A PILOT STUDY TO INVESTIGATE SHORT-COURSE LOVASTATIN THERAPY IN VIETNAMESE ADULTS WITH DENGUE

PRINCIPAL INVESTIGATORS

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BACKGROUND

Dengue is the most common vector-borne viral infection of humans, with around 100 million infections reported each year and some 2 billion people living in areas of risk.[1] A wide variety of clinical disease manifestations are seen, ranging from asymptomatic infection to life-threatening hypovolaemic shock due to systemic vascular leakage. Among symptomatic patients the clinical manifestations evolve from an initial non-specific viral syndrome, through a critical period when complications may occur, followed by a spontaneous recovery phase. At present clinical management is limited to fluid resuscitation and supportive care.

Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, also known as statins, were introduced into clinical practice in the late 1980s and were quickly established as an effective drug for lipid lowering and mortality reduction in cardiovascular disease.[2] They are currently one of the most widely prescribed classes of drugs globally. Statins have an excellent safety profile.[3] The most common adverse effects are elevation of the liver transaminases and myopathy, however these effects are rare and appear to be dose related.[4, 5]. There is increasing evidence of their safety is patients with liver disease and some evidence that they may have a beneficial therapeutic effect in patients with hepatitis C.[6-8] More recent research has demonstrated that statins have effects beyond their lipid-lowering properties. These pleiotropic effects include the restoration or improvement of endothelial cell function, increasing production of nitric oxide, reducing the release of cytokines and acute phase proteins and reducing inflammatory responses within the vessel wall.[9, 10] One of the major features of both dengue and sepsis is widespread vascular endothelial disruption resulting, in part, from exposure to inflammatory mediators.[11-13] This suggests that in view of their pleiotropic effects, it is plausible that statins may favourably augment the pathophysiological mechanisms of these two conditions. A study in healthy volunteers showed that the inflammatory response to endotoxin was suppressed in those receiving a statin at a high dose, perhaps explaining its beneficial effect observed in sepsis.[10, 14] Furthermore, *in vitro* work has demonstrated that lovastatin may have an anti-viral effect in dengue by reducing virion assembly.[15, 16] Various observational studies have suggested that statin therapy may result in improved outcomes for a diverse range of conditions including sepsis and pneumonia.[17-25] For example, a retrospective cohort study of 120 patients with multi-organ dysfunction admitted to intensive care suggested that prior statin therapy may be associated with a more favourable outcome;[19] a retrospective cohort study of 5353 patients admitted with bacteraemia showed that statin therapy was associated with a significantly lower 31 – 180 day mortality;[23] and a retrospective cohort analysis of 69168 patients with cardiovascular disease demonstrated that statin therapy was associated with a significantly reduced risk of subsequent sepsis.[18] In addition, a nested cohort study including 763 patients exploring the association between statin therapy and mortality in critically ill patients showed that statin therapy was associated with a reduction in all-cause mortality.[26] This association was more evident in high-risk groups, including those aged >58 years, diabetics, patients with a reduced Glasgow Coma Score, patients requiring vasopressor therapy and those classified as having severe sepsis. The mortality reduction was mainly observed with statin doses equivalent to <u>> 40 mg</u>

simvastatin (equivalent to 80 mg lovastatin).[26] A number of randomised clinical trials are currently underway assessing the potential beneficial role of statins in sepsis and acute lung injury (NCT00528580, NCT00676897, NCT00979121). These trials are investigating doses of statins equivalent to 80mg lovastatin. This dose has the maximum potential to show a beneficial clinical effect in patients with dengue fever and therefore has been selected for the proposed trial. There are no identified safety concerns with this dose that contraindicate its use in dengue fever.

Typically severe vascular leakage and shock occur around the fifth day of illness in patients with dengue. It is possible that initiation of statin therapy early in the course of the illness may prevent or favourably modulate these effects. As approximately 5% of patients admitted with dengue will develop shock or other complications a very large trial would be required in order to demonstrate a benefit. However a pilot study would be an opportunity to formally assess the safety of using statins in this group. In addition such a study will also provide an opportunity to investigate the effect of statin usage on the immune response to dengue and to generate preliminary data likely to be helpful in planning a large trial in the future.

While there is extensive experience of using statins as a lipid-lowering agent and growing observational data from their use in critically ill patients, this would be the first study looking at the use of statins in dengue. We propose to investigate the effect of lovastatin for 5 days in adult dengue patients presenting in the first 72 hours of illness. As this is the first study investigating statin therapy in dengue with a particular focus on safety we propose a dose-escalation study investigating 40mg lovastatin versus placebo with a safety review after recruitment of the first 30 patients. If this review is satisfactory we will increase the lovastatin dose to 80mg and conduct a further safety review after the next 30 and the next 100 patients.

Relatively little is known about dengue disease pathogenesis, particularly in relation to the mechanisms responsible for the systemic vascular leak syndrome. However, there is evidence to indicate that severe disease is associated with higher viral loads, and to implicate immune response mechanisms in pathogenesis.[27, 28] The clinical signs suggestive of imminent or current shock in dengue have been well described.[29] The use of clinical scoring systems based on deranged physiological observations has developed as a tool to help diagnose and predict prognosis in critically ill patients.[30-33] Moreover, continuous monitoring of physiological parameters such as heart rate variability, oxygen saturation, respiratory rate and blood pressure appears to have prognostic significance in the critical care setting.[32, 34] While it is intuitive that clinical scoring systems will have applicability in dengue patients, they have not been validated in this group. In addition, the use of intensive non-invasive physiological monitoring has not been studied in dengue. It is plausible that physiological changes that can be measured non-invasively may be surrogate markers of the profound capillary leak observed in severe disease, and they may prove to be useful markers of drug efficacy or adverse effect.

OBJECTIVES:

This study is intended to provide preliminary information to assist in designing a possible future trial powered to assess efficacy as well as safety in the same population, and likely to require a large number of participants.

PRIMARY OBJECTIVE:

The primary objective is to assess the safety of a short course of lovastatin therapy used early in the course of acute dengue infections

SECONDARY OBJECTIVES:

- To assess if there is an effect of lovastatin therapy in relation to clinical and virological safety parameters.
- To determine the effect of lovastatin therapy on measures of the immune response to dengue infection
- To determine the effect of lovastatin therapy on the clinical physiological responses in dengue

SUBSTUDY OBJECTIVE:

- In a subset of patients to use intensive, non-invasive monitoring to explore the impact of dengue, and lovastatin therapy, on physiological parameters that could potentially be used as markers of disease severity or predictors of progression

STUDY ENDPOINTS:

PRIMARY ENDPOINT:

The primary objective of the study is to evaluate the safety and tolerability of lovastatin in adult patients with dengue including:

- Rate of adverse events in each cohort

SECONDARY ENDPOINTS:

We will compare the following between the cohorts:

- Disease progression as defined by 1 or more of the following:
    - Admission to intensive care unit
    - Diagnosis of shock (see definition)
    - Development of severe bleeding (see definition)
    - CNS involvement
    - Death
- Fever clearance time (defined as the time from enrolment to the first time the temperature falls to $< 37.5\ ^{o}$ C and remains below this level for 48 hours)
- Plasma viraemia – AUC day 3 – 6 (log10-transformed)

- To determine the effect of lovastatin therapy on T and B cell responses to dengue infection. The magnitude of CD8+ T cell responses and in parallel the magnitude and durability of the anti-dengue antibody response will be compared between treatment arms

- Quality of life scores from visual analog scale during treatment (quantifiable self-measurement of quality of life in relation to personal health)

EXPLORATORY INVESTIGATIONS:

- Haematological, biochemical and physiological abnormalities:
    - Platelet nadir between day 3 and 8 of illness
    - Maximum haematocrit between day 3 and 8 of illness
    - Percentage increase in haematocrit between day 3 and 8 of illness from baseline
    - Maximum ALT and CK recorded between day 3 and 8 of illness
    - Lowest oxygen saturation recorded between day 3 and 8 of illness
    - Number of patients in each group requiring colloid
- Virological safety parameters:
    - Duration from enrolment to the first undetectable viremia measurement
    - Duration from enrolment to first negative NS1 measurement

Baseline haematocrit was defined as the value recorded at day 28 of study, or a mean of age- and sex-matched healthy population value.

CLINICAL DEFINITIONS:

SHOCK:

This is defined as cardiovascular decompensation (presence of a weak pulse, and/or narrowing of pulse pressure, and/or hypotension with cold-clammy skin, increased capillary refill time, peripheral cyanosis or skin mottling) that is considered by the attending clinicians to be a result of plasma leakage, and to require fluid resuscitation.

SEVERE BLEEDING:

Bleeding is considered clinically severe if it results in haemodynamic instability that requires fluid resuscitation for shock and/or requires a blood transfusion. This assessment is made by the clinicians managing the patient according to the particular clinical status of the patient and/or the decrease in haemoglobin and/or haematocrit. Any bleeding which results in death is also considered severe. Similarly any life threatening bleed, e.g. intracranial bleeding, is considered severe even if shock resuscitation is not required.

STUDY DESIGN

This study is a randomized, placebo-controlled, double-blind trial of lovastatin therapy in Vietnamese adults with dengue infection. The trial will be conducted in two phases with an escalation of dose between phases if the results of an interim data review show no safety concerns within the first cohort of patients treated with the lower dose.

The study will enroll up to 330 evaluable patients into a maximum of two sequential cohorts. Up to 165 patients will be randomized to receive lovostatin and up to 165 patients randomized to receive placebo. All cohorts will be randomized in a 1:1 fashion (lovostatin:placebo). The dose will begin at 40 mg per day in Cohort 1 and may continue to 80 mg per day in Cohort 2. All patients will be treated for up to 5 days. Dose progression, cohort initiation and continuation will be determined and defined after designated safety reviews within each cohort and between cohorts. See Appendix - Study safety flow chart for safety review schedule

COHORT 1 (40mg lovostatin per day or matched placebo):
The first 30 patients enrolled will be treated with 40mg lovostatin or placebo at a 1:1 ratio for up to 5 days.

A Data Safety and Monitoring Board (DSMB) review will take place when day 6 data is available from the 30th patient enrolled to this cohort. Based on a review of all reported serious adverse events and unblinded summary tables of enrolment characteristics, adverse events and overall clinical outcomes, the DSMB will recommend to: A) stop recruitment in cohort 1 and begin recruitment in cohort 2 OR, B) continue recruitment in cohort 1 OR C) stop the study. The results of the DSMB review will be forwarded to the Viet Nam Ministry of Health for consideration. Approval to begin recruitment in Cohort 2 (80mg) will be required by the Viet Nam Ministry of Health.

If recruitment continues on cohort 1, a second review by the Data Safety and Monitoring Board (DSMB) will be planned to take place on or before day 6 data is available from the 100th patient enrolled to this cohort.

COHORT 2 (80mg lovostatin per day or matched placebo):
Patients in cohort 2 will be treated with 80mg lovostatin or placebo at a 1:1 ratio for up to 5 days.

A Data Safety and Monitoring Board (DSMB) review will take place when day 6 data is available from the 30th patient enrolled to this cohort. Based on a review of all reported serious adverse events and unblinded summary tables of enrolment characteristics, adverse events and overall clinical outcomes, the DSMB will recommend to: A) continue recruitment in cohort 2 OR, B) stop recruitment on cohort 2 and continue recruitment in cohort 1 OR C) stop the study.

If recruitment continues on cohort 2, a third review by the Data Safety and Monitoring Board (DSMB) will be planned to take place on or before day 6 data is available from the 100th patient enrolled to this cohort.

If recruitment stops on cohort 2 and continues on cohort 1, a third review by the Data Safety and Monitoring Board (DSMB) will be planned to take place on or before day 6 data is available from the 100th patient enrolled to this cohort.

A maximum total of 330 patients will be enrolled. This will include a minimum of 30 patients in cohort 1 and a maximum of 300 patients in cohort 2. The exact number of patients in each cohort will depend on the results of the safety reviews.

This is a study focusing primarily on safety, however we have confidence based on expert opinion that lovastatin has the potential to be an effective therapeutic for dengue. In view of this we are keen to recruit enough patients to ensure the study has the power to effectively explore its potential efficacy. We intend to enrol over 2 dengue seasons aiming to meet the recruitment targets.

STUDY POPULATION AND ENROLMENT

Patients with dengue will be identified in the out-patient clinic or upon admission to the specialist dengue wards at the Hospital for Tropical Diseases, Ho Chi Minh City (Wards D and C). After phase 1 of the study is complete and safety reviews are satisfactory, recruitment will commence at Tien Giang Hospital, Tien Giang Province

Trained study physicians will assess all potential patients for the following inclusion and exclusion criteria:-

INCLUSION CRITERIA

- Age > 18
- Case definition of suspected dengue infection (based on WHO 2009 Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control - *Fever and 2 of the following criteria: Nausea, vomiting, Rash, Aches and pains, Tourniquet test positive, Leukopenia, Any warning signs)*
- <72 hours of fever
- Positive rapid test for dengue non-structural protein 1
- Informed consent or assent to participate in the trial

EXCLUSION CRITERIA

Patients with one or more of the following criteria at enrolment will be excluded from the study:

- Signs or symptoms suggestive of any other acute infectious disease
- ALT >150 U/L
- CK >1000 U/L
- Platelets < 50 x 10^9/L
- Myopathy
- Cirrhosis

- Use of statins within 1 week
- Chronic use of medication contraindicated for use with lovastatin (cholestyramine, isradipine, warfarin, amiodarone, azole antifungals, fibrates, colchicine, ciclosporin, danazol, macrolides, nefazodone, niacin (high doses), protease inhibitors, verapamil, diclofenac, doxycycline, imatinib, isoniazid, nicardipine, propofol, quinidine, and diltiazem)
- Pregnancy and lactation (all females of childbearing potential must provide urine for a βHCG test)

ALT will be used to determine inclusion as this enzyme is more specific for liver pathology. Both dengue and statin therapy can affect the levels of ALT, however if levels exceed 250 U/L (5 times the upper limit of normal) during the trial the study drug will be stopped.
There is some evidence that statin therapy can affect platelet function.[35] In view of this and in light of the Ministry of Health dengue treatment guidelines (2012) patients with a platelet count less that 50 x 10^9/L will not be enrolled. In addition, the study drug will be stopped if the platelet count falls below 5 x 10^9/L or the patient develops gastrointestinal bleeding.

IDENTIFICATION AND ENROLMENT

Patients presenting to the out-patients department or in-patient wards with a clinical suspicion of dengue and less than 72 hours of fever will be identified to study staff. Dengue will be confirmed by NS1 rapid test for patients with less than 72 hours of fever. Patients with <72 hours of fever and a positive rapid test will be approached by study staff, offered an informed consent form to read and told about the study. The patient will be invited to ask questions and study staff will ensure that the details of the study are well understood. The patient may consider participation in the study for a period of up to 24 hours from approach provided they remain within the recruitment time window . If patients agree to participate they will need to be admitted to hospital for at least 48 hours after the initiation of the study drug. If the patient gives consent the patient will be allotted the next consecutive study number and enrolled to the study.

MEDICINAL PRODUCTS

RANDOMIZATION AND BLINDING

Randomization to either treatment arm described below will be 1:1 and stratified according to the ward of recruitment. A randomization list using block randomization with variable block size will be prepared and maintained confidentially from study staff by the Clinical Trials Pharmacist.
A chronological log of all enrolled patients will be maintained and the next available sequential study code will be assigned to each patient as they enroll. The assigned number will correspond to a coded, sealed, pre-packaged bottle containing six doses of either lovastatin or visually matched placebo. Drug appearance and administration schedules will be identical to maintain blinding amongst the attending physicians and nurses.

TREATMENT

Patients will be assigned to one of two treatment arms:

- Active Medicinal Product:
- COHORT 1 - 40mg lovastatin once daily for up to 5 days;
- COHORT 2 - 80mg lovastatin once daily for up to 5 days

OR

- Placebo: visually matched placebo once daily for up to 5 days

The first dose will be given as soon as practically possible after enrolment. If patients are discharged before the completion of 5 days, study drug will be stopped on this day.

DISPENSING, STORAGE AND ACCOUNTABILITY

Bottles will be prepared centrally by an unblinded study pharmacist and distributed to the site in batches as required. Drugs will be stored in accordance with the manufacturers' recommendations in a secure area. All movements of study medication will be recorded. Both individual subject and overall drug accountability records will be kept up to date by the study staff.

COMPLIANCE AND REDOSING

Study drugs will be administered as Directly-Observed-Therapy. If the patient vomits, within 30 minutes of taking the treatment one replacement dose will be given. Replacement doses can be given on multiple occasions, provided that occasions are separated by 24 hours. Dosing and redosing will be recorded in the CRF.

DISCONTINUATION OF STUDY TREATMENT

The investigator may discontinue treatment for a participant if he or she considers it necessary for any reason including:

- An adverse event which requires discontinuation of the study medication (Lovastatin) or results in inability to continue to comply with study procedures
    - ALT >250 U/L (ALT> 5 x ULN) Patients will have to stop the study drug
    - Palatelets < 5 x 10^9/L
    - Development of gastrointestinal bleeding (refer to definition above and MOH dengue guidelines 2011)
- Disease progression which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Intolerance of study medication including a vomiting both an initial dose and a redose of medication within 30 minutes of swallowing each dose.

If treatment is discontinued due to an adverse event, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilised. Patients who discontinue study treatment will continue to be followed as per protocol and will not be withdrawn from the study.

UNBLINDING

The Clinical Trials Pharmacist will hold the unblinded randomization list with details of the contents of each bottle. This list will be accessed only in the case of emergency unblinding authorized by an investigator as per standard operating procedures. Emergency unblinding will be performed in the case of an adverse event when the knowledge of the identity of the treatment will contribute to the treating physician's ability to care for the patient.

CONCOMITANT MEDICATION

Throughout the study, investigators and treating physicians may prescribe any concomitant medications or treatments deemed necessary (e.g. antipyretics, analgesics or anti-emetics) to provide adequate supportive care.

According to manufacturer's recommendations, the following drugs should not be prescribed in combination with lovastatin: cholestyramine, isradipine, warfarin, amiodarone, azole antifungals, fibrates, colchicine, ciclosporin, danazol, macrolides, nefazodone, niacin (high doses), protease inhibitors, verapamil, diclofenac, doxycycline, imatinib, isoniazid, nicardipine, propofol, quinidine, and diltiazem. If a patient is regularly taking any of these drugs they will not be eligible for entry into the study.

Citrus fruits are not recommended during treatment.

Any medication, other than the study medication taken during the study will be recorded in the CRF.

EVALUATION

CLINICAL EVALUATION

Patients will be followed by a study physician daily until discharge, and all signs and symptoms recorded in the case report form. Blood samples will be obtained according to the schedule below, and an ultrasound scan will be performed on day 6 of illness to detect signs of plasma leakage. Clinical management decisions will remain in the hands of the attending ward doctors. In the event that shock or any other serious complication develops the patient will be transferred to the appropriate ICU where they will continue to be followed by one of the study physicians. Details of all adverse events will be recorded on specific forms, together with an assessment as to whether the event is likely to be related to any treatment received, and all serious adverse events will be reported promptly to the Data and Safety Monitoring Board (DSMB).

Quality of life will be measured by questionnaire and visual analog scale daily.
Patients who are fit to discharge on or after study day 3 may be followed as an out-patient until study day 6. All patients will be asked for attend a follow-up visit for review after 4 weeks (+/- 5 days). At this visit they will have repeat blood sampling. This will provide the baseline haematocrit from which to calculate the % increase observed during the illness. Should participants not return for follow-up the % increase will be calculated from a population baseline obtained from the results of other study participants.

LABORATORY EVALUATION

Haematocrit, platelet and total cholesterol measurements will be carried daily or more frequently if clinically indicated. These tests will be repeated at the follow-up visit.

Renal and liver function tests, electrolytes and coagulation profiles, will be carried out at enrolment, 48 hours later, day 5-6 of illness and at the follow up visit. Measuring the total cholesterol will allow us to assess whether the drug is being absorbed. These results will not be returned to the treating physicians in order to maintain blinding. If the ALT measured 48 hours after enrolment is greater than 250 U/L the study drug will be discontinued.

Conventional serological and virological tests will be carried out to confirm dengue infection and indentify the infecting serotype. Plasma samples collected at daily intervals until discharge (and daily until day 6 if discharged before day 6) will be assessed for viremia levels, NS1 levels, and concentrations of various pro- and anti-inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-10). CD8 and dengue IgG/IgM will also be assessed to evaluate immune response.

DNA will be extracted from residual blood samples and genotyped for genetic variants known to be associated with severe dengue, e.g. MICB and PLCE1.[36] Assays for whole blood gene expression will be performed at day 1 and day 2. These tests will require sending the samples overseas (Genome Institute of Singapore, Singapore).

An approximate sampling schedule is given in the table below. If patients are discharged before the completion of 5 days they would have the scheduled sampling in addition to the planned discharge sampling taken on this day. In this case, day 6 blood tests will be done as outpatients and will include FBC, viral load/NS1 serology and cholesterol.

Test	**Vol**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6/ Discharge**	**Day 28(+/-5)/ follow-up**
FBC (incl HCT + PLT)	2ml	x*	x*	x*	x*	x*	x*	x
Viral load/ NS1 Serology (day 1 & 6)	5ml	x	x	x	x	x	x	x
Immunology/genetics		x					x	
Cholesterol	2ml	x	x	x	x	x	x	x
Biochemistry*		x*		x*			x*	x
Coagulation	4ml	x*		x				x
Whole blood gene expression assay	5ml	X	x					
Urine myoglobin**			x	x	x	x	x	

Note:
**These tests are routine blood tests for clinical care*
*** Do not perform >24 hours after study drug is stopped*
Biochemistry: urea, creatinine*, sodium*, potassium*, bilirubin*, ALT*, AST*,CK*
Coagulation tests: PT, APTT, INR
FBC: full blood count

RADIOLOGICAL EVALUATION

On the 6th day of illness study participants will have an ultrasound performed to assess for evidence of capillary leak. The sonographer will record the presence or absence of ascites, pleural effusion, pericardial effusion, gall bladder thickening or hepatomegaly.

SAFETY REPORTING

The safety profile of Lovastatin is well documented for healthy persons and in a diverse range of conditions. The drug is licensed for use in Viet Nam by a number of manufacturers. The doses used in this study (40mg or 80mg per day) are approved doses for other indications. Dengue fever is not an approved indication for lovastatin.

Safety monitoring in this trial will include close observation while patients are in hospital, recording and treating any intercurrent illnesses or drug-related side effects and reporting serious adverse events to the DSMB and relevant ethical committees.

DEFINITIONS

Adverse Event (AE) An unfavourable or unexpected sign, including an abnormal laboratory finding that is temporally associated with the use of an investigational product, whether on not considered related to the product. Events will be considered Adverse Events only if they occur after the first dose of study drug is given. Pre-existing conditions that worsen after the first dose of study drug are reported as Adverse Events.

Significant laboratory abnormalities should be entered on the AE form if they meet one or more of the following conditions:

- Accompanied by clinical symptoms
- Leading to a change in the study drug (dose modification or withdrawal)
- Requiring a change in concomitant therapy (e.g. dose adjustment or discontinuation)

Stopping of study drug for any clinical indication will be recorded as an adverse event.

Serious Adverse Event

A serious adverse event is an AE that:

- Results in death or
- Is life-threatening i.e. the patient was at risk of death at the time of the AE or
- Requires prolongation of existing hospitalization or
- Results in persistent or significant disability/incapacity or

- Is a congenital anomaly/birth defect or
- Requires acute medical or surgical care to prevent one of the outcomes listed above

REPORTING PROCEDURES FOR SERIOUS ADVERSE EVENTS

All AEs and SAEs will be recorded on the patient CRF.

The intensity of clinical AEs will be recorded on a three-point scale:

Mild	Discomfort noticed but no disruption of normal daily activities
Moderate	Discomfort sufficient to reduce or affect daily activity
Severe	Inability to perform daily activity

The relationship of the AE to the treatment should be assessed and recorded as:

- Definitely related to study drug
- Likely related to study drug
- Unlikely related to study drug
- Very unlikely related to study drug

All SAEs will be reported to the Data Safety Monitoring Board, the Oxford Tropical Research Ethics Committee and the Viet Nam Ministry of Health as soon as possible. Partially unblinded summary tables of all AEs and SAEs will be reviewed by the trial's independent Data and Safety Monitoring Committee after the enrolment of 30 patients in cohort 1 of the study. Further reviews will occur after enrolment of the first 30 and first 100 patients in cohort 2. Additional safety reviews will be at the discretion of the DSMB based on available data and ongoing reporting. All DSMB reports will be sent to the Oxford Tropical Research Ethics Committee and the Viet Nam Ministry of Health.

Any pregnancy must be reported to the DSMB. The PI must take all reasonable efforts to discover the outcome of the pregnancy. If there is a congenital abnormality or a still born baby, this will be reported as a serious adverse event.

DSMB

An independent Data Safety and Monitoring Board (DSMB) will be set up consisting of qualified volunteers with the necessary knowledge of clinical trials and statistics. The DSMB will review the protocol and agree to a data review schedule and reporting requirements before the study commences. All data reviewed by the DSMB will be in the strictest confidence. A DSMB charter will outline its responsibilities and how it will operate.

The DSMB will perform a safety review after day 6 data is available for the first 30 patients enrolled in cohort 1. This review will include unblinded summary tables of SAEs, AEs or event reports submitted to the DSMB. An analysis of overall clinical outcome will be performed. The report from the DSMB will be forwarded to the Viet Nam Ministry of Health whose approval will be required in order to begin recruitment in cohort 2 using 80mg lovastatin. An additional safety review will be

performed after the enrolment of 100 patients on cohort 1 or cohort 2 (whichever enrolls 100 patients first), and at the discretion of the DSMB based on available data and reports.

The DSMB will be notified as soon as possible when an Investigator becomes aware of the occurrence of a Serious Adverse Event.

The DSMB members are:
- Professor Danny McAuley, Professor of Intensive Care Medicine Queen's University Belfast and Co-Director of Research UK Intensive Care Society
- Dr Sue Lee, Statistician, Oxford Tropical Programme Thailand
- Dr Nguyen Thanh Hung, Vice-Director Children's Hospital 1, HCMC, Vietnam
- Professor Nguyen Tran Chinh, Clinician, Hospital for Tropical Diseases, HCMC, Viet nam
- Dr Tim Cook, Intensive Care Physician, Bristol UK

DATA AND ANALYSIS

SOURCE DATA

Source documents are original documents, data, and records from which participants' study data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, microfiches, radiographs, and CRFs.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). In this study the CRF will be used as the source document for some data points. The CRF may be paper or electronic format.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent form, the participant will be referred to by the subject number and initials, not by name. Data recorded on paper will be entered on site on the database in accordance with standard operating procedures and monitored for accuracy.

SAMPLE SIZE

This is a study focusing primarily on safety, however based on published evidence suggesting the potential efficacy of statins for sepsis and other syndromes, we have confidence that lovastatin has the potential to be an effective therapeutic for dengue. In view of this we are keen to recruit enough patients to ensure the study has the power to effectively explore its potential efficacy. Therefore a sample size of 330 patients has been determined.

If the results of this pilot study are satisfactory a larger trial will be conducted to assess efficacy in preventing shock and other severe complications.

ANALYSIS

All randomized patients will be included according to the intention-to-treat principle.

Pre-defined endpoints and exploratory investigations will be compared between the two treatment arms based on linear regression for continuous endpoints, logistic regression for binary endpoints, and Cox regression for time-to-event endpoints including fever clearance time. For laboratory markers, analyses will be adjusted for the pre-dose value of the respective marker; plasma viremia and NS1-endpoints will additionally be adjusted for dengue serotype.

The clinical, virological and immunological findings will also be correlated with MICB and PLCE1 genotype using descriptive statistical methods. The microarray immune response data and the continuous physiological data will be analysed using appropriate software packages by staff familiar with these techniques.

QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, Viet Nam Guidelines for Good Clinical Practice and study standard operating procedures. The OUCRU Clinical Trials Unit will be engaged in assuring good governance, regulatory compliance and QA/QC of study execution.

MONITORING

This trial will be monitored to ensure that data are generated, documented and reported in compliance with the protocol, standard operating procedures and the appropriate regulatory requirements. Monitoring will be performed according to procedures defined by the OUCRU Research Governance Team and the agreed monitoring plan.

ETHICAL CONSIDERATIONS

DECLARATION OF HELSINKI

The Investigator will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki (Seoul 2008).

ETHICAL REVIEW

The study protocol and its associated documents will be submitted to the ethical committees of the Hospital for Tropical Diseases, Ho Chi Minh City, the Viet Nam Ministry of Health, the Oxford University Tropical Research Ethics Committee (OxTREC) and the London School of Hygiene and Tropical Medicine (LSHTM). The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

INFORMED CONSENT

The study staff will discuss the study with all potential adult participants or, with the accompanying parent/guardian. Study staff will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. The patient will be invited to ask questions which will be answered by study staff, and they will be provided with appropriate numbers to contact if they have any questions subsequently. If the patient agrees to participate, they will be asked to sign an informed consent form. A copy of the form will be given to them to keep. If required, the patient will be given up to 24 hours to consider the study or up until 72 hours of illness has passed at which point the patient is no longer eligible for the study. In addition to the procedures above, illiterate signatories will have the Informed Consent Form read to them in the presence of a witness who will sign to confirm this. Adults without the mental competency to understand the ICF may be consented by a parent/guardian. All patient information sheets and consent forms will be written in the local language and will use terms that are easily understandable.

RISKS

This study will use a drug that has been studied thoroughly and its toxicities are well described. In general, the drug is well tolerated. Rare side effects of statins include myopathy, rhabomyolysis and liver disease. These are rare even in those on long-term therapy, however given the nature of dengue it is plausible that they may be observed more frequently. The incidence of rhabomyolysis has been estimated to be 0.44 per 10,000 person years for patients on statin monotherapy.[37] Patients will have measurements of creatine kinase and urine tests for myoglobinuria while on treatment with lovastatin to assess for this adverse event. Patients will be closely monitored for all adverse events and treated as per standard of care. Patients will have additional blood samples taken for research purposes, which pose a small risk of bruising and infection.

PATIENT BENEFITS & COMPENSATION

Trial patients will have the medical costs associated with diagnosis and treatment of dengue or any trial related procedures covered by the study. Patients will be reimbursed the cost of local transport to attend for the follow up visits.

ALTERNATIVES TO STUDY PARTICIPATION

Subjects are able to decline freely participation in this study. If so, they will receive standard care for their condition.

WITHDRAWAL OF PARTICIPANTS FROM THE STUDY

Each participant has the right to withdraw from the study at any time.
The reason for withdrawal will be recorded in the CRF.

CONFIDENTIALITY

Participants will be assured that all information generated in this study will remain confidential. The trial staff will ensure that the participants' anonymity is maintained. Participant's names will be recorded at the time of enrolment to allow

for their identification at follow-up visits. However identifiable information will be linked to stored data or samples only by a protected Master List. This list will not be shared outside the study staff. All documents will be stored securely as per Viet Nam guidelines and be accessible to trial staff and authorised personnel only. Direct access will be granted to authorised representatives from the host institution and the regulatory authorities, if applicable, to permit trial-related monitoring and inspections.

SAMPLE SHARING AND STORAGE

Samples collected will be used for the purpose of this study as stated in the protocol and stored for future use. Consent will be obtained from subjects for sample storage and/or shipment of specific samples to collaborating institutions for investigations that cannot be performed locally. Any proposed plans to use samples other than for those investigations detailed in this protocol will be submitted to the relevant ethics committees prior to any testing.

The participants will be identified by a study specific participant number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file. Data may be used alone or in combination with data from related studies in secondary analyses.

SPONSORSHIP AND INSURANCE

The University of Oxford has appropriate insurance-related arrangements in place in respect of the University's role as research sponsor for this study.

SUB-STUDY

Intensive monitoring:

Objective:

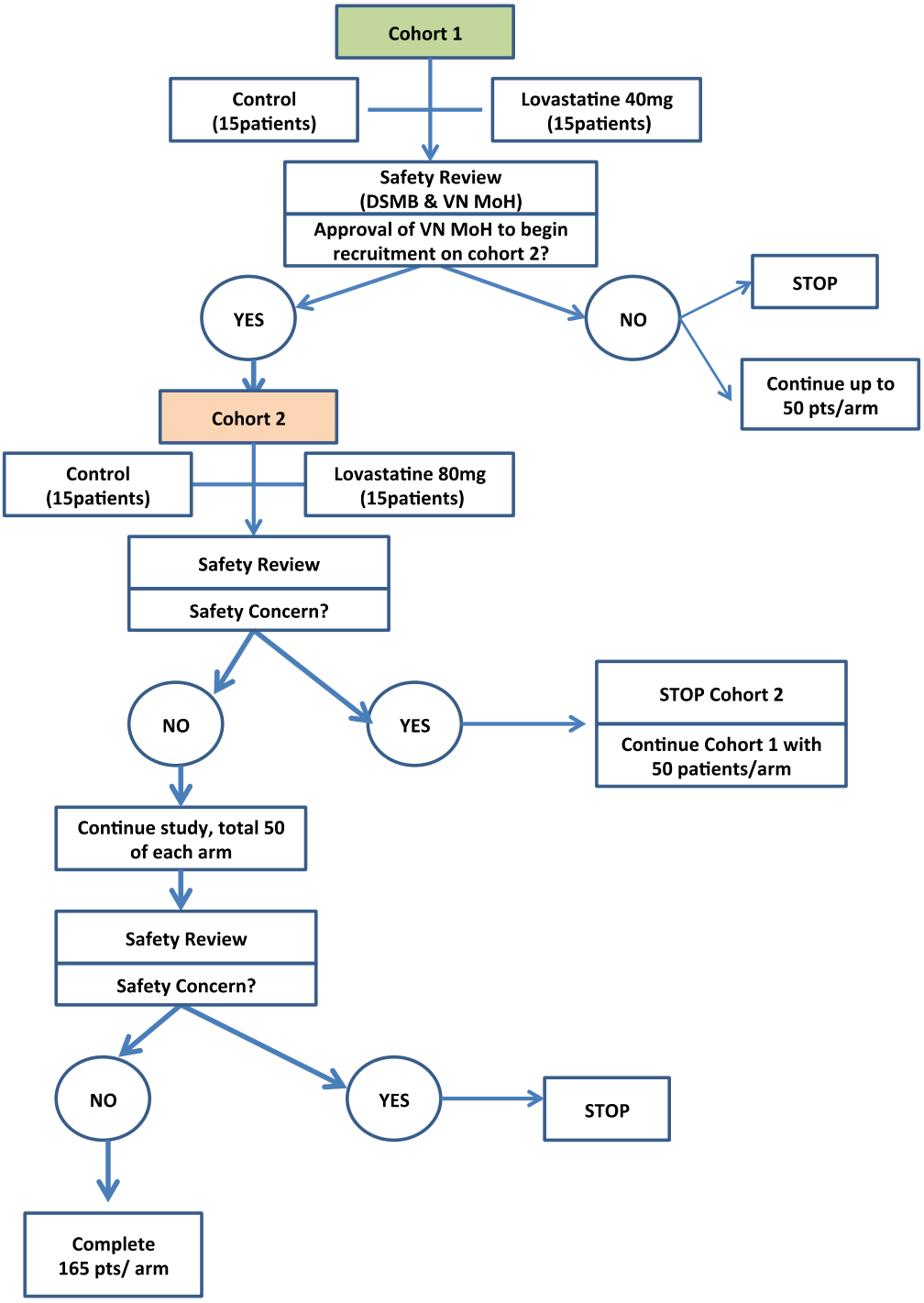
- To use intensive, non-invasive monitoring in a subset of each arm of the study to explore the impact of dengue, and statin therapy, on various physiological parameters that could potentially be used as markers of disease severity or predictors of progression

We plan to intensively monitor 40 randomised participants and therefore make observations on ~20 subjects in each treatment arm. Intensive monitoring will involve continuous measurement of respiratory rate, blood pressure at 15 minute intervals, oxygen saturation and continuous electrocardiography through a single lead Holter device. This component of the study is observational and aims to establish whether physiological changes can be useful surrogates for disease progression and treatment effect.

The subset from each arm who undergo intensive monitoring will be assessed by the study physician and receive the study medication as detailed above. They will be fitted with an ECG monitor, a continuous blood pressure recorder, an adhesive

respiratory rate sensor and an oxygen saturation probe. Data from these devices will be captured for a 72-hour period from enrollment.

APPENDIX 1: STUDY SAFETY FLOW CHART



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