

STUDY NO. NC10/C01
PROTOCOL NO.: Version 8.2 dated 22 July 2018

**“Phase IIa, Prospective
Study to evaluate the safety and measure efficacy of anti-
Chlamydomphila antibiotic combination (ACAC) therapy comprising
100mg doxycycline, 500mg azithromycin and 300mg rifabutin in the
treatment of patients with Coronary Heart Disease (CHD)”**

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APPENDICES

APPENDIX 1 STUDY LABORATORY TESTS

APPENDIX 2 LIST OF PROHIBITED MEDICATIONS

APPENDIX 3 SERIOUS ADVERSE EVENT FORM

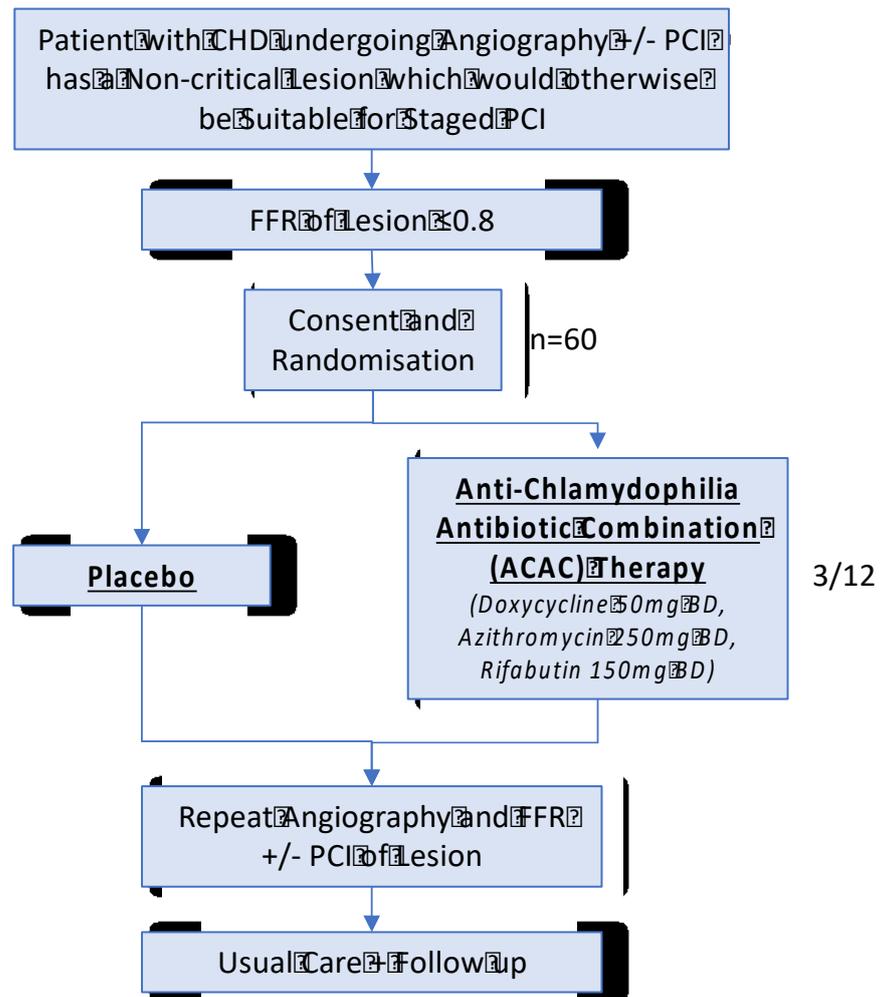
APPENDIX 4 DEFINITIONS OF CAUSALITY AND SEVERITY OF ADVERSE EVENTS

APPENDIX 5 DECLARATION OF HELSINKI 2008

PROTOCOL SYNOPSIS

Title	A Phase IIa, prospective study to evaluate the safety and efficacy of anti- <i>Chlamydophila</i> antibiotic combination (ACAC) therapy comprising 100mg doxycycline, 500mg azithromycin and 300mg rifabutin in the treatment of patients with Coronary Heart Disease (CHD)
Study Sites	Liverpool Hospital/ Sydney Southwest Private Hospital
Objectives	<p><u>Primary objectives:</u></p> <ol style="list-style-type: none"> 1. To evaluate the effect of an antibiotic combination therapy on fractional flow reserve (FFR) (coronary arterial flow) in participants with Coronary Heart Disease <p><u>Secondary objectives:</u></p> <ol style="list-style-type: none"> 1. To evaluate the effect of an antibiotic combination therapy on plaque morphology and volume as assessed with intra-coronary imaging (IVUS and/or OCT) in culprit and non-culprit arteries in a substudy 2. To evaluate angiographic stenoses changes using Quantitative Coronary Angiography (QCA) during ACAC therapy. 3. To evaluate morbidity and clinical improvement during the ACAC therapy. 4. To record major adverse clinical events (MACE). Including death, recurrent myocardial infarction, stroke and major bleeding. <p><u>Exploratory objectives:</u></p> <ol style="list-style-type: none"> 1. To evaluate the use of loop-mediated isothermal amplification (LAMP) for the detection of <i>Chlamydophila pneumoniae</i> in blood samples, compared with standard titers and cell culture 2. To evaluate changes in indices of pressure and flow (as measured with coronary pressure/flow guidewire) in culprit and non-culprit coronary arteries
Design	Phase IIa, multicenter, randomised, placebo-controlled trial
Population	Subjects with symptomatic coronary heart disease on medical therapy who have a culprit artery lesion suitable for percutaneous coronary intervention (PCI) and another stenosis potentially suitable for staged PCI, with FFR ≤ 0.80 . Or subjects who have a single non-culprit artery of intermediate severity with a clinically indicated FFR ≤ 0.80 which is suitable for staged PCI.
Sample Size	60 subjects
Treatment	All subjects will be assigned to either active treatment or matching placebo. They will receive ACAC in conjunction with standard medical therapy for 3 months.
Efficacy Data	FBC, ESR, Electrolytes/Urea/Creatine, Liver Function tests, Lipid Studies ,CK-MB, High Sensitivity Troponin T, <i>Chlamydophila pneumoniae</i> titer, <i>Chlamydophila pneumoniae</i> macrophage culture, <i>Chlamydophila pneumoniae</i> (<i>c. pneumoniae</i>) DNA detection (exploratory), hs CRP, symptomatology, Functional Status – New York Heart Association Class, Quantitative Coronary Angiography (QCA); FFR and Coronary pressure/flow indices, plaque volume, plaque composition, fibrous cap thickness, macrophage content.
Safety data	Adverse event reports, Blood tests (standard haematology, serum chemistry and pregnancy) and Physical Examination, compliance and major adverse cardiac events MACE
Statistical procedures	Results will be presented as mean +/-SD for continuous normally distributed variables, as median (interquartile range) for continuously non-normally distributed data and as percentages for categorical data. Significant changes in clinical symptom improvement, arterial flow and biochemical variables over time will be assessed using ANOVA for repeated measurements. A <i>P</i> value < 0.05 will be considered statistically significant.
Study duration & dates	Total of 24-month recruitment period. From Q1 - 2018 to Q3 - 2019. Study duration is 6 months from the initial angiographic procedure

PATIENT FLOW DIAGRAM



STUDY SCHEDULE

Assessments and Recordings	V1	V2	Phone Call	V3	Phone Call	Phone Call	V4
	Screening and Baseline	Day 30 (+7 days)	Day 60	Day 90 (+21 days)	Day 120	Day 150	Day 180 (+ 21 days)
Subject Information	X						
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Demographics	X						
Medical History	X						
Confirmation of eligibility	X						
Laboratory tests							
<i>FBC</i>	X	X		X			
<i>ESR</i>	X	X		X			
<i>EUC</i>	X	X		X			
<i>Liver Function Tests</i>	X	X		X			
<i>Lipid Studies</i>	X	X		X			
<i>CK-MB</i>	X	X		X			
<i>High Sensitivity Troponin T</i>	X	X		X			
<i>High Sensitivity CRP</i>	X	X		X			
<i>Pregnancy testing</i>	X						
<i>C. pneumoniae macrophage cultures</i>	X			X			
<i>C. pneumoniae titers</i>	X			X			
<i>C. pneumoniae DNA detection</i>	X			X			
Physical Examination	X	X		X			
Vital Signs	X	X		X			
Quantitative Coronary Angiogram	X			X			
ECG	X			X			
New York Heart Association Functional Class	X	X		X			
Fractional Flow Reserve, Pressure/flow wire measurements and intracoronary imaging (IVUS + OCT)	X			X			
Concomitant medications	X	X	X	X	X	X	X
Concomitant illnesses	X	X	X	X	X	X	X
Medication dispense/collection	X			X			
Medication review/Accountability		X		X			
Participant Symptomatology Diary	X	X		X			X
Adverse events	X	X	X	X	X	X	X

ABBREVIATIONS AND DEFINITIONS

ACAC	Anti-Chlamydomphila Antibiotic Combination
ACS	Acute Coronary Syndrome
AE	Adverse Event
CANTOS	Canakinumab Antiinflammatory Thrombosis Outcome Study
CDD	Centre for Digestive Diseases
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CK-MB	Creatine Kinase MB
CRF	Case Report Form
CRP	C-Reactive Protein
CTN	Clinical Trials Notification (Scheme)
ECG	Electrocardiogram
EOT	End of Trial
EUC	Electrolytes, Urea, Creatinine
FBC	Full Blood Count
FFR	Fractional Flow Reserve
GCRP	Good Clinical Research Practice
HREC	Human Research Ethics Committee
HMR	Hyperaemic Microvascular Resistance
HSR	Hyperaemic Stenosis Resistance
ICH	International Conference for Harmonisation
IMR	Index of Microvascular Resistance
IVUS	Intra-Vascular Ultrasound
JUPITER	<u>J</u>ustification for the <u>U</u>se of statins in <u>P</u>rimary prevention: an <u>I</u>ntervention <u>T</u>rial <u>E</u>valuating <u>R</u>osuvastatin
LAMP	Loop-mediated isothermal amplification (of DNA)
LDL	Low density lipoprotein

MACE	Major Adverse Cardiovascular Events
NHMRC	National Health and Medical Research Council
OCT	Optical Coherence Tomography
PCI	Percutaneous Coronary Intervention
SAE	Serious Adverse Event
SD	Standard Deviation
TGA	Therapeutic Goods Administration
TWAR	Taiwan Acute Respiratory Agent
QCA	Quantitative Coronary Angiography
V1	Visit 1
V2	Visit 2
V3	Visit 3
V4	Visit 4

For Laboratory tests please see Appendix 1.

1. INTRODUCTION AND STUDY RATIONALE

1.1 Introduction

Coronary artery disease (CAD) describes the pathologic process of accumulation of atheromatous plaques within the walls of arteries responsible for supply of oxygen and nutrients to the myocardium. These plaques usually consist of cholesterol deposits, calcium minerals and various amounts of connective tissue and inflammatory cells, and this atheroma eventually reduces the rate of blood flow to the heart¹. Atheromatous plaques originate from the chronic inflammatory responses to many cytokine stimuli, including oxidized Low-Density Lipoprotein (LDL) particles coming into contact with arterial walls. The consequent damage and accumulation of cholesterol results in atherosclerosis and stenosis of arteries².

CHD events result in approximately 6.9 million deaths each year worldwide. In Australia, CHD remains the leading cause of death and is a major contributor to permanent disability. CHD accounted for approximately 48,500 deaths in Australia in 2008 (34% of all mortalities)³. Whilst CHD is usually diagnosed in its advanced stages, a majority of subjects with CAD remain asymptomatic and demonstrate no visible evidence of the disease before a major cardiac event occurs. The prevalence of CHD increases with age and is gender and lifestyle-related. Available data has shown a higher incidence of CHD amongst those aged over 40 years, and men have been shown to be twice as likely to present with CHD compared to women. Tobacco smoking, hypertension, hypercholesterolaemia, family history, diabetes, lack of physical exercise and significant weight gain are associated with an increased risk of developing CHD⁴.

The presence of cholesterol in plaque has directed research efforts towards the development of various groups of drugs to treat hypercholesterolaemia, together with diets and lifestyle changes. Though increased C-reactive protein (CRP) levels have been associated with increased rates of events, there is debate as to whether the JUPITER [Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin] trial supports the concept of therapeutic intervention to “reduce inflammation”⁵. The landmark Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial which compared Canakinumab, a monoclonal antibody to the pro-inflammatory cytokine interleukin-1 β , with placebo, showed that anti-inflammatory therapy lead to a significant reduction in cardiovascular events compared to placebo⁶. Certainly there is an established and growing body of evidence suggesting that atherosclerosis is a chronic inflammatory disease. This has led to the exploration of potential instigators and propagators of inflammation being considered, including the concept of a chronic infection in the arterial wall leading to chronic inflammation. Though antigens of several candidate infective agents have been identified antigenically, only *Chlamydia pneumoniae* TWAR has been identified in viable form. Furthermore, *C. pneumoniae* has been shown to be a mediator of foam cell formation laden with lipopolysaccharide, as an infective periphenomenon of the *Chlamydia* infection⁷. Chlamydia terminology has recently been refined and *Chlamydia pneumoniae* has been moved to *Chlamydophila pneumoniae* genus. For the present both terminologies are being used interchangeably.

Chlamydophila pneumoniae

Chlamydophila pneumoniae is an obligate intracellular pathogen which requires another cell in order to complete its replication. *C. pneumoniae* exists as a non-biologically active ‘elementary body’ outside a host, only transforming into a ‘reticulate body’ in endosomes upon phagocytosis and utilises the host’s replication mechanisms for proliferation. The consequent replicate reticulate bodies are converted back into elementary bodies and released for further infection into new host cells⁸.

The presence of *C. pneumoniae* in atheromatous plaque specimens and in vitro animal models have implicated *C. pneumoniae* as a potential infectious agent in the pathogenesis and progression of CAD particularly as there is no commensal form of *C.pneumoniae*. *C. pneumoniae* is a common cause of pneumonia and respiratory infection worldwide and has been shown to infect and propagate in human

smooth muscle and endothelial cells of coronary arteries which can be cultured from circulating macrophages. Antibodies to *C. pneumoniae* have been detected in elevated levels in subjects with atherosclerosis though this is non-specific. Some suggest that coronary arterial infection stems from the migration and proliferation of infected macrophages into the intimal layer of the arterial wall after an initial functional alteration or injury to the endothelium⁹.

While numerous studies have demonstrated the presence of *C. pneumoniae* in atherosclerotic arteries and endarterectomy specimens, a lack of standardisation of current diagnostic methods may be responsible for inconsistent detection of *C. pneumoniae*. Indeed detection methods have been largely concentrated on serological studies due to the complexity and difficulty in obtaining tissue cultures. However, accumulating evidence indicates that detection through serological investigations has not been as specific as previously assumed¹⁰. Although cell culture studies remain the gold standard in *C. pneumoniae* detection, isolation and proliferation of *C. pneumoniae* in tissue samples remain a difficult problem resulting in inconsistent results¹¹.

Current Treatment

In subjects with stable coronary heart disease (CHD) and inducible ischemia, percutaneous coronary interventions (PCI) can improve symptoms. As coronary angiography is often inexact in accurately determining the functional significance of non-critical coronary stenoses, fractional flow reserve (FFR) can assess their physiological significance¹². For subjects with acute coronary syndromes and multivessel disease undergoing an early invasive strategy, performing PCI in the culprit vessel and 'staging' possible treatment allows measurement of non culprit FFR at initial angiography. While non-culprit FFR in Acute Coronary Syndrome (ACS) subjects may worsen acutely due to endothelial dysfunction, randomisation should minimise confounding. Return for planned staged PCI as guided by angiography has been standard practice in such subjects, and increasingly, revascularisation is FFR guided. We plan to perform another FFR study to evaluate flow changes over a three month period.

The coronary circulation is comprised of two major components: the major epicardial coronary arteries which have a capacitance function; and the microvascular circulation comprised of pre-arterioles and arterioles which provide a metabolic interface with cardiac myocytes.¹³ Both components of the circulation have been implicated in coronary ischaemia. By measuring pressure and flow in the epicardial coronary artery, indices to assess the microcirculation can be calculated such as CFR, IMR, HSR, HMR at the same time as calculating the FFR value. This provides a comprehensive physiological assessment of the entire coronary circulation¹⁴. We plan to record pressure and flow indices at the time of measurement of FFR in the culprit and non-culprit arteries.

Intra-coronary imaging such as Intravascular Ultrasound (IVUS) and Optical Coherence Tomography (OCT) allow direct visualisation and characterisation of coronary plaque in vivo.¹⁵ Due to the unique characteristics of the energy source utilised for image acquisition, the resolution and penetration capabilities of IVUS (ultrasound) and OCT (infra-red light) differ. The combination of the two modalities allow for comprehensive assessment of atherosclerotic plaque including: atheroma volume, cap thickness, arterial remodelling, calcification, lipid pool/necrotic core, macrophage accumulation and neovascularisation.¹⁵ Intracoronary imaging has been shown to be safe in routine practice and is able to predict clinical events according to lesion characteristics and improve outcomes following PCI however its routine use is limited by cost and reimbursement.^{15,16} We plan on assessing plaque with OCT and IVUS at the time of initial and staged PCI in the culprit and non-culprit coronary arteries in a sub-study.

Other current treatment methodologies have been directed at reduction of cholesterol accumulation in arteries and hence reduction of the associated inflammation. As such, lipid-modifying therapies have reduced mortality and clinical events¹⁷.

Antibiotics and coronary heart disease

Previous trial designs examining the effects of antibiotics in subjects with CHD have been focused on treatment studies with serological and clinical symptoms as primary endpoints. However previous

trials have not yielded reproducible changes in clinical outcomes, likely due to small subject populations, inadequate dosing, and particularly the *use of a single antibiotic* rather than a multiple treatment drug regimen, coupled with a short duration of treatment¹⁸⁻²².

Results of human trials and meta-analyses performed demonstrate that the use of single antibiotics has little or no beneficial effect in reducing subsequent acute cardiac events¹⁹⁻²². In CLARICOR trial of 13,702 subjects in Denmark, there was evidence of significantly higher mortality rates in subjects who received two weeks of treatment with clarithromycin (Odds ratio 1.45, 95% confidence intervals 1.09 to 1.92; P=0.01)¹⁹. On the contrary, results from the only multiple antibiotic STAMINA trial reported the treatment of 325 subjects directed against *C. pneumoniae* and *Helicobacter pylori* showed a significant 36% reduction in the incidence of coronary events at 12 weeks and 12 months. The effect was independent of the presence of antibody to *C. pneumoniae* or to *H. pylori*²². However the use of combined multiple antibiotics in subjects with CHD has not been investigated. Thus, one cannot entirely exclude the importance of antibiotics in the role of CHD treatment.

1.2 RATIONALE

Chlamydomphila pneumoniae has been evaluated as one potential underlying cause of CHD. The strongest evidence supporting this hypothesis comes from multiple investigators who enrolled over 19,217 subjects in 'Anti-Chlamydia trials'¹⁷. However, these studies can be questioned on the basis of the type and duration of therapy. The recent change of the genus no longer permits treatment of *C. pneumoniae* as if it were *Chlamydia trachomatis* - which can generally be cured with a short course of macrolides [azithromycin, roxithromycin or clarithromycin] used in the majority of subjects analysed in this meta-analysis²³. *C. pneumoniae* is largely intra-cellular and its preferred locations (arterial muscle cells and macrophages) may contribute to its persistence in the body¹⁷. *C. pneumoniae* can be refractory to antibiotic treatment²⁴ in spite of in vitro susceptibility to various macrolides and ansamycins likely due to the use of sub-optimal dosages, which has been shown to encourage or induce persistence of *C. pneumoniae in vitro*²⁵. Rupp et al (2009) reported *C. pneumoniae* to infiltrated apoptotic neutrophils that are subsequently taken up by monocyte-derived macrophages, thereby preventing clearance by the body's immune system and preventing susceptibility in the presence of antibiotics²⁶.

The previously reported trials have used short-term or recurrent treatment²³. The rationale for combined antibiotic long-term therapy stems from experience with other intracellular bacteria e.g. *Mycobacterium tuberculosis*, characterized by its dormant forms and affinity for developing resistance. The presence of infection in arterial cells with associated fibrosis and calcification for a considerable time may warrant prolonged treatment to enable antibiotic penetration into infected spaces. The occurrence of dormant forms also requires long-term treatment to anticipate the 'awakening' of dormant bacterial cells so that antibiotics can be effective during their division phase. The use of a combination therapy rather than a single agent to address dormant forms is concurrent with experience with other chronic and multiple resistant strain infections such as *H. pylori*, *M. tuberculosis*, and *Mycobacterium avium paratuberculosis* which are treated with combination antibiotics to minimise the development of resistant strains. Indeed, in the *C. pneumoniae*/CHD trials, the only trial that showed marked improvement in primary end points was the trial that used multiple antibiotics albeit for 7 days²². Hence, it would be reasonable to trial an appropriate multiple-antibiotic regimen in CHD

In this trial, antibiotics against *C. pneumoniae* known to be active within cells will be used. The three drugs will be administered simultaneously to minimise resistance development, and these will be used for a minimum of 3 months. At this stage ideal duration of treatment is not known.

In preliminary clinical experience of 5 subjects, with 3-6 month treatment using clarithromycin, rifabutin and doxycycline, subjects noted reduced shortness of breath, angina episodes, and marked improvement in claudication. The dosage schedule for this trial will be initiated as half doses in the first week followed by full dosages from week two onwards. This is designed as to minimize the onset of potential adverse effects in subjects. The dose-escalating schedule allows introduction of the medications into the body and maximizing bioavailability yet minimizing the potential adverse events.

The experience with long term use of a combination of three antibiotics in *Mycobacterium avium paratuberculosis* and Crohn's disease has been largely in younger subjects who did not receive anti-platelet agents as concomitant therapies, and thus, macrolide anti-platelet agent interactions have not been observed. In the proposed group of subjects with CHD disease, clarithromycin has a known interaction with anti-platelet agents and therefore in this group, clarithromycin will be substituted by azithromycin, a similar macrolide that demonstrates the same intracellular activity against *C.*

pneumoniae but does not exhibit such interaction with anti-platelet agents. The azithromycin dose will be reduced due to its longer half-life, resulting in longer bioavailability duration in the body. Due to reported occasional QT-prolongation at high doses of azithromycin, ECG monitoring of the QT-interval will be carried out.

2. STUDY OBJECTIVES

2.1 Primary Objectives

1. To evaluate the effect of antibiotic combination therapy on objective measures of improvement in coronary flow as determined by fractional flow reserve (FFR) in subjects undergoing PCI with non-critical lesions in non-culprit arteries.

2.2 Secondary Objectives

1. To evaluate the effect of an antibiotic combination therapy on plaque morphology and volume as assessed with intra-coronary imaging (IVUS and OCT) in a substudy.
2. To evaluate angiographic stenoses changes (QCA) during the ACAC trial.
3. To examine safety and major adverse clinical events (MACE) during the ACAC therapy.
4. To record major adverse clinical events (MACE). Including death, recurrent myocardial infarction, stroke and major bleeding.

2.3 Exploratory Objectives

1. To evaluate the use of LAMP for the detection of *C. pneumoniae* in blood samples.
2. To evaluate the changes in indices of pressure and flow (as measured with the pressure/flow FFR wire) in the culprit and non-culprit coronary arteries

3. STUDY DESIGN

This is a Phase II a, prospective study comprising of 60 subjects referred to Liverpool Hospital and Sydney Southwest Private Hospital who have paired FFR assessments. To achieve this number, assuming a dropout rate of 25%, approximately 80 subjects will be approached. These subjects will be undergoing investigation for **symptomatic CHD**. Once consent has been obtained and the subject has met all the inclusion criteria without exclusions, they will be included in the study. The study is a randomised, placebo controlled trial at a randomisation rate of 1:1.

The primary endpoint will be a 20% reduction in the rate of FFR <0.80 in subjects planned to undergo staged PCI at 3 months among those randomly assigned to ACAC therapy. That is a difference of 6 or more in the ACAC group who had an FFR <0.80 at onset will have a FFR ≥0.80 at 3 months than in control group.

4. SELECTION OF STUDY POPULATION

4.1 Inclusion Criteria

The subject must meet all of the following criteria to be eligible for inclusion in the study:

1. Males and females (without childbearing potential as evidenced by hysterectomy, tubal ligation or at least one year post-menopause) aged 18 to 80 years, inclusive.
2. Ability to provide written informed consent to participate in the study.
3. Subjects with documented recent acute coronary syndrome (ACS) or evidence of myocardial ischemia.
4. Subjects who have a culprit lesion suitable for PCI, and a non-critical lesion in another vessel suitable for staged PCI with an FFR of ≤ 0.80 , for subjects undergoing diagnostic angiography and FFR without ad hoc PCI. Or subjects who have a single non-critical culprit lesion of intermediate severity with a clinically indicated FFR ≤ 0.80 which is suitable for staged PCI.
5. No serious co-morbidities, which might interfere with the subject's ability to enter the study.
6. Able to communicate effectively with the study team and to comply with the protocol.

4.2 **Exclusion Criteria**

The subject must be excluded for any of the following reasons:

1. Females that are of child bearing potential
2. Subjects without a non-culprit lesion considered appropriate to plan a staged PCI.
3. Clinically significant haematologic, hepatic, metabolic, renal, rheumatologic, anaphylactic reactions, neurological or psychiatric disease
4. Clinical evidence of any other disease, which might interfere with the subject's ability to enter the trial.
5. Concomitant administration of medications that may interfere with treatment as assessed by the Investigator, including allergy to any component of the therapy.
6. Concomitant administration of any medication prohibited for use during this study (e.g. colchicine)
7. Male subjects consuming greater than 60g alcohol per day, or female subjects consuming greater than 40g alcohol per day.
8. Evidence of any recent history of, or current recreational drug abuse
9. Serious adverse reaction or hypersensitivity to therapeutic drugs.
10. Unable and to comply with the study requirements.
11. Subjects who have been involved in an experimental drug protocol within the past four weeks.

A list of subjects screened but deemed ineligible will be maintained indicating reason(s) for exclusion.

If a subject becomes pregnant during the course of the study, they will be immediately withdrawn and treated in the way least likely to harm both subject and foetus.

4.3 Recruitment

Two centre's will participate, enrolling subjects until 60 have completed study participation defined as completing paired FFR assessments per protocol. Subjects meeting the diagnostic criteria for myocardial ischemia or a recent acute coronary syndrome whose cardiologist recommends angiography will be eligible.

Subjects presenting to the study centre/s will be invited to participate. All eligible subjects who are willing to participate will be given the opportunity to ask questions, and will be informed about the study before signing consent. Subject characteristics will be registered on a case report form that contains basic demographics and clinical characteristics age, sex, medical history, smoking and drinking habits, concomitant medications and current symptoms. In addition, a physical examination will be performed by the Investigator or sub-investigators including height, weight and vital signs.

Subjects who fail the inclusion/exclusion criteria will be informed that they are not eligible for inclusion into the study and will be treated by the Investigator as per standard clinical practice.

4.4 Number of Subjects

A total of 60 subjects will complete study participation. Withdrawn and delayed exclusions may be replaced, see Section 9.2.

4.5 Randomisation and Randomisation Schedule

For the purposes of this study, participants will be randomised at screening visit.

Subjects recruited into the study who are then deemed eligible for Randomisation at Screening Visit will be randomised in a double-blinded 1:1 manner, either to active study medication or matching placebo.

Randomisation will be performed centrally by CDD. Sites will need to contact CDD to randomise study participants. CDD will be responsible for allocation of study medication based on the randomisation number. Randomisation numbers will be given out to site over the phone and confirmed electronically via signed, emailed randomisation confirmation sheet.

CDD is not a clinical site for the conduct of the trial so study investigators involved in any study participant recruitment, assessment and eventual follow-up will remain blinded to the medication therapy assignment.

Investigators/Sub-Investigators will be instructed to contact CDD on the number below;

CDD
Research Department
02 9713 4011 then dial '2' for the Research Department

4.6 Blinding Procedures

In order to eliminate a placebo effect, the subject and clinical study investigators / staff will be blinded to the allocation of treatment in a double-blinded fashion.

Unblinded CDD staff will allocate subject numbers according to the treatment arm to which the subject is assigned. Medication will be dispensed by site staff as allocated by subject numbers provided by CDD.

5. STUDY PRODUCT

5.1 Dosage Schedule

The treatment will be administered as 50mg capsules of doxycycline, 250mg capsules of azithromycin and 150mg of rifabutin twice daily (morning and evening). The total number of capsules taken is 3 capsules a day in the first week then 6 capsules a day to be taken after food, with plenty of fluids, until the end of dosing period.

The treatment is to be on a dose escalation schedule, taken as per the following schedule:

Week 1 (Day 1 to Day 7)

Time	Doxycycline	Azithromycin	Rifabutin
Breakfast	50mg	250mg	150mg
Dinner			

Week 2 until EOT (Day 8 to Day 90)

Time	Doxycycline	Azithromycin	Rifabutin
Breakfast	50mg	250mg	150mg
Dinner	50mg	250mg	150mg

5.2 Supplies and Accountability

The Sponsor will be responsible for supplying the Investigators/institutions with the study drug. The Sponsor will not supply an Investigator/institution with the study drug until the Sponsor obtains all required documentation from the HREC and regulatory authorities. The Sponsor will ensure timely delivery of investigational product(s) to the Investigator(s) and maintain records that document shipment, receipt, disposition, return, and destruction of the study drug.

The Principal Investigator is responsible for study drug accountability, reconciliation, and record maintenance at his/her site. The Principal Investigator or designated, trained study site personnel will document all aspects of drug receipt, inventory, dispensing, return, reconciliation, and shipment.

All study medication must be kept in a locked area with access restricted to designated study site personnel. At each visit, a supply of study medication sufficient to last until the next clinic visit will be dispensed (plus a small additional quantity to account for the visit window). A list of the exact supply of study treatment dispensed to each Subject at each visit will be included on the Investigational Product Accountability Log that will need to be completed at each study visit. Used and unused product will be returned by the Subject to the clinic in the packaging in which it was provided to the Subject. The Investigator or delegated staff will reconcile all study units with those recorded as being dispensed and account for any inconsistencies in writing in the Investigational Product Accountability Log or similar document maintained in the Investigator study file.

At the conclusion of the study, all unused medication and all medication containers will be returned to the Sponsor. The Sponsor will ensure that a final report of drug accountability is prepared and that unused medications are appropriately destroyed.

5.3 *Packaging and Labeling*

Study medications will be packed and supplied by the Sponsor. Medication will be labelled with dosing instructions, the protocol number, subject number and will indicate that the supplies are for clinical trial use only. Study medications will be dispensed in study specific bottles, appropriately labeled. Packs must only be allocated to the subject whose number is borne on that pack. Subjects will be asked to return all partly used packs at each clinic visit. Further supply will be prescribed at visit suitable for the treatment period.

5.4 *Compliance*

Compliance will be assessed by capsule counts conducted by the Investigator or delegated staff at each visit. Compliance will be calculated as a percentage (%) of expected and the value will be entered on the appropriate page of the CRF at each study visit. Secondary 'on treatment' analyses will be performed. Participants will be deemed 'non-compliant' if compliance percentage falls below 80%, at which point, study staff are required to re-educate the participant on study medication dosing.

6. PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

6.1 *Prior and Concomitant Illnesses*

Participants who have provided consent will have their files reviewed and be required to provide details to document any illnesses present at the time of sample collection. Illnesses present at the time of informed consent, will be regarded as concomitant illnesses and will be documented on the appropriate pages of the Case Report Form (CRF) - see Section 8. Adverse Events

6.2 *Prior and Concomitant Treatments*

All treatments being taken by the subjects on entry to the study or at any time during the study are regarded as concomitant treatments and must be documented preferably by generic name on the appropriate pages of the CRF.

Changes to concomitant medications should be kept to a minimum during the study. However they may be altered at the discretion of the investigator according to Good Clinical Practice.

6.3 *Prohibited medication*

A list of drugs prohibited for use during the study is listed in Appendix 2.

7. INVESTIGATIONAL PROCEDURES AND SCHEDULE

7.1 *Description of Study Schedule*

7.1.1 Visit 1 Screening and Baseline

The subject will be approached regarding the study and will be asked to read the Subject Information and Consent document, encouraged to ask any questions, which will be answered to best of the Investigators ability. If the subject agrees to participate then they will be asked to provide written informed consent. Subject will be asked to provide details of their medical history, a description of their current symptoms and list all current medications. Subjects will also be given a Diary to collect symptoms relating to coronary artery disease and this will remain with the participant for the duration of the study. The Diary will be reviewed at each visit. Blood samples will be collected for laboratory tests. Pregnancy testing will be completed to ensure pregnant women are not included into the study. The following tests and assessments will be performed:

- FBC, ESR and EUC
- LFT and Lipid Studies
- CK-MB, high sensitivity Troponin T
- High sensitivity CRP
- *Chlamydophila pneumoniae* macrophage cultures
- *C. pneumoniae* titer measurement
- *C. pneumoniae* DNA detection using LAMP

- Pregnancy Testing
- Physical examination
- Vital Signs
- Symptomatology record
- ECG
- Angiogram
- Fractional Flow Reserve Study and pressure/flow measurements
- IVUS and OCT of culprit and non-culprit arteries (sub-study)
- Functional status – New York Heart Association Class
- Concomitant medications/ concomitant illness record
- Cardiac events record

The Subject's GP will be informed of the Subjects participation in the study, unless the subject does not consent to this disclosure.

Subjects will be dispensed with investigational product with instruction on correct administration. The investigational product will be dispensed in specifically labelled bottles.

7.1.2 Visit 2 (Day 30 ± 7)

At this visit the subject will be asked to report on current symptoms, changes to concomitant medications, and adverse events. A blood sample will be collected for laboratory tests. Subject will undergo a complete physical examination. The following assessments will also be performed:

- FBC, ESR and EUC
- LFT and Lipid Studies
- CK-MB, high sensitivity Troponin T
- High sensitivity CRP
- ECG
- Physical examination
- Vital Signs
- Symptomatology record
- Functional status – New York Heart Association Class

Subjects will be required to bring their medication with them for assessment of compliance.

7.1.3 Visit 3 – End of Treatment (Day 90 ± 21)

At this visit the Subject will be asked to report on current symptoms, changes to concomitant medications, and adverse events. A blood sample will be collected for laboratory tests. Subject will undergo a physical examination. The following assessments will also be performed:

- FBC, ESR and EUC
- LFT and Lipid Studies
- CK-MB, high sensitivity Troponin T
- High sensitivity CRP
- *Chlamydophila pneumoniae* macrophage cultures
- *C. pneumoniae* titer measurement
- *C. pneumoniae* DNA detection using LAMP
- Symptomatology record
- Physical examination
- Vital Signs
- ECG
- Angiogram
- Fractional Flow Reserve Study and pressure/flow measurements
- IVUS and OCT of culprit and non-culprit arteries (sub-study)
- Functional status – New York Heart Association Class

The Subject will be required to return all unused medication, and all used empty packaging for assessment of compliance. Subjects will also be required to return diaries for review and collection.

7.1.4 Visit 4 Final Follow up

Subjects will be asked to return a final time to the clinic at Day 180 (+21 days), where changes to symptoms, concomitant medications and adverse events will be recorded.

This will mark the end of subject participation.

7.1.5 Phone Contact (Day 150)

At this time, the participant will be contacted by phone to assess symptoms and any Adverse Events after discontinuation of antibiotic therapy and to check the use of concomitant medications.

7.2 Methods of Data Collection

7.2.1 Efficacy data

Response to antibiotic therapy will be assessed through a set of specified measurements:

7.2.1.1 Laboratory Studies

Efficacy of combination therapy will be assessed using blood samples collected at each visit. The following tests will be performed: FBC, ESR, lipid studies, liver function. In addition the following will be performed:

High Sensitivity C-Reactive protein (Hs-CRP) is a protein in the blood that increases in the presence of inflammation. Hs-CRP will measure the small amounts of CRP produced by the long-term inflammation from atherosclerosis.

- 4ml of blood will be collected in an EDTA tube. This sample will then be transferred to the laboratory for a full blood count
- 4ml of blood will be collected in an SST/Gel tube and transferred to the laboratory for liver function tests, Cholesterol, triglyceride studies and hs-CRP.
- 4ml of blood will be collected in a lithium heparin tube. Sample will then be transferred to the laboratory for CK-MB, Troponin T tests and UEC testing.
- 5ml of blood will be collected in a special ESR collection tube. This will then be sent to the laboratory to test the ESR
- 2 x 4ml of blood will be collected in citrated tubes. This sample will be transferred to the laboratory for *C. pneumoniae* detection by macrophage culture and *C. pneumoniae* titer determination (Visit 1 and 3 only).
- 3 x 4ml (i.e. 12 ml) of blood will be collected in an EDTA tube. This sample will be transferred to the laboratory for *C. pneumoniae* DNA detection by LAMP (Visit 1 and 3 only).

The total blood drawn on at Visit 1 and Visit 3 will be approximately 40mls. The total blood drawn at Visit 2 will be approximately 20 ml.

Blood samples will be tested by an experienced pathologist at Lavery Pathology for all tests excluding *C. pneumoniae* DNA detection by LAMP (exploratory research) and *C.pneumoniae macrophage cultures*.

7.2.1.2 Symptomatology

At each study visit, Subjects will be asked about their symptoms as reported during the Screening period and Visit 1. Changes in symptoms severity will be recorded in the source documentation.

7.2.1.3 Other

At each visit subjects will be asked to complete a Functional status – New York Heart Association Class questionnaire. This questionnaire will be used to assess changes in their quality of life.

7.2.1.4 Electrocardiogram (ECG)

An ECG will be performed for safety at Visit 1 and Visit 3.

7.2.1.5 Fractional Flow Reserve (FFR) Study

The results will assess the physiological effect of arterial stenosis.

Fractional Flow Reserve study is a guide wire based procedure to measure changes in blood pressure and flow across non-critical lesions in the coronary arteries performed at the time of a diagnostic or interventional coronary procedures. The results will assess the degree of arterial stenosis. This is a common clinically indicated procedure, which will be performed at

baseline and at Visit 3. Additional measurements of coronary flow as measured with the FFR pressure/flow guidewire will be recorded in the culprit and non-culprit artery at the time of FFR measurement.

7.2.1.6 Intra-coronary Imaging (IVUS and OCT) Sub-study

The results will assess the anatomic extent and morphology of atherosclerosis and response to treatment.

Intra-coronary imaging (IVUS and OCT) are catheter based imaging techniques allowing direct visualisation and characterisation of coronary plaque in vivo in addition to assessment of coronary arteries and stent expansion pre and post stent implantation. Indications for use are to determine lesion characteristics and to guide stent implantation. The results will be used to assess plaque volume and morphology and response to therapy.

7.2.2 Safety data

7.2.2.1 Laboratory Studies

A blood sample will be collected at each visit. The following blood tests will be performed: Full blood count, Full biochemistry as per detailed in 7.2.1.1. Additional tests may also be requested by the physician, but will not be part of the study unless related to adverse events.

The reference ranges for normal laboratory values and current certificate of NATA accreditation will be obtained from the relevant laboratories before the study commences.

Values outside the normal range will be noted in the CRF and those considered to be of clinical significance, particularly those related to the study medication, will be recorded as adverse events. Values outside the normal range will be monitored until they have been stabilised.

Comparison of pre and post treatment C. pneumoniae parameters will be assessed to show efficacy of the ACAC combination.

7.2.2.2 Adverse Events

Adverse events will be assessed at each visit throughout the study by open general questioning from the Investigator or study staff and entered into the CRF. The use of other medications during the study will also be noted along with the reason for use in order to capture other potential adverse effects.

8. ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse event

The term adverse event (AE) covers any sign, symptom, syndrome, or illness that appears or worsens in a subject during the period of observation in the clinical study and that may impair the well being of the subject. The term also covers laboratory findings or results of other diagnostic procedures that are considered to be clinically relevant (e.g. that require unscheduled diagnostic procedures or treatment measures, or result in withdrawal from the study). Undesirable experiences including intercurrent events (or diseases), drug reactions and clinical abnormalities or clinically significant laboratory test abnormalities, which occur during the course of a trial, are also included.

The AE may be:

- A new illness
- Worsening of a sign or symptom of the condition under treatment or of a concomitant illness
- An effect of a comparator drug
- Unrelated to participation in the clinical study
- A combination of one or more of these factors

Thus, no causal relationship with the study medication is implied by the use of the term “adverse event”. Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an adverse event. Planned surgical measures permitted by the study protocol and the conditions leading to these measures are not adverse events. (See Appendix 4 - Definitions of causality and severity of adverse events).

Interviews concerning AEs must be conducted with regard to objectivity. Questions must be phrased so that they do not, by suggestion, lead the subject into giving information, which is not valid. The investigator must carefully evaluate the response and comments of the subject in order that he or she may judge the true nature and severity of the adverse event.

8.2.2 Serious adverse event

A serious adverse event (SAE) is any AE that occurs at any dose of the study medication or at any time during the period of observation that:

- Results in death
- Is immediately life threatening
- Requires or prolongs hospitalisation
- Results in persistent or significant disability or incapacity
- Occurs with overdose
- Involves cancer
- Involves congenital anomaly/birth defects
- Is medically important
- Requires medical intervention to prevent permanent impairment or damage

“Medically important” events are events that may not be immediately life threatening or result in death or hospitalisation, but may jeopardise the subject or require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse. Such events should be documented and reported as SAEs.

Events “requiring medical intervention to prevent permanent impairment or damage” are events where the investigator believes that medical or surgical intervention is necessary to preclude permanent impairment of a body function or to prevent damage to a body structure.

As there is an overlap between the terms “medically important event” and events requiring medical intervention to prevent permanent impairment or damage, it is left to the discretion of the investigator to select the more applicable of the two criteria when completing the “Serious adverse event form”.

All AEs observed by the investigator or reported by the subject whether or not considered related to the test medication will be followed up by the investigator until resolution, stabilisation or lost to follow up.

All AEs will be reported in the adverse event forms of the CRF and assessed by the investigator in terms of severity and relationship to the test medication. The outcome of the adverse event as well as the measures taken as a result of this event will be described in the adverse event form of the case record form.

8.2 Period of observation

AEs fall into the categories non-serious and serious (see section 8.1.2 serious adverse event). The period of observation for adverse events extends from the time the subject gives his informed consent, until he/she undergoes the final clinical examination scheduled in the study. All AEs unresolved at the final clinical examination will be monitored until they resolve or are clearly determined to be due to a subject's stable or chronic condition, or intercurrent illness(es).

Adverse events occurring after this period of observation are to be reported if the investigator feels that there is a causal relationship to the test medication.

8.3 Documentation and Reporting of Adverse Events by the Investigator

All AEs that occur after the subject has signed the informed consent document must be documented on the pages provided in the CRF. The adverse events must also be recorded in the subject's medical records.

The following approach will be taken to documentation:

- All SAEs must be documented on "Serious Adverse Event" forms.
- Non-serious AEs must be documented in the adverse event forms supplied in the CRF.
- Subjects should be encouraged to note adverse events and discuss them at their next visit or to report any event of concern to the investigator immediately.
- Every attempt should be made to describe the AE in terms of a diagnosis. If appropriate, component symptoms should also be listed below the diagnosis. If only non-specific signs or symptoms are present, these should be recorded as a diagnosis.
- For each event occurring after the first dose of study medication/randomisation, the investigator will classify whether the event is to be considered treatment emergent and whether there is a reasonable possibility that the event was associated with the use of the study medication.

An AE that occurs during the study after the first dose of study medication/randomisation will be considered as treatment emergent if (1) it was not present at the time of the first dose of study medication/randomisation and is not a chronic condition that is part of the subject's history, or (2) it was present at the time of the first dose of the study medication/randomisation or as part of the subject's medical history, but its intensity (severity or frequency) has worsened after the first dose of study medication/randomisation.

AEs that fulfil the criteria for seriousness (section 8.1.2 serious adverse event) have to be reported to the sponsor immediately (for procedure see Section 8.4 Immediate reporting by investigator to sponsor).

AEs that do not fulfil the criteria for immediate reporting will be supplied to the sponsor in the same way as all other CRF pages.

All subjects who have AEs -whether considered associated with the use of the study medication or not-must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice- even after the end of the period of observation-until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

All questions on the completion and supply of adverse event forms and any further forms issued to the investigator at a later date to clarify unresolved issues should be addressed to the sponsor.

8.4 Immediate Reporting by Investigator to Sponsor

Serious adverse events must be documented on "Serious adverse event" forms and supplied to the sponsor within 24 hours or at the latest on the following working day. The investigator must also inform the site monitor in all cases. The sponsor will ensure all legal reporting requirements are met.

Serious Adverse Events should be reported to:
Prof Thomas Borody
CDD
02 97134011 or 0417 733 707

The initial report must be as complete as possible, including details of current illnesses and (serious) adverse events and assessment of the causal relationship between the event and the study medication. Copies of the case report form pages containing the following information (if not already supplied to the sponsor) must be sent with the "Serious adverse event" form:

- Demography
- Medical and surgical history
- Previous and concomitant medication
- Study medication administration record

The forms documenting all non-serious adverse events that have occurred up to the time of occurrence of a serious adverse event, even if still incomplete, must also be sent with the "Serious adverse event" form.

Information not available at the time of the initial report (e.g. an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up "Serious adverse event" form that carries the number(s) of the initial report(s).

The principal investigator at each site will be responsible for reporting SAE's at their site. The principal investigator at Liverpool Hospital (lead site) will be responsible for reporting all SAE's to the Human Research Ethics Committee.

8.5 Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) will monitor participant safety, data quality and evaluate progress of the study. The DSMB will be composed of 3 members including a biostatistician. They monitor the safety of the study and will advise on the conduct and the ongoing validity of the trial.

Dr Ross Grant will be the nominated DSMB contact and will be responsible for communication between the DSMB and the Sponsor, Study Sites (and relevant personnel), Ethics committee and Investigators.

9. WITHDRAWALS AND DEFAULTERS

Subjects may be withdrawn from study medication for the following reasons:

- Determined to be necessary as per investigator, including:
 - Due to AE or SAE
 - Development of any exclusion criteria may be cause for discontinuation
 - Treatment with prohibited medications
 - Female participants fall pregnant or female partners of male participants fall pregnant
 - Subject lost to follow up (does not complete all Study visits and Study staff have failed to make contact with the subject nor arrange a successful visit for a period of 1 month past a scheduled Study visit).
 - If, in the investigators opinion continuation in the study would be detrimental to the subjects well-being

- Subjects are free to withdraw consent at any time.

In all cases the reason for withdrawal, if given, must be recorded in the Case Report Form and in the subject's medical records. The subject must be followed up to establish whether the reason was an adverse event, and if so, this must be reported in accordance with the procedures in section 8 Adverse Events.

Every effort will be made to follow up subjects who withdraw or are withdrawn from the study. All subjects withdrawn or who choose to withdraw consent will be asked to return to the study site to undergo final EOT study procedures, or as far as possible, all safety examinations scheduled for the final EOT visit for all subjects who taken at least one dose of the study drug/s. This is especially important for those subjects who withdraw due to AEs or SAEs, in order to allow for collection of detailed safety data in accordance with the procedures in section 8 Adverse Events. These subjects should be given appropriate care under medical supervision until the symptoms of any AE resolve, or the subject's condition becomes stable. Subjects may then be contacted at the end of the study for final safety monitoring procedures.

While primary analysis will be of all consenting subjects, a secondary 'on-treatment' analysis will be performed on subjects who remain compliant at the end of the 90-day endpoint.

10. EMERGENCY PROCEDURES

10.1 *Emergency Sponsor Contact*

In emergency situations, the subject should phone the number listed on the subject information sheet.

10.2 *Emergency Treatment*

During and following a subject's participation in the trial, the investigator/institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator/institution should inform the subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware. Any participant with potential cardiac emergency will be directed to the nearest hospital Emergency Room.

11. STATISTICAL PROCEDURES

11.1 *Analysis Variables*

Analysis of efficacy will be performed according to intention-to-treat method and include all subjects who received the combination antibiotic therapy. However as the primary efficacy analysis is of subjects with paired FFR assessments, only such subjects will be included in the primary analysis. All other subjects will be included in secondary analyses. Numerical data will be presented as mean \pm SD for normally distributed variables and as median with interquartile range for non-normally distributed data. Non-normally distributed data will be log-transformed (e.g. CRP levels) before being used for comparisons. Significant changes in subjects' arterial flow, clinical symptoms and biochemical variables over time compared with that baseline will be assessed using an analysis of covariance. Differences between treatment and control groups in the occurrence of FFR \geq 80 will be assessed using Fisher's exact test. All tests will be 2-tailed with a 5% significance level.

11.2 *Interim Analysis*

A DSMB directed interim analysis is planned when 60 paired FFR data are available. At this time point, the trial would declare the combination therapy as effective if the p value is less than 0.05. However, the trial might still continue to collect extra data to accurately characterise side effects as advised by the DSMB.

11.3 *Sample Size Justification*

This is a feasibility study designed to provide preliminary observations and generate hypothesis for future studies. The sample size of 60 subjects with paired FFR was not defined on the basis of a clinical endpoint hypothesis but rather to provide information about antibiotic combination therapy efficacy and safety. The sample size required is established by assessment of the minimum number of subjects needed to provide reliable and non-trivial results. The sample size is in the range of the intervention group in the azithromycin clinical trial of subjects with CAD (n=60).

12. ETHICAL AND LEGAL ASPECTS

12.1 Good Clinical Practice

The procedure set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor and Investigator abide by the principles of the NIH Good Clinical Practice (GCP) guidelines of the Therapeutic Goods Administration (TGA) published in CPMP/ICH/135/95 July 2000 and the ethical principles laid down in the current revision of the Declaration of Helsinki 2004 (Appendix 5) and the NHMRC National Statement on Ethical Conduct in Human Research 2007. The study will also be carried out in keeping with local legal and regulatory requirements.

12.2 Delegation of Investigator Responsibilities

The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the study treatments, and their trial-related duties and functions.

The investigator should maintain a list of co-investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

12.3 Subject Information and Informed Consent

Before being admitted to the clinical study, the subject must consent to participate after the nature, scope, and possible consequences of the trial have been explained in a form understandable to him or her.

An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. The document must be in a language understandable to the subject and must specify who informed the subject. The person who informs the subject must be a physician.

After reading the informed consent document, the subject must give consent in writing. The subject's consent must be confirmed at the time of consent by the personally dated signatures of; the subject, person conducting the informed consent discussions, and a witness.

It is recommended that the investigator inform the subjects' primary physician (GP) about the subjects' participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

12.4 Confidentiality

Only the Subject Number and Subject initials will be recorded in the Case Report Form, and if the Subject name appears on any other document (e.g. pathologist report), it must be obliterated before a copy of the document is supplied with the CRF. Study findings stored on a computer will be stored in accordance with local data protection laws. The subjects will be told that representatives of the Sponsor, HREC, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The Investigator will maintain a confidential Subject identification list (Subject numbers with the corresponding Subject names) to enable records to be identified.

12.5 Protocol Amendments

Neither the investigator nor the sponsor will alter this study protocol without obtaining the written agreement of the other. Once the study has started, amendments should be made only in exceptional cases. The changes then become part of the study protocol.

12.6 Approval of the Study Protocol and Amendments

Before the start of the study, the study protocol, informed consent document, and any other appropriate documents will be submitted to the institutional ethics committee (HREC) with a cover letter or a form listing the documents submitted, their dates of issue, and the site for which approval is sought. If applicable the documents will also be submitted to the authorities, in accordance with local legal requirements (CTN).

Study medication can only be accessible to the investigator for study use after documentation on **all** ethical and legal requirements for starting the study has been received by the sponsor. This documentation must also include a list of the members of the HREC and their occupations and qualifications. If the HREC will not disclose the names of the committee members to the Sponsor, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP and NHMRC guidelines. Formal approval by the HREC should preferably mention the study title, study code, study site, and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member.

Before the first subject is enrolled in the study, all ethical and legal requirements must be met. The HREC and, if applicable, the authorities must be informed of all subsequent amendments, in accordance with local legal requirements. Amendments must be evaluated to determine whether formal approval must be sought and whether the informed consent document should also be revised. If formal HREC approval is required then the amendments must not be implemented until approval is granted.

The investigator must keep a record of all communication with the HREC and, if applicable between a coordinating investigator and the HREC. This also applies to any communication between the investigator (or coordinating investigator, if applicable) and the authorities.

12.7 Ongoing Information for HREC

If required by legislation or the HREC, the investigator must submit to the HREC:

- Information on serious or unexpected adverse events as soon as possible.
- Periodic reports on the progress of the study.

12.8 Premature Closure of the Study

The sponsor or the investigator has the right to close this study at any time. As far as possible, this should occur after mutual consultation. The HREC must be informed, if required by legislation.

Should the study be closed prematurely, all study materials (completed, partially completed, and blank case report forms, study medication, etc) must be returned to the sponsor, as if the study had been completed.

12.9 Record Retention

The following records must be retained by the investigator for a *minimum* of 15 years after the completion or termination of the study:

- Signed informed consent documents for all subjects
- Subject identification code list, screening log (if applicable), and enrolment log
- Record of all communications between the investigator and the HREC
- Composition of the HREC (or other applicable statement as described in section 12.6 Approval of the study protocol and amendments)

- Record of all communications between the investigator and sponsor
- List of sub investigators and other appropriately qualified persons to whom the investigator had delegated significant trial-related duties, together with their roles in the study and their signatures
- Copies of case record forms and of documentation of corrections for all subjects
- Drug accountability records
- Records of any body fluids or tissue samples obtained
- All other source documents (subject records, hospital records, laboratory records etc)

Normally, these records will be held in the investigator's archives. If the investigator is unable to meet this obligation, he or she must ask the sponsor for permission to make alternative arrangements. Details of these arrangements should be documented.

12.10 Liability and Insurance

The sponsor will underwrite any legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

12.11 Ongoing Access to Study Drug

According to the Declaration of Helsinki (Revision 2008), at the conclusion of the study, every subject entered into the study should be assured of access to the best proven, in this case, therapeutic method identified by the study. As such, this study is assessing the efficacy of a combination therapy. If this therapy should be deemed to be beneficial to the subject, the Sponsor will provide the subject with access to the study drug from the time at which the study has concluded through to either the Study drug being registered for use in Australia, the application for registration in Australia is rejected by the TGA, the Sponsor determines that the Study drug will not succeed in gaining registration, or through further studies determines that it is not the best proven therapy.

13. ADMINISTRATION

13.1 Case Report Forms (CRFs)

A CRF will be provided by the sponsor for each subject.

All protocol-required information collected during the study must be entered by the investigator, or designated representative, in the case report form. Details of case report form completion and correction will be explained to the investigator. If the investigator authorises other persons to make entries in the case report form, the names, positions, signatures, and initials of these persons must be supplied to the sponsor.

The investigator, or designated representative, should complete the case report form pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The completed case report form must be reviewed and signed by the investigator named in the study protocol or by a designated sub-investigator.

The sponsor will retain the originals of all case report forms. The investigator will retain a copy of all completed CRF pages.

13.2 Monitoring

Monitoring and auditing procedures developed by the sponsor (or delegate) will be followed, in order to comply with GCP guidelines. On-site checking of the case record forms for completeness and clarity, cross checking with source documents, and clarification of administrative matters will be performed.

13.2.1 Study monitoring

Monitoring will be done by personal visits from a representative of the sponsor (site monitor) who will review the case record forms and source documents. By frequent communications (letter, telephone and fax), the site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements.

All unused study materials are to be returned to the sponsor after the clinical phase of the trial has been completed.

13.3 Source Data Verification and On-site Audits

Regulatory authorities, the HREC, and/or the sponsor's clinical quality assurance group may request access to all source documents, case record forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must supply support at all times for these activities.

13.4 Archiving

All study documents (including source documents) will be archived for a period of 15 years following completion of the study (according to the requirements of GCP). The archive boxes must be labelled clearly to identify the study/subject/documents as well as the 15-year archiving requirement.

The Investigator must store these in a secure, limited access area.

13.5 Use of Study Findings

All information concerning the product as well as any matter concerning the operation of the sponsor such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by and are unpublished, are confidential and must remain the sole property of the sponsor. The investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from sponsor is obtained.

The original case report forms will be stored for 15 years completed as part of the study.

By signing the study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

13.6 Report and Publications

The sponsor will prepare the final report on the study 12-16 weeks after the close of the database and the close out meeting. The investigator (or co-investigator) will be required to

sign a statement that he/she has read the report and confirms that, to the best of his or her knowledge, it accurately describes the conduct and results of the study.

The Final Study Report may be made available to the HREC, at the HREC's request. The HREC Final Study Report will be sent to the HREC once the study has been closed. This document includes information on how the study was performed, number of AE's and SAE's and their resolution status.

The findings of the study may be published in a scientific journal or presented at a scientific meeting. Before submitting the results of the study for publication or presentation, the investigator will allow the sponsor 30 days in which to review and comment on the manuscript. For any publication manuscript prepared by the sponsor, the sponsor reserves the right to select the investigators who will be authors and review the manuscript. The sponsor will allow the selected investigators 30 days for full review of the manuscript before publication.

14. STUDY DURATION AND DATES

The duration of this study is to be a period of 24 months with subject recruitment proposed to start in Q1 2016 and ending in Q2 2018.

15. DECLARATION OF SPONSOR AND INVESTIGATOR

15.1 Declaration of Sponsor

The study protocol was subject to critical review and has been approved by the appropriate protocol review committee of the sponsor. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product.
- The moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of GCP.

The investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Authorised Representative

Date: ___/___/___ Signature: _____

Name (block letters): _____

Statistician

Date: ___/___/___ Signature: _____

Name (block letters): _____

15.2 Declaration of Investigator

I _____

- have received approved product information for the combination therapy consisting of rifabutin, azithromycin and doxycycline.
- have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol.
- will not enrol the first subjects in the study until I have received approval from the appropriate HREC and until all legal requirements in have been fulfilled.
- agree the study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the GCP guidelines of the TGA.
- agree to obtain, in the manner described in this study protocol, written informed consent or witnessed verbal informed consent to participate for all subjects enrolled in this study.
- am aware of the requirements for the correct reporting of serious adverse events, and I undertake to document and to report such events as requested.
- agree with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals.
- agree to keep all source documents and case report forms as specified in Section 12.9 Record retention, of this protocol.
- will provide curriculum vitae before the study starts, which may be submitted to regulatory authorities.

Investigator

Date: ___/___/___ Signature: _____

Name (block letters): _____

16. REFERENCES

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APPENDIX 1: STUDY LABORATORY TESTS

Blood Examination

BIOCHEMISTRY

LFTs

Albumin
Bilirubin
GGT (Gamma Glutamyl Transferase)
ALP (Alkaline Phosphatase)
ALT (Alanine Aminotransferase)
AST (Aspartate Aminotransferase)

CK-MB

High sensitivity Troponin T

Hs-CRP

Electrolytes, urea, creatinine

Serum lipids

HAEMATOLOGY

Haemoglobin
Red Cell Count
Haematocrit
MCV
MCH
MCHC
RDW
WCC
Neutrophils
Lymphocytes
Eosinophils
Basophils
NRBC
Platelets
ESR

Chlamydomphila pneumoniae macrophage cultures

Chlamydomphila pneumoniae titers

Chlamydomphila pneumoniae DNA detection by LAMP (exploratory)

APPENDIX 2 – LIST OF RESTRICTED MEDICATIONS

The below list includes the trade names of restricted medications. However it cannot be assumed that this is a complete list. As such, all concomitant medications should be checked, regardless of their presence/absence on the below list.

INR monitoring will be conducted on any participant taking Warfarin

Closer clinical and blood-test monitoring on those with the following concomitant therapies:

- Calcium channel blockers
- Beta blockers
- Amiodarone
- Anti-depressants
-

Statins and ezetimibe can cause abnormal liver function tests. In such patients slower initiation of antibiotics may be required

Absolutely Contraindicated

- Colchicine (Azithromycin interaction)

Relatively Contraindicated

- Theophylline
- Terfenadine
- Tolterodine

APPENDIX 3: SERIOUS ADVERSE EVENT REPORT FORM

- Initial Report: Date ___/___/___
- Follow-up Report (1): Date ___/___/___
- Follow-up Report (2): Date ___/___/___
- Follow-up Report (3): Date ___/___/___

SITE INFORMATION					
Protocol No.	Site No.	Investigator Name	Reporter Name	Reporter Phone No.	Sponsor
PATIENT INFORMATION					
Patient No.	Patient Initials	D.O.B	Sex	Height (at time of event)	Weight (at time of event)
		___/___/___	Male / Female	cm	kg
EVENT					
Event Onset Date	Resolution Date	Severity of Event	Relationship to Product	Treatment Required	Outcome of Event
___/___/___	___/___/___	<input type="checkbox"/> Mild <input type="checkbox"/> Moderate <input type="checkbox"/> Severe	<input type="checkbox"/> Definitely related <input type="checkbox"/> Probably related <input type="checkbox"/> Possibly related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Not related (If cause known, specify _____) <input type="checkbox"/> Unassessable	<input type="checkbox"/> No <input type="checkbox"/> Yes (If yes, specify in comments and con meds)	<input type="checkbox"/> Recovered without treatment <input type="checkbox"/> Recovered with treatment <input type="checkbox"/> Event ongoing without treatment <input type="checkbox"/> Event ongoing & controlled with treatment <input type="checkbox"/> Event ongoing & not controlled with treatment <input type="checkbox"/> Patient died
Did the SAE occur during Lead-In (Pre-randomisation) Phase					YES / NO
EVENT CATEGORY (Check all that apply)					
<input type="checkbox"/> Fatal <input type="checkbox"/> Life Threatening <input type="checkbox"/> Required Inpatient Hospitalisation or Prolongation of Existing Hospitalisation <input type="checkbox"/> Resulted in Permanent or Significant Disability <input type="checkbox"/> Congenital Anomaly / Birth Defect		<input type="checkbox"/> Other Significant Medical Hazard (Medically Important) <input type="checkbox"/> Necessitates medical or surgical intervention to preclude permanent impairment of body function / structure <input type="checkbox"/> Necessitates medical or surgical intervention to relieve unanticipated, temporary impairment of body function <input type="checkbox"/> Results in permanent impairment of a body function or permanent damage to a body structure			

STUDY DRUG INFORMATION						
Product Name	Form	Strength	Dose	Route	Date of First Intake	Date of Last Intake

					__/__/__	__/__/__
					__/__/__	__/__/__
					__/__/__	__/__/__
					__/__/__	__/__/__

ACTION TAKEN

- None
- Dose Reduced to : _____ Date: ____/____/____
- Dose Increased to : _____ Date: ____/____/____
- Drug Interrupted : Date: ____/____/____
- Drug Restarted : Date: ____/____/____
- Drug Discontinued Permanently

CONCOMITANT MEDICATIONS
(Exclude study drugs and medications used to treat this SAE)

Medication Name	Form	Strength	Dose	Date Started	Date Stopped	Reason
				__/__/__	__/__/__	
				__/__/__	__/__/__	
				__/__/__	__/__/__	
				__/__/__	__/__/__	
				__/__/__	__/__/__	

RELEVANT SAE LABORATORY DATA

Test	Date of Baseline Test	Value of Baseline Test	Date of Study Test	Value of Study Test
	__/__/__		__/__/__	
	__/__/__		__/__/__	
	__/__/__		__/__/__	
	__/__/__		__/__/__	
	__/__/__		__/__/__	
	__/__/__		__/__/__	

APPENDIX 4: DEFINITIONS OF CAUSALITY AND SEVERITY OF ADVERSE EVENTS

1. *Criteria for determining relationship of clinical adverse events to test drugs*

a. **Not Related**

This category applies to those adverse experiences that, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).

b. **Unlikely** (must have two of the following)

In general, this category can be considered applicable to those adverse experiences, which, after careful medical consideration at the time in which they are evaluated, are judged to be unrelated to the test drug. An adverse experience may be considered unlikely related if or when:

- i. It does not follow a reasonable temporal sequence from administration of the drug.
- ii. It could readily have been produced by the participant's clinical state, environment or toxic factors, or other modes of therapy administered to the patient.
- iii. It does not follow a known or expected response pattern to the test drug.
- iv. It does not reappear or worsen when the drug is re-administered.

c. **Possibility** (must have two of the following)

This category applies to those adverse experiences which, after careful medical consideration at the time they are evaluated, a connection with the study medication appears unlikely but can not be ruled out with certainty. An adverse experience may be considered possibly related if or when:

- i. It follows a reasonable temporal sequence from administration of the drug.
- ii. It could not readily have been produced by the participant's clinical state, environment or toxic factors, or other modes of therapy administered to the patient.
- iii. It follows a known response pattern to the test drug.

d. **Probably** (it must have three of the following)

This category applies to adverse experiences which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study medication. An adverse experience may be considered probably related if or when:

- i. It follows a reasonable temporal sequence from administration of the drug.
- ii. It could not readily have been produced by the participant's clinical state, environment or toxic factors, or other modes of therapy administered to the patient.
- iii. It disappears or decreases on cessation or reduction in dose. There are important exceptions when the adverse event does not disappear upon discontinuance of the drug, yet drug-relatedness clearly exists; eg. a) bone marrow depression, b) fixed drug eruptions, and c) tardive dyskinesia.
- iv. It follows a known response pattern to the test drug.

e. **Definitely** (Must have all four of the following)

This category applies to those adverse experiences which the investigator feels are incontrovertibly related to the study medication. An adverse experience may be considered definitely related if or when:

- i. It follows a reasonable temporal sequence from administration of the drug.
- ii. It could not readily have been produced by the participant's clinical state, environment or toxic factors, or other modes of therapy administered to the patient.
- iii. It disappears or decreases on cessation or reduction in dose and recurs with re-exposure to the drug. This is not to be construed as requiring re-exposure to the drug. However, a category of definitely related can only be used when recurrence is observed.
- iv. It follows a known response pattern to the test drug.

3. *Criteria for rating the severity of adverse events:*

“Adverse Events”: Any adverse change or experience from the patient’s baseline (pre-treatment) condition which occurs during the course of a clinical study, after starting treatment, whether considered treatment related or not. “Treatment” includes all investigational agents administered during the course of the study.

“Severity of adverse events”: All adverse events must be rated on a three-point scale of increasing severity.

- a. **Mild:** symptoms barely noticeable to the patient; does not influence performance or function. Prescription drugs not ordinarily needed for relief of symptoms but may be given because of the personality of the patient.
- b. **Moderate:** symptoms of a sufficient severity to make patient uncomfortable; performance of daily activities is influenced; patient is able to continue in the study; treatment for symptoms may be needed.
- c. **Severe:** symptoms cause severe discomfort. May be of such severity that patient can not continue performance of daily activities. Severity may cause cessation of treatment with study drugs; treatment for symptoms may be given and/or patient hospitalised.

APPENDIX 5 – DECLARATION OF HELSINKI 2008

1

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens. DoH/Oct2008

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy DoH/Oct2008volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed. DoH/Oct2008

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be

declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication. DoH/Oct2008

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or

Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.