Oxford Haemodynamic Adaptation to Reduce Pulsatility Trial:

Randomised, placebo controlled, double-blind crossover study of effects of sildenafil on cerebral arterial pulsatility in patients with cryptogenic or lacunar stroke and small vessel disease

(OxHARP)

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I declare that the Chief Investigator or other investigators have no conflict of interest

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. KEY STUDY CONTACTS

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2. SYNOPSIS

Study Title	Oxford Haemodynamic Adaptation to Reduce Pulsatility: Randomised, placebo controlled, crossover study of effects of sildenafil on cerebral arterial pulsatility in patients with cryptogenic or lacunar stroke and small vessel disease
Internal ref. no. (or short title)	OxHARP
Study Design	Randomised, placebo-controlled, double-blind, crossover study

Study Participants	Cryptogenic or lacunar stroke or TIA, with mild to moderate small vessel disease changes on MRI or CT	
Planned Sample Size	75	
Treatment duration	3 weeks per treatment (12-14 weeks in total)	
Follow up duration	11 to 15 weeks	
Planned Study Period	36 months	
	Objectives	Outcome Measures
Primary	Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) reduces cerebral arterial pulsatility on transcranial ultrasound, compared to placebo in patients with chronic vascular injury to the brain (small vessel disease)	Gosling's MCA pulsatility index on Transcranial ultrasound (MCA-PI)
Secondary	1) Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) alters cerebral arterial reactivity to carbon dioxide on transcranial ultrasound, compared to placebo 2) Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) has non-inferior effects on cerebral arterial pulsatility and reactivity to carbon dioxide on transcranial ultrasound, compared to cilostazol (a PDE 3 inhibitor	1) and 2) Change in mean flow velocity on TCD before vs during CO2 challenge
Tertiary Objectives 1) Assess whether 3 weeks of treatment with sildenafil or cilostazol increases cerebral arterial reactivity to carbon dioxide on MRI, compared to placebo 2) Biochemical / genetic predictors of response to treatment	Reactivity of BOLD signal on MRI to CO2 challenge Markers of endothelial function (e.g. ICAM, VEGF, vWF) and genetic targets of the medications in the trial	After 3 weeks of treatment with sildenafil and cilostazol versus placebo Analysed in the 2 years after the last visit of the last participant.

Investigational Medicinal Product(s)	Sildenafil Cilostazol
Formulation, Dose, Route of Administration	Sildenafil: Oral 25 - 50mg tds, tablet Cilostazol: Oral, 50 - 100mg, BD, tablet (with mid-day placebo) Placebo: Oral, TDS

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CI	Chief Investigator
CO2	Carbon dioxide
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computed Tomography (brain scan)
CTA	Clinical Trials Authorisation
CTRG	Clinical Trials and Research Governance
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
etCO2	End-tidal carbon dioxide
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IRB	Independent Review Board
MCA-PI	Middle cerebral artery pulsatility index
MHRA	Medicines and Healthcare products Regulatory Agency
N2	Nitrogen
NHS	National Health Service
NRES	National Research Ethics Service

PDE5	Phosphodiesterase 5 inhibitor
PI	Principal Investigator
MCA-PI	Middle cerebral artery pulsatility index (Gosling's)
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Study Master File
TSG	Oxford University Hospitals NHS Foundation Trust / University of Oxford Trials Safety Group

4. BACKGROUND AND RATIONALE

Chronic injury to the small vessels of the brain ('small vessel disease') is associated with acute stroke,[1] progressive cognitive decline,[2] late-onset refractory depression,[3] functional impairment in daily living[4] and increased mortality.[5] Originally defined on pathological grounds, it is now recognised primarily on brain imaging by a pattern of white matter hyperintensiities on T2-based imaging in the periventricular and deep white matter, or acute lacunar strokes, cerebral microbleeds and dilated perivascular spaces.[6] Despite accounting for more than 30% of strokes and 40% of dementia,[7] the underlying mechanism of small vessel injury is unclear and may reflect systemic haemodynamic influences on the cerebral vasculature, such as increased transmission of pulsatile blood pressure to the brain[8] or inherent dysfunction of the small vessels and their endothelium.[9] However, white matter hyperintensities are highly prevalent in the population, affecting the majority of patients over the age of 65 and many patients with advanced imaging changes of SVD remain functionally independent.[10] As such, it is important to target novel treatments at those patients with SVD who are at an increased risk of morbidity.

The underlying cause of many strokes remains undefined despite intensive investigation ('cryptogenic' stroke), indicative of a major gap in our understanding of the pathophysiology of stroke and resulting in significant excess morbidity.[11] In patients with small vessel disease, acute stroke due to small vessel occlusion (lacunar stroke) is commonly recognised as the principle type of stroke, but cryptogenic stroke remains common. Furthermore, classification of lacunar stroke relies upon clinical and imaging criteria rather than demonstration of an in situ occlusion of a perforating small vessel. This can therefore result from multiple aetiologies and rates of misclassification of lacunar events are high. Given this aetiological uncertainty, the diffuse cerebral arteriopathy of small vessel disease is likely to contribute to both lacunar stroke and cryptogenic strokes.[12] Lacunar and cryptogenic strokes are therefore a marker of an increased risk of morbidity in the context of chronic SVDs, and identification of an effective treatment for the associated arteriopathy may also offer a novel intervention for a large number of cryptogenic strokes that currently have no specific treatment option.[12]

Hypertension is the strongest modifiable risk factor for small vessel disease, but there is only limited evidence that reduction of blood pressure alone significantly reduces progression of development of SVD.[13] However, pulsatility of blood flow in the brain is associated with small vessel disease,[8] resulting from increased transmission of the pulsatile aortic waveform to the brain through stiff conduit vessels. Secondly, there is significant endothelial dysfunction in SVDs, with increased breakdown of the blood-brain barrier detected by increased extravasation of contrast material on MRI in both white matter hyperintensities and normal appearing white matter.[14] This

may be secondary to factors such as hypertension or arterial pulsatility, or it may reflect an underlying, primary endotheliopathy.

Aortic pulsatility, aortic stiffness and transmission of the aortic waveform are increased by reflection of the outgoing aortic pressure wave from the distal small vessels back towards the aorta, increasing aortic pulsatility. The resulting enhanced pulsatility of blood reaching the low resistance small vessels in the brain causes increased shear stress during systole and potential hypoperfusion of tissues during diastole.[15] This is potentially modifiable by vasodilating medications that delay the site and severity of wave reflection, reducing aortic pulsatility. Such medications may also act upon muscular conduit vessels (distal ICA / MCA) to increase elasticity, improve dampening of the aortic waveform and reducing pulsatility at distal vessels.

Endothelial dysfunction is associated with abnormalities of vasodilation and vasoconstriction, controlled through the endothelial nitric oxide – cGMP pathway.[16] This can be tested by measuring the cerebral blood flow response to carbon dioxide,[17] with either transcranial ultrasound or MRI imaging. A number of agents have the potential to effect the endothelium. Phosphodiesterase inhibitors reduce breakdown of cGMP, potentiating the nitric oxide driven vasodilation of small arterioles. Phosphodiesterase 5 inhibitors (sildenafil, tadalafil) have been widely used in erectile dysfunction and in another occlusive vasculopathy (pulmonary arterial hypertension), with an established safety profile and tolerability. A number of studies have demonstrated a potential benefit of sildenafil on endothelial function,[18] but this has not been assessed specifically in cerebral small vessel disease. Cilostazol is a phosphodiesterase 3 inhibitor that has been tested in clinical trials of patients with stroke with a reduction in recurrent events during follow up,[19] potentially explained by a reduction in cerebral arterial pulsatility.[20] Polymorphisms in the PDE3 gene have also been shown to be associated with stroke risk in genome-wide association studies.[21] However, it has an additional antiplatelet effect, complicating understanding of how it's pharmacological effects translates into clinical outcomes, and complicating its use in clinical practice.

This study therefore aims to test the physiological effect of sildenafil, a phosphodiesterase 5 inhibitor, on cerebral arterial pulsatility, with secondary assessment of effects on cerebrovascular endothelial function. This will be tested at doses consistent with those tolerated in pulmonary arterial hypertension. It will be tested in recovered patients with a previous cryptogenic or lacunar stroke or probable TIA requiring treatment, and mild-moderate small vessel disease. This will allow assessment of effects on cerebrovascular physiology in the presence of small vessel injury, but prior to establishment of likely irreversible changes. Secondly, patients will then receive cilostazol, to compare the relative effect of PDE5 inhibition and PDE3 inhibition on cerebrovascular haemodynamics as a secondary outcome measure.

5. OBJECTIVES AND OUTCOME MEASURES

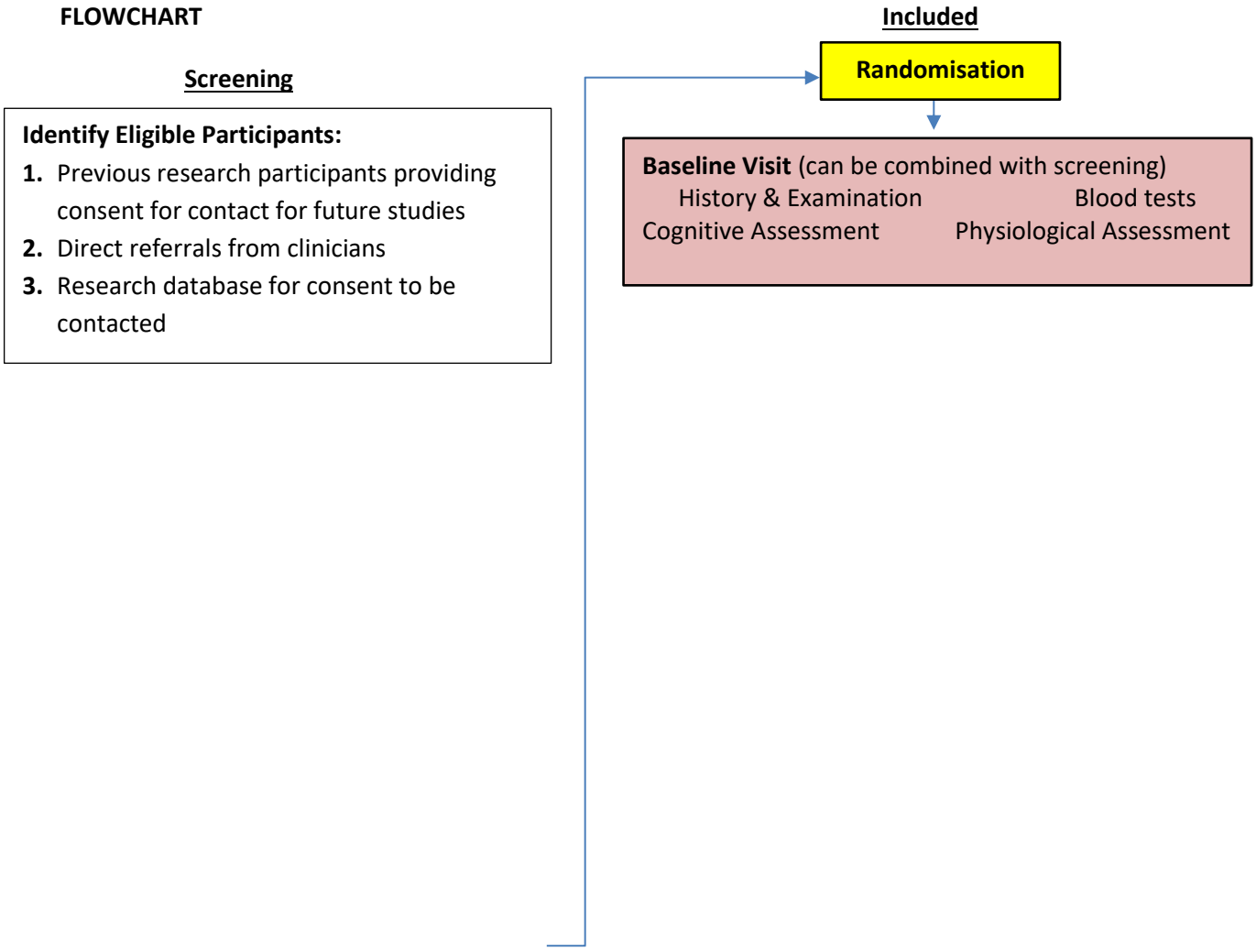
Objectives	Outcome Measures	Timepoint(s)
Primary Objective Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) reduces cerebral arterial pulsatility on transcranial ultrasound, compared to placebo in patients with chronic vascular injury to the brain (small vessel disease)	Middle cerebral arterial pulsatility index ((Gosling's PI = peak systolic velocity – end diastolic velocity)/(mean flow velocity)) on transcranial ultrasound	Baseline, after 3 weeks of treatment with placebo versus 3 weeks of sildenafil
Secondary Objectives 1) Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) alters cerebral arterial reactivity to carbon dioxide on transcranial ultrasound, compared to placebo 2) Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) has non-inferior effects on cerebral arterial pulsatility and reactivity to carbon dioxide on transcranial ultrasound, compared to cilostazol (a PDE 3 inhibitor)	Percentage increase in MCA velocity on 6% CO2 vs medical air As above	As above
Tertiary Objectives 1) Assess whether 3 weeks of treatment with sildenafil (a PDE5 inhibitor) or cilostazol increases cerebral arterial reactivity to carbon dioxide on MRI, compared to placebo 2) Determine biochemical / genetic predictors of response to treatment	Reactivity of BOLD signal on MRI to CO2 challenge Markers of endothelial function (e.g. ICAM, VEGF, vWF) and genetic targets of the medications in the trial	After 3 weeks of treatment with sildenafil or cilostazol versus placebo Analysed in the 2 years after the last visit of the last participant.

6. STUDY DESIGN

This is an investigator-led, randomised, placebo and actively controlled, double-blind (patient and clinician), assessor-blinded, crossover design study. 75 participants will be consented and assessed at a baseline visit, undergo a clinical assessment, followed by a research assessment including transcranial ultrasound to determine cerebral arterial pulsatility and reactivity. They receive three treatments in

randomised sequence: placebo (one tablet TDS, titrated to 2 tablets after 1 week), cilostazol (50mg bd, titrated to 100mg bd, with midday placebo) or sildenafil (25mg tds, titrated to 50mg tds)- NB titration up only happens if the participant is not on omeprazole. After each treatment, the will undergo a repeat clinical and physiological assessment, followed by at least 1 week washout before starting the next treatment. However, the washout may be prolonged if unavoidable (ie due to the COVID-19 pandemic). A subgroup of 60 participants will undergo MRI imaging when on sildenafil and placebo to assess arterial pulsatility on MRI and cerebrovascular reactivity, whilst 30 will undergo MRI on all three treatments.

FLOWCHART



Sequence	Drug 1	Drug 2	Drug 3
1	Sildenafil	Placebo	Cilostazol

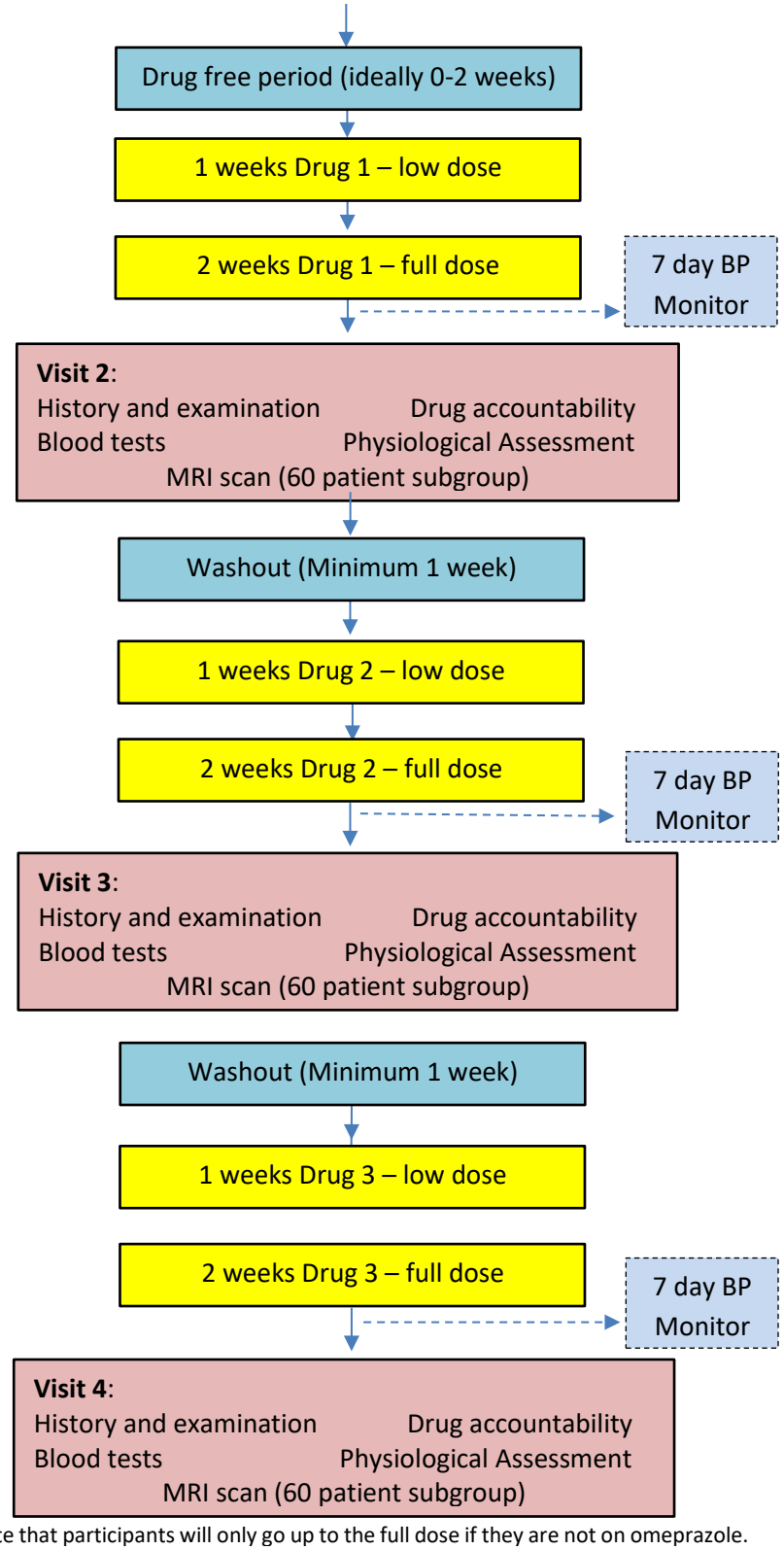
2	Sildenafil	Cilostazol	Placebo
3	Cilostazol	Sildenafil	Placebo
4	Cilostazol	Placebo	Sildenafil
5	Placebo	Sildenafil	Cilostazol
6	Placebo	Cilostazol	Sildenafil

Screening phone-call ,patient invited and PIS sent in advance

Screening Visit:
Review eligibility
Detailed explanation of trial

CONSENT

Medical History & Examination
Blood tests / Pregnancy Test / ECG
Review medical records (if necessary)



Drug Allocation

*Note that participants will only go up to the full dose if they are not on omeprazole.

7. PARTICIPANT IDENTIFICATION

7.1. Study Participants

75 patients with a history of a cryptogenic or lacunar stroke or probable TIA requiring treatment more than 1 month previously and mild-moderate small vessel disease.

7.2. Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Male or Female, aged 18 years or above.
- Can record MCA waveform on at least one side ('useable TCD window')
- Non-disabling, ischaemic stroke or probable TIA requiring treatment, >1 month prior to randomisation, of either cryptogenic or lacunar aetiology, confirmed clinically or on brain imaging
- White matter hyperintensities on MRI (Fazekas scale) or CT (Blennow scale) consistent with cerebral small vessel disease:
    - Age <60: MRI - Fazekas score 1 – 3 (max 2 points in periventricular or deep score)
      CT – Blennow score 1 – 3 (max 2 points in periventricular or deep score)
    - Age >60: MRI - Fazekas score 1 – 4 (max 2 points in periventricular or deep score)
      CT – Blennow score 1 – 4 (max 2 points in periventricular or deep score)

7.3. Exclusion Criteria

- Pregnant or breastfeeding women, women of childbearing age not taking contraception.
    - Acceptable contraception in women of childbearing age is a "highly effective" contraceptive measure as defined by the Clinical Trials Facilitation Group (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf) and includes combined (oestrogen and progesterone containing) or progesterone-only contraception associated with inhibition of ovulation, or intrauterine device or bilateral tubal occlusion
- Other major neurological or psychiatric conditions affecting the brain and interfering with the study design (e.g. multiple sclerosis)
- Other causes of stroke such as
    - ≥50% luminal stenosis (NASCET) in large arteries supplying the infarct area

  - major-risk cardioembolic source of embolism (permanent or paroxysmal atrial fibrillation, sustained atrial flutter, intracardiac thrombus, prosthetic cardiac valve, atrial myxoma or other cardiac tumours, mitral stenosis, recent (<4 weeks) myocardial infarction, left ventricular ejection fraction less than 30%, valvular vegetations, or infective endocarditis)
  - other specific causes of stroke (e.g. arteritis, dissection, drug misuse)
- Large vessel occlusion on MRA or CTA (carotid, basilar or MCA)
- Modified Rankin Score >3 (requires assistance to walk)
- Unable to swallow
- Renal impairment (eGFR <35ml/min)
- Significant biochemical abnormalities (sodium <130, K+ <2.5 or >5.5, LFTs >3 x upper limit of normal range)
- Life expectancy <2 years
- Contraindication to active agents
  - Concurrent use of alphablocker
  - Regular use of nitrate (ISMN, GTN, other)
  - Heart failure (NYHA 2-4)
  - Severe aortic stenosis
  - Bilateral renal artery stenosis
  - Uncontrolled arrhythmias
  - Previous priapism
  - Anatomical deformation of the penis
  - Recent myocardial infarction (within 6 months)
  - Unstable angina
  - History of non-arteritic ischaemic optic neuropathy
  - Hypotension: BP <90/60
  - Haemodynamically significant aortic / mitral valve disease
  - Sickle cell disease, myeloma, leukaemia
  - Uncontrolled hypertension (BP >180/110 despite treatment with 3 antihypertensives)
- Scheduled elective surgery or other procedures requiring general anaesthesia during the study.
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study or the participant's ability to participate in the study.

- Participants who have participated in another research study involving an investigational product in the past 12 weeks.
- Use of an anticoagulant (warfarin, dabigatran, rivaroxaban etc) or more than one antiplatelet drug.
- Predisposition to intracerebral haemorrhage (previous ICH, likely cerebral amyloid angiopathy) or intraocular haemorrhage (uncontrolled diabetic retinopathy or neovascularisation)
- Allergy to constituents of medications or components of placebo / overencapsulation
- Use of CYP induces that interact with study medications (ketoconazole, erythromycin).

Exclusion criteria specific for MRI substudy:

- Not able to transfer to MRI scanner
- Active respiratory illness (such as moderate to severe asthma or COPD) such that they are unable to tolerate MRI or unable to lie flat
- Claustrophobia
- Contraindication to MRI scan (pacemaker, aneurysm clip etc)
- Other significant brain disorder that may confound interpretation of MRI imaging (multiple sclerosis, brain tumour)

8. STUDY PROCEDURES

Participants will be recruited from multiple sites within travel distance of the John Radcliffe Hospital in Oxford, but the study visits will take place in Oxford (see below).

Each participant will undergo a screening visit, a baseline visit (which can be combined with the screening visit if the test results required for determining eligibility are available) and 3 'on-medication' visits. The screening visit will incorporate an assessment of eligibility and detailed trial explanation, followed by informed consent. They will then have a full medical history and examination, review of medical records, ECG (if one is not available from the previous year) and blood tests (if none available in the previous 3 months). They will then have a baseline study visit, either combined with the screening visit, if the availability of screening results permit, or within 1 month. At the baseline visit, they will undergo a repeat medical history for any events since the screening visit, repeat screening blood tests or ECG (if indicated), a formal cognitive assessment and a physiological assessment. If all eligibility criteria are fulfilled, they will be then assigned a study ID and associated randomisation schedule, followed by provision of allocated medication by the clinical trials pharmacy. Participants will then take each of placebo, sildenafil or cilostazol for 3 weeks each, in random order, at low dose for 1 week followed by titration to full dose for 2 weeks (minimum 11 days). Patients on omeprazole will not increase the dose after one week (due to an interaction with cilostazol). After visits 1 and 2, there will be a minimum of a 1 week washout period prior to starting the new medication. On-treatment visits will include review for SAEs and a repeat core physiological assessment. All participants, up to a maximum of 60, will additionally be invited to undergo an MRI scan at follow ups on placebo and sildenafil (with up to thirty also being scanned on cilostazol).

For participants who are unable to attend an on-treatment study visit once their medication has been started (for example due to the COVID-19 pandemic), then they will be able to restart treatment at the

point of stopping treatment, if still during the titration phase, but otherwise will restart three weeks of treatment from the beginning. The washout period can be prolonged as long as is necessary during the COVID-19 outbreak, before the next treatment phase is started.

Activity / assessment	**Screening** -21 to 0	**Baseline Day 0**	**Visit 1 Day 28**	**Visit 2 Day 56**	**Visit 3 Day 84**
Informed Consent	X				
Eligibility Criteria	X				
Demographics	X				
Medical/ History	X	X			
Vital Signs	X		X	X	X
Physical Exam	X		X	X	X
Cognitive Assessment		X			
Pregnancy Test***	(X)	(X)	(X)	(X)	(X)
Laboratory Tests *	X	X	X	X	X
ECG	X				
Randomisation		X			
7 days home BP monitoring twice daily for 1 week		X	X	X	X
Study drug dispensation		X	X	X	
mRS (disability)		X			
NIHSS (stroke severity) only for stroke patients		X			
Drug accountability			X	X	X
Concomitant Medication	X	X	X	X	X
Adverse Events (AE)		X	X	X	X
Pulse wave velocity/ pulse wave analysis		X	X	X	X
Transcranial (TCD) ultrasound scan		X	X	X	X
Beat-to-beat BP monitoring		X	X	X	X
Peripheral Vascular Reactivity		X	X	X	X
MRI assessment (60 patients)			X	X	X

* Laboratory Tests: FBC, U+Es, LFTs (screening and monitoring). Baseline tests will also include gamma-GT, HbA1C, lipid profile, BNP or NT-proBNP.
*** Pregnancy tests will only be applicable to women of child-bearing potential

8.1. Recruitment

Stroke clinicians and members of the clinical team at each Participation Identification Centre (including research staff associated with the clinical research network) will be asked to recommend potentially eligible patients, including patients who have previously been seen by the clinical service. Research nurses from the clinical research network will screen potential patients for eligibility, discuss the study with them face-to-face if attending for clinical follow-up or send a letter to patients at other times. Eligible patients will be provided invitation letters and the patient information sheet, including the contact details of the OxHARP core team at the Centre for Prevention of Stroke and Dementia, University of Oxford, and invited to contact the core team to discuss the study further. In all centres, patients who have provided specific

consent to be approached for further research studies, either as part of research databases or through previous clinical studies, will be contacted directly by managers of these research databases to invite them to participate in this study using their own materials.

8.2. Screening and Eligibility Assessment

Initial screening against eligibility criteria will be carried out against recorded demographic and clinical information, and then during the screening telephone call / visit. The patient information sheet will be sent to the potential participant prior to the baseline visit, with a minimum 1 week interval prior to the face-to-face assessment. At the screening visit, the study will be explained in detail, and the patient's demographic details and clinical history reviewed, which may include review of the their medical notes (if necessary if the participant is unable to be detailed enough, done by secure correspondence with the clinical care team). After informed consent is provided, an ECG, physical exam and laboratory tests will be performed for screening purposes prior to randomisation, except where an ECG is available from the past year, or blood tests (U+Es, FBC) are available from the past 3 months, without a clinical event that would require repeat testing.

8.3. Informed Consent

Informed consent will be taken by the chief investigator, or a delegated study physician, compliant with GCP best practice. The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed. Potential participants from Patient Identification Centres will be asked to contact the OxHARP clinical team and discuss the study first by telephone prior to arranging to visit the John Radcliffe for a formal screening visit.

Written and verbal versions of the Participant Information Sheet and Informed Consent will be presented to the participants detailing no less than: the precise nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the study site in the study master file, with a copy in the patient's medical notes.

8.4. Randomisation, blinding and code-breaking

Randomisation will be performed by the Huddersfield PMU at the point of overencapsulation of medication in advance of provision of treatments to the Oxford Clinical Trial pharmacy. Study numbers will then be provided in sequence to participants at the Oxford Clinical Trial pharmacy. Each sequential study ID will be randomly allocated to one of six different treatment schedules, as outlined in the drug allocation

table on page 11, to produce three groups of 12 and three groups of 13 patients. The randomisation schedule will be generated by Huddersfield PMU.

The study will be blinded to the participant, blinded to the core study team (Dr Webb, Ms Amy Lawson, Dr Karolina Wartolowska) and blinded to the endpoint assessors (TCD measurement and MRI analysis). In patients consenting to the MRI substudy, the Trials pharmacy (or Dr Louise Silver, who is otherwise independent of the study), will identify the visit at which the participant will be taking cilostazol so that the MRI scans can be arranged, but will not inform the patient or core team when sildenafil or placebo are assigned.

If clinical need requires unblinding of a specific patient, this will be done by the study pharmacist who will retain the randomisation schedule, following formal discussion with the Chief Investigator. Due to the low risk level associated with the study medication, and no specific medical intervention indicated for any potential complication that requires prior knowledge of current study treatment, emergency out of hours code-breaking is not required.

8.5. Baseline Assessments

Clinical Assessment

Following assessment of eligibility and full informed consent at the screening visit, the participant will be interviewed for demographic details, medical history, a review of the their medical notes (if necessary if the participant is unable to be detailed enough) since screening and undergo a medical examination:

- Vital signs (sitting blood pressure x2 with a BHS validated device, heart rate, oxygen saturations, temperature)
- Cardiovascular examination (heart sounds, postural blood pressure)
- Respiratory examination
- Gastrointestinal examination
- Neurological examination
- National Institute of Health Stroke Scale (NIHSS)
- Modified Rankin Score (mRS)
- Montreal Cognitive Assessment (MoCA)
- Fluid Intelligence and matching pairs cognitive assessments (Biobank protocol)

Clinical Tests

If an ECG within the previous year is not available, or the medical history indicates that a potential cardiac event has occurred since the last ECG, this will be performed prior to randomisation. In addition, if relevant blood tests are not available from the last 3 months, clinical blood tests (~10mls blood) will be performed for FBC, U+Es, LFTs, CRP, BNP or NT-proBNP, gamma-GT, lipid profile, HbA1C. In women of child-bearing potential, a pregnancy test will be performed. These initial clinical tests will be performed for screening according to the inclusion criteria (FBC, U+Es, LFTs), and used for demographic baseline data (CRP, BNP or NT-proBNP, gamma-GT, lipid profile, HbA1C). At each follow-up visit, we will repeat FBC, U+Es, LFTs for monitoring effects of initiating medications.

All patients that have consented to the study will also have twenty millilitres of blood taken at baseline for storage and later analysis. In patients this will be drawn, where possible, at the same time as the screening visit bloods. Serum, plasma, buffy coat and whole blood samples are prepared and transferred to cryotubes. Each cryotube is labelled with a unique identification number, recorded in the specimen logbook and frozen at –85 ^{0}C. All bloods samples are stored in freezers in laboratories in the Wolfson Centre for Prevention of Stroke and Dementia for up to 2 years after final recruitment to the study, to allow for analysis of the samples. Samples will be tested for biomarkers that predict response to the randomized treatment allocation in the study, and for correlation with severity of baseline white matter hyperintensities (to determine interaction with treatment effect). Blood will be stored and then analysed at completion of the study, as the extent of testing will depend upon the demonstrated effect of medications in the study, whilst allowing for assessment of new biochemical and genetic markers reported during the study, in this rapidly developing field. As a minimum, markers of endothelial function (ICAM, VEGF, vWF) and genetic targets of the medications in the trial, shown to be associated with small small vessel disease (ie PDE3 polymorphisms), will be measured. These will not specifically include tests of direct clinical relevance to the participant and are very unlikely to have clinical implications. In the unlikely circumstance that incidental biomarker or genetic results that could be relevant to the participant are identified, these will be assessed by an independent physician (Peter Rothwell), the participant informed and a referral made to an appropriate specialty, where clinically indicated.

Physiological Assessment

Central Aortic Blood Pressure / Aortic Pulse Wave Velocity (10 minutes)

Radial artery pulse waveforms will be recorded using a pressure tonometer and designated software (SphygmoCor, At-Cor Medical, Sydney, Australia). Briefly, mean values of approximately 10 radial pulse waves will be used to generate a corresponding central aortic pressure waveform with a validated mathematical transfer function. A minimum of 10 radial artery pulse waveforms are required by the software to calculate the AI_x and to also derive an operator index (indicating the quality and reproducibility of the arterial signal (SphygmoCor, At-Cor Medical, Sydney, Australia).

Aortic arterial stiffness will be assessed by the gold standard technique of carotid-femoral pulse wave velocity. This requires recording of the pulse wave by applanation tonometry with the SphygmoCor device from both carotid and femoral arteries, and the speed of transmission between the two sites used to estimate the velocity of the pulse wave. This provides a measure of the stiffness of the central arteries.

Transcranial Doppler Ultrasound (TCD)

TCD will be performed with DiaMon 2MHz probes, firstly with a handheld probe to best identify the middle cerebral and basilar arteries, with application of ultrasound gel over the temporal (in front of the ear) and suboccipital windows (at the nape of the neck). The M1 segment will be identified between 45-55mm depth as the segment of the artery with blood flow towards the probe, slightly superficial to a site of bifurcation into another vessel with flow away from the probe (the anterior cerebral artery), with blood flow towards the probe ideally extending for at least 1cm within the skull. The basilar artery will be idenfied via the trans-foramen magnum bone window, identifying blood flowing away from the probe at >75mm. The maximal velocity in these segments will be identified and peak, trough and mean blood flow velocities will be derived from the wave form envelope, taking the average of at least 5 cardiac cycles. From these measures Gosling's pulsatility index will be derived as the primary measure of cerebral pulsatility. Where bone windows are adequate (ie MCA blood flow can be insonated via the temporal bone), bilateral

monitoring insonation of the middle cerebral artery will be established with 2 probes held to the head a with a comfortable headset, with spectra acquired by a DWL Doppler Box to a dedicated laptop. This waveform will be acquired over 10 minutes with concurrent heart rate and blood pressure monitoring (see below). End-tidal carbon dioxide monitoring will be performed with AD Instruments Gas Analyser ML206. All waveforms will be simultaneously acquired with a AD Instruments Powerlab 8/35.

Continuous non-invasive blood pressure monitoring

Continuous ECG and BP will be recorded using a Finometer (BP monitor which allows continuous non-invasive assessment of finger arterial pressure). After a period of at least 5 minutes of rest and after achievement of a satisfactory BP signal from the monitor and the stabilization of BP at the same level, recordings will be performed for up to 10 minutes (minimum 5 minutes). The outputs from the Finometer and simultaneous surface ECG recordings will be analysed to estimate cerebral autoregulation and transmission of arterial pulsatility using in-house analysis programs written in Matlab, according to both sequential and spectral methods. The system will be allowed to establish a stable internal calibration before this function is turned off for the monitoring period. A standard brachial blood pressure will be obtained twice with a calibrated device (A&D UA-767 BT) from the contralateral arm twice, and the Finometer trace calibrated to this reading by 2 point calibration.

Cerebral Autoregulation (10 minutes)

Once established, bilateral TCD, blood pressure, ECG and end-tidal CO2 will be acquired simultaneously for 5 minutes after a minimum of 5 minutes of supine rest. Continuous systolic, diastolic and mean values will be derived and related to each other by transfer function analysis using in-house software written in Matlab. This allows estimation of baroreceptor sensitivity and cerebral autoregulation.

CO2 reactivity (10 minutes)

Following the period of monitoring, reactivity to CO2 will be assessed by 3 minutes of inhalation of 6% CO2, delivered via a respiratory circuit with a non-invasive ventilation mask and harness, supplied with 2 alternating periods of medical air versus 6%CO2/21%O2/balance N2. The resulting increase in MCA mean flow velocity on TCD will be related to the change in end-tidal CO2 to determine the vasodilatory response, adjusted for any change in blood pressure. Secondly, the participant will be instructed to hyperventilate at 1 breath per second for 30 seconds to reduce etCO2 and estimate change in mean flow velocity per unit change in etCO2.

Peripheral Vascular Reactivity (10 minutes)

Effects of medication on peripheral endothelial function will be estimated by assessment of flow-mediated slowing (Vicorder, Skidmore Industries). This determines the change in speed of flow of the pressure waveform between two sites in the arm, before and after a short period of occlusion. This induces endothelium dependent vasodilatation, which results in slowing of the speed of blood flow. If the previous tests last >60 minutes to achieve accurate measurements, or if the participant finds this procedure too uncomfortable this element may be omitted at follow up visits.

Blood pressure monitoring

Patients will be provided with a home blood pressure monitor to record their blood pressure twice daily (on waking and evening), recorded three times at each sitting, for 7 days prior to their subsequent study visit (A&D UA-767 BT). Patients will be asked to record their blood pressure readings and return them for

assessment of how allocated treatment affects blood pressure, and how this may interact with the primary outcome measure. If potential symptoms of hypotension occur, patients may be requested to perform additional readings for clinical safety purposes. Participants will be asked to record any side-effects on the same diary card as used for blood pressure monitoring.

Medication Dispensation

Study medication / placebo will be dispensed by the Oxford University Clinical Trials Pharmacy after randomisation.

8.6. Subsequent Visits

Participants will be reviewed at visits 2, 3 and 4 (3, 6-7 and 11-13 weeks respectively) following sequential, blinded treatment in random order with the study drugs, sildenafil and cilostazol, and placebo for 3 weeks. At each follow up visit they will undergo assessment of potential side effects or unwanted effects (SAEs/SUSARS – see below), including repeat clinical examination and repeat blood tests, as at the baseline visit. Concomitant medications will be recorded, and compliance with study drugs will be assessed by questionnaire and by counting of returned study drug.

Following clinical assessment, the physiological assessment will be repeated as the primary study outcome (pulsatility index). Then the next drug will be dispensed, and any residual drug will be returned.

8.7. MRI Sub-Study

In 60 consenting patients an additional MRI assessment will be performed at each of the follow-up visits on sildenafil and placebo, and up to 30 on cilostazol, to assess cerebrovascular haemodynamics in greater detail. This may involve the following sequences, with a minimum requirement to have FLAIR and SWI images at a minimum of one timepoint and T1, arterial spin labelling and CO2 reactivity scanning at both timepoints :

At first MRI visit:

- T1 structural image (volume 3D acquisition, 1mm isotropic)
- FLAIR (for quantification of white matter hyperintensities)
- Susceptibility weighted imaging
- Arterial Spin Labelling : 4 vessel selective, non-contrast perfusion imaging (pcASL), to estimate baseline absolute cerebral blood flow
- High frequency, multiband BOLD imaging, optimised for pulsatility of blood flow (pulse-MB, TR 0.4) – 7 minutes monitoring.
- Standard multiband BOLD imaging (according to BioBank imaging protocol) with 5 minutes of fixation on a blank screen, viewing intermittent blank screens then a radial flashing checkerboards.
- Carbon dioxide challenge with inhalation of 6% CO2 in medical air in 3 minute blocks, interposed with 2 minute blocks of breathing medical air, delivered via an open respiratory circuit with one way valves. Throughout imaging, the participant will have physiological blood pressure, end-tidal CO2 and oxygen saturation monitoring.

At second / third MRI visits:

- T1 structural image (volume 3D acquisition, 1mm isotropic)
- Diffusion tensor imaging (DTI – 64 directions, for estimation of mean diffusitivity and fractional anisotropy as sensitive measures of white matter injury) (only at second visit)
- Arterial Spin Labelling : 4 vessel selective, non-contrast perfusion imaging (pcASL), to estimate baseline absolute cerebral blood flow
- High frequency, multiband BOLD imaging, optimised for pulsatility of blood flow (pulse-MB, TR 0.4) – 7 minutes monitoring.
- Standard multiband BOLD imaging (according to BioBank imaging protocol) with 5 minutes of fixation on a blank screen, viewing intermittent blank screens then a radial flashing checkerboards.
- Carbon dioxide challenge with inhalation of 6% CO2 in medical air in 3 minute blocks, interposed with 2 minute blocks of breathing medical air, delivered via an open respiratory circuit with one way valves. Throughout imaging, the participant will have physiological blood pressure, end-tidal CO2 and oxygen saturation monitoring.

8.8. Sample Handling

All clinical blood tests will be taken for clinical screening and safety purposes and processed via the Oxford University Hospitals NHS Trust laboratory, after which the samples will be disposed of according to local procedures. For research samples, serum, plasma, buffy coat and whole blood samples are prepared and transferred to cryotubes. Each cryotube is labelled with a unique identification number, recorded in the specimen logbook and frozen at –85 ^{0}C. All bloods samples are stored in freezers in laboratories in the Wolfson Centre for Prevention of Stroke and Dementia

8.9. Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Withdrawal of Consent
- Loss to follow up

Any data acquired up to the point of withdrawal from the study will be retained except in circumstances where the participant explicitly withdraws consent for this. Participants will not be replaced.

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

If an individual experiences side-effects on the first treatment, such that they can not continue, they will still be eligible to proceed to the next stage. Similarly, if patients are unable to tolerate study procedures related to secondary study outcomes (ie CO2 reactivity testing or MRI imaging), they will be able to continue with the study for the purposes of the primary outcome measures (TCD pulsatility index) but not undergo the non-tolerated component. This option will only be provided when the participant expresses a desire to withdraw from the study due to intolerability of the test. If participants withdraw from the study due to clinically significant adverse events, then they will continue to be monitored by the study team until resolution of the adverse event.

8.10. Definition of End of Study

The end of active patient management is the date of the last visit of the last participant (LVLP), but blood will be stored for up to 2 years after LVLP for analysis,

9. INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

9.1. IMP Description

The principal IMP is oral sildenafil, given at 25mg TDS for one week, then automatically increased to 50mg TDS for a further 2 weeks, if tolerated, as determined by its provision in labelled dispensing packs. If side-effects occur at the higher dose, the dose will be returned to the lower dose. Sildenafil is a phosphodiesterase 5 inhibitor, widely used for erectile dysfunction and used in selected patients with primary pulmonary hypertension.

The comparator will be placebo, also taken three times daily.

The secondary comparator IMP is cilostazol, as a positive control, provided at 50mg bd and titrated to 100mg bd after one week, to continue for 2 further weeks. Cilostazol is a phosphodiesterase 3 inhibitor used at this dose in peripheral arterial disease, and used in large randomised controlled Trials of patients with previous stroke, with a beneficial effect compared to aspirin.[19] It is expected to improve cerebral reactivity[22] and pulsatility[20] and is included as a positive control to assess whether sildenafil has a similar magnitude of physiological effect, to estimate if a sufficient magnitude of clinical effect would be expected, to justify larger clinical trials. In order to preserve the blinding of this study, whilst taking cilostazol twice a day participants will be given placebo to take at midday. This means that, from the participant's perspective, they are always taking the study medication three times daily.

Participants will start sildenafil and cilostazol at lower doses for a week before being titrated up to the high dose. The high dose is similar to the medication's use in usual care, given more frequently in the case of sildenafil, but the titration is necessary to settle the participants on the new medication.

Medications will be overencapsulated, overlabelled and blinded by the Huddersfield NHS Pharmacy Manufacturing Unit, and dispensed by the Oxford University Clinical Trials pharmacy. Medications will be provided in 'dosette' type packaging providing dosing times, such that throughout the study patients will take TDS medication (sildenafil TDS, placebo TDS, cilostazol – placebo – cilostazol). Code-break will be

provided to and retained by the Oxford University Clinical Trials Pharmacy, with individual code-breaking allowed on request of the CI (Dr Alastair Webb) when clinically indicated.

For each treatment, medication will be provided as one tablet three times a day for one week, followed by 2 tablets 3 times per day. If side effects occur on the higher dose, the participant will decrease back to one tablet 3 times per day, on discussion with an OxHARP physician. Participants will not be moved on to the higher dose if they are also taking omeprazole due to an interaction with cilostazol.

Labelling and quality control will be performed by Huddersfield PMU according to Volume 4. Good Manufacturing Practices, Annex 13. Manufacture of investigational medicinal products, July 2010.

9.2. Storage of IMP

Medication and placebo will be stored in the Oxford University Clinical Trials pharmacy in recommended conditions, according to pharmacy Standard Operating Procedures. All medications are stable at room temperature for ease of storage by participants.

9.3. Compliance with Study Treatment

Participants will be required to return any unused medications and associated packaging for drug reconciliation at visits 2 and 3. The packaging will incorporate a dosing schedule allowing accurate accounting of compliance.

9.4. Accountability of the Study Treatment

All study medications will be dispensed by the Oxford University Clinical Trials Pharmacy, who will take principal responsibility for drug accountability. Dispensed medications will be documented, and participants will return all study medications to the study pharmacy after each study visit. Number of returned medication will be recorded.

9.5. Concomitant Medication

All concomitant medications will be listed at each study visit. Concomitant use of other PDE5 inhibitors (sildenafil, vardenafil) or other PDE inhibitors (cilostazol) will not be allowed. Similarly, concomitant use of alpha-blockers, nitrates (GTN, ISMN, nicorandil) or direct vasodilators (hydralazine, naftidofuryl) will not be allowed. Use of anticoagulants or more than one antiplatelet agent will also be contraindicated. If a clinical need for these medications arises, the participant will be withdrawn from the study.

9.6. Post-study Treatment

The IMP will not be provided beyond the duration of the study.

10. SAFETY REPORTING

10.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the study treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the study in question.

NB: to avoid confusion or misunderstanding of the difference between the terms “serious” and “severe”, the following note of clarification is provided: “Severe” is often used to describe intensity of a specific event, which may be of relatively minor medical significance. “Seriousness” is the regulatory definition supplied above.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of “serious”.

Safety data will be routinely collected at each follow-up, including clinical history of side effects or adverse events, clinical examination and blood tests (FBC, U+Es, LFTs). Patients will also be provided a telephone number to contact in case of side effects, and if the side effects suggest possible hypotension, may be asked to perform additional BP monitoring.

10.2. Causality

The relationship of each adverse event to the study medication must be determined by a medically qualified individual according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from study medication administration. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant’s clinical state or by other modes of therapy administered to the participant.

10.3. Procedures for Recording Adverse Events

All AEs occurring during the study that are observed by the Investigator or reported by the participant, will be recorded on the CRF, whether or not attributed to study medication.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs considered related to the study medication as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator’s clinical judgment to decide whether or not an AE is of sufficient severity to require the participant’s removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant will undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

10.4. Reporting Procedures for Serious Adverse Events

SAEs will be recorded and deemed reportable for up to 30 days following last administration of a trial drug, whether occurring after the end of the trial or between trial phases following a prolonged washout period.

All SAEs (other than those defined in the protocol as not requiring reporting) must be reported on the SAE reporting form to the DSMB within 24 hours of the Site Study Team becoming aware of the event. It will also be reviewed at the next DSMB meeting. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and emailed to the DSMB as requested. The DSMB will review all AEs and SAEs on a yearly basis.

10.5. Expectedness

Expectedness will be determined according to the Summary of Product Characteristics by the study principal investigator.

10.6. SUSAR Reporting

All SUSARs will be reported by the CI to the sponsor and the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days. Events reported to the REC will use the HRA report of serious adverse event form (see HRA website).

Treatment codes will be un-blinded for specific participants.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current study.

10.7. Safety Monitoring Committee

A Data Safety and Monitoring Board will conduct a review of all SAEs for the study reported during the quarter and cumulatively. The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To seek additional advice or information from investigators where required
- To evaluate the risk of the study continuing and take appropriate action where necessary

The Data and Safety Monitoring Board have reviewed the study protocol, will be informed of SAEs yearly, and will perform an interim safety assessment after 30 patients have been recruited (or 12 months have elapsed). If recruitment is excessively slow, the DSMB may recommend either that the study is stopped, or that the cilostazol phase of the study is removed and subsequent patients are only randomised to sildenafil vs placebo in random order. With removal of the third arm of the study, the overall population size would also be reduced to 50 patients.

11. STATISTICS

11.1. Description of Statistical Methods

A statistical analysis plan will be signed off prior to analysis.

Primary outcome :

Change in Gosling's Pulsatility Index from baseline to follow up, utilising the visually-assessed, better quality TCD recording (defined by blinded assessor) will be compared between sildenafil and placebo phases by paired T-test, comparing change in MCA-PI from baseline to after 3 weeks of treatment.

Secondary outcome:

The non-inferiority analysis will be based on the 95% CI for the mean change difference between sildenafil and cilostazol. If the upper 97.5% confidence limit is less than 0.08 units (2/3 of the clinically estimated overall cilostazol effect), non-inferiority will be declared.

Differences in reactivity to CO2 from baseline in each of three states (sildenafil, cilostazol, placebo) will be compared by mixed-effect general linear models, with post-hoc pairwise comparison.

Tertiary outcomes

Equivalent statistical techniques will be used to determine responses to each of the further physiological measures for sildenafil versus placebo as the primary comparison (paired t-test for continuous outcomes), and mixed-effect linear models across the three groups. MRI imaging outcomes will be compared by analysis tools within the FSL software library, utilising linear models for interaction between drug allocation and BOLD-response to carbon dioxide. Secondly, the relevance of derived physiological indices including home blood pressure, arterial stiffness, cerebral autoregulation, peripheral vascular reactivity and beat-to-beat blood pressure variability will be determined both as predictors of cerebral pulsatility and autoregulation in linear models, and for the interaction between drug class and these indices on cerebral pulsatility and reactivity.

Individuals missing either sildenafil or placebo interventions will be excluded from the analysis corresponding to the primary objective but included in the safety analysis. Participants without cilostazol exposure will be included in the sildenafil-placebo comparison, but not in the - secondary and tertiary analyses.

Safety data will be presented for all patients who are randomised and receive at least one dose of one treatment. Safety data will be summarised descriptively.

Genetic / biochemical predictors of response to treatment will be assessed by mixed linear models for continuous outcomes, and paired t-test for binary measures.

11.2. The Number of Participants

75 patients will be recruited to the study. All participants will be invited to optionally participate in the MRI substudy of whom at least 30 will be imaged on sildenafil and placebo alone, and up to 30 on all three treatments. Participants who leave the study before taking any trial medication will be replaced. Participants unable to tolerate the MRI scan will continue in the main study, but will be replaced by new recruitment to the substudy, where necessary.

11.3. The Level of Statistical Significance

At a power level of 0.9, with a 2-sided significance of 5%, a clinically relevant 0.12 unit change in pulsatility index (equivalent to a ~20% difference in risk of recurrent stroke), and conservatively allowing for a standard deviation of differences in PI between repeated measures of 0.2, gives an estimated minimum sample size of 32 patients (paired t-test). Allowing for a 15% drop-out rate, 38 patients would be required. A sample size of 66 achieves 90% power to detect the non-inferiority of sildenafil c.f. cilostazol using a non-inferiority margin of 0.08 (and mean of paired differences 0) at α=0.025 (for a 95% CI) with a within-subject variance of 0.02 (equivalent to a SD of differences 0..[23,24] This equates to 75 patients in total with a 12% drop out rate.

A two-sided 5% significance level will be used for all hypothesis tests.

11.4. Criteria for the Termination of the Study

A Data and Safety Monitoring Board will be established. They will review the data after 30 patients have been recruited to the study, or 12 months after the first visit of the first patient (whichever is sooner), or at the request of the sponsor or CI. If a decision to terminate the study is contemplated, this will be discussed with the CI and the sponsor prior to a final decision being made.

The advisory recruitment threshold is set at projected recruitment of <35 patients in 2 years, allowing for any delays resulting from the COVID-19 pandemic. No threshold is defined for efficacy for study cessation. Any statistically significant excess of SAEs in the treatment arm, or occurrence of unexpected treatment-related SAEs of sufficient severity (in the view of the DSMB), may also result in early cessation of the study. If after 12 months the recruitment falls below the rate expected to complete the study in 3 years, allowing for delays due to the COVID-19 outbreak, the overall study size can be reduced to 50 participants. Any data collected to that date, including on cilostazol, will be retained and analysed according to the original study plan. An interim statistical analysis plan will be signed off prior to the interim analyses.

11.5. Procedure for Accounting for Missing, Unused, and Spurious Data.

All physiological data will reviewed and cleaned by automated and manual processes to reduce artefactual data. Data will be defined as artefactual or missing. The statistical analysis plan will describe the procedure for accounting for missing or spurious data. The completeness and correctness of the data will be monitored as per the monitoring plan

11.6. Inclusion in Analysis

All possible data will be used on an intention-to-treat basis, but where patient withdrawal results in missing data, the final primary analysis will only include patients where primary outcome data is available (Gosling's MCA-PI).

11.7. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

All deviations from the statistical analysis plan for the study will be reported in the final report of the study.

12. DATA MANAGEMENT

12.1. Source Data

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

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

12.2. Access to Data

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

12.3. Data Recording and Record Keeping

All study data will be entered and stored on paper CRFs. The participants will be identified by a unique study specific number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

13. QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

Regular monitoring will be performed according to GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

14.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

14.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

14.4. Reporting

The CI shall submit once a year throughout the clinical study, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

14.5. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so, and the General Data Protection Regulation to ensure transparency and confidentiality of data use.

14.6. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

14.7. Other Ethical Considerations

Potential incidental findings detected on brain imaging will be reviewed by the study Chief Investigator (Dr Alastair Webb). If deemed to be potentially clinically significant, this will be they will then be reviewed under the fMRIB SOP for incidental findings, including referral to an independent neurologist (Prof Martin Turner), review by a neuroradiologist if indicated and an independent clinical appointment arranged for the patient with the independent neurologist to discuss the findings and any implications. If this process is required, the chief investigator will inform the participant that an incidental finding has been identified on their brain scan and that they will have the opportunity to discuss this with an independent neurologist if they wish.

Incidental findings identified on physiological testing or clinical examination will be reviewed by the study chief investigator. If any clinical action needs to be taken, the chief investigator will discuss this with the participant and contact the participants GP to initiate an appropriate clinical response.

If patients lose capacity during the study, they will be withdrawn from the study and their GP will be informed. If this occurs as a result of study procedures or treatments, they will be followed up by the study

team until resolution of the adverse event or until they are clinically stable. Data collected up to the point of their withdrawal from the study will be retained.

Finally, male participants will be asked if they have a deformity of the penis which is a contraindication to use of sildenafil due to an increased risk of priapism. This is a personal question but will be asked sensitively by an experienced physician.

15. FINANCE AND INSURANCE

15.1. Funding

The study is funded through a Wellcome Trust Clinical Research Career Development Fellowship, held by the Chief Investigator (AW).

15.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

16. PUBLICATION POLICY

Any publications resulting from this study will be authored by the chief investigator and any additional study physicians recruited to the study in the future. In addition, any substantive contribution in recruiting and monitoring patients at non-core sites (Reading, High Wycombe, Swindon) will be recognised by authorship / acknowledgement, depending upon the level of involvement. There is no expectation that this study will generate intellectual property.

17. REFERENCES

1. Lau KK, Li L, Schulz U, et al. Total small vessel disease score and risk of recurrent stroke: Validation in 2 large cohorts. *Neurology.* 2017;88(24):2260-2267.
2. Smith EE, Egorova S, Blacker D, et al. Magnetic resonance imaging white matter hyperintensities and brain volume in the prediction of mild cognitive impairment and dementia. *Arch Neurol.* 2008;65(1):94-100.
3. Teodorczuk A, Firbank MJ, Pantoni L, et al. Relationship between baseline white-matter changes and development of late-life depressive symptoms: 3-year results from the LADIS study. *Psychol Med.* 2010;40(4):603-610.
4. Inzitari D, Pracucci G, Poggesi A, et al. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ.* 2009;339:b2477.
5. Rensma SP, van Sloten TT, Launer LJ, Stehouwer CDA. Cerebral small vessel disease and risk of incident stroke, dementia and depression, and all-cause mortality: A systematic review and meta-analysis. *Neurosci Biobehav Rev.* 2018;90:164-173.
6. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* 2013;12(8):822-838.
7. Gardener H, Wright CB, Rundek T, Sacco RL. Brain health and shared risk factors for dementia and stroke. *Nature Reviews Neurology.* 2015;11(11):651-657.

8. Webb AJ, Simoni M, Mazzucco S, Kuker W, Schulz U, Rothwell PM. Increased cerebral arterial pulsatility in patients with leukoaraiosis: arterial stiffness enhances transmission of aortic pulsatility. *Stroke.* 2012;43(10):2631-2636.
9. Molina C, Sabin JA, Montaner J, Rovira A, Abilleira S, Codina A. Impaired cerebrovascular reactivity as a risk marker for first-ever lacunar infarction: A case-control study. *Stroke.* 1999;30(11):2296-2301.
10. Simoni M, Li L, Paul NL, et al. Age- and sex-specific rates of leukoaraiosis in TIA and stroke patients: population-based study. *Neurology.* 2012;79(12):1215-1222.
11. Li L, Schulz UG, Kuker W, Rothwell PM, Oxford Vascular S. Age-specific association of migraine with cryptogenic TIA and stroke: Population-based study. *Neurology.* 2015;85(17):1444-1451.
12. Li L, Yiin GS, Geraghty OC, et al. Incidence, outcome, risk factors, and long-term prognosis of cryptogenic transient ischaemic attack and ischaemic stroke: a population-based study. *Lancet Neurol.* 2015;14(9):903-913.
13. van Middelaar T, Argillander TE, Schreuder F, Deinum J, Richard E, Klijn CJM. Effect of Antihypertensive Medication on Cerebral Small Vessel Disease: A Systematic Review and Meta-Analysis. *Stroke.* 2018;49(6):1531-1533.
14. Topakian R, Barrick TR, Howe FA, Markus HS. Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. *J Neurol Neurosurg Psychiatry.* 2010;81(2):192-197.
15. Markus HS, Lythgoe DJ, Ostegaard L, O'Sullivan M, Williams SC. Reduced cerebral blood flow in white matter in ischaemic leukoaraiosis demonstrated using quantitative exogenous contrast based perfusion MRI. *J Neurol Neurosurg Psychiatry.* 2000;69(1):48-53.
16. Gomez-Vallejo V, Ugarte A, Garcia-Barroso C, et al. Pharmacokinetic investigation of sildenafil using positron emission tomography and determination of its effect on cerebrospinal fluid cGMP levels. *J Neurochem.* 2016;136(2):403-415.
17. Blair GW, Doubal FN, Thrippleton MJ, Marshall I, Wardlaw JM. Magnetic resonance imaging for assessment of cerebrovascular reactivity in cerebral small vessel disease: A systematic review. *J Cereb Blood Flow Metab.* 2016;36(5):833-841.
18. Olmestig JNE, Marlet IR, Hainsworth AH, Kruuse C. Phosphodiesterase 5 inhibition as a therapeutic target for ischemic stroke: A systematic review of preclinical studies. *Cell Signal.* 2017;38:39-48.
19. Shinohara Y, Katayama Y, Uchiyama S, et al. Cilostazol for prevention of secondary stroke (CSPS 2): an aspirin-controlled, double-blind, randomised non-inferiority trial. *Lancet Neurol.* 2010;9(10):959-968.
20. Han SW, Lee SS, Kim SH, et al. Effect of cilostazol in acute lacunar infarction based on pulsatility index of transcranial Doppler (ECLIPse): a multicenter, randomized, double-blind, placebo-controlled trial. *Eur Neurol.* 2013;69(1):33-40.
21. Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet.* 2018;50(4):524-537.
22. Blair GW, Appleton JP, Flaherty K, et al. Tolerability, safety and intermediary pharmacological effects of cilostazol and isosorbide mononitrate, alone and combined, in patients with lacunar ischaemic stroke: The LACunar Intervention-1 (LACI-1) trial, a randomised clinical trial. *EClinicalMedicine.* 2019;11:34-43.
23. Julious SA, Campbell MJ, Altman DG. Estimating sample sizes for continuous, binary, and ordinal outcomes in paired comparisons: practical hints. *J Biopharm Stat.* 1999;9(2):241-251.
24. Julious SA. Sample sizes for clinical trials with normal data. *Stat Med.* 2004;23(12):1921-1986.

18. APPENDIX D: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2.0	14th April 2020	A.Webb	See document 'OxHARP Amendment 1_14_4_20.docx'

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee or MHRA.