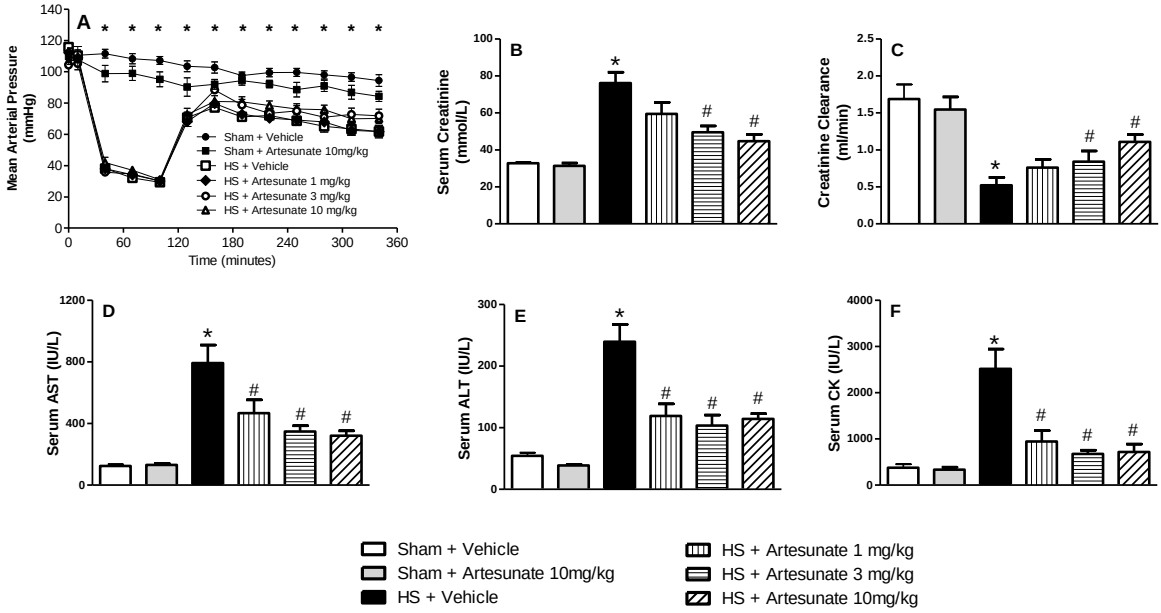


Figure 1: Effect of Artesunate on (A) mean arterial pressure monitored throughout haemorrhage and resuscitation, (B, C) renal dysfunction; serum creatinine levels and creatinine clearance, (D, E) liver injury; serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, and (F) neuromuscular injury; serum creatine kinase levels were measured subsequent to sham-operation (Sham + Vehicle, n=10; Sham + Artesunate 10 mg/kg, n=10) or haemorrhagic shock (HS + Vehicle, n=10; HS + Artesunate [1 mg/kg i.v.], n=10; HS + Artesunate [3 mg/kg i.v.], n=10); HS + Artesunate [10 mg/kg i.v.], n=10. Data represent mean ± SEM for n observations, **P*<0.05 *vs.* Sham + Vehicle, #*P*<0.05 *vs.* HS + Vehicle.

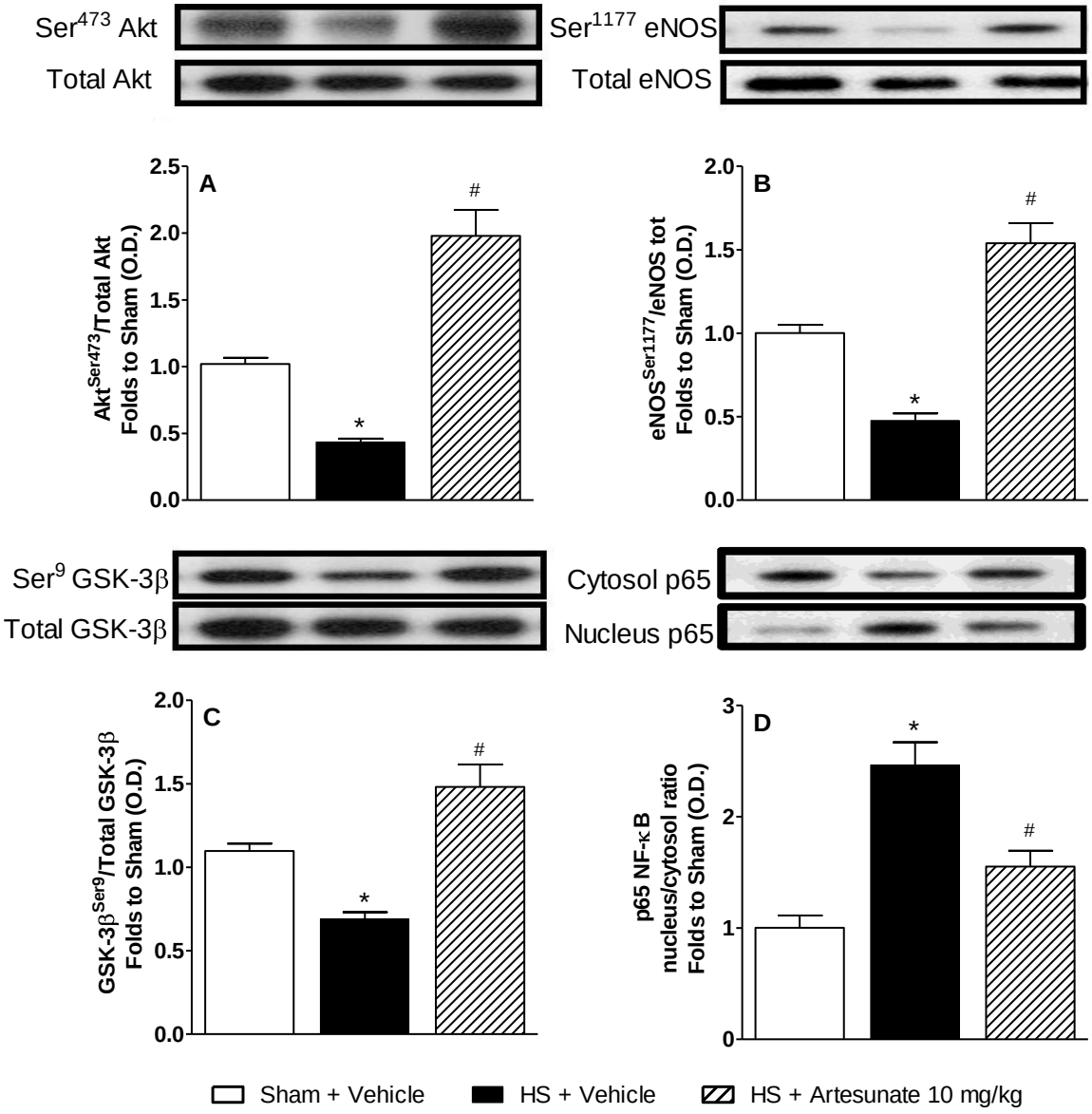


Figure 2: Effect of Artesunate on the phosphorylation of (A) Ser^{473} on Akt in the liver, (B) Ser^{1177} on eNOS in the liver, (C) Ser^{9} on GSK-3β in the liver, and (D) nuclear translocation of p65 (NF-κB activation) subsequent to subjected to sham-operation (Sham + Vehicle, n=3) or haemorrhagic shock (HS + Vehicle, n=10); and HS + Artesunate 10 mg/kg, n=3. Data represent mean ± SEM for n observations, **P*<0.05 *vs.* Sham + 10% DMSO, #*P*<0.05 *vs.* HS + 10% DMSO.

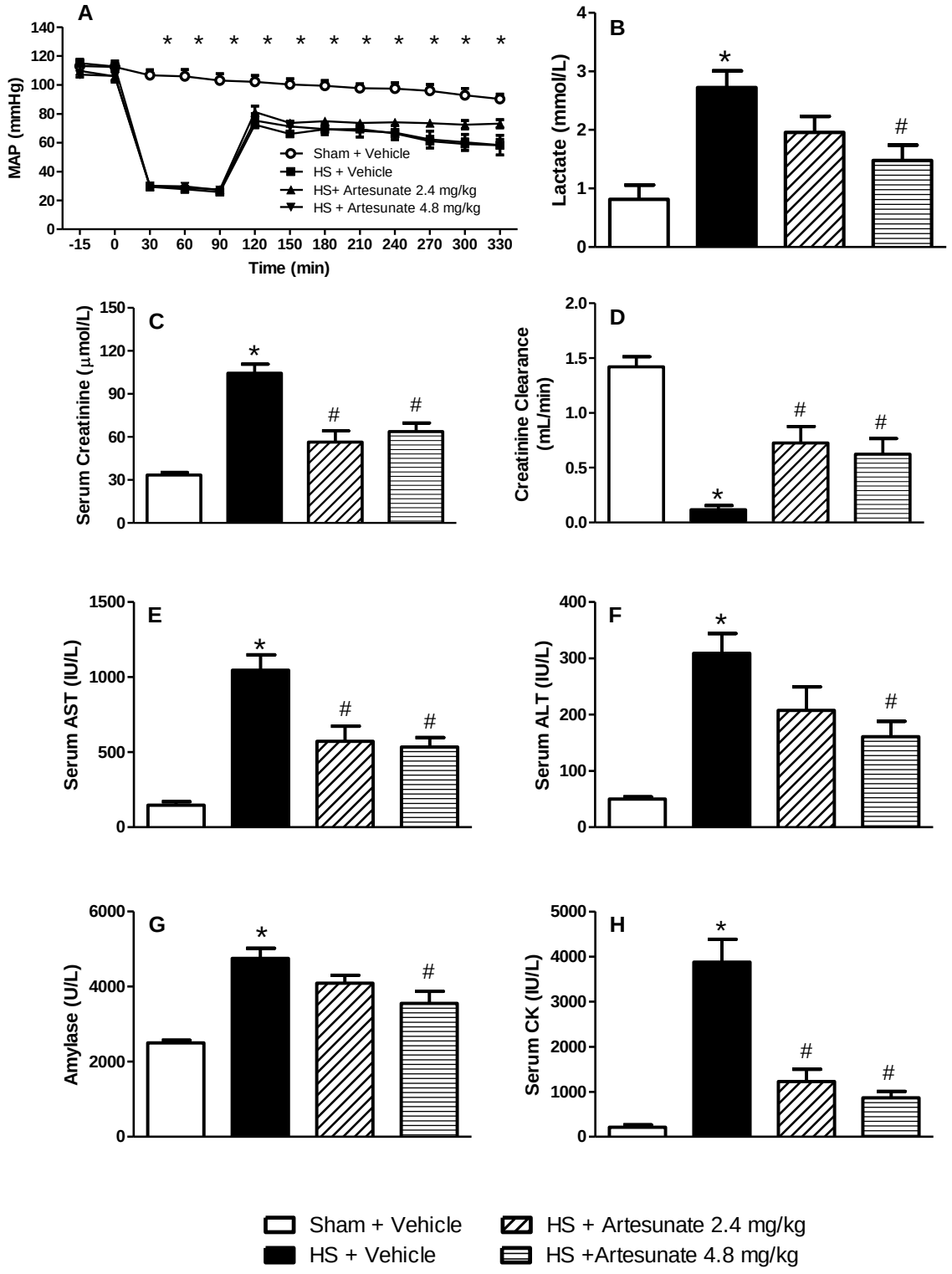


Figure 3: Effect of Artesunate on (A) mean arterial pressure monitored throughout haemorrhage and resuscitation, (B) global organ perfusion; lactate levels, (C, D) renal dysfunction; serum creatinine levels and creatinine clearance, (E, F) liver injury; serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, (G) pancreas injury; amylase levels, and (H) neuromuscular injury; serum creatine kinase levels were measured subsequent to sham-operation (Sham + Vehicle, n=7) or haemorrhagic shock (HS +)

Clinical Data

Following an in-depth review of the literature, there are no human studies demonstrating use of Artesunate as a treatment for reducing multiple organ failure resulting from severe haemorrhage and injury. However, due to widespread use of Artesunate for the treatment of severe malaria, it has been available outside of the UK (and 'off-licence' inside the UK) in various non-GMP formulations (parenteral, oral, and rectal suppository) for a number of years. Therefore, there is a large volume of published information about clinical experiences with the drug, including in critically ill malaria patients. Collectively these studies have involved thousands of subjects. Several peer reviews have summarised various aspects of Artesunate trials, and have found Artesunate to be safe and well-tolerated (Riberio & Olliaro 1998, McIntosh & Olliaro 2000, Jones *et al.* 2007, Nosten & White 2007, Hess *et al.* 2010, Sinclair *et. al* 2012).

In a review of 188 (published and unpublished) trials, including 9241 subjects treated with Artesunate for malaria, Riberio & Olliaro (1998) found no serious adverse events or severe significant toxicity in a safety analysis of adverse events. In this review the most commonly reported adverse experiences were gastrointestinal symptoms. Occasional neutropenia (1.3%) reticulocytopenia (0.6%) and elevated liver enzymes (0.9%) were reported, and occasional instances of bradycardia/prolonged QT intervals (1.1%). Reports of neuropsychiatric disorders were reported in 4 patients on however they were all on con-current mefloquine.

McIntosh & Olliaro (2000) examined 41 trials of Artesunate in over 5000 patients and concluded that there was no evidence to suggest artemesinin drugs were more harmful than standard treatment for uncomplicated malaria. Similarly, Sinclair et al. (2012) examined 8 trials of Artesunate in severe malaria involving 1664 adults and 5765 children and reported no evidence of adverse effects of Artesunate, except for transiently worse neurological sequale in children.

Although artemisinins are recognized as a family of exceptionally safe drugs there have been four cases of IgE-mediated anaphylactic reactions to oral and intravenous Artesunate (Leonardi *et al.* 2001, Mohapatra *et al.* 2009, Dube *et al.* 2012). These allergic reactions to artemisinins are considerably rare.

With respect to pregnancy, there are Cochrane database reviews of uncomplicated malaria treatment in pregnant women that discuss Artesunate (Orton & Garner 2005, Orton & Omari 2008). The current guidance suggests Artesunate may be given in the second and third trimesters for treatment of malaria, however due to insufficient evidence, it is not currently recommended for use in the first trimester unless benefits are considered to outweigh the risks (WHO, 2010).

With regards to the increased dose (4.8 mg/kg) we similarly do not expect significant adverse effect based on safety studies demonstrating that Artesunate can be safely administered up to doses of 8 mg/kg; Healthy volunteer Phase 1 studies have shown that single and multiple doses of intravenous Artesunate at the highest dose tested (8 mg/kg) were well tolerated with no serious side effects observed, and showed no dose-dependant increase in any adverse events, apart from a dose-related decrease in reticulocyte counts four days after dosing (Li *et al.* 2009, Miller *et al.* 2012).

9.2 Assessment and management of risk

Artesunate has been extensively used in the treatment of both child and adult malaria including use in critically ill patients. They are very well tolerated, with a well-documented safety profile (see

previous section). As patients will receive all other clinically indicated treatments, there is no risk of ineffective therapy by administration of the trial treatment.

Whilst there is a risk of adverse events occurring as a result of oral or intravenous Artesunate, including gastrointestinal symptoms, neutropenia, reticulocytopenia, liver enzyme disturbance, bradycardia and prolonged QT intervals (Riberio & Olliaro 1998), these risks are low (all occurring in under 1.5% of recipients) and unlikely to result in significant detriment to the subject particularly in light of the serious nature of their injuries. Moreover, anaphylactic reactions to Artesunate are extremely rare.

The potential benefits of Artesunate in reducing multiple organ failure outweigh the risk of adverse events associated with the medication. Artesunate will be administered as an intravenous preparation due to the critical illness of the subjects enrolled that will preclude oral administration. Intravenous Artesunate has been more widely studied within the literature compared to intramuscular Artesunate (Jones *et al.* 2007) and is therefore the preferred route of administration in this trial.

This trial is categorised as: Phase 2a

9.3 Rationale for study design

This study aims to investigate whether administration of a single intravenous bolus dose of Artesunate reduces multiple organ failure in severely injured patients with trauma haemorrhage. The rationale for the study include:

1. Pre-clinical data provide both clinical and mechanistic evidence of the potential benefit of Artesunate in reducing multiple organ failure.
2. There are currently no other specific therapeutic agents that are proven to effectively attenuate multiple organ failure and its detrimental consequences in human studies (Lord et al., 2014)
3. Artesunate has a well-documented favourable safety profile, including in other critical illnesses.

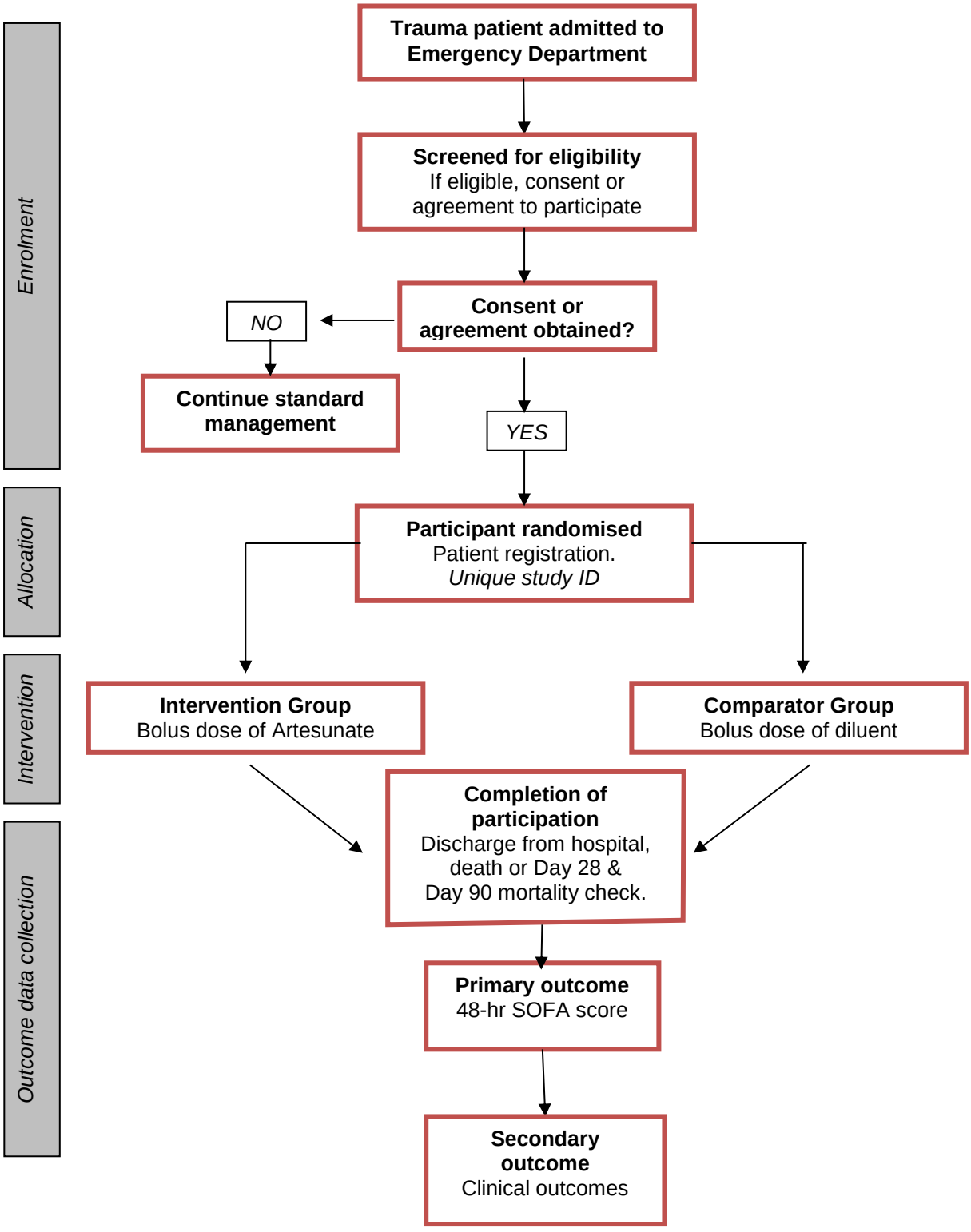
The patient will receive the usual standard of care regardless of whether or not they receive Artesunate. With regards to dosage, 2.4mg/kg as a bolus is the recommended treatment does for malaria (a bolus which is repeated 12 hours later). There is published safety data for Artesunate using intravenous boluses up to 8mg/kg (Li *et al.* 2009, Miller *et al.* 2012).

In our study, due to the dose-dependent benefit seen in the pre-clinical data, we wish to determine whether an increased dose of 4.8mg/kg Artesunate is more efficacious than 2.4mg/kg in reducing multiple organ failure following major haemorrhage in trauma. We do not expect a significant increase in serious adverse events in relation to this increased dose, although this will be closely monitored.

A placebo is justified in this trial to reduce effects of potential investigator bias when collecting follow-up data (including on organ failure) and potential bias arising from differential treatment by clinicians on account of knowing the patient's enrolment in the trial.

Please see section 9.2 for further assessment of the risks and benefits of the study.

10.0 Trial Flowchart



11.0 Trial Objectives and Design

11.1 Primary Objective/s

The aim of this study is to determine whether treatment with Artesunate compared with placebo improves the outcome in severely injured trauma subjects with haemorrhage.

11.2 Secondary Objective/s

The secondary objectives of the study are, to determine the effects of Artesunate on organ failure, lung injury, kidney injury, infection, ventilator requirements, hospital stay, critical care stay and mortality.

The safety objective is to evaluate the safety of Artesunate in the treatment of severely injured trauma subjects.

11.3 Endpoints

11.3.1 Primary Endpoint

The primary efficacy endpoint is reduction in SOFA score at 48 hours for Artesunate treatment arm versus placebo.

11.3.2 Secondary Endpoint

The secondary endpoints are:

- Maximum SOFA score: Maximum SOFA score over 7 days.
- Mean SOFA Score (Days 2-5).
- Prolonged organ failure: Defined as SOFA>5 on Day 7.
- Days on organ support: As per the CTCOFR score (Nadel *et al*., 2007).
- Incidence of acute lung injury: As per Berlin definition (ARDS Task Force, 2012).
- Incidence of acute kidney injury: As per the RIFLE score (Bellomo *et al*., 2004).
- Incidence of infection: As per definitions of infection by (Cole *et al.*, 2014).
- Number of ventilation free days.
- Total length of hospital stay.
- Total length of critical care stay.
- Mortality (discharge, 28 day and 90 day).
- Alive and free of multiple organ failure (defined as SOFA$\leq$5) at 48 hours.

The safety endpoint is the serious adverse event rate in the first 28 days.

11.4 Exploratory or Tertiary endpoints/outcomes

N/A

11.5 Objectives and End Points Summary

Primary Objective	**Primary Endpoint**	**Outcome Measures**
To determine whether artesunate improves outcomes in patients with trauma haemorrhage	48-hour SOFA score	SOFA Score
Secondary Objective	**Secondary Endpoints**	**Outcome Measures**
To determine effects of Artesunate on organ failure	Maximum SOFA score Prolonged organ failure Days on organ support **Mean SOFA score (Day 2-5)**	SOFA Score CTCOFR score
To determine effects of Artesunate on lung injury	Incidence of acute lung injury	Berlin Definition of ALI
To determine effects of Artesunate on kidney injury	Incidence of acute kidney injury	RIFLE Score
To determine effects of Artesunate on infection	Incidence of infection	Cole et *al.* Definitions
To determine effects of Artesunate on ventilator requirements	Number of ventilation free days	Number of ventilation free days
To determine effects of Artesunate on hospital stay	Total length of hospital stay	Total length of hospital stay.
To determine effects of Artesunate on critical care stay	Total length of critical care stay	Total length of critical care stay
To determine effects of Artesunate on mortality	Mortality (discharge, 28 day and 90 day).	Mortality (discharge, 28 day and 90 day).
To determine effects of Artesunate on patient safety	Serious adverse event rate in first 28 days	SAE rate in first 28 days
To determine the impact of Artesunate on early survival	Alive and free of multiple organ failure (defined as SOFA$\leq$5) at 48 hours	48-hour SOFA score 48-hour mortality

11.6 Trial Design

This is a single-centre, randomized, placebo-controlled, parallel group study with a sequential group-dosing regimen (adaptive design). Its aim is to evaluate the safety and efficacy of Artesunate treatment compared to placebo, in addition to standard treatment, in severely injured trauma patients with traumatic hemorrhage. All subjects will be managed in accordance with local guidelines for traumatic hemorrhage.

Subjects will be assessed for eligibility upon arrival to the hospital. Due to the urgent/emergency nature of the intervention subjects will be recruited within 4 hours of their injury and within 2 hours of admission to the accident and emergency department. Eligible patients therefore need to be consented very soon after admission. As most, but potentially not all, subjects will be incapacitated at time of eligibility, consent can be obtained from relatives or if no relatives are present two

doctors (one independent of the trial) will decide on consent. If and when the subject regains physical and mental capacity they will be provided written information on the trial and will be given the opportunity to ask questions, informed consent will be sought for continuation in the trial. For any subject included but did not regain full capacity, consent will be sought from a relative or other appropriate representative for continuation in the trial.

Subjects will be randomized to one of three groups:

- Placebo arm (n = 35)
- Low Dose intervention arm: active drug 2.4mg/kg (n=35)
- High Dose intervention arm: active drug 4.8mg/kg (n=35)

The trial will be divided into two stages. The first stage will enroll patients into the Placebo (n=17) and Low Dose intervention arms (n=35), following which an interim analysis will be performed. The analysis will be limited to an analysis of safety data between the two groups. Provided there are no issues regarding safety or toxicity, the study will continue with the second phase enrolling patients into the high dose intervention arm (n=35) or placebo (n=18).

Each subject will be dosed and then remain in the study for 28 days or until they are discharged. There will be a 90-day follow-up though this will be conducted using data obtained via the NHS spine services (Health and Social Care Information Centre).

Subjects who do not complete the primary outcome end-point at 48-hours (e.g. due to death or discharge) will be replaced. Variables will be evaluated at different time points from screening through day 28 as per the schedule of events.

Adverse events (AEs) and serious adverse events (SAEs) will be reviewed by the trauma research fellow (who is a clinician) on a daily basis from the point of dosing throughout the 28-day subject participation in the trial. Please see Section 17 for further details.

The following will be determined at various points throughout the trial SOFA score, renal injury, lung injury, infection rate, the number of mechanical ventilation days, RRT days, vasopressor days, CTCOFR score, ACCU length of stay and the hospital length of stay.

11.7 Study Setting

A single-centre study conducted in an NHS Major Trauma Centre.

12.0 Eligibility Criteria

12.1 Inclusion Criteria

A subject will be eligible for the study if they meet the following criteria:

- Adult male and female trauma patients over 16 years of age.
- Activation of the local massive haemorrhage protocol (activation criteria are: systolic blood pressure <90 mmHg, AND suspected haemorrhage, AND minimal response to small volume fluid resuscitation).
- Patients with active, ongoing haemorrhage.
- Agreement is provided on behalf of incapacitated patients by Personal Legal Representative or Professional Legal Representative (i.e. trauma team leader).

12.2 Exclusion Criteria

A patient will not be eligible for the study if they meet any one of the following criteria:

- Time of admission more than 2 hours after the time of injury.
- Time of drug administration not attainable within 4 hours of injury.
- Subject not expected to survive more than 48 hours.
- Evidence of severe traumatic brain injury (GCS 3 at scene).
- Known pregnancy.
- Suspected non-haemorrhagic cause of shock.
- Massive haemorrhage protocol activation more than one hour after arrival.
- Concurrent participation on another Clinical Trial of an IMP.
- Breastfeeding females.
- Known allergy to Artesunate.

NOTE: Participants are considered not of child bearing potential if they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are postmenopausal.

13.0 Trial Procedures

13.1 Recruitment

A total of 105 adult male and female patients will be enrolled in this trial. Research personnel will identify potential subjects upon admission to the emergency department.

13.2 Participant identification

The trauma research fellow will attend all trauma admissions to the accident and emergency resuscitation department in order to screen participants for potential enrolment according to the study inclusion and exclusion criteria.

The trauma research fellow is a clinician by training, but not a member of the clinical team attending to the participant. This is due to the critical condition of participants, meaning that it is unlikely that a treating clinician will concurrently be able to provide the necessary care for the patient whilst also undertaking the research screening and enrolment procedures.

However, once the trauma research fellow deems a participant to be eligible for enrolment, they will confirm eligibility with the clinician leading the care for the patient (i.e. the trauma team leader) prior to enrolling the participant into the study. Our study centre and research team has experience in conducting similar screening procedures successfully in previous studies.

13.3 Informed Consent Procedures

Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial and are out-side standard routine care at the participating site (including the collection of identifiable participant data unless the trial has prior approval from the Confidentiality Advisory Group (CAG) and the Research Ethics Committee (REC)).

13.3.1 Responsibility for obtaining consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants at their site and must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki. If delegation of consent occurs then details should be provided in the Site Delegation Log.

The trauma research fellow, who is a clinician by training, will be responsible for obtaining consent.

13.3.2 Consent Considerations

The right of a participant to refuse participation without giving reasons will be respected.

The participant must remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment and must be provided with a contact point where he/she may obtain further information about the trial. Where a participant is required to re-consent, for example if during the trial new Research Safety Information becomes available, or following an

amendment that affects the patient, or new information needs to be provided to a participant, it is the responsibility of the PI to ensure this is done in a timely manner.

13.3.3 Population

The study population will consist of adult male and female trauma patients with suspected active or ongoing haemorrhage.

13.3.4 Vulnerable participant's considerations

The PI takes responsibility for ensuring that all vulnerable subjects are protected and participate voluntarily in an environment free from coercion or undue influence.

13.3.5 Written/ reading / translation considerations

Participants who cannot read or write, require translators, or have cognitive impairment will be supported during the informed consent process. This includes allowing a witness to sign on a participant's behalf (in the case of problems with reading or writing) or allowing someone to date the form on behalf of the participant. In the case of participants who require verbal information to be provided in a different language, this will be done via telephone translation services or a hospital or personal interpreter.

13.3.6 Participants lacking capacity

Most, but potentially not all, subjects will be incapacitated at the time of eligibility (critical injury, mechanical ventilation, sedation), such that the Mental Capacity Act (England; 2005) and Declaration of Helsinki (World Medical Association; 2013) provide guidance. Patients will be enrolled within four hours of their injury and within two hours of admission to the accident and emergency department. This is a particularly stressful time for relatives and families, an important consideration when the intervention under investigation is time sensitive. This study requires the IMP to be given rapidly, thereby necessitating that eligible patients are consented very soon after hospital admission. As injury is an unexpected event, it is uncommon for relatives to be present at the time of hospital admission.

As stipulated by GCP the subject and/or their Personal LR (e.g. next of kin) should be given ample time to consider giving their consent for the study. It is felt that 24 hours is sufficient time for the patient and/or Personal LR to consider participation and give informed consent. However, the need for urgent treatment in this trial means implementation of the research cannot be delayed and it would be inappropriate to delay treatment until fully informed consent can be obtained from the patient or other nominated representative. Patients incapable of giving consent in emergency situations are an established exception to the general rule of informed consent in clinical trials.

As the timeframe required for subject or Personal LR consent is not compatible with the time sensitivity of this trial, several approaches to obtaining informed consent will be used. All are consistent with the Mental Capacity Act (England; 2005) and Declaration of Helsinki (2013).

Consent for initial enrolment in the trial will be sought from a Professional LR in the form of independent clinicians (trauma lead for the subject), who are familiar with the consenting process and present at every trauma call.

If Personal LRs are present, bearing in mind the clinical situation and their level of distress, they will be provided with brief information about the trial either verbally or in writing. Specifically, the investigator will explain to the relatives that the patient will receive the usual emergency treatments for traumatic haemorrhage but that in addition to these, the patient has been enrolled in a research study that aims to improve the outcome of patients with this condition.

It will be explained the study is being done to see whether using a drug called Artesunate will improve patients' outcome by protecting against organ failure. The relative will be informed that the patient will be given a single dose of Artesunate or placebo. The investigator will explain that whilst we hope that Artesunate will improve outcome after trauma haemorrhage, at present we cannot be sure about this. If the relative or representative objects their wishes will be respected. If no relatives are present, two doctors (one independent of the trial) will consider the patient's eligibility criteria and any known views of the patient about trial participation. Together they will decide whether or not to enrol the patient into the trial.

We do not propose to include a telephone contact with relatives/personal consultees in order to minimise stress and anxiety at a difficult time.

If and when patients regain physical and mental capacity to give consent, information will be provided to them and written informed consent will be sought for continuation in the trial. If a patient or representative declines to give consent for continuation at this stage, his/her wishes will be respected. For any patient included that did not regain full capacity, consent will be sought from a relative or other appropriate representative for continuation of the trial.

There is potential for a small group of research subjects to exist for whom a Personal LR is not available and who are subsequently unable to give consent due to death or permanent disability. In such cases Professional LR permission still stands in this situation and these subjects can remain within the trial.

In cases where the subject dies before the investigator has discussed the trial with the patient or the Personal LR, the patient can remain in the trial under the Professional LR consent from the Trauma Team Leader. We will not attempt future telephone contact with relatives/Personal LR in order to minimise stress and anxiety associated with the unexpected and traumatic death of their relative / next of kin. In cases where the investigator has made initial contact with the Personal LR prior to the subject's death but written agreement has not yet been obtained, then we will make a maximum of three further attempts (by any combination of telephone, e-mail or letter) to contact the Personal LR and obtain consent. If after these further attempts the Personal LR has not been contacted, then the subject shall remain in the trial based on the Professional LR consent.

In summary, we believe these approaches are justified under the conditions of the Mental Capacity Act (England; 2005) for the following reasons:

1. The urgent/emergency nature of the intervention.

2. The fact the IMP administered is already widely used in humans.

3. The proposed recruiting centre has used this consenting procedure for previous randomised controlled trials in trauma (MP4OX Ph2b - EudraCT: 2010-023129-39; Cryostat Ph2a – ISRCTN55509212) and the ongoing ACIT-2 observational study (REC ref: 07Q0603/29) that has successfully recruited more than 1300 patients to date.

Prior to any study related activities and subsequently for continuation when the subject is capable or from a relative or representative, a copy of the Informed Consent Form approved by the Research Ethics Committee will be signed and dated. The original signed forms will be retained by the site in the study file, together with any subsequent approved amended versions. Copies of the original will be given to the subject or Personal LR for retention in their own records, and placed in the medical care records.

13.3.7 Minors

N/A

13.3.8 Consenting process

The trauma research fellow will be responsible for the consenting process, which will be conducted in the manner outlined in section 13.3.6. An assessment of capacity of the participant will be made according to the following criteria:

- Ability to understand the purpose and nature of the research.
- Ability to understand what the research involves, its benefits (or lack of benefits), risks and burdens.
- Ability to understand the alternatives to taking part.
- Ability to retain the information long enough to make an effective decision.
- Ability to make a free choice.
- Ability to make this particular decision at the time it needs to be made (though their capacity may fluctuate, and they may be capable of making some decisions but not others depending on their complexity)
- Where participants are capable of consenting for themselves but are particularly susceptible to coercion, it is important to explain how their interests will be protected.

The trauma research fellow will subsequently have a discussion with the participant (if and when they regain capacity) or his/her Personal LR (in the case of participants without capacity) about the nature and objectives of the trial, and the possible risks associated with their participation. They will also be provided with written information about the trial.

As stipulated by GCP, the participant should be given ample time to consider giving their consent for the study. 24 hours is the minimum period of time for an individual to consider their participation within the study. If for any reason, less than 24 hours is to be given, it will be documented why this is the case along with justification for this decision. The date that the Patient Information Sheet (PIS) is given to the participant or their Personal LR must be documented within the patient's notes to ensure that sufficient time is given.

13.3.9 Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies

Blood samples will be drawn from participants at 6 time points for PK/PD analysis (See Section 13.8.3). Each draw will consist of a maximum draw of 10 mls. Any excess blood from this draw that is not required for PK/PD analysis will be processed and stored for future ethically approved research, provided the participant gives consent.

Specifically, SST® 5 mL vacutainer tubes (BD biosciences, UK) will be used to collect serum at each time point, which will be stored for later biomarker analysis and co-incubation with healthy donor peripheral blood mononuclear cells. This would allow exploration of mechanistic pathways behind

treatment with Artesunate and the influence that it has on circulating immunologically active mediators.

PAXgene® 2.5 mL vacutainer tubes (PreAnalytiX, Germany) will also be collected at 4 and 12 hour time points. Data generated from a rat haemorrhage model treated with Artesunate demonstrated increases in survival pathways (*Annals of Surgery, under review*). Genomic patient data generated will allow reverse translational research to further explore this phenomenon with our validated animal model. All samples will be labelled and stored in catalogued cryoboxes at -80°C immediately following processing.

Participants will be given the option to consent for these samples to be used for future research related to the conditions under investigation, including genetic research (but not research related to hereditary diseases). Samples and data will be de-identified prior to being shared with collaborators involved in this future research.

The decision to opt out of ancillary research will not affect a participant's ability to remain in the trial. Withdrawal from ancillary research will result in disposal of any obtained samples in accordance with Barts Health NHS Trust policy, and a note of their withdrawal will be made on the consent log.

13.4 Screening Procedures

Patients will be assessed for eligibility to enter the trial according to the defined inclusion/exclusion criteria upon arrival at the hospital. This will include a pregnancy test for all females of childbearing age. If patients are assessed to be eligible, consent for entry into the study will be sought. Data will be collected on all patients who are screened for eligibility on the study screening-log by the Trauma Research Fellows.

A screening log will be completed, which will record all patients considered for eligibility to the trial. The log will include date and time of screening, and the participant age, gender, and reasons for non-enrolment. The screening log will include patients approached but in whom consent was not obtained for the trial (with reasons). The screening log data will be reviewed at regular intervals.

The trauma research fellow and research assistant are responsible for completing the screening log after an eligible patient is identified and considered for participation in the trial

13.5 Patient Allocation

13.5.1 Randomisation Method

A simple randomisation method will be used as detailed below:

- First stage: subjects will be randomised to Low dose active or placebo on a 2:1 ratio.
- Second stage: subjects will be randomised to High dose active or placebo on a 2:1 ratio.

13.5.2 Randomisation Procedure

Once a subject is determined eligible for the study and informed consent has been obtained, each subject will be enrolled as soon as possible and will be assigned a unique study identifier; this will be used throughout the subjects' participation in the study, and will be documented on the enrolment

log. This number will be a numerical value commencing at '001', and as the study is single centre it will ascend sequentially thereafter.

Randomisation codes will be generated and secured by an independent statistical consultant from Fisher Clinical Services. The Investigator or designee will open a pre-sealed envelope containing the randomised treatment group allocation.

Randomisation must take place within two hours of hospital admission. Randomisation details will be documented on the enrolment log.

In the event that replacement supplies are required (e.g. due to more than one hour passing following reconstitution of the IMP) the investigator or designee should immediately contact Barts Health Pharmacy, who will quarantine the unused IMP as per their standard operating procedures. As the randomisation allocation is provided in pre-sealed envelopes and the IMP administrator is un-blinded, replacement IMP will be prepared as per the original randomisation allocation provided in the pre-sealed envelope.

13.5.3 Cohort allocation/sequential allocation

N/A

13.6 Blinding

The trial participant, clinical team providing treatment (including pharmacy), and research investigators will be blinded to treatment group allocation. In addition, outcome assessors and data monitoring committees will also remain blind to group allocation.

However, due to the slight difference in opalescence of the IMP solution and placebo, and the requirement to visualise the IMP to confirm adequate re-constitution (see Section 15.12), it is not possible to blind the IMP administrator. Therefore, the IMP administrator, who will prepare the active ingredient IMP or placebo (diluent only), will be un-blinded.

In order to maintain blinding of the subject and clinical team, the IMP and placebo will be prepared in a separate room to the clinical team (Section 15.12). The administrator will simulate the time taken to re-constitute the IMP (6 minutes) when preparing the placebo, and administer it in a similar manner (i.e. as an intravenous injection over 1-2 minutes, given within the specified time-frame of one hour).

In order to maintain blinding of research investigators collecting follow-up data, a rota will be drawn up such that the investigator who administered the IMP/placebo will not be involved in collecting follow-up information for that subject. Our centre and its research team operate to a rota that has successfully conducted such clinical trial protocols previously.

Final unblinding of all trial participants will occur after creation of a locked analysis data set.

13.7 Unblinding

Unblinding of the treatment allocation is only allowed for safety concerns in an emergency situation.

Un-blinding or code breaks is the responsibility of the PI or Co-Investigator (medically qualified delegated deputy). However due to the emergency nature of the trial, it is important that the treating physician, has rapid unblinding access.

Sealed unblinding envelopes will be provided to the sites along with the study pack. The sealed unblinding envelopes will be stored in the Trauma Research Office in a secure area.

The treating physician will have the authority to request and un-blinding if the Safety of the participant is at risk. Any research team member can perform an un-blinding on the authority of a treating physician.

On receipt of the treatment allocation details the PI or treating physician will continue to deal with the participant's medical emergency as appropriate.

It is important that the unblended staff only divulge the treatment allocation to individuals on a strictly need to know basis.

Any un-blinding episode is classes as adverse event. The investigator must assess the relationship of the AE/SAE to the study drug. In all cases the CI and monitor must be notified within 24 hours after the code has been broken.

The breaking of the code (but not the result) and the reasons for doing so must be documented in the CRF, and medical notes. Written information will be disseminated to the Data Safety Monitoring Committee for review in accordance with the DMC Charter.

The blind will be maintained, where appropriate, for staff involved in data analysis and interpretation. It is important that the PI only divulges the treatment allocation to individuals on a strictly need to know basis.

The un-blinding envelopes will be monitored to ensure security throughout the study.

13.8 Trial Schedule

13.8.1 Schedule of Treatment for each visit

The following procedures will be conducted after arrival of the subject at the hospital.

Screening:

- Assess eligibility (refer to inclusion/exclusion criteria).
- Check that written agreement has been given to participate in the study in the form of a signed and witnessed informed consent form.
- Review medical and trauma history.
- Pregnancy test: by either urinary, serum, or point-of-care (see Fromm *et al.* 2012) testing of human chorionic gonadotrophin (hCG) for women of childbearing age.
- Vital signs (heart rate, blood pressure, respiration rate, oxygen saturations, body temperature, Glasgow Coma Score - GCS).
- Dispense IMP in accordance with the randomization sequence.

Dosing:

- Body weight

- IMP administration
- Obtain blood samples for PK/PD analysis
- Monitor AEs and SAEs.

The following procedures will be conducted after dosing of the subject.

0 to 12 hours

- Blood sampling for PK/PD analysis
- Record baseline arterial blood gas.
- Monitor and record AEs and SAEs.
- Sequential organ failure assessment (SOFA) score.
- Renal injury, as per the risk, injury, failure, loss of function, endstage renal failure (RIFLE) score.
- Lung injury as per the Berlin definition.
- Infection rate.
- Number of ventilation free days.
- Renal Replacement Therapy (RRT) days.
- Vasopressors days.
- Composite time to complete organ failure (CTCOFR) score.
- Total length of critical care stay.
- Total length of hospital stay.

0 to 48 hours:

- Monitor and record AEs and SAEs.
- Record safety blood results.
- Record 24 hour fluid and transfusion requirements.
- Record concomitant medications.
- Sequential organ failure assessment (SOFA) score.
- Renal injury, as per the risk, injury, failure, loss of function, endstage renal failure (RIFLE) score.
- Lung injury as per the Berlin definition.
- Infection rate.
- Number of ventilation free days.
- Renal Replacement Therapy (RRT) days.
- Vasopressors days.
- Composite time to complete organ failure (CTCOFR) score.
- Total length of critical care stay.
- Total length of hospital stay.
- Record whether patient is alive and free of multiple organ failure (SOFA$\leq$5) at 48 hours

49 hours to Day 7:

- Monitor AEs and record SAEs.
- Record safety blood results.
- SOFA score.
- Renal injury, as per the RIFLE score.
- Lung injury as per the Berlin definition.
- Infection rate.
- Number of ventilation free days.
- RRT days.

- Vasopressors days.
- CTCOFR score.
- Total length of critical care stay.
- Total length of hospital stay.

Day 8 to Day 14:

- Monitor AEs and record SAEs.
- Record safety blood results (day 14 +/- 1).
- Renal injury, as per the RIFLE score.
- Lung injury as per the Berlin definition.
- Infection rate.
- Number of ventilation free days.
- RRT days.
- Vasopressors days.
- CTCOFR score.
- Total length of critical care stay.
- Total length of hospital stay.

Day 15 to Day 28:

- Monitor AEs and record SAEs.
- Record safety blood results (day 27 +/- 1)
- Renal injury, as per the RIFLE score.
- Lung injury as per the Berlin definition.
- Infection rate.
- Number of ventilation free days.
- Total length of critical care stay.
- Total length of hospital stay.
- Mortality status.

Until discharge:

- Number of ventilation free days.
- Total length of critical care stay.
- Total length of hospital stay.
- Mortality status.

Day 90 (Follow-up):

- Mortality status.

13.8.2 Schedule of Assessment (in Diagrammatic Format)

ASSESSMENT	SCREENING	RANDOMISATION/DOSING	FOLLOW-UP ASSESSMENTS						
	0-4HRS	0-4 HRS	0-12 HRS	13-48 HRS	49 HRS -DAY 7	DAY 7- 14	DAY 15 - 28	DISCHARGE	DAY 90**
Confirm eligibility	X								
Informed Consent	X								
Demographic Information	X								
Pregnancy Test	X								
Medical History	X								
Vital signs	X								
Body Weight		X							
Study Treatment		X							
PK/PD Sampling*		X	X						
Safety bloods sampling*			X	X	X	X	X		
Monitor AEs			X	X	X	X	X		
Monitor SAEs			X	X	X	X	X		

Concomittant medications			X	X					
SOFA Score			X	X	X				
Renal injury			X	X	X	X	X		
Lung injury			X	X	X	X	X		
Infection rate			X	X	X	X	X		
Mechanical ventilation days			X	X	X	X	X	X	
RRT days			X	X	X	X			
Vasopressors days			X	X	X	X			
CTCOFR Score			X	X	X	X			
ACCU length of stay			X	X	X	X	X	X	
Hospital length of stay			X	X	X	X	X	X	
Mortality Status							X	X	X
Alive and free of MOF				X					

*Please See section 13.8.3 for detailed PK sampling and clinical bloods sampling time points.
**End of Study

13.8.3 Trial assessments

Medical History

The medical and trauma history will be conducted at screening and should include (but not be limited to) demographic information, subjects' medical history with an emphasis on the trauma, current medication, past medication and allergies. This will be ascertained as part of the subjects' initial clinical assessment by the trauma team, and the Trauma Research Fellow. The known medical history must be documented in the subject's notes (the on-site source document) prior to IMP administration and also recorded in the CRF. Any further medical history ascertained subsequent to the initial assessment will be derived from the subjects' notes during the assessment phase.

Vital signs

Vital signs will be recorded at baseline and at PK/PD sampling points. They will also be monitored for 28 days as part of the SAE assessment process.

Systolic and diastolic blood pressures will be recorded using a standard sphygmomanometer reading or by invasive arterial monitoring (where this has been inserted as part of routine clinical care). Pulse rate and oxygen saturations will be measured by pulse oximetry or by arterial monitoring as above. Respiration rate will be assessed by observation as breaths per minute; and a tympanic body temperature measure will be used, unless other methods (e.g rectal monitoring) are in place for continuous clinical monitoring. GCS will be recorded from the subject notes or assessed clinically.

Pregnancy test

Prior to administration of any IMP a pregnancy test will be undertaken on all women of childbearing potential.

Note: The subject must not be dosed if any tests are positive.

Body weight

Body weight will be estimated to the nearest 10kg.

PK/PD Blood samples

Subjects will have 10mls of blood drawn to determine the plasma levels of Artesunate and its metabolite DHA (PK-PD study in man) at the following time points:

- At hour 0 (within 10 minutes) immediately prior to IMP dosing
- 5 (±1 minute) minutes after the start of IMP infusion
- 30 (±2 minutes) minutes after the start of IMP infusion
- 1 (±5 minutes) hours after the start of IMP infusion
- 4 (±20 minutes) hours after the start of IMP infusion
- 12 (±30 minutes) hours after the start of IMP infusion

The total 60 mls of blood sampled equates to approximately 1% of the total circulating blood volume over the 12-hour period. As recruited patients will be receiving blood transfusions as part of their standard clinical care during this time, it is not anticipated that the samples taken will have any

adverse effects on clinical outcomes. Blood that is collected and not used for PK/PD analysis will be processed and stored for use in ancillary research (See Section 13.3.9)

Clinical Blood Sampling

In order to calculate SOFA and RIFLE scores, ascertain presence of infection, and degree of shock on admission, record will be made the following parameters as part of routine clinical bloods (i.e. no additional specific research blood draws will be taken):

- Platelets
- Creatinine
- Estimated Glomerular Filtration rate (eGFR)
- Bilirubin
- Total white blood cells
- Neutrophils
- Lymphocytes
- C-reactive protein
- Arterial or venous blood gas result (admission result only)

In addition, in order to ensure adequate safety of the IMP, the following will be monitored and for the purposes of safety monitoring for AEs and SAEs:

- Haemoglobin
- Haemocrit
- Red cell count
- Mean cell volume
- Mean corpulscular haemoglobin (MCH)
- Red blood cell distribution width (RDW)
- Mean corpuscular haemoglobin concentration (MCHC)
- White cell count
- Platelet count
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Alkaline phosphate
- Gamma-glutanyl transpeptidase (GGT),
- Bilirubin
- Creatinine
- Urea
- Sodium
- Potassium
- Calcium
- Albumin
- Prothrombin Time
- International Normalised Ratio (INR)

These parameters will be collected daily for days 1-7, and subsequently on day 14±1, day 21±1, and day 27±1, provided the subject is still in hospital. Where possible, these data will be collected from

routine clinical bloods. In the event they have not been undertaken as part of routine clinical bloods, the research fellow will take the bloods at the specified times (unless the subject declines).

Radiological Investigation Results

Radiological investigations (including Chest X-rays and CT scans) undertaken as part of a subjects' clinical course, will be examined for calculation of ARDS scores as well as for assessment of adverse events. No specific radiological investigations will be undertaken for research purposes.

Outcome Measures

Please see Appendix 2 for a full description of the outcome measures that will be recorded, including: SOFA Score, Renal injury (RIFLE Score), Lung Injury (ARDS Score), Infection rate, and days of organ failure (CTCOFR score).

Ventilator free days will be calculated by the subtracting the number of days spent on mechanical ventilation from 28.

Renal replacement therapy days will be calculated as the number of days spent on haemodialysis or haemofiltration.

Vasopressor days will be calculated as the number of days spent on ionotropic drugs, including for instance noradrenaline, dobutamine, vasopressin.

Length of stay will be recorded in days, for the total number spent in ACCU and in Hospital.

Mortality will be recorded at 28 days, discharge and 90 days.

At 48 hours it will be recorded whether the patient was alive and free of multiple organ failure (defined as SOFA$\leq$5).

13.8.4 Follow-up Procedures

There are no specific follow-up procedures for subjects once they have been discharged or completed the 28-day trial period. Trauma Research Fellows will collect study information including AEs and SAEs by as per the Schedule of Assessment (Section 13.8.2).

In order to ensure completion of follow-up for SAEs for subjects who are discharged from hospital prior to 28 days, an investigator will attempt to contact the subject or their General Practitioner by telephone on or around 28 days to encourage reporting of any SAEs occurring following discharge. Subjects will be provided with details of whom to contact if they have any questions regarding the trial or wish to provide further information.

Study patient 90-day mortality follow-up shall be conducted using data obtained via the NHS spine services (Health and Social Care Information Centre). It is not practicable to attempt to approach subjects to request "their" survival data; such actions are precluded because there is sufficient potential to do harm and cause distress by inadvertently approaching the families of deceased study subjects.

13.9 Withdrawal criteria

Every reasonable effort will be made to maintain protocol compliance and to retain patient participation in the study consistent with the provisions of informed consent and good clinical practice. The following are potential reasons why a patient may be withdrawn from the study:

1. Withdrawal of consent: the patient, the patient's authorised representative, independent physician, or designated individual who had provided initial consent to enter the study may withdraw consent at any time throughout the duration of the trial, without prejudice to future medical care and treatment.
2. Retrospective exclusion: If a patient is deemed to not meet one or more of the inclusion/exclusion criteria in retrospect they will be withdrawn from the study.
3. Major protocol deviation from the study design by the subject, observed or suspected by the investigator.
4. Adverse event.
5. Administrative: the sponsor or monitoring committees decide to terminate or discontinue the study.
6. The subject's health would be jeopardised by continued participation and is withdrawn at the discretion of the investigator.

Data Collection And Follow-Up For Withdrawn Subjects

If a patient is withdrawn from the study before receiving IMP but after randomisation, any ongoing SAE experienced by the patient, which are not injury- or standard-treatment related, will be followed to resolution but no other safety or efficacy assessments will be conducted.

Patients who withdraw from the study after receiving IMP should be followed for safety by conducting safety assessments through to the end of day 28. If a patient who withdraws has develops an SAE during the monitoring period, every effort must be made to follow such events until satisfactory resolution is obtained or until further follow-up is no longer warranted.

Any samples obtained from withdrawn participants will be discarded in accordance with the Bart's Health NHS Trust standard procedures.

Replacement Of Subjects

Subjects who do not complete the primary outcome end-point at 48-hours (e.g. due to death or discharge) will be replaced. Subjects who withdraw from the study at any time point will also be replaced. Withdrawn participants will not be permitted to re-enter the study.

13.10 Early withdrawal

The study withdrawal form will be completed for these patients and a reason for withdrawal captured. All subject's withdrawn from the study will be managed in accordance with the hospital's standard procedures.

13.11 End of trial (EOT)

The end of study definition is when all surviving Subjects complete 90-day mortality check.

The CI is delegated the responsibility of submitting the EOT notification to REC and MHRA once reviewed by sponsor. The EOT notification must be received by REC and MHRA within 90 days of the end of the trial.

If the study is ended prematurely, the Chief Investigator will notify the Sponsor, REC & MHRA including the reasons for the premature termination (within 15 days).

14.0 Laboratories and samples

14.1 Central Laboratories

Trauma Research Laboratory, Ward 12D, The Royal London Hospital, Barts Health NHS Trust & The Blizard Institute, Queen Mary, University London will perform the preparation of blood samples taken for the PK/PD study, as well as for any ancillary studies.

Professor Nick White, Mahidol Oxford Tropical Medicine Research Unit, Bangkok will perform liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) on plasma samples collected for the PK/PD study.

14.2 Local Laboratories

Department of Haematology and Department of Clinical Biochemistry, The Royal London Hospital, Barts Health NHS Trust, will perform the analysis of routine clinical blood samples taken.

14.3 Sample Collection/Labelling/Logging

Blood for PK sampling (up to 10 mls per sampling time point) will be drawn from either a central, arterial or large bore peripheral line sited for purposes of patient care or, from the femoral vein or antecubital fossa. The sample will be processed in the Trauma Research Laboratory to extract plasma for the PK/PD study. Plasma samples will stored at $-80^0C \pm 10^0C$ prior to analysis.

Guidelines for sample preparation, storage, packaging and shipping are provided to the study site by the independent analytical laboratory (See Appendix 1). Once blood is drawn, it will be collected in two sodium fluoride/potassium oxalate tubes (1 ml in each tube) and placed on wet ice. In the Trauma Research Laboratory, 200 uL from one of the tubes will be transferred to tubes containing potassium dichromate 0.4M to stabilise the blood samples and frozen at $-80^0C \pm 10^0C$. The second sodium fluoride/potassium oxalate tube will be centrifuged within 15 minutes of collection at 2000xg for 7 minutes and a temperature of 4^0C, after which the plasma will be transferred into a screw cap cryovial and frozen at $-80^0C \pm 10^0C$.

For excess blood not used for PK sampling, consent will be sought to store serum and whole blood samples for ethically approved future research studies. Serum will be collected in a clot activator gel separated 5 ml tube (BD Biosciences, UK) and centrifuged at 1000xg for 10 minutes at room temperature. Whole blood will be collected in PAXgene 2.5 ml (PreAnalytiX, Germany) tubes. PAXgene tubes and serum will be catalogued and stored immediately after processing at $-80^0C \pm 10^0C$ at Trauma Research Laboratory, The Royal London Hospital, Barts Health NHS Trust & The Blizard Institute, Queen Mary, University London.

All samples collected for use in the central laboratories will be labelled and recorded on the biological sample log detailing the subject unique study ID, date of collection, sample number, sample storage temperature and date of shipment.

All samples will be de-identified.

14.4 Sample Receipt/Chain of Custody/Accountability

Samples for PK analysis will be transferred from the Trauma Research Laboratory, Barts Health NHS Trust & The Blizard Institute, Queen Mary, University of London, to the central laboratory at Mahidol Oxford Tropical Medicine Research Unit, Bangkok. The research team will be responsible for ensuring samples are packaged appropriately in a clearly labelled primary container and placed in a leak proof secondary container. Samples will be frozen in an upright position and the labelling of boxes and each sample will be clear and freeze resistant. Qualified couriers will be used for transportation of samples to the Mahidol Oxford Tropical Medicine Research Unit with ensured cold chain control.

Upon arrival at the analytical laboratory samples will be logged on the sample receipt log detailing the unique subject ID, date of arrival, sample number and physical integrity of the sample. If the sample has been compromised in transit the study team as well as the sponsor will be informed.

14.5 Sample Analysis Procedures

Determination of the plasma levels of Artesunate and DHA will be performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) as described below. The analysis will be carried out in a blinded fashion by an independent laboratory (Mahidol Oxford Tropical Medicine Research Unit, Bangkok), which has helped to develop the PK-PD assays to measure Artesunate and DHA in human plasma (Hanpithakpong et al., 2008; Lindegardh et al., 2008; Hendriksen et al., 2013) as well as the External Quality Control program for anti-malarial drugs organised by the Word-Wide Antimalarial Resistance Network (WWARN) and has a significant expertise in the determination of Artesunate and DHA in animals and man.

The plasma concentrations of Artesunate and DHA will be measured using liquid chromatography–tandem mass-spectrometry (see Hanpithakpong *et al.*, 2008 for extensive methodology). Quality control samples at low, middle, and high concentrations will be analyzed in triplicate within each analytical batch to ensure accuracy and precision during the analysis. The total coefficients of variation are <8% for all quality control samples. The lower limit of quantification was set at 1.2 ng/ml for ARS and 2.0 ng/ml for DHA (Hendriksen *et al.* 2013).

14.5.1 The arrangements for sample analysis

Samples for analytic studies of PK/PD analysis will be shipped to the Mahidol Oxford Tropical Medicine Research Unit, Bangkok, either as a single shipment at the end of the study, or with an additional half-way batch (approx. n=50) during the recruitment phase of the project, as agreed with the sub-contractor. The storage conditions and shipment of the samples will be conducted according the specialist laboratory instructions (see Appendix 1)

Samples will be shipped in a clearly labelled primary container and placed in a leak proof secondary container. Samples will be frozen in an upright position and the labelling of boxes and each sample will be clear and freeze resistant. Qualified couriers will be used for transportation of samples to the Mahidol Oxford Tropical Medicine Research Unit with ensured cold chain control. The research team will be responsible for arranging couriers. Please see Appendix 1 for a summary of the sample analysis and shipping arrangements as provided by the laboratory.

The Mahidol Oxford Tropical Medicine Research Unit does not supply sample collection kits for PK/PD analysis to the recruitment site. The study site will procure all sample collection tubes.

Ancillary studies related to the trial may involve the analysis of participant DNA (See Section 14.3 and 13.3.9), provided the subject has given informed consent. Information regarding samples taken for genetic research will be disseminated in the participant information sheet.

Samples taken for PK/PD analyses will initially be processed on the recruitment site to extract plasma (see Section 14.3), and shipped samples will consist of small volumes (less than 1 ml per sample), so we do not envisage any remaining sample following analysis. However, in the event there is sample remaining following analysis, these will be discarded in accordance with the HTA Code of Practice.

Samples used for ancillary studies will be stored at The Blizard Intitute, Queen Mary University of London, in accordance with HTA regulations.

We do not anticipate that the research would produce findings of clinical significance for individual donors or their relatives.

14.5.2 Sample Storage Procedures

Samples will be stored at the Trauma Research Laboratory, Ward 12D, Barts Health NHS Trust and The Blizard Institute, Queen Mary University London prior to transfer to and analysis by the Mahidol Oxford Tropical Medicine Research Unit, Bangkok. Samples will be placed under storage conditions immediately after processing in catalogued cryoboxes.

Samples for PK/PD analysis can be stored for the duration of the study period until analysis either at the end of the study or at the halfway point, as per the specialist laboratory instructions. Serum and PAXgene samples collected for ethically approved future research studies can be stored long-term and will be stored in an ethically approved tissue bank at the Blizard Institute, Queen Mary, University of London in accordance with the Human Tissue Act.

If a participant withdraws consent, the Tissue Custodian, who is the CI, will be informed and give approval to the lab to discard any samples in accordance with the Human Tissue Act and Barts Health NHS Trust policy. This will be documented and accounted for in the accountability/sample destruction log.

Samples will be stored in specialist freezers at $-80^0C \pm 10^0C$. They will be stored in linked-anonymised form using the unique study ID. Only the research team will be able to link stored samples to personal identifiable information. The central analytical laboratory in Mahidol Oxford Tropical Medicine Research Unit, Bangkok, in addition to any collaborators involved with future ethically approved research will only have access to the de-identified samples coded by the unique study ID.

14.6 Sample and Data Recording/Reporting

Data collection will be the responsibility of the principal investigator. Data will be recorded on paper CRFs, which will subsequently be entered onto a secure, password protected study database, (Discovere) by the research team. Queen Mary, University of London, will host the database and

physical storage will occur at a Cerner contracted Data Center owned and operated by Equinix, located in Slough, UK. Equinix are Cerner's Data Center supplier of choice in the UK.

All participant data recorded in the Discovere database will be de-identified, using the study unique study ID. No person identifiable information will be stored in the database. Sample data will be recorded on biological specimens log, which will be password protected and stored on the Barts Health NHS Trust secure server.

Data will be shared with collaborators in a de-identified form, except for safety concerns (to the sponsor or DMC) or with collaborators responsible for the analysis of study results (to the statistician, PK/PD sampling) in which case linked-anonymised (study ID) data will be used. Only the research team will be able to link the unique study ID to personal identifiable information.

14.7 End of study

At the end of the study, any remaining samples from the PK/PD analysis will be destroyed after analysis in accordance with the HTA's Code of Practice.

Any samples obtained for ancillary studies (pending ethical approval), will be stored at The Blizard Institute, Queen Mary University of London, under the institutions storage license from the HTA.

15.0 Trial Medication

15.1 Name and description of investigational medicinal product(s)

Artesunate provided as a white crystalline powder in a glass vial containing 110mg, supplied with a second glass vial of reconstitution diluent which is a sterile phosphate buffer comprised of a mixture of sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous and water for injection to achieve a 0.3 M solution with a pH of 8.0 (7.9-8.1).

The placebo comprises the same, buffered diluent drawn up to the volume that would be necessary to reconstitute active IMP according to the subjects' weight. The sterile phosphate buffer is provided in a glass vial and is comprised of a mixture of sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous and water for injection to achieve a 0.3 M solution with a pH of 8.0 (7.9-8.1).

15.2 Legal status of the drug

Artesunate is currently not licensed for use in the UK. The trial is being carried out under a Clinical Trial Authorisation (CTA). The drug is therefore only to be used by the named investigators, for the participants specified in this protocol, and within the trial.

15.3 Investigator Brochure

The IB, version 2.0 release date 15th March 2016, will be used in this specific trial. Updated versions of the IB will be assessed by the research team and sponsor for potential implications for study participants and the conduct of the trial, and if necessary advice will be sought from the TSG/TMG/DMC. If an amendment to the study protocol is required on the basis of the updated information in the IB, then this will be submitted to the ethics committee as a formal amendment.

15.4 Drug storage and supply

Please refer to Section 15.7 -15.9, and Appendix 1 for procedures regarding the shipment, receipt, distribution, return and destruction of IMPs including placebo.

15.5 Supplier

The IMP supplier is SIgma Tau Industrie Farmaceutiche Riunite S.p.A, 47 Viale Shakespeare, Rome, Italy. The IMP is provided free of charge for use in the trial. Trial stock supplies will be utilised.

15.6 Manufacturer

Name and address of the manufacturing source:
Sanofi-Aventis S.p.A., Garessio Site, Via R. Lepetit, 142, 12075 - GARESSIO (CN), Italy

Name and address of the administrative source:

Sanofi-Aventis Viale Bodio 37/b, 20158 – MILANO, Italy

Manufacture (sterile filtration of artesunate), testing and release of sterile drug substance:
Farmabios S.p.A. Via Pavia, 1, 27027 Gropello Cairoli (PV) , Italy

Sterility testing of the sterile artesunate:
Eurofins Biolab S.r.l., Via Buozzi, 2, 20090 Vimodrone (MI), Italy

15.7 How the drug should be stored

Upon receipt of the IMP shipment, Barts Health NHS Trust Pharmacy will conduct an inventory and return an acknowledgement that all IMP was received as stated and undamaged. Five doses of the IMP will be stored in a secured limited-access area, in the Trauma Sciences Lab situated on Ward 12D in the Royal London Hospital, Barts Health NHS Trust. This is to allow immediate access of the IMP to Trauma Research Fellows' upon enrollment of a subject as administration time critical. Trial team members will perform a daily check on the Ward IMP supply to ensure stock is maintained. The Trauma Research Fellow/Assistant will be responsible for contacting pharmacy for re-supply, and this will also be recorded.

Barts Health NHS Trust Pharmacy (Royal London Hospital) and trial team members storing IMP in the Trauma Sciences Lab (Ward 12D, Royal London Hospital, Barts Health NHS Trust) will be responsible for storing the IMP in line with local procedures and excursion management. If the IMP does experience an excursion outside the ranges as stipulated by the manufacturer the product will be quarantined and guidance will be sought from the IMP manufacturer (Sigma Tau), who will confirm whether the supplies are suitable for use, or whether they require replacement.

All study dry-fill Artesunate and phosphate buffer solution must be stored in a secure limited-access area, at a controlled temperature of up to 25 degrees Celsius, which will be monitored and recorded and protected from light. Once reconstituted the Artesunate should be administered as soon as possible, and used within one hour. The investigator will maintain accurate records of the disposition of all IMP supplies received during the study and complete the accountability log for all IMP assigned to a subject.

15.8 Details of accountability

The investigator or designee will be responsible for IMP accountability. Barts Health NHS Trust Pharmacy (Royal London Hospital) is responsible for maintaining records of the products delivery to the trial site, supply to the Trauma Sciences Lab (Ward 12D, Royal London Hospital, Barts Health NHS Trust) and reconciliation of all IMP received by the site. Records will include dates of receipt, transfer and reconciliation, quantities, batch/serial numbers and expiration dates.

Trial team members have responsibility for maintaining the IMP stock in the Trauma Research Lab (Ward 12D, Royal London Hospital, Barts Health NHS Trust), and administering of IMP to subjects enrolled in the study. All empty or unused IMP vials will be returned to the Pharmacy within 24 hours of administration (or the next working day if out-of-hours) by the trauma research fellow for reconciliation. Records will include dates of receipt, administration to trial participants and of return of empty or unused vials to the Pharmacy for reconciliation quantities. Record will be made of batch/serial numbers, expiration dates, and the study ID assigned to the IMP and trial subjects.

15.9 Medication destruction/return and Recall

All IMP that is to be destroyed will be documented and accounted for in accountability/drug destruction logs. No IMP will be discarded or destroyed until it has been reconciled by the study monitor. IMP will be destroyed on-site by Barts Health NHS Trust Pharmacy in accordance with local guidelines. The certificate of destruction of the unused quantities will be supplied to the IMP provider.

No IMP will be provided to subjects or leave the control of the hospital.

The clinical study team will retain all empty or unused vials of IMP for reconciliation and ensure return of the vials to the site pharmacy within 24 hours of administration (or the next working day if out-of-hours).

In the event of an IMP recall by the supplier, all IMP will be quarantined by Barts Health NHS Trust Pharmacy, halting the chance that the IMP is administered to patients.

15.10 Prescription of IMP / Placebo/NIMP

Subject weight will be estimated on arrival to the nearest 10kg and the dose derived from this. Barts Health NHS Trust pharmacy department will hold and provide investigational product to the Trauma Sciences research laboratory where the next five patient doses (un-mixed) will be held in a monitored storage area (see also Section 15.7). The pharmacy will be provided with a current site delegation log to ensure they are aware of who the assigned prescribing healthcare professionals working on the study are.

15.11 Preparation and labelling of IMP

Packaging and labelling of the IMP and diluent will be performed by Fisher Clinical Services, in accordance with regulations outlined in Annex 13 (Manufacture of Investigational Medicinal Products). However, the IMP will not be pre-labelled with individual patient details by pharmacy prior to provision to the research team. This is required due to the time-critical nature of the study necessitating immediate access of the IMP to research members enrolling patients into the study (See also sections 15.7 and 15.14).

Artesunate powder will be supplied in a clear 17 ml type I glass vial secured with a chlorobutyl rubber stopper and a snap-off aluminium seal. Phosphate buffer solution will be supplied in a clear 11.8 ml type I glass vial, secured with a bromobutyl rubber stopper and flip-off cap. Please see Section 15.12 for details on how the drug and placebo are to be prepared.

15.12 Preparation and Administration of IMP

The dose of Artesunate will be 2.4mg/kg or 4.8mg/kg depending on the stage of the study or placebo and is administered as a single dose.

It is necessary to reconstitute the IMP (by mixing of the phosphate buffer solution and the dry-filled Artesunate) immediately prior to administration. This is due to the inherent instability of Artesunate in aqueous solutions.

The sterile phosphate solution is packaged as 11.5 mL in 11.8 mL vials to allow clinicians to withdraw a full 11.0 mL for injection into vials containing Artesunate (110 mg/vial). The result in the drug vials is 11 mL of drug solution at a concentration of 10 mg/mL. For treatment purposes, 10 mL can be easily withdrawn from the prepared vials. All solubilized drug should be used within one hour of preparation.

The IMP will be prepared as follows:

1. Intravenous Artesunate is a dry fill product requiring administration within one hour of drug reconstitution. Each vial contains 110 mg of Artesunate powder.

2. Before initiating mixing, the required dose (total mg of Artesunate) is calculated using the patient's weight (in kilograms). The volume of Artesunate solution (10 mg/mL) to be used can be calculated by dividing the necessary dose by 10.

3. The ampoule of 11.5 mL of sterile phosphate solution should be opened and 11 mL of the solution drawn into a 20-mL syringe labeled with the patient's name and study number, date, time, and total dose of IMP.

4. The buffer is slowly injected into the Artesunate vial (against the wall) and gently inverted for approximately 6 minutes to ensure complete dissolution (an Artesunate solution of 10 mg/mL). **After reconstitution, the drug shall be used within 1 hour.**

5. Using the same syringe, the required amount of the 10 mg/mL Artesunate solution should be drawn back into the same syringe.

6. Following reconstitution of the active IMP, the administering investigator will confirm adequate reconstitution (i.e. no particulate matter visible in the vial and/or discolouration).

7. The IMP will subsequently be administered to the patient into an established intravenous access line through an 8µm (or below) syringe filter, over 1-2 minutes. The drug should be used within one hour of reconstitution.

The Placebo will be prepared as follows:

1. The required volume of placebo (phosphate buffer solution only) to be given is calculated to the same volume as if the patient were receiving a dose of active IMP (See Step 2 above).

2. The ampoule of 11.5 mL of sterile phosphate solution should be opened and the required volume of the solution drawn into a 20-mL syringe labeled with the patient's name or study number, date, time, and equivalent dose of active IMP.

3. After a period of 6 minutes, the sterile phosphate buffer solution will be administered as into an intravenous line using an 8µm (or below) syringe filter, as an injection over 1-2 minutes, and within one hour of preparation.

4. Blinding will be maintained as far as possible as described in Section 13.6.

The research fellow will record the start and stop time of all dosing. All details of the active IMP or placebo infusion will be recorded, including any interruption and the reason. Any dose reductions or delays will be recorded.

15.13 Dosage schedules

Artesunate will be administered as an intravenous preparation due to the critical illness of the subjects enrolled that will preclude oral administration. Intravenous Artesunate has been more widely studied within the literature compared to intramuscular Artesunate (Jones *et al.* 2007) and is therefore the preferred route of administration in this trial.

Participants will be dosed after randomisation has taken place. A 2.4mg/kg or 4.8mg/kg single bolus dose of artesunate solution or placebo will be calculated depending on the stage of the trial. The bolus dose will be administered once as an intravenous injection over 1-2 minutes, within 4 hours from the time of injury and within 2 hours of admission.

15.14 Dispensing of IMP

Each member of staff who administers the IMP will sign the local pharmacy (Barts Health Pharmacy, Royal London Hospital) dispensing-log in order to track the IMP. All trial team members will have had study specific training in order to administer the IMP, which will be recorded in a delegation log.

Five doses of the IMP will be stored in a secured limited-access area, in the Trauma Sciences Lab situated on Ward 12D in the Royal London Hospital, Barts Health NHS Trust. This is to allow immediate access of the IMP to Trauma Research Fellows' upon enrollment of a subject as administration time critical.

Trial team members will perform a daily check on the Ward IMP supply to ensure stock is maintained. The Trauma Research Fellow/Assistant will be responsible for contacting pharmacy for re-supply, and this will also be recorded.

15.15 Dosage modifications

The IMP will be administered as a single bolus dose based on the participant's weight, and no further dose modifications are required in patients with co-morbid conditions (e.g. renal or liver dysfunction) or concomitant therapies.

If the patient experiences an adverse toxicity reaction to the IMP, whilst the single bolus dose is being administered, administration of the IMP will be stopped and no further IMP will be administered. Any dose reductions or delays occurring for other reasons (e.g. problems with intravenous access, etc.) will clearly be documented on the CRF.

There are no rescue medications in the event of toxicity reactions. Treatment will consist of general supportive measures.

Experience of acute overdose with Artesunate is limited. A case has been documented in a 5-year-old child who was inadvertently administered rectal Artesunate at a dose of 88 mg/kg/day over 4

days, representing a dose more than 7-fold higher than the highest recommended Artesunate dose (Campos *et al*., 2008). The overdose was associated with pancytopenia, melena, seizures, multiorgan failure and death. Treatment of overdose should consist of general supportive measures.

15.16 Known drug reactions and interaction with other therapies

Artesunate is rapidly and extensively converted to dihydroartemisinin (DHA), the active metabolite, primarily by plasma and erythrocyte esterases. DHA elimination is also rapid (half-life approximately 45 min) and the potential for drug-drug interactions appears limited. In vitro drug-interaction studies have demonstrated minimal effects of artesunate on cytochrome P450 isoenzymes. Few clinical drug-drug interaction studies have been performed, however no clinically significant interactions have been identified (See Investigators Brochure, Version 1.0, Dated 6th January 2016).

There are no prohibited concomitant medications or therapies. Contraindications include:

- Serious adverse reaction or hypersensitivity to artesunate or any other artemisinin.
- Pregnancy due to the risk of spontaneous abortion, especially during the first trimester.

15.17 Prior and Concomitant medication

There are no restrictions on prior therapies taken by a subject and concomitant therapies and they are permitted concurrently with the trial medication. Investigational drugs, devices, procedures or therapies (other than the trial medication) will not be used during the subject's participation in the study because of their potential to confound the results. Enrollment into a strictly observational trial is permitted, as long as the study assessments in this trial are not compromised.

Prior therapies taken by a subject and concomitant therapies subject receives for the first 48-hours will be captured in the CRF.

15.18 Trial restrictions

There are no trial restrictions.

15.19 Assessment of compliance

There is no compliance measurement requirement for this trial. The IMP is administered as a single dose by qualified clinically trained research fellows at the hospital.

15.20 Name and description of each Non-Investigational Medicinal Product (NIMP)

N/A

15.21 Arrangements for post-trial access to IMP and care

There are no arrangements for post-trial provisions after completion of the trial.

17 Pharmacovigilance

17.1 General Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death. • Is life-threatening. • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity. • Consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "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.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, judged by either the reporting investigator or the Sponsor as having a reasonable causal relationship to a medicinal product.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Reference Safety Information (RSI): • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product. • In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

17.2 Site Investigators Assessment

The Principal Investigator is responsible for the care of the participant, or in his/her absence an authorised medic within the research team is responsible for assessment of any event for:

- Seriousness
  Assessing whether the event is serious according to the definitions given in section 17.1.

- Causality
  Assessing the causality of all serious adverse events/reactions in relation to the trial treatment according to the definition given. If the SAE is assessed as having a reasonable causal relationship, then it is defined as a SAR.

- Expectedness
  Assessing the expectedness of all SARs according to the definition given. If the SAR is unexpected, then it is a SUSAR.

- Severity
  Assessing the severity of the event according to the following terms and assessments. The intensity of an event should not be confused with the term "serious" which is a regulatory definition based on patient/event outcome criteria.

    - **Mild**: Some discomfort noted but without disruption of daily life
    - **Moderate**: Discomfort enough to affect/reduce normal activity
    - **Severe**: Complete inability to perform daily activities and lead a normal life

17.3 Reference Safety information

IB (Version 1.0, Section 6.10, Dated 6th January 2016)

17.4 Notification and reporting Adverse Events or Reactions

If the AE is not defined as SERIOUS, the AE is recorded in the study file and the participant is followed-up by the research team.

Death as a result of disease progression and other events that are primary or secondary outcome measures are often not considered to be SAEs and can be reported in the normal way on the CRF.

17.5 Notification of AEs of special interest

N/A

17.6 Adverse events that do not require reporting

The period for AE reporting is from dose administration to 28 days. The following SAEs are clinical outcome measures and are expected due to the nature of the injuries sustained:

- Deaths
- Infection
- Organ failure (single or multi-)
- Acute lung injury
- Acute kidney injury

The following SAEs are not clinical outcome measures but are expected SAEs due to the nature of injuries sustained:

- Thromboembolic events (deep vein thrombosis, pulmonary embolism)
- Ischaemic events (myocardial infarction, stroke, transient ischaemic attack)
- New-onset major bleeding

New onset major bleeding refers to a second episode of major bleeding which is temporally and clinically separate from the major haemorrhage that led to the entry of the participant into this study.

Any event meeting the definition of an SAE and not listed above will be treated as an 'unexpected SAE' and will require notification as outlined in Section 17.7.

17.7 Notification and Reporting of Serious Adverse Events & SUSARs

All Serious Adverse Event (SAEs) will be recorded in the participants' notes, the CRF, electronic database, the sponsor SAE form and reported to the sponsor (Joint Research Management Office)/IMP provider (as per IMP supply agreement) within 24 hours of the PI or co-investigators becoming aware of the event (excepting those specified in this protocol – Section 17.6, as not requiring reporting). Nominated co-investigators will be authorised to sign the SAE forms in the absence of the PI at the participating site.

Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur during the trial will be reported to the JRMO/IMP provider (as applicable IMP supply agreement) within 24 hours of the PI or co-investigator becoming aware of the event. SUSARs should be reported to the sponsor (JRMO) within 24 hours.

If the AE is not defined as SERIOUS, the AE is recorded in the study file and the research team will follow up the subject.

17.8 Sponsor Medical Assessment

Sponsor has delegated the responsibility for oversight of IMP safety profile and medical assessment of SAEs and SUSARs to the CI. The CI must review all SAEs within 24 hours of receipt. This review should encompass seriousness, relatedness and expectedness. Day 0 for all SUSARs is when the SAE/SUSAR is received by the CI and /or coordinating team and /or sponsor whichever is first.

It is expected that the CI will achieve oversight of IMP safety profile through trial committees as per section 28.0.

It is noted that the CI can upgrade an event to 'related' or 'unexpected' but cannot downgrade the PI or delegate assessment of an event to 'unrelated' or 'expected'. If there is disagreement between CI and PI (or delegate) assessment, it is the CI's responsibility to liaise with the Site PI or delegate before CI's final decisions. The CI and PI/delegate assessment can differ.

17.9 Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health and safety, in accordance with Regulation 30. The measures should be taken immediately. In this instance, the approval of the Competent Authority prior to implementing these safety measures is not required. However, it is the responsibility of the CI to attempt, where possible, to discuss the proposed changed with the Sponsor and Medical Advisor at the MHRA (via telephone) prior to implementing the change if possible.

The CI has an obligation to inform both the MHRA and Research Ethics Committee **in writing within 3 days**, in the form of a substantial amendment. The sponsor (JRMO) must be sent a copy of the correspondence with regards to this matter as soon as it is sent.

17.10 Procedures for reporting blinded SUSARs

The CI as sponsor medical assessor will assess the event blinded for all possible IMPs/combinations.

17.11 Pregnancy

If a patient becomes pregnant whilst involved in a CTIMP, it is not considered to be an SAE or an AE. However, it is an event that requires reporting, monitoring and follow up. If a participant, or participant's partner, becomes pregnant whilst or after taking an IMP the sponsor should be notified immediately (within 24 hours of site becoming aware of the pregnancy) using the sponsor pregnancy form. The pregnancy reporting procedure will be the same as the SAE reporting route.

The CI in conjunction with the site PI/delegate should determine if the foetus has been exposed to an investigational medicinal product. The PI has the responsibility to ensure that the pregnancy form is completed and sent to the sponsor within the agreed timelines. The initial report should be sent within 24 hours and follow up information submitted as and when it becomes available up to agreed follow up time after birth.

Participants are screened for pregnancy prior to enrolment in the trial (and administration of the single IMP bolus dose) and excluded in the event they are pregnant. If a participant subsequently becomes pregnant whilst enrolled in the trial they are they can continue on the study for follow-up assessment and monitoring. The manufacturer will be informed.

Wherever possible, any pregnancy will be followed to term, any premature termination reported, and the status of the mother and child will be reported to the sponsor after delivery. Any events to the mother or child that occur during this time that could be considered to be a SAE must be reported to the sponsor in line with section 17.7, utilising the sponsor SAE reporting form.

18.0 Annual reporting

Development Annual Safety Update (DSUR)
The DSUR will be written by the CI (using Sponsor template) and submitted to the sponsor for review prior to submission to the MHRA. The DSUR is due for submission within 60 days of the end of the reporting period. The reporting period is annually from the date on the "notice of acceptance letter" from the MHRA. As delegated Sponsor Medical Assessor the CI will carry out a risk benefit analysis of the IMPs encompassing all events having arisen on the trial. REC will be sent a copy of the DSUR.

Annual Progress Report (APR)
The APR will be written by the CI (using HRA template) and submitted to the sponsor for review prior to submission to the REC. The APR is due within 30 days of the anniversary date of the "favourable opinion" letter from the REC.

19.0 Statistical and Data Analysis

19.1 Sample size calculation

The primary objective of this trial is to determine the signs of potential efficacy of Artesunate in reducing multiple organ failures and complications. To establish this, the primary comparison of interest will be on the SOFA score at 48 hours.

Each of the Artesunate dose level groups will be compared to placebo. For each dose level the null hypothesis will be that there is no difference in the response rates between Artesunate and placebo, the alternative hypothesis being that the response rate is higher for Artesunate than for placebo.

This is a Phase 2a study and we are looking for safety and efficacy data with which to appropriately power a subsequent definitive 2b trial. A formal sample size calculation is not possible given the lack of existing human efficacy data.

However if Artesunate leads to a clinically relevant 2-point reduction in 48-hour SOFA score, this study will have a power of 80% to detect this difference with 70 intervention and 35 control subjects (α=0.05; σ=3.43) by unpaired t-test.

The intervention arms will consist of 35 subjects receiving Artesunate at 2.4 mg/kg and 35 receiving Artesunate at 4.8 mg/kg. We shall also perform two one-sided t-tests on each of the two intervention arm does levels against the placebo where 35 patients in each arm will detect a 2-point reduction in 48-hour SOFA (α=0.05; σ=3.43) with 78% power.

This should provide enough data on safety profile and potential clinical efficacy to determine whether progression to a 2b study is justified and to develop an appropriate study protocol.

19.2 Planned recruitment rate

This single site study will be performed at the Royal London Hospital Major Trauma Centre, Barts Health NHS Trust. The hospital sees approximately 2200 trauma patients a year, of which 550 are severely injured. In 2014 the number of trauma patients who activated the major haemorrhage protocol (either pre-hospital or in the emergency department) was 143 over the 12-month period.

Our department has previous experience with randomizing patients with major haemorrhage into interventional trials. The Cryostat study (Curry *et al.* 2015), conducted in 2012-13, successfully randomized 31.8% of potentially eligible adult trauma patients with major haemorrhage over a 15-month period. 68% of all major haemorrhage protocol activations were considered eligible for recruitment in that study. Of the randomized patients, only one patient (0.02%) withdrew consent.

Recruitment for the Cryostat study was between the hours of 8 am and 8-10pm, and the reason for non-enrolment of 69% of eligible patients was they fell outside of recruitment hours. For TOP-ART, recruitment hours will be extended to recruit eligible patients 24 hours a day, 7 days a week. Based on the Cryostat study and the extended recruitment hours, it is anticipated that 24 months will provide sufficient duration to recruit the planned number of participants, taking into consideration potential screen failures and participant withdrawals, and the duration of funding.

At present there are no other ongoing interventional trials competing for the same cohort of patients within or outside the department. In the event another competing study is commenced

within the same department, priority will be given to the TOP-ART trial to ensure that recruitment is not compromised. The clinical trial coordinator and lead clinical fellow are responsible for tracking and driving recruitment rates.

19.3 Statistical analysis plan (SAP)

Full details of planned statistical summaries and analyses will be described in a separate Statistical Analysis Plan. Any deviations from the statistical analysis plan will be fully documented in the clinical study report, with the reasons for doing so. A brief summary of the SAP is provided below (see also Sections 19.4-19.13).

An interim analysis will be performed after recruitment of the first 52 patients. The analysis will be limited to an analysis of safety data between the two groups. Provided there are no issues regarding safety or toxicity, the study will be continued with an increased dose in the intervention arm.

The final analysis will be performed according to standard trial best-practice criteria. All data analysis will be performed before the breaking of the randomization code. Standard tests of two-group comparison will be used throughout. One- and two-way ANOVA will be used to analyse time-dependent (pKa) data. Safety data will be re-analysed with the final cohort as outlined for the interim analysis (see Section 19.9).

19.4 Summary of baseline data and flow of patients

Demographic information and baseline characteristics will be summarised for each treatment group by type of variable; categorical data (including gender) using counts and percentages, and continuous variables (including age, ISS, blood products and fluids in the first 24 hours) by means, standard deviations, medians, minima, maxima, and numbers of subjects, as appropriate. Baseline comparability of demographic and baseline characteristics for the three treatment groups will be performed.

A CONSORT flow diagram will be provided to illustrate how the study population was recruited and handled during the course of the study. The number (%) of subjects who withdraw from the study and their reasons for withdrawal will be displayed by treatment group.

19.5 Primary outcome analysis

The primary endpoint is the SOFA score at 48 hours; placebo and treatment groups will be analysed using standard tests of two-group comparison depending on data normality. Intention to treat and per protocol analyses will be performed. The main analysis will be the per protocol analysis. There are no pre-determined subgroup analyses.

19.6 Secondary outcome analysis

Secondary Outcome Analysis

The secondary endpoints that are continuous variables include: maximum SOFA score, mean SOFA Score (Days 2-5), number of days on organ support, number of ventilation-free days, number of days

in hospital, and number of days in critical care. Placebo and treatment groups will be analysed using appropriate standard tests of two-group comparison depending on data normality.

The secondary endpoints that are catagorical variables include: incidence of acute kidney injury as per the RIFLE classification. Secondary endpoints that are nominal variables include: incidence of prolonged organ failure, incidence of acute lung injury, incidence of infection, mortality rate (28-day, 90-day and discharge), and 'alive and free of multiple organ failure' at 48 hours. Placebo and treatment groups will be analysed using appropriate standard tests of two-group comparison.

19.8 Adjusted analysis

The main analysis will be performed on unadjusted data. Continuous variables will be tested for normality and appropriate tests of comparison will be chosen accordingly.

19.9 Interim analysis and criteria for the premature termination of the trial

An interim analysis will be performed after recruitment of the first 52 patients. The analysis will be limited to an analysis of safety data between the two groups. Provided there are no issues regarding safety or toxicity, the study will be continued with an increased dose in the intervention arm.

At the interim analysis, a qualitative assessment of all recorded AEs and SAEs will be undertaken by the DMC to determine if the trial should be discontinued on account of safety concerns. AEs will be coded by system organ class (SOC) and preferred term (PT), using MedDRA. An overview of all AEs, SAEs, relationship to IMP, intensity and AEs leading to withdrawals or death will be presented by treatment group. AEs will be summarised using descriptive statistics. Treatment emergent AEs will be analysed separately.

The number and percentage of subjects with abnormal vital signs and/or laboratory values will be presented by treatment group. The data may also be presented as shift tables (normal, abnormal and not clinically significant, abnormal and clinically significant) with screening values as a reference.

The trial will be terminated following the interim analysis based on the safety data. The criteria for termination (e.g. total SAEs per group) shall be defined by the DMC. Outside the planned interim analysis the study will be terminated only on the grounds of new safety concerns or unforeseen recruitment issues.

The sponsor retains the right to stop the study, should the need arise.

19.10 Subject population

The analysis populations will be defined as follows:

Intent-to-Treat (ITT) Population

The ITT population is defined as all patients randomised according to their treatment allocation irrespective of whether they received that treatment or not.

Per Protocol (PP) Population

The PP population is defined as all patients randomised and treated according to the protocol i.e. based on treatment that patients actually received**.** The primary efficacy analysis will be performed on this study population.

Safety Population

The safety population is defined as all patients randomised who received any partial dose of IMP and will be analysed based on treatment that patients actually received.

19.11 Procedure(s) to account for missing or spurious data

The study team will follow participants daily in order to maximise capture of follow-up data. Missing outcome data will be imputed where appropriate. Reasons for missing data will be recorded on the CRF during data capture where possible (e.g. observation not performed, sample not taken).

19.12 Other statistical considerations.

Any deviations from the original statistical plan will be reported in the clinical study report with reasons for doing so. Participants who withdraw from the study will be replaced. Evaluable patients are defined as those who meet study inclusion and exclusion criteria, provide consent for participation, are randomised to either treatment or comparator group and treated according to protocol, and reach the primary endpoint (SOFA score at 48 hours). Evaluable patients will analysed in the 'per protocol' analysis for comparison against the intention-to-treat analysis.

20.0 Data Handling & Record Keeping

20.1 Confidentiality

The Principal Investigator has a responsibility to ensure that participant anonymity is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval. All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) and all subsequent amendments as defined in the JRMO SOP 20 Archiving.

The Chief Investigator and the study team will adhere to these parameters to ensure that the Participant's identity is protected at every stage of their participation within the study. To ensure this is done accordingly, at time of consent each participant will be allocated a unique screening number by either the PI or a member of the study team before undergoing any screening procedures.

The subject's initials (the first letter of their first name and the first letter of their last name) will be used as a means of pseudo-anoymising parameters. This information will be kept on a screening log, and updated accordingly throughout the study. Once the patient has completed screening procedures and is enrolled onto the study, they will be allocated a unique study ID/randomisation number by the investigator.

Subject identifiable information (name, date of birth, hospital number) will be recorded for the purposes of consent (on the consent log) and data collection including AEs and SAEs (hardcopy Case Report Form). In addition, contact information for the subject (address, telephone number and General Practitioner Details) will be recorded in the consent log in the event an investigator may need to contact the subject or their GP with regards to the trial. Access to all identifiable information will be limited to the study investigators.

If any subject information needs to be sent to a third party (including correspondence/ communication to central laboratories, CROs, sponsor) the PI and the study team will adhere to patient pseudo-anonymous parameters. This includes the patient initials, date of birth, gender as well as the unique study ID/randomisation number. Any information that is to be collected by these third parties will utilise these coded details for any relevant documents as well as maintaining databases.

As Investigator, you agree that all information communicated to you by the sponsor is the exclusive property of the sponsor and you will ensure that the same shall be kept strictly confidential by you or any other person connected with the work and shall not be disclosed by you or such person to any third party without the prior written consent of the sponsor. You shall communicate the results of the work promptly to the sponsor.

All rights and interests worldwide in any inventions, know-how or other intellectual or industrial property rights which arise during the course of and/or as a result of the clinical study which is the subject of the protocol or which otherwise arise from the information or materials supplied under this agreement, shall be assigned to, vest in and remain the property of the sponsor.

All investigators and trial site staff must comply with the requirements of the Data Protection Act 1998 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

20.2 Data Custodian Details

The Chief Investigator Professor Karim Brohi, is the 'Custodian' of the data research data (Address: Centre for Neuroscience and Trauma, Blizard Institute, Barts & The London School of Medicine & Dentistry, Queen Mary University of London, 4 Newark Street, London, E1 2EF. Phone: 077 0319 0545. Email: k.brohi@qmul.ac.uk). Barts Health NHS Trust and Queen Mary University of London policy is that study data should be kept for 20 years following completion of the study.

Subject identifiable information (name, date of birth, hospital number) will be recorded for the purposes of consent and data collection including AEs and SAEs (hardcopy Case Report Form) which will be stored in a secured locked room on site, accessible only to members of the study team. This data will also be stored in a secure, electronic password protected consent log stored on the Barts Health NHS Trust server, along with contact information for the subject (address, telephone number and General Practitioner Details) in the event an investigator may need to contact the subject or their GP with regards to the trial. Access to all identifiable information will be limited to the study investigators.

If any subject information needs to be sent to a third party (including correspondence/ communication to central laboratories, CROs, sponsor) the PI and the study team will adhere to patient pseudo-anonymous parameters. This includes the patient initials, date of birth, gender as well as the unique study ID/randomisation number. Any information that is to be collected by these third parties will utilise these coded details for any relevant documents as well as maintaining databases.

Participants will be anonymised with regards to any future publications relating to this study.

20.3 Psuedononymisation

The participants initials (the first letter of their first name and the first letter of their last name) will be used as a means of pseudo-anoymising parameters. This information will be kept on the screening log, which should be updated accordingly throughout the study. A copy of the screening log will be stored on the Barts Health NHS Trust server as a back-up in the event recovery of the document is required. Once the participant has completed screening procedures and is enrolled onto the study, the participant will be allocated a unique study ID/randomisation number by the PI.

20.4 Transferring/Transporting Data

Any personal data will remain coded and stored securely within the information technology services of Barts Health NHS Trust. De-identified data requiring electronic transfer between sites will be done so in accordance with the UK Data Protection Act 1998, and will be encrypted.

Samples provided to third party laboratories for analysis will be de-identified via the use of code−labels and the code−index will not be divulged to any parties outside of our local research group. Explicit consent will be sought from participants for samples to be sent to the central laboratory based outside the EEA (Mahidol Oxford Tropical Medicine Research Unit, Bangkok).

Anonymous data may be sent to other parties for help with statistical analysis. No patient identifiers will be sent in this case. No un-coded identifiable personal patient information will leave the local research group.

20.5 Data collection tools and source document identification

The trauma research fellow (who is a clinician by training) will be responsible for data collection and for identifying source documentation. Patients who are enrolled into the study will have the relevant study data entered onto a paper CRF using the source documents and materials described in Section 20.6. Study data will subsequently be entered by members of the research team into a secure password protected electronic database (Discovere), in addition to the relevant associated study files such as the consent and sample logs, stored securely on the Barts Health NHS Trust server. Patients who are screened, but not enrolled in the study will have pseudoanonymised data recorded on a screening log.

20.6 Source Data

ICH E6 section 1.51, defines source data as "All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)." ICH E6 1.52, defines source documents as "Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial)."

All documents will be stored safely in confidential conditions.

The following items will be used as source data in this study:

- Subject medical notes including pharmacy dispensing records and clinical observation charts.
- Barts Health NHS Trust electronic Care Records Service for access to clinical laboratory test results, radiology results, general practitioner or subject contact information.
- Subject monitoring equipment (vital signs).
- NHS Spine database for confirmation of 90-day mortality status.
- Correspondences (telephone or writing) with the General Practitioner or Subject.

Data will be collected from source documents, such as weight, vital signs, heart rate, temperature, pregnancy test and recorded on the CRF by the trauma research fellow. Source documents will be examined for the relevant data by trauma research fellows who are clinicians and therefore familiar with handling confidential patient records. Every effort will be made to maintain a participant's confidentiality. Any electronic care records will only be accessed using a password protected log-in on Barts Health NHS Trust computers (Royal London Hospital site). Medical notes and pharmacy/observation charts will be examined on the ward or at the participant's bedside wherever possible.

20.7 Case Report Form

Data collection for this study will be accomplished using a paper case report form (CRF) to capture data prospectively and transferred to an electronic data capture system (Discovere). CRFs are required and will be completed for each randomized subject. It is the Investigator's responsibility to ensure the accuracy, completeness and timeliness of the data reported on the subjects CRF. CRFs will be completed in a timely fashion to support the study timelines. Source documentation supporting the CRF data will indicate the subject's participation in the study and document the dates and details of informed consent and study procedures. Data collected on the CRF will be verified against the source documentation.

Elements included on the CRF are:

- Registration/randomisation number.
- Eligibility/exclusion criteria checklist.
- Visit details.
- Date.
- Drug/dose.
- Any dose reductions/delays.
- AEs.
- Withdrawal from study.
- Follow up of outcomes.
- Death, prior/current medication.
- SAE/SUSAR form.
- Pregnancy form.
- If biosamples have been collected.

20.8 CRFs as Source Documents

The protocol allows data to be entered directly onto the case report forms (CRF), for example, vital signs taken from monitoring equipment. Therefore the CRF is considered a source document. If the CRF is transmitted to the sponsor, the trial site will retain a copy to ensure that the principal investigator can provide access to the source documents to a monitor, auditor, or regulatory agency.

20.9 Data handling and record keeping

The investigator /institutions will keep records of all participating participants (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages. CRF data will be uploaded to a secure database, Discovere. The database will be programmed to alert the user to values falling outside of pre-determined reference ranges prompting them double check the data for potential errors. The database has no interaction with other systems. Documented training will be provided to investigators entering data onto the CRF and electronic database.

It is the responsibility of the Chief Investigator (CI) to maintain adequate records for the study including completed CRFs, signed Informed consent documents, drug disposition records and all correspondence with the REC and the sponsor. Original documents will be retained as part of the audit trail. Any amendments or corrections to original documents will be signed and dated.

The CI must make study data accessible to the monitor, other authorised representatives of the Sponsor, and Regulatory Agency (e.g. MHRA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent form and the Investigator's copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All paper records including the CRF and consent forms will be stored in a secure locked room at the recruiting site (Royal London Hospital, Barts Health NHS Trust). Electronic data containing patient identifiable information will be stored securely in password protected files on the Barts Health NHS Trust server and backed-up regularly. Only de-identifed information, as required by the study protocol and captured on the CRF, will be uploaded onto the Discovere database.

20.10 Access to Data, Source Data and Documents

The Chief Investigator, the Clinical Trial Coordinator and those trauma Research Fellows (who are also clinicians, some of whom may be involved with the direct clinical care of participants) and Research Assistants directly involved in the study will have access to trial documents including those containing personal data.

Direct access will also be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

21.0 Archiving

During the course of research, all records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the Research Governance Framework and Trust Policy that the records are kept for a further 20 years.

Site files from other sites must be archived for 20 years at the originating site external site and cannot be stored at the Barts Health Modern Records Centre or within Queen Mary University of London.

Destruction of essential documents will require authorisation from the Sponsor.

Archiving will be authorised by the sponsor following submission of the end of study report.

For trials involving Barts Health NHS Trust patients, undertaken by Trust staff, or sponsored by Barts Health NHS Trust or Queen Mary, University of London, the approved repository for long-term storage of local records is the Trust Modern Records Centre, which is based at 9 Prescot Street.

22.0 Monitoring, Audit and Inspection

22.1 Monitoring

A Trial Monitoring Plan will be developed and agreed by the Sponsor and Chief investigator based on the sponsors trial risk assessment, this will include on site monitoring. Monitoring procedures are detailed in the Trial Monitoring Plan.

22.2 Auditing

Sponsor retains the right to Audit any trial, trial site or central facility. In addition, any part of the trial may be inspected by the regulatory bodies and funders where applicable.

22.3 Notification of Serious Breaches to GCP and/or the protocol

The Site Principal investigator is responsible for reporting any serious breaches to the sponsor (JRMO) **within 24 hours.**

The Chief Investigator is responsible for reporting any serious breaches to the sponsor (JRMO) **within 24 hours**.

The sponsor will work with the CI to investigate any potential breach and notify and report to the MHRA (as applicable) within 7 working days of becoming aware of the serious breach.

A "serious breach" is a breach which is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase. The sponsor will notify the licensing authority in writing of any serious breach of:

- the conditions and principles of GCP in connection with that trial; or
- the protocol relating to that trial, within 7 days of becoming aware of that breach.

22.4 Compliance

The CI will ensure that the trial is conducted in compliance with the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), and any subsequent amendments of the clinical trial regulations, current Research Governance Framework, GCP guidelines, the World Medical Association Declaration of Helsinki (1996), the Sponsor's SOPs, and other regulatory requirements as amended.

22.5 Non-Compliance

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used e.g. it is not acceptable to enrol a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol.

Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

Systematic failure of both the CI and the study staff adhering to SOPs/protocol/ICH-GCP and UK regulations, which leads to prolonged collection of deviations, breaches or suspected fraud.

Non-compliances may be captured from a variety of different sources including monitoring visits, CRFs, communications and updates. The sponsor will maintain a log of the non-compliances to ascertain if there are any trends developing which need to be escalated.

CI and the coordinating team should assess the non-compliances and action a timeframe in which they need to be dealt with. This assessment should include the need to escalate to the sponsor. Any event with the potential to affect participant safety or data integrity should be reported to the sponsor within 24 hours of the Coordinating team becoming aware.

Where applicable corrective and preventative actions APA should be assigned. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the Sponsor will agree an appropriate action, including an on-site audit.

22.6 Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA.

The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.

Before any site can enrol participants into the trial, the Principal Investigator or designee will apply for NHS permission from the site's Research & Development (R&D) department.

For any amendment that will potentially affect a site's NHS permission, the Principal Investigator or designee will confirm with that site's R&D department that NHS permission is ongoing (note that both substantial amendments, and amendments considered to be non-substantial for the purposes of REC and/or MHRA may still need to be notified to NHS R&D).

This study does not involve ionising radiation.

23.0 Financial and other competing interests for the chief investigator, PIs at each site and committee members for the overall trial management

There are no competing interests.

24.0 Ethical and Regulatory Considerations

Before the start of the trial, approval will be sought from the Research Ethics Committee (REC) and MHRA for the trial protocol, informed consent forms and other relevant documents e.g. advertisements and GP information letters.

Decision whether an amendment constitutes a minor or substantial amendment lies with the sponsor.

Substantial amendments that require review by the Sponsor and REC and MHRA (where relevant) will not be implemented until the REC and or MHRA grants a favourable opinion for the study (note that amendments may also need to be reviewed and accepted by the MHRA and/or NHS R&D departments before they can be implemented in practice).

All correspondence with the Sponsor, REC and MHRA will be retained in the Trial Master File at the lead site and Investigator Site File at each site.

The Chief Investigator will notify the REC, MHRA and Sponsor of the end of the study.

25.0 Peer review

The main scientific peer review was through the Wellcome Trust grant review. In addition, the single-site trial protocol underwent internal Trust peer review chaired by an expert in Critical Care Medicine, who is independent from the trial research group (see approval, dated 12/03/2015).

26.0 Public and Participant Involvement

The Trial Steering Committee will include a patient representative, who will partake in management of the research.

27.0 Indemnity

27.1 Amendments

Under the Medicines for Human Use (Clinical Trials) Regulations 2004, the sponsor may make a non-substantial amendment at any time during a trial. If the sponsor wishes to make a substantial amendment to the CTA or the documents that supported the original application for the CTA, the sponsor must submit a valid notice of amendment to the licencing authority (MHRA) and to the REC for consideration. The MHRA and/or the REC will provide a response regarding the amendment within 35 days of receipt of the notice. It is the sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the MHRA and/or REC.

If applicable, other specialist review bodies (e.g. CAG) need to be notified about substantial amendments in case the amendment affects their opinion of the study.

Amendments also need to be notified to NHS R&D departments of participating sites to assess whether the amendment affects the NHS permission for that site. Note that some amendments that may be considered to be non-substantial for the purposes of REC and/or MHRA may still need to be notified to NHS R&D (e.g. a change to the funding arrangements). For studies with English sites processed in NIHR CSP the amendment should be submitted in IRAS to the lead CRN, which will determine whether the amendment requires notification to English sites or may be implemented immediately (subject to REC/MHRA approval were necessary).

Any amendment will be categorised according HRA definitions and tracked to the most recent protocol version.

27.2 Access to the final trial dataset

The PI/CI, the trial statistician, and delegated study investigators (pending approval by the PI/CI), will have access to the final trial dataset.

28.0 Trial Committees

Trial Management Group

A Trial Management Group (TMG) comprising the Chief Investigator, Clinical Trials Coordinator and lead Clinical Research Fellow will be responsible for the day-to-day running and management of the trial. The Trial Management Group (TMG) will meet regularly to ensure all practical details of the trial are progressing and working well and everyone within the trial understands them.

Trial Steering Committee

The role of the Trial Steering Committee (TSC) is to provide overall supervision for the trial and provide advice through its independent chairperson. In accordance with the Trial Terms of Reference for the TSC, the TSC is responsible for periodically reviewing safety data and liaising with the DMC regarding safety issues. The ultimate decision on continuation or discontinuation of the trial lies with the TSC.

Members of the TSC will include membership from the TMG and independent members including:

Independent Chair
Independent Lead Clinical Experts
Lay-person/Patient Representative
Clinical Trial Coordinator
Co-optable Members including the trial chief investigator, trial statistician and trial collaborators

Data Monitoring (and ethics) Committee

In accordance with the Trial Terms of Reference for the DMC, the DMC are responsible for periodically reviewing un-blinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis. The Regulations require that all Serious Adverse Events (SAEs), unless excluded in the protocol are reviewed by an appropriate Safety Monitoring Committee.

Members of the DMC will be completely independent from the running of the trial. For the purposes of this study, an independent member who can provide expert disease specific advice will join the core DMC.

The group will be called at the interim review for a formal analysis of safety data as outlined in Section 19 of this protocol. The TSG may also request further reviews by the DMC regarding any safety concerns or issues regarding trial management arising outside of the interim review period. The DMC may also provide advice to the Chair of the TSC and can recommend premature closure of the trial.

29.0 Publication and Dissemination Policy

29.1 Publication

An End of Trial Declaration will be submitted to the MHRA within 90 days of the trial end point. Following this a Trial Summary Report, formatted according the CONSORT (Consolidated Standards of Reporting Trials) 2010 guidelines, will be submitted to the MHRA and ethics committees within 6-12 months.

The results will published as soon as possible after trial completion. After discussion with the TSC, a Trial Writing Group will be formed for this purpose by the Chief Investigators, which will include key members of the Trial Management Group. The TSC will oversee the timely analysis, writing up and publication of the main Trial results. Investigators and independent members of the TSC and DMC will be given the opportunity to read and comment on the main trial findings before submission for publication.

The sponsor retains the right to review all publications prior to submission or publication. Responsibility for ensuring accuracy of any publication from this study is delegated to the Chief Investigator.

The full study report will be accessible via Eudra CT.

29.2 Dissemination policy

For the main report of this Phase 2b study submitted for publication, together with associated methodology, we will use the International Committee of Medical Journal Editors definitions of Authorship and Contributorship http://www.icmje.org/ethical_1author.html). The members of the TSC and DMC will be listed with their affiliations in the Acknowledgements/Appendix of the main publication and the support of the Sponsor (Queen Mary University of London) and Funder (Wellcome Trust) acknowledged.

The usual academic channels will be used to disseminate findings e.g. publication in peer reviewed journals, presentation at academic meetings and through patient organisations. We will seek advice from out-patient panels about other ways of disseminating findings to patient groups. Participants can be provided with results of the study on request following completion of the trial. This is highlighted in the Participant and Personal LR Information Sheets.

30.0 References

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This protocol is based on JRMO CTIMP Protocol Template June 2015 version 4.0.

Appendices

APPENDIX 1: Core Lab Instructions to Investigators
APPENDIX 2: Scoring Systems and Definitions
APPENDIX 3: Definitions of Thrombo-embolic and Ischaemic Events

APPENDIX 1: CORE LAB INSTRUCTIONS TO INVESTIGATORS

The purpose of this section is to describe requirements for specimen collection and shipping procedures to the Department of Clinical Pharmacology, MORU in Bangkok, Thailand. This should be considered as a recommendation, aiming to ensure the highest possible sample quality in the pre-analytical phase of collection and shipping during clinical trials.

GENERAL RECOMMENDATIONS

Different matrices require different procedures as specified below.

Whole Blood

Prepare tubes containing anticoagulants. Disinfect area and let dry before venipuncture. Put on tourniquet if required. The tourniquet should be released before withdrawal of blood begins.

Prolonged venous occlusion can change concentrations of blood constituents. It has been shown that the total protein concentration increases significantly in serum if the tourniquet is kept on during a five minute long sampling procedure. Use appropriate needle (size 19G to 22G). If the needle has too small internal diameter, it may lead to haemolysis. Haemolytic samples might exhibit false concentration of the analyte in the plasma and serum if the analyte is present inside the red blood cells. If a syringe is used to withdraw the blood, take off the needle before transferring blood into tubes with anticoagulant. To assure proper mixing with anticoagulant, the tube should be well mixed after blood collection by gently inverting the tube about ten times. Store blood samples at -80 degrees Celsius as soon as possible.

Plasma

Plasma is obtained after centrifugation of whole blood after anticoagulants has been added. Too low centrifugal force (i.e. ~200g) will result in platelet rich plasma. The blood should be cooled and centrifuged as soon as possible and plasma should be separated immediately after centrifugation. The white cell layer (i.e. the buffy coat) should not be transferred with the plasma. Store plasma samples at -80 degrees Celsius (if no drug specific recommendations are available) as soon as possible.

DRUG SPECIFIC SAMPLING RECOMMENDATIONS - ARTESUNATE/DHA

Whole blood

Collect 1 ml whole blood in appropriate collection tubes containing sodium fluoride/potassium oxalate as anticoagulant. The tubes should be pre-chilled on wet ice prior to use. After collection of blood, the tube should be placed on wet ice and processed as soon as possible after collection. Transfer exactly 200 µL blood to tubes containing 40 µL potassium dichromate 0.4M to stabilize the blood samples. Freeze the samples at or below -80 degrees Celsius in a laboratory freezer. **Do not thaw samples after freezing. Record the clock time of sampling and storing.**

Plasma

Collect 1 ml whole blood in appropriate collection tubes containing sodium fluoride/potassium oxalate as anticoagulant. The tubes should be pre-chilled on wet ice prior to use. After collection of blood, the tube should be placed on wet ice and processed as soon as possible after collection (the

samples can be placed in the ice bath for 5 to 10 minutes prior to centrifugation to allow the blood to chill in the tube). Centrifuge blood within 15 minutes of collection. Centrifuge whole blood at (4 degrees Celsius) 2000 x g for 7 minutes to obtain plasma. Immediately after centrifugation, transfer plasma into a screw cap cryovial (Corning No.: 430659 or suitable alternative) and freeze the plasma samples at or below - 80°C in a laboratory freezer. **Do not thaw samples after freezing. Record the clock time of sampling and storing and note any sign of haemolysis.**

Note: Degradation of these drugs (DHA in particularly) is highly dependent upon temperature. Keeping a low temperature (i.e. pre-chilled tubes and ice) as soon as possible after collection and until the sample has been put into the freezer significantly reduces degradation.

SHIPMENT OF SAMPLES

Biological sample shipments that do not contain infectious agents (i.e. plasma, whole blood) should be sent to the address below. Samples should have a clearly labelled primary container placed in a leak proof secondary container and shipped with ensured cold chain control. It is recommended to use a qualified courier e.g. World courier for transportation. Samples must be frozen in upright position and the labelling of boxes and each sample must be clear and freeze persistent.

Consignee address:
Dr. LIJIANG SONG
C/O MAHIDOL-OXFORD TROPICAL MEDICINE RESEARCH UNIT
FACULTY OF TROPICAL MEDICINE
420/6 RAJVITHI ROAD
BANGKOK 10400, THAILAND
Tel. no. 66 2 203-6333 ext. 7308
Fax no. 66 2 354-6018

A scanned or faxed copy of airway bill and invoice/packing list should be sent to the logistic manager before the shipment is dispatched. A detailed sample inventory list in Excel should be sent by mail to Dr. Lijiang Song prior to dispatch of the shipment.

The inventory list should be organized according to a template, *L042 Sample list template*, which can be obtained from the Pharmacology Laboratory.

CONTACT DETAILS

Ms. Pornjarus Sukhapiwat, Logistics manager
Tel. no. 66 2 203-6333 ext. 6336
Fax no. 66 2 354-8000
Email: jiab@tropmedres.ac

Mr. Winai Kaewkong, Logistics officer
Tel. no. 66 2 203-6333 ext. 6313
Fax no. 66 2 354-8000
Email: winai@tropmedres.ac

Dr. Lijiang Song, Head of Laboratory
Tel. no. 66 2 203-6333 ext. 7308
Fax no. 66 2 354-6018
Email: lijiang@tropmedres.ac

APPENDIX 2: SCORING SYSTEMS AND DEFINITIONS

1. **SOFA Score Parameters (Vincent et al. 1998)**

PARAMETER	SCORE BREAKDOWN				
	0	1	2	3	4
PaO2/FiO2 ratio	>53.3	≤53.3	≤40	≤26.7	≤13.3
Platelets	≥150	<150	<100	<50	<20
Bilirubin	<20	20-32	33-101	102-204	>204
CVS	MAP ≥70	MAP<70	Dopamine ≤5 or any dose dobutamine	Dopamine >5 or adrenaline ≤0.1 or noradrenaline ≤0.1 mcg/kg/min	Dopamine >15 or adrenaline >0.1 or noradrenaline >0.1 mcg/kg/min
GCS	15	13-14	10-12	6-9	<6
Creatinine	≤109	110-170	171-299	300-440 or urine <500ml/day	>440 or urine <200ml/day

2. **CTCOFR Scoring parameters (Nadel et al. 2007)**

PARAMETER	DESCRIPTION
Last day vasoactive agent	dobutamine <5 μg/kg/min or cessation of adrenaline/noradrenaline/phenylephedrine or other vasoactive agent
Last day mechanical ventilation	IPPV, CPAP, Bimodal
Last day renal replacement therapy:	peritoneal dialysis, haemodialysis, ultrafiltration, haemofiltration
For each parameter score 1 point per day up to 14 days Total CTCOFR Score if composite >14 days = 15 points Total CTCOFR Score if death during study (28 days) = 16 points.	

3. **RIFLE Score Parameters (Bellomo et al. 2004)**

	EGFR/SERUM CREATININE (Cr)	URINE OUTPUT
Risk	Cr x 1.5 or; eGFR decrease by >25%	<0.5ml/kg/h for 6 hours
Injury	Cr x 2 or; eGFR decrease by >50%	<0.5ml/kg/h for 12 hours
Failure	Cr x 3 or; GFR decrease by >75% or; If baseline Cr >353.6 μmol/L, increase of 44 μmol/L	<0.3ml/kg/h for 24 hours or; anuria for 12 hours
Loss of function	Complete loss of function >4 weeks	
End-stage kidney disease	Complete loss of function > 3 months	

4. Acute Lung Injury Definitions (ARDS Task Force, 2012)

THE BERLIN DEFINITION OF ACUTE RESPIRATORY DISTRESS SYNDROME	
TIming	<1 week of a known clinical insult or new or worsening respiratory symptoms
Chest Imaging[a]	Bilateral opacities not fully explained by effusions, lobar/lung collapse, nodules
Origin of Oedema	Respiratory failure not fully explained by cardiac failure or fluid overload. Need objective assessment (e.g. echocardiography) if no risk factor present.
Oxygenation: **Mild** **Mod** **Severe**	 200mmHg <PaO2/FiO2 ≤300mmHg with PEEP or CPAP[b] ≥5 cmH2O 100mmHg <PaO2/FiO2 ≤200mmHg with PEEP ≥5 cmH2O PaO2/FiO2 ≤100mmHg with PEEP ≥5 cmH2O
a: Chest radiograph or Computed Tomography scan b: This may be delivered non-invasively in the mild acute respiratory distress syndrome PEEP: Positive End Expiratory Pressure; CPAP: Continuous Positive Airway Pressure; FiO2: Fraction of inspired Oxygen; PaO2: Pressure of arterial Oxygenation	

5. Infection Definitions (Cole et al. 2014)

LOCATION	CRITERIA
Bacteraemia	Recognised pathogen cultured from one or more blood cultures *or* Common skin contaminants cultured from two or more blood cultures on two separate occasions within a 48 hour period.
Respiratory infection*	VAP. Consider if: increased inflammatory markers, pyrexia ≥38.^{0}c or <36.^{0}c, Inotrope requirements increased (NA>0.1), new onset purulent sputum, increased ETT secretions, increased Fi02 since previous day, increased PEEP since previous day, new or progressive infiltrates on chest x-ray, alternative source of infection excluded. Confirmed organisms isolated from sputum. Pneumonia/RTI. Confirmed organisms isolated from sputum, *and* increase or change in character of sputum with increased suction requirements, *and/or* new onset cough, *and/or* dyspnoea or tachypnoea, *and/or* pyrexia ≥38^{0}c, WBC <4,000 or >12,000mm^3/increased inflammatory markers, *and/or* worsening gaseous exchange, *and/or* new or progressive infiltrates on chest x-ray.
Urinary tract infection	Positive urine culture (collected using appropriate sterile technique), *and* elevated WBC count and inflammatory markers, *and/or* pyrexia ≥38^{0}c, *and/or* dysuria, *and/or* abdominal/flank pain.
Vascular catheter line infection	Organisms cultured from site swab or catheter tip. Signs and symptoms of phlebitis *and/or* purulent discharge from line site *and/or* pyrexia ≥38^{0}c.
Traumatic wound infection	Localised heat, redness, swelling, tenderness, pyrexia ≥38^{0}c. and at least one of the following: Organisms isolated from open, accidental wounds, *or* Organisms isolated from contaminated wounds, following cleansing and possibly closure but not requiring surgical intervention, *or* Organisms isolated from purulent wound drainage.

Surgical site infection (SSI)	Localised heat, redness, swelling, tenderness, pyrexia ≥38^{0}c. *and* at least one of the following: Organisms isolated from wound, discharge or tissue of surgical incision(s), *or* Surgical wound dehiscence *and/or* visual evidence of SSI diagnosed by a surgeon.

The following conditions are not infections:
Colonisation, which means the presence of microorganisms on skin, on mucous membranes, in open wounds, or in excretions or secretions but are not causing adverse clinical signs or symptoms.
Inflammation that results from tissue response to injury

VAP: Ventilator Associated Pneumonia, NA: Noradrenaline, ETT: Endotracheal Tube, PEEP: Positive End Expiratory Pressure, RTI: Respiratory Tract Infection, WBC: White Blood Cells
**Not all criteria are required for diagnosis of VAP. Daily review of clinical VAP chart in patient notes*.

APPENDIX 3: DEFINITIONS OF THROMBOEMBOLIC & ISCHAEMIC EVENTS

1. Definitions of venous thromboembolic events.

For a VTE to be diagnosed, there must be clinical symptoms and definitive radiological evidence as set out in the table below:

TYPE OF VENOUS THROMBOEMBOLISM	DIAGNOSIS
Deep venous thrombosis	Accepted methods of diagnosis include: • compression ultrasound • venography
Pulmonary embolism	Accepted methods of diagnosis include: • CT pulmonary angiogram (CTPA) • Ventilation-Perfusion scan (V/Q or Q scan as per local guidelines)

2. Definitions of ischaemic events:

ISCHAEMIC EVENT	DIAGNOSIS

Myocardial infarction	The term myocardial infarction should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischaemia. Under these conditions presence of one of the following criteria meets the diagnosis of MI:
	
	• Detection of rise of troponin with at least one value above the 99th percentile of the ULN, plus evidence of myocardial ischaemia with at least 1 of the following:
	  • symptoms of ischaemia
	  • ECG changes indicative of new ischaemia [ST-T changes, or LBBB]
	  • development of pathological Q waves in ECG
	  • imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
	
	• Sudden, unexpected cardiac death, often with cardiac symptoms, and accompanied by new ECG changes, but before blood tests could be taken or death occurred before the appearance of cardiac biomarkers in the blood
	
	• Pathological findings of acute MI

Ischaemic stroke	Clinical report of brain imaging consistent with an ischaemic stroke in association with new onset focal or generalised neurological deficit (defined as deficit in motor, sensory or coordination function).

The definition for MI is taken from a consensus document published on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction, 2007.