

STUDY PROTOCOL

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Cerebral blood flow and cognition after 3 months tadalafil treatment in small vessel disease (ETLAS-2): study protocol for a randomized controlled trial

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Abstract

Background Targeted treatment is highly warranted for cerebral small vessel disease, a causal factor of one in four strokes and a major contributor to vascular dementia. Patients with cerebral small vessel disease have impaired cerebral blood flow and vessel reactivity. Tadalafil is a specific phosphodiesterase 5 inhibitor shown to improve vascular reactivity in the brain.

Methods The ETLAS-2 trial is a phase 2 double-blind, randomized placebo-controlled, parallel trial with the feasibility of tadalafil as the primary outcome. The trial aims to include 100 patients with small vessel occlusion stroke or transient ischemic attacks and signs of cerebral small vessel disease more than 6 months before administration of study medication. Patients are treated for 3 months with tadalafil 20 mg or placebo daily and undergo magnetic resonance imaging (MRI) to evaluate changes in small vessel disease according to the STandards for Reporting Vascular changes on nEuroimaging (STRIVE) criteria as well as cerebral blood flow, cerebrovascular reactivity, and neurovascular coupling in a functional MRI sub-study. The investigation includes comprehensive cognitive testing using paper–pencil tests and Cambridge Neuropsychological Test Automated Battery (CANTAB) tests in a cognitive sub-study.

Discussion The ETLAS-2 trial tests the feasibility of long-term treatment with tadalafil and explores vascular and cognitive effects in cerebral small vessel disease in trial sub-studies. The study aims to propose a new treatment target and improve the understanding of small vessel disease. Currently, 64 patients have been included and the trial is estimated to be completed in the year 2024.

Trial registration Clinicaltrials.gov, NCT05173896. Registered on 30 December 2021.

Keywords Stroke, Dementia, ASL-MRI, BOLD-dependent MRI, Blood–brain barrier, Cognitive testing

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Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see <http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/>).

Title {1}	Cerebral blood flow and cognition after three months tadalafil treatment in small vessel disease (ETLAS-2): study protocol for a randomized controlled trial.
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Name and contact information for the trial sponsor {5b}	Department of Brain and Spinal Cord Injury, Neuroscience Centre, Copenhagen University Hospital – Rigshospitalet, Valdemar Hansens Vej 13, 2600 Glostrup, Denmark & Neurovascular Research Unit, Department of Neurology, Copenhagen University Hospital – Herlev and Gentofte, Borgmester Ib Juuls Vej 1, 2730 Herlev, Denmark. Sponsor delegate person: Christina Kruuse Email: ckruuse@dadlnet.dk
Role of sponsor {5c}	This trial is an investigator initiated clinical trial. The sponsor delegate person is the initiator of the study, involved in study design, MRI design, data analysis, and writing of reports. The sponsor can decide on submitting reports for publication and has authority over data and manuscript submission. The funders are not involved in the study design and in conducting the trial. Funders have no right to data from this trial.

Introduction

Background and rationale {6a}

In this trial, we explore a treatment targeting cerebrovascular regulation in cerebral small vessel disease (CSVD). CSVD is the underlying cause of 25% of ischemic cerebral strokes and is involved in most hemorrhagic strokes [1, 2]. Further, CSVD is the primary cause of vascular dementia, the most frequent dementia aside from Alzheimer's disease [2–4]. Finding a treatment for CSVD will significantly impact the burden of stroke and dementia.

CSVD is defined by changes in the small vessel structure and function with reduced cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) [5–9]. Its pathophysiology involves endothelial dysfunction which is associated with impaired endothelial nitric oxide (NO) production and reduced cyclic guanosine monophosphate (cGMP) within the smooth muscle cells of the vascular wall [10–12]. The NO-cGMP signaling is key to regulating blood flow in the body and a disruption of this signaling is likely to be involved in CSVD pathology [13–15]. Phosphodiesterase 5 (PDE-5) inhibitors, such as tadalafil, are pharmacological agents that reduce the breakdown of cGMP and therefore augment the NO-cGMP signaling [16]. PDE-5 inhibitors are currently used to treat erectile dysfunction and pulmonary hypertension [16]. Since the PDE-5 enzyme is also expressed in human brain and brain vasculature, PDE-5 inhibitors also act on the cerebral vasculature [17–19].

Improving CBF and CVR with tadalafil could be an attractive mechanism for slowing or reverting the effects of CSVD and reducing the risk of recurrent stroke and dementia. We have previously shown in a pilot study

that a single dose of tadalafil 20 mg vs. placebo increased blood oxygen saturation in the cerebral cortex, using a bedside method to estimate regional CBF [20]. Tadalafil and other PDE-5 inhibitors are well-tested, and their side-effect profile is well-known and often tolerated [21–23].

Objectives {7}

The purpose of this study is to test the feasibility of daily treatment with tadalafil in CSVD patients and explore the effect on CBF and CVR with magnetic resonance imaging (MRI) and changes in cognition. The main hypothesis is that daily treatment with oral tadalafil (20 mg) is feasible and at least 90% of patients in both treatment arms will reach the target dose. Target dose is defined as the intake of study drug in at least 90% of the trial period. The secondary outcomes on CBF, CVR, and cognition are hypothesis generating in exploratory sub-studies to identify the effect on vascular reactivity and cognition. The overall hypothesis is that tadalafil can be a disease-modifying treatment for CSVD.

Trial design {8}

The ETLAS-2 trial is an investigator-initiated, randomized, placebo-controlled, double-blind, parallel-arm, phase 2 clinical trial in the Capital Region of Denmark. The allocation ratio is 1:1 between tadalafil and placebo. The framework applied is exploratory.

Methods: participants, interventions, and outcomes

Study setting {9}

The trial is conducted at the Department of Neurology, Copenhagen University Hospital—Herlev and Gentofte (HGH) in collaboration with Danish Research Centre for Magnetic Resonance (DRCMR), Copenhagen University Hospital—Amager and Hvidovre (AHH), Department of Neurology, Copenhagen University Hospital—Rigshospitalet (RH), Department of Neurology, Copenhagen University Hospital—Bispebjerg and Frederiksberg (BFH), and Department of Neurology, Copenhagen University Hospital—North Zealand (NZH). Patients that have signs of CSVD and late-phase small vessel occlusion stroke/transitory ischemic attack (TIA) (within the previous 5 years) can be included with the start of intervention earliest 6 months post-stroke. The cutoff of 6 months has been chosen because PDE-5 inhibitors are currently contraindicated in the acute phase of stroke based on case

reports of cerebral hemorrhage and lack of experience. The aim of this trial is not to treat the acute ischemia but to study the underlying vascular pathophysiology of CSVD Tables 1 and 2.

Eligibility criteria {10}

Who will take informed consent? {26a}

Investigators in the trial obtain oral and written informed consent from trial participants after an information visit is done. The information visit is carried out in an uninterrupted room. During the information visit, the trial is explained in detail regarding background, methods, potential side-effects, and what the participants can gain from participating in the trial.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

We collect blood samples for a biobank for future research as a separate voluntary study. A separate informed consent is obtained from trial participants who agree to participate.

Interventions

Explanation for the choice of comparators {6b}

The trial is designed as a placebo-controlled study with a 1:1 randomization rate between tadalafil and placebo. As we test the feasibility of daily tadalafil treatment in patients with cerebral small vessel disease and its effect on cerebrovascular and cognitive outcomes, placebo treatment was chosen as a comparator.

Intervention description {11a}

The intervention is a daily intake of tadalafil 20 mg or placebo together with guideline medication for approximately 3 months. Tadalafil or placebo

Table 1 Inclusion criteria

1	MRI/computed tomography (CT) evidence of small vessel occlusion stroke(s)/lacunar stroke(s) (involving ≤ 2 cm in the acute phase and ≤ 1.5 cm in the late phase) and/or white matter hyperintensities (\geq grade 2 on Fazekas's scale)
2	Clinical evidence of CSVD: (a) small vessel occlusion stroke (lacunar stroke) syndrome with symptoms lasting > 24 h, occurring < 5 years ago OR (b) transient ischemic attack (TIA) with symptoms lasting < 24 h AND an initial MRI diffusion-weighted imaging (DWI) showing a small vessel occlusion stroke, occurring < 5 years ago OR (c) TIA with symptoms lasting < 24 h AND no acute MRI-DWI lesion but MRI/CT evidence of CSVD with old small vessel occlusion stroke(s) and/or WMH (\geq grade 2 on Fazekas's scale), occurring < 5 years ago
3	Age ≥ 50 years

Table 2 Exclusion criteria

1	Known diagnosis of dementia or under investigation for dementia
2	Pregnancy or nursing
3	Women of childbearing age not taking contraception
4	Known cortical infarction (> 1.5 cm maximum diameter)
5	Known carotid artery stenosis \geq 50% with Doppler ultrasound, CT angiography, or MRI angiography diagnosed within the last 5 years
6	Known carotid or vertebral dissection as a cause of stroke
7	Stroke after carotid or heart surgery
8	Known hypercoagulable disease
9	Systolic BP < 90 and/or diastolic BP < 50
10	Known severe renal impairment (eGFR < 30 ml/min)
11	Known severe hepatic impairment (Child–Pugh > B)
12	History of non-arthritic anterior ischemic optic neuropathy
13	Concomitant use of PDE-5 inhibitors during trial period
14	Patients receiving nicorandil and nitrates, e.g., isosorbide mononitrate, isosorbide dinitrate, and glyceryl trinitrate
15	History of acute myocardial infarction in the last three months before trial intervention
16	Body weight > 130 kg
17	Known cardiac failure (NYHA \geq II)
18	Known persistent or paroxysmal atrial fibrillation/flutter
19	History of “sick sinus syndrome” or other supraventricular cardiac conduction conditions such as sinoatrial or atrioventricular block (second or third degree)
20	Other known cardiogenic cause of stroke
21	Contraindication to CO ₂ challenge, e.g., severe respiratory disease
22	MRI not tolerated or contraindicated
23	Known monogenic causes of stroke, i.e., CADASIL
24	Unable to provide informed consent
25	The participant does not wish to be informed about the results from the MRI

(lactose monohydrate and potato starch) are included in 96 opaque capsules for each participant to ensure blinding. Participants are instructed to take study medication in the morning. The dose of 20 mg tadalafil was based on our previous study [20], a collaborative study conducted at St George’s University of London, UK [24], and that 20 mg is common in other conditions, such as pulmonary hypertension and erectile dysfunction.

Criteria for discontinuing or modifying allocated interventions {11b}

Participants who repeatedly fail to comply with study agreements can be discontinued. Discontinuation will also occur if the first MRI cannot be conducted as this is considered an important outcome of the trial. In case of serious adverse events due to study medication, the intervention will be stopped. Participants who request to stop study medication, e.g., due to side effects, will be allowed to do so without being discontinued. If study medication is stopped, the participants are encouraged to continue the study without study medication as the feasibility of the treatment is the main outcome. The reason for terminating the study medication is noted.

Strategies to improve adherence to interventions {11c}

Participants are contacted by a phone call every week for the first month after the study medication has been initiated to promote motivation for the study and to assess side effects and adverse events.

Relevant concomitant care permitted or prohibited during the trial {11d}

Apart from nitrates (e.g., isosorbide mononitrate, isosorbide dinitrate, and glyceryl trinitrate) and PDE-5 inhibitors, any treatment or medication is permitted. Nitrates are prohibited due to their interaction with PDE-5 inhibitors. Nitrates and concomitant use of PDE-5 inhibitors during the trial period are listed as exclusion criteria.

Provisions for post-trial care {30}

Damages to participants caused by the study are covered, as described in the Danish Law.

Outcomes {12}

Outcomes will be analyzed in a main study and the following predefined sub-studies: functional MRI

exploratory sub-study, cognitive exploratory sub-study, and biomarker exploratory sub-study. All outcomes in the main study and sub-studies are assessed twice, initially at the baseline visit before study medication is initiated and then at the 3-month follow-up visit at the end of the trial period comparing changes from baseline to follow-up.

Main study

Primary outcome: feasibility The proportion of participants achieved full target dose of tadalafil/placebo by the end of the trial period. We allow a 10% failure of tablet intake in the trial period which still counts as full completion of medication. Assessment is done by tablet count and a questionnaire.

Secondary outcome: MRI Cerebral MRI is performed on a 3 T Philips Achieva scanner (Philips, The Netherlands). The following MRI sequences are used for anatomical evaluation: T1w, T2w, fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI), susceptibility-weighted imaging (SWI), and time of flight (TOF). MRIs will be assessed by two independent neuroradiologists according to the STRIVE guidelines from 2013, including white matter hyperintensity (WMH), lacunes, microbleeds, enlarged perivascular space, atrophy, and recent infarcts [25]. We also evaluate the presence of cortical superficial siderosis which is included in the recent STRIVE guidelines from 2023 [26]. WMH volume is assessed using an automatic solution with manual correction based on FLAIR and T1w images [27].

Secondary outcome: cognition Informant Questionnaire on Cognitive Decline in the Elderly (IQCOED) and Montreal Cognitive Assessment (MoCA) are used to assess cognitive function in the main study.

Secondary outcome: depression, fatigue, and mental well-being questionnaires The degree of depression is assessed using Becks Depression Inventory II (BDI-II). Fatigue Severity Scale (FSS) is used to examine fatigue, and WHO-5 Well-Being Index to assess mental well-being.

Secondary outcome: safety and adverse events Adverse events are registered according to good clinical practice from the day of inclusion until the safety follow-up visit is done.

Secondary outcome: blood pressure, heart rate, and electrocardiogram Blood pressure, heart rate, and electrocardiogram are performed.

Secondary outcomes: blood samples Routine blood samples are performed (hemoglobin, hematocrit, leukocytes, platelets, liver-, kidney-, and metabolic function, and high sensitivity C-reactive protein). To control for intake of study medication, plasma levels of tadalafil will be analyzed after the trial is completed.

Secondary outcomes: registered-based follow-up Register-based outcome assessment will be done with a composite measure of death, any ischemic event, hemorrhagic event, or dementia per patient registry after 3 and 5 years, respectively, from the end of the trial. These outcomes will be published separately.

Functional MRI exploratory sub-study

Calibrated functional MRI

We perform dual-echo pseudo-continuous arterial spin labeling (pCASL) to measure cerebral blood flow (CBF) and blood oxygen level-dependent (BOLD) signals during interleaved normocapnia and hypercapnia (5% CO₂, 20% O₂ in N₂) with/without visual contrast-reversing checkerboard stimulation [28]. From this, we estimate baseline cerebral blood flow (if possible, gray/white-matter perfusion), CVR using hypercapnia, stimulation-induced increase in cerebral metabolic rate of oxygen (CMRO₂) and perfusion, and the neurovascular coupling index (NVC) defined as the ratio of those two [29].

BOLD functional MRI (fMRI)

We assess changes in the regional event-related BOLD response during a peripheral sensory stimulation of the dominant index finger.

Diffusion-prepared pCASL (DP-pCASL)

We perform a sequence set of DP-pCASL scans to map the water exchange across the blood–brain barrier (BBB) [30–33]. The outcome is a global brain water coefficient (k_w).

See Supplementary material 1 for MRI acquisition and stimulation details (Table 3).

Cognitive exploratory sub-study

The participants undergo a 1.5-h cognitive assessment evaluating the domains of processing speed, verbal and visual working memory, attention, learning and memory, executive functions, and an estimation of premorbid intelligence by the Danish Adult Reading Test (DART). The following paper-and-pencil and Cambridge Neuropsychological Test Automated Battery (CANTAB) tests are done:

Table 3 fMRI outcomes

MRI sequence	Outcome	Details
Calibrated fMRI	CBF/perfusion	Baseline perfusion, gray/white-matter perfusion
	CVR	Increase in perfusion and BOLD signal during hypercapnia
	Visual stimulation response	Visual stimulation-induced increases in CMRO ₂ and CBF
	NVC	Ratio of visual stimulation-induced ΔCMRO ₂ and ΔCBF
BOLD fMRI	BOLD response	Changes in BOLD response to a sensory stimulation
DP-pCASL	BBB water exchange	Water exchange across BBB as a rate constant (k_{tr})

Paper-and-pencil: symbol digit modalities test (SDMT), trail making tests A and B, fluency (animal, F-A-S), and digit span forward, backward, ordering, and letter number sequence (from WAIS-IV).

CANTAB: motor screening (MOT), spatial working memory (SMW), paired associates learning task (PAL), reaction time (RTI), one-touch stockings of Cambridge (OTS), and rapid visual information processing (RVP).

Biomarker exploratory sub-study

Blood samples are centrifuged and stored as plasma, serum, and full blood at -80 °C until the last patient, last visit. Multiplex enzyme-linked immunosorbent assay (Mesoscale, MD, USA) will be used to assess; vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin 1beta (IL-1β), E-selectin, vascular endothelial growth factor, and specific micro-RNAs [20].

Participant timeline {13}

Participants complete five on-site study visits. Information, inclusion, and the baseline visit can be conducted on the same day. The second visit includes the first MRI

examination of the brain (MRI-1), after which the participants can start the study medication. After 1 month of study medication, a follow-up visit is done to assess the side effects. After 3 months of study medication, the second MRI examination (MRI-2) is conducted followed by the 3-month visit after which the study medication is stopped. The participants are contacted by telephone once weekly for the first 3 weeks after the study medication is initiated and after 2 months to assess the side effects. A safety follow-up telephone visit is performed within 2 weeks after the end of the study medication. Visits are performed at HGH and MRIs are done at DRCMR, AHH. See Fig. 1 and Table 4 for visit details.

Sample size {14}

We hypothesize that 90% of patients in both arms will reach the target dose and complete the trial. By non-inferiority test with a power of 80% and significance level of 0.05, we can detect a difference of 20% between those reaching the target dose on tadalafil compared to placebo with a total sample size of 64 patients. A difference of 20% compared to placebo and 30% in absolute percentage not being able to comply with treatment is considered relevant if to be used in

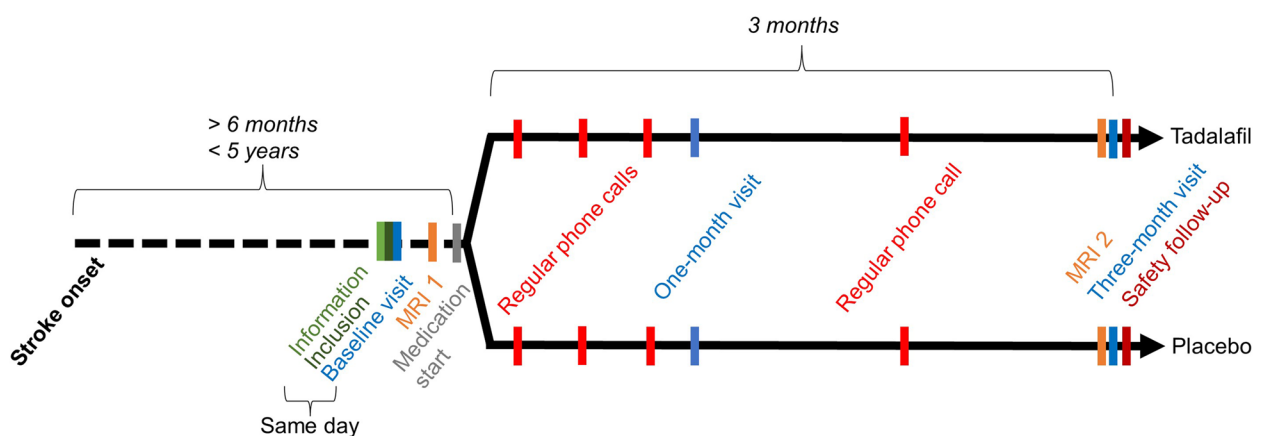










Fig. 1 Trial flow chart

Table 4 Schedule and timing of standard protocol assessments

Study procedures	Information and inclusion 	Baseline visit 	MRI-1 	Regular phone calls 	One-month visit 	MRI-2 	Three-month visit 	Safety follow-up 
Information	X							
Informed consent	X							
Medical history		X						
Concomitant medication		X			X		X	
Demographics		X						
Modified Rankin Score (mRS)		X					X	
National Institute of Health Stroke Scale (NIHSS)		X					X	
Cognitive testing		X					X	
MRI			X			X		
Dispensing study medication		X			X			
Study medication tablet count					X		X	
Blood pressure		X			X		X	
Electrocardiogram		X			X		X	
Blood samples: same-day analysis		X					X	
Blood samples: biomarkers		X					X	
Adverse events assessment		X	X	X	X	X	X	X

clinical settings. Sample size calculation was also performed for CBF based on 3 T arterial spin labeling (ASL)-MRI for healthy individuals since few 3 T ASL data in patients with CSVD existed when the study was designed. A baseline perfusion of 25 (± 5) ml/100 g/min (mean \pm SD) in subcortical white matter, 55 (± 10) ml/100 g/min in cortical gray matter, and 45 (± 10) ml/100 g/min in deep gray nuclei is used for sample size calculations [34–39]. To detect a treatment effect of 15% with a statistical power of 80%, a total sample size of 58 is required in subcortical white matter, 50 for cortical gray matter, and 72 for deep gray nuclei. Based on previous studies, we expect a 15% dropout and 10% of patients are estimated not to reach the target dose. To account for a drop-out of up to 25%, we aim to include 100 patients in total. The sample size is calculated using R software (version 3.6.0).

Recruitment {15}

Patients are identified by screening hospital records of previously admitted patients in the neurological departments (HGH, BFH, RH, and NZH). Invitation to participate and trial information is sent to the electronic post-box of eligible patients. The written invitation is followed by a phone call after 1–2 weeks to identify interest in participation. Interested patients are invited to the information and inclusion visit.

Assignment of interventions: allocation

Sequence generation {16a}

Patients are randomized consecutively to tadalafil or placebo treatment. Randomization procedure and packing of medication are performed by the Capital Region Pharmacy, Copenhagen, Denmark. Randomization is performed 1:1 in variable block sizes. The possibility of block size variation is known to the investigators who enroll participants. However, the blocks are in random order and that order is concealed from the investigators. During inclusion, each participant receives a consecutive randomization number, and the participant is handed out study medication with a matching randomization number.

Concealment mechanism {16b}

Tadalafil or placebo are packed in 96 opaque capsules in identical appearing containers to conceal which medication the participant has been randomized to. The investigator holds a set of sealed envelopes for each participant where randomization is stated. The envelopes are kept secured and unavailable to study personnel.

Implementation {16c}

The allocation sequence is performed by the Capital Region Pharmacy, Copenhagen, Denmark. The investigators who enroll participants and conduct intervention are not involved in the randomization procedure.

Assignment of interventions: blinding

Who will be blinded {17a}

The trial participants, care providers, and the research team are blinded throughout the whole study from the first patient to the last patient, last visit. Unblinding will be performed after all the trial participants have completed the trial and analyses are done.

Procedure for unblinding if needed {17b}

In case of adverse events (AE), the trial participants are instructed to contact the study group/investigator. In case of a serious adverse event (SAE), the investigator will assess whether the SAE is related to the study drug as soon as the person becomes aware of the SAE. In this case, the investigator can unblind immediately if necessary. Unblinding can be done by breaking the sealed randomization envelopes.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Assessment and collection of outcomes are performed by the study group at the different visits. The medical history of each participant can be collected from the patient's electronic medical file. Every study delegate who collects data is trained by a qualified trainer for each outcome to ensure the quality of outcomes. We use the following study instruments: MRI scans, neurological examination, questionnaires, cognitive tests performed as paper–pencil tests and computer-based tests, blood samples, blood pressure, electrocardiogram (ECG), and study medication tablet count. A summary of all outcomes is described in the outcomes section.

Plans to promote participant retention and complete follow-up {18b}

Participant retention is promoted by having close contact with each participant through weekly phone calls for the first months of the study period. Participants are handed out contact information to the study group and are informed that they can contact us at all times in case of questions and emergencies. Participants are also educated about the importance of completing all the visits to be able to assess if the study intervention has any effect on the measured outcomes. If participants discontinue the study, they are asked if we may access their medical records for the next 5 years as a registered-based follow-up to check if the patients have had cerebrovascular events or developed dementia. A participant is not considered lost to follow-up until multiple attempts have been made trying to contact the participant. Each attempt is registered in the electronic case report form (eCRF).

Data management {19}

All data recorded are kept in the eCRF, REDCap (Vanderbilt University, USA) including medical history, concomitant medication, paper–pencil cognitive tests, questionnaires, adverse events, and blood samples. CANTAB cognitive tests are stored in a logged secure server at the Capital Region of Denmark and MRI data is stored locally at a secure server at DRCMR, AHH. Paper–pencil cognitive tests and questionnaires are performed physically and are therefore also stored in paper format in a physical case report form (CRF). The in-house study visits are also documented in the patients' electronic medical record except for MRIs which are documented locally in a logbook at DRCMR, AHH. All data are checked for entry and range errors in REDCap after each visit and MRI data are visually checked for quality and artifacts directly after each MRI sequence is performed. After full completion of each subject, the primary investigator signs all eCRFs confirming that they are complete and accurate.

Confidentiality {27}

Personal information about each participant is stored in REDCap where only authorized study personnel have access. The physical CRFs and the trial master file (TMF) are stored behind two locks to protect against unauthorized access. When exporting data from REDCap to the statistical software, personal identifiers will be removed and data will be pseudo-anonymized. Re-identification can be carried out by the investigator if needed. After the end of the trial and final analysis, data will be fully anonymized.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

After the collection of blood samples, they are centrifuged and immediately stored in a local freezer at -80° at the HGH site behind two locks. After analysis of blood samples, they will be stored in a biobank (BIOSEK) at the HGH site. No genetic analyses are planned to be carried out.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

All outcomes are assessed twice, at baseline, and after 3 months, comparing the two treatment arms. Data will be analyzed for both the intention-to-treat population and the treatment group in the main study. In sub-studies, only the treatment group will be analyzed. The primary outcome will be evaluated by comparing participants achieving target dose by pharmacy count, using a binary logistic

regression model with and without adjusting for age, sex, blood pressure, and CSVD score according to STRIVE criteria. MRI outcomes will be analyzed using ANCOVA general linear regression model. MRI data will be adjusted for different factors, such as age, sex, blood pressure, and hematocrit. Cognitive outcomes will likewise be compared using ANCOVA test. All tests, except for the main study outcomes, will be treated as hypothesis-generating, and therefore no multiple comparison correction will be done. A two-sided *P*-value of 0.05 is considered statistically significant. Data is planned to be analyzed using *R* (version 4.3), Microsoft Excel, and REDCap.

Interim analyses {21b}

An interim analysis will not be done.

Methods for additional analyses (e.g., subgroup analyses) {20b}

No additional analyses are planned to be performed that are not described in Sect. 20a.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Data from participants who discontinue the trial before the last visit will be used when possible. Outcomes that are not assessed due to the patient's withdrawal from the study will be noted as missing data. Missing data will not be imputed.

Plans to give access to the full protocol, participant-level data, and statistical code {31c}

Fully anonymized data and statistical code will be made available after the trial in public databases.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

The trial is a single-center trial based at the HGH site with multiple collaboration partners. The steering committee includes the primary investigator (PI)/sponsor (CK), the leader at DRCMR, AHH (HRS), MRI post-doc (KNM), professor in psychology (BF), and sub-investigator (SI) and PhD-student (JÖ). All parts of the steering committee provide organizational support, monitor progress, discuss issues, and provide input to the trial. CK and JÖ are responsible for the practical part of running the trial including participant recruiting, data gathering, and analysis of data. HRS, KM, and JÖ are responsible for performing the MRI scans and analyzing MRI data, and BF and JÖ are responsible for collecting and analyzing cognitive data. Quarterly meetings are performed within the steering committee. Weekly meetings are held between the PI and SI. There is no Stakeholder and Public Involvement Group in this trial.

Composition of the data monitoring committee, its role, and reporting structure {21a}

This trial is monitored by the Good Clinical Practice (GCP) unit in the Capital Region of Denmark. The GCP unit is independent from the sponsor and has no competing interests. The monitor from the GCP unit monitors every aspect of the trial, including participant inclusion, data gathering, and safety of the trial. Frequency of monitor visits depends on enrollment rate and are held approximately for every ten subjects recruited. A written report is generated after each monitor visit describing which participants that have been monitored and findings from the monitoring including data results, adverse events, and safety.

Adverse event reporting and harms {22}

In case of events and side effects, the trial participants will be instructed to contact the study group/investigator. All side effects and adverse events (AE) during the trial will be recorded in the eCRF and assessed by the PI/SI for causality with the study drug. The Danish summary of product characteristics for Tadalafil Stada is used as a reference document for the assessment of events. All SAEs will be reported to the sponsor within 24 h and once yearly to the Ethics Committee in the Capital Region of Denmark and the Danish Medicines Agency. Suspected unexpected serious adverse reactions (SUSAR) that are life-threatening or fatal will be reported to the Danish Medicines Agency within 7 days. Other suspected unexpected serious adverse reactions that are not life-threatening or fatal will be reported within 15 days. In case of SAEs, the investigator may unblind immediately if necessary.

Frequency and plans for auditing trial conduct {23}

Direct access to the original data and patient details upon monitoring, auditing, or inspection will be made accessible to the legal authorities (the National/Regional Ethics Committee, The Danish Medicines Agency, The Danish Data Protection Agency, and the GCP-unit). The process can be conducted independently from the investigators and sponsor.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Any changes in study design and activities will require a protocol amendment. All amendments must be approved by the Ethics Committee in the Capital Region of Denmark and the Danish Medicines Agency before implementation. Trial registries will also be updated with the amendment.

Dissemination plans {31a}

Results will be published in peer-reviewed international scientific journals according to the CONSORT

guidelines. Results will also be presented at relevant national and international conferences. Trial registers will be updated when there is new information in the trial. All participants will receive a lay summary with the main findings from the trial once the trial is completed and the results have been analyzed. Further lay summary of findings will be provided to patient organizations.

Discussion

In this randomized, placebo-controlled, double-blind parallel-arm phase 2 clinical trial, we assess the feasibility of daily treatment with tadalafil 20 mg or placebo for 3 months in CSVD patients. Further, we assess tadalafil's impact on functional cerebrovascular MRI markers of CSVD and cognitive outcomes.

Tadalafil was chosen over other PDE-5 inhibitors since it has the longest plasma half-time of the available PDE5i and due to its documented brain penetrance in rodents [40]. Further, it has been tested in two single-dosing cross-over trials in CSVD patients with good tolerability [20, 24]. We previously found that a single dose of tadalafil increased cortical blood oxygenation measured with near-infrared spectroscopy in CSVD patients, indicating that tadalafil improves cortical perfusion [20]. However, the PASTIS trial found no effect of a single dose of tadalafil on CBF using pCASL-MRI [24]. In the LACI-2 trial, the NO donor isosorbide mononitrate and the PDE-3 inhibitor cilostazol were tested in CSVD patients. They found that treatment was feasible for 1 year with both drugs. Isosorbide mononitrate reduced recurrent stroke risk and cognitive decline. Cilostazol reduced dependence and a combination of both drugs reduced the composite outcome defined as recurrent stroke, cognitive impairment, functional impairment, and death [41]. Hence, this trial showed that it was possible to reduce stroke risk and cognitive decline in CSVD patients by targeting the NO-cGMP pathway.

Feasibility of treatment was selected as the primary outcome since it is essential when repurposing known medication. The MRI protocol was designed to examine key pathological features of CSVD, including the STRIVE criteria [25]. The calibrated fMRI measurements were chosen since it is a non-invasive method to assess CBF, CVR, and NVC [42]. Consequently, these calibrated fMRI measurements may provide proof of concept that tadalafil improves CBF and vessel reactivity. A previous study showed improved event-related BOLD response after sildenafil treatment in patients with Becker Muscular Dystrophy which indicates that PDE-5 inhibitors might only improve on-demand perfusion and not baseline perfusion [43]. NVC addresses changes in local perfusion due to changes in neuronal activity and is therefore an important outcome of this trial [44].

Cerebral BOLD fMRI with sensory stimulation was chosen since it is another method to assess changes in perfusion due to neuronal activity. DP-pCASL is a novel MRI method to address BBB permeability that does not require gadolinium. This outcome was chosen since it is generally accepted that CSVD patients have impaired BBB integrity [45]. The cognitive testing battery was specifically designed to target domains that can be impaired in CSVD patients, e.g., processing speed, verbal and visual working memory, attention, learning and memory, and executive functions [46].

Trial status

The current protocol version is number 6 dated to 17 May 2022. The first patient was included on 14 June 2022, and on the date of submission of this manuscript (3 April 2024), 64 patients have been included. The trial is estimated to end in late 2024. Three serious adverse events have been reported so far, however, none are adjudicated as caused by trial medication or methods.

Abbreviations

AE	Adverse events
AHH	Amager Hvidovre Hospital
ANCOVA	Analysis of covariance
ASL	Arterial spin labeling
BBB	Blood-brain barrier
BDI-II	Becks Depression Inventory II
BOLD	Blood oxygen level-dependent
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBF	Cerebral blood flow
cGMP	Cyclic guanosine monophosphate
CMRO ₂	Cerebral metabolic rate of oxygen
CRF	Case report form
CSVD	Cerebral small vessel disease
CVR	Cerebrovascular reactivity
DART	Danish Adult Reading Test
DP-pCASL	Diffusion prepared pseudo-continuous arterial spin labeling
DRCMR	Danish Research Centre for Magnetic Resonance
DWI	Diffusion-weighted imaging
ECG	Electrocardiogram
eCRF	Electronic case report form
FLAIR	Fluid-attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
FSS	Fatigue severity scale
GCP	Good clinical practice
HGH	Herlev Gentofte Hospital
ICAM-1	Intercellular adhesion molecule-1
IL-1β	Interleukin 1beta
IL-6	Interleukin-6
IQCODE	Informant Questionnaire on Cognitive Decline in the Elderly
MoCA	Montreal cognitive assessment
MOT	Motor screening
MRI	Magnetic resonance imaging
NO	Nitric oxide
NVC	Neurovascular coupling-index
NZH	North Zealand Hospital
OTS	One-touch stockings of Cambridge
PAL	Paired associates learning task
pCASL	Pseudo-continuous arterial spin labeling
PDE-5	Phosphodiesterase 5
PI	Primary investigator
RH	Rigshospitalet
RTI	Reaction time

RVT	Rapid visual information processing
SAE	Serious adverse event
SDMT	Symbol digit modalities test
SMW	Spatial working memory
STRIVE	STandards for Reporting Vascular changes on nEuroimaging
SUSAR	Suspected unexpected serious adverse reaction
SWI	Susceptibility weighted imaging
TIA	Transitory ischemic attack
TMF	Trial master file
TNF- α	Tumor necrosis factor alpha
TOF	Time of flight
VCAM-1	Vascular cell adhesion molecule 1
WHO-5	World Health Organization 5
WMH	White matter hyperintensity

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13063-024-08402-4>.

Supplementary Material 1.
Supplementary Material 2.

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Authors' contributions {31b}

JÖ wrote the first draft of this manuscript and is involved in the study design, patient recruitment, data gathering, MRI acquisition, and data analysis. KNM is involved in the design, acquisition, and analysis of MRI data. NN and MMN are involved in MRI analysis. BF is involved in designing the cognitive testing battery and analysis of cognitive data. HC, HKI, and MBP are involved in the study design and patient recruitment. HRS is involved in designing the study with focus on MRI, MRI analysis, and is the leader at DRCMR, AHH. CK is the initiator of the study, involved in study design, MRI design, data analysis, and is the leader at HGH. All authors have contributed to and approved the final version of the manuscript.

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Availability of data and materials {29}

The investigators will have access to an anonymized final trial dataset containing study results which will be made available in public databases permitted by Danish Law and upon reasonable request directly from the investigators. MRI data can be made available upon reasonable request. Data from medical files and participants are confidential and will remain unavailable.

Declarations

Ethics approval and consent to participate {24}

Ethical approval for this study was obtained from The Ethics Committee in the Capital Region of Denmark (H-20031301), the Danish Medicines Agency (EudraCT-number: 2020-002329-27), and Capital Region at Knowledge Center for Data Reviews (P-2020-1193). Written informed consent will be obtained from all subjects before the study.

Consent for publication {32}

Not applicable—no identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form are available from the corresponding author on request. Permission for publication of anonymized data is given by patients in the consent form.

Competing interests {28}

HRS has received honoraria as a speaker and consultant from Lundbeck AS, Denmark, and as editor (Neuroimage Clinical) from Elsevier Publishers, Amsterdam, The Netherlands. He has received royalties as book editor from Springer Publishers, Stuttgart, Germany, Oxford University Press, Oxford, UK, and Gyldendal Publishers, Copenhagen, Denmark. HC has received honoraria as a speaker from Bayer, Pfizer, and BMS, and as senior guest editor from American Heart Association (Stroke). She has received royalties from Gyldendal Publishers, Copenhagen, Denmark. The other authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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