Perfusion by Arterial Spin Labelling following Single Dose Tadalafil in Small Vessel Disease (PASTIS): study protocol for a randomized controlled trial

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Abstract

Background. Cerebral small vessel disease is a common cause of vascular cognitive impairment in older people, with no licensed treatment. Cerebral blood flow is reduced in small vessel disease. Tadalafil is a widely-prescribed phosphodiesterase-5 inhibitor that increases blood flow in other vascular territories. The aim of this trial is to test the hypothesis that tadalafil increases cerebral blood flow in older people with small vessel disease.

Methods. PASTIS is a phase II randomised double-blind crossover trial. In two visits, 7-30 days apart, participants undergo arterial spin labelling to measure cerebral blood flow and a battery of cognitive tests, pre and post-dosing with oral tadalafil (20 mg) or placebo.

Sample size: 54 participants are required to detect a 15% increase in cerebral blood flow in subcortical white matter ($p<0.05$, 90% power).

Primary outcomes are cerebral blood flow in subcortical white matter and deep grey nuclei. Secondary outcomes are cortical grey matter cerebral blood flow and performance on cognitive tests (reaction time, information processing speed, digit span forwards and backwards, semantic fluency).

Discussion. Recruitment started on 4th September 2015 and 25 participants have completed to date (8th November 2016). No serious adverse events have occurred. All participants have been recruited from one centre, St George's University Hospitals NHS Foundation Trust.

Trial registration. European Union Clinical Trials Register (EudraCT number 2015-001235-20) registered: 13th May 2015.

Keywords tadalafil cerebral blood flow vascular cognitive impairment vascular dementia phosphodiesterase MRI arterial spin labelling

Background

Cerebral small vessel disease (SVD) is a frequent cause of vascular cognitive impairment (VCI) in older adults [1-4]. There is currently no licensed treatment for SVD or for VCI [1, 2]. There is evidence from some previous studies to suggest that cerebral blood flow (CBF) is reduced in SVD, particularly in subcortical white matter [5-10]. We hypothesize that increasing CBF has potential to be both a symptomatic and a disease-modifying treatment for SVD and VCI.

Phosphodiesterase-5 inhibitors (PDE5i) such as sildenafil and tadalafil are well-established pharmacological vasodilators, causing enhanced nitric oxide-cGMP signalling in peripheral small arteries [11-13]. PDE5i are widely-used in treatment of erectile dysfunction and pulmonary hypertension [13]. PDE5 mRNA and protein are also found in human brain tissue [12, 14, 15]. Side-effect profiles are well-known and the drugs are well-tolerated in the target population [16-18]. In a meta-analysis of 28 placebo-controlled trials [18] overall incidence of myocardial infarction, cardiovascular death, or cerebrovascular death in tadalafil-treated patients did not differ from placebo. Incidence of these adverse events was independent of dosing regimen and duration of tadalafil therapy (up to 27 months) [18]. The choice of tadalafil (over other PDE5i) was based on long plasma half-life (17 h in healthy adults) [16, 17] and established brain penetration (brain:plasma ratio 1:10 in rodents and primates) [12, 19]. This study will test whether single dose tadalafil increases CBF in older people with neuroradiological and clinical evidence of SVD.

Methods

Objectives

The aim of this study is to test the hypothesis that tadalafil increases cerebral blood flow in subcortical areas in older people with symptomatic small vessel disease.

Design of the Study

PASTIS is a phase II double-blind crossover trial. Participants are randomised to order of treatment (tadalafil 20 mg, placebo; oral administration). Two visits are performed 7-30 days apart, with perfusion MRI and a battery of cognitive tests pre and 3-5 hours post dosing (see Figure 1).

***** **Figure 1 near here**

A SPIRIT checklist is appended (see Table 1).

Table 1. Schedule of enrolment, interventions, and assessments in the PASTIS trial. From PASTIS Protocol v4, 27 Jan 2016.

Study Procedures	Visit 0	Visit 1 – Day 1(< 60 day window)			Visit 2 (7 - 30 days later)		
	Screening	before IMP dose	IMP dosing	3-6 hrs post IMP dosing	before IMP dose	IMP dosing	3-6 hours post IMP dosing
Informed consent	x						
Inclusion/exclusion criteria	x						
Medical history	x						
Demographics	x						
Screening	x						
Modified Rankin Score	x						
MRI		x		x	x		x
Neuropsychological Test Batteries see Appendix 3	x	x		x	x		x
Dispensing/Administration of IMP			x			x	
Concomitant Medication	x	x			x		
Measure blood pressure	x	x		x	x		x
FBC*		x		x	x		x
plasma samples for Tadalafil drug levels				x			x

*Sample for full blood count (FBC) to be taken immediately following 1st scan and 2nd scan on both visits.

Trial Endpoints

The primary endpoints are change in regional CBF in two sub-cortical brain areas (deep white matter and deep grey nuclei). The secondary endpoints are i) change in regional CBF in cortical grey matter, ii) change in neuropsychological test performance, iii) plasma tadalafil concentration-dependence of any changes observed.

Setting of the Study

Participants are recruited from St George's University Hospital NHS Foundation Trust and local Participant Identification Centre (PIC) sites. All patient visits, data management and trial coordination are performed at St George's. PASTIS has been adopted on to the UK NIHR Clinical Research Network Portfolio.

Characteristics of Participants

Participants are older people (men and women) without diagnosis of dementia who have radiological and clinical evidence of symptomatic SVD. Following Informed consent the following activities will occur at a screening visit (see Figure 1).

1. Trial eligibility criteria check
2. Medical history
3. Concomitant medication checklist: medications, dose and frequency
4. MRI suitability/contraindication checklist
5. Participant demographics, including ethnic origin.
6. Next of kin and GP contact details to be recorded if not already in medical notes or check if still current and up-to-date.

7. Affix Clinical Trials Alert sticker to front of the medical notes
8. Complete Case Report Form screening page ensuring the participant trial ID is included.
9. Test of premorbid functioning (TOPF) – to establish estimated levels of cognitive functioning pre-illness
10. NIH Stroke Scale (NIHSS)
11. Montreal Cognitive Assessment (MoCA) to establish estimated levels of cognitive functioning
12. Record the Modified Rankin Score (mRS)

Inclusion Criteria

1. Radiological evidence of cerebral SVD defined as: MRI evidence of lacunar infarct(s) (≤1.5 cm maximum diameter) and/or confluent deep WMH (≥grade 2 on Fazekas scale)

2. Clinical evidence of SVD, including:

a) lacunar stroke syndrome with symptoms lasting >24 hours, occurring at least 6 months prior to visit 1; <u>or:</u>

b) transient ischaemic attack (TIA) lasting < 24 hours with limb weakness, hemi-sensory loss or dysarthria at least 6 months previously and with MRI Diffusion Weighted Imaging performed acutely showing lacunar infarction, <u>or</u> if MRI is not performed within 10 days of TIA, lacunar infarct in an anatomically-appropriate area

3. Age ≥ 50 years.

4. Imaging of the carotid arteries with Doppler ultrasound, CT angiography or MR angiography in the previous 12 months, demonstrating <70% stenosis in both internal carotid arteries or <50% stenosis in both internal carotids if measured in previous 12-60 months.

Exclusion Criteria

1. Known diagnosis of dementia
2. Cortical infarct (>1.5 cm maximum diameter)
3. Systolic BP <90 and/or diastolic BP <50 mmHg
4. Creatinine Clearance <30 ml/min
5. Severe hepatic impairment
6. History of lactose intolerance
7. Concomitant use of PDE5i e.g. sildenafil, tadalafil, vardenafil
8. receiving nicorandil or nitrates e.g. isosorbide mononitrate, GTN
9. Weight >130 kg
10. Uncontrolled cardiac failure
11. Persistent or paroxysmal atrial fibrillation
12. History of gastric ulceration
13. History of 'sick sinus syndrome' or other supraventricular cardiac conduction conditions
14. Uncontrolled COPD

15. Stroke or TIA within 6 months

16. MRI not tolerated or contra-indicated

17. Known monogenic causes of stroke e.g. CADASIL

18. Unable to provide informed consent

Randomization

The randomisation list will be generated by Sharp Clinical Services, Crickhowell, Powys, UK (http://www.sharpservices.com/our-facilities/sharp-clinical-services-wales/) and will be in blocks as detailed in the Client Study Information form kept in the Sponsor Site File. The participants will be acting as their own controls. Each participant will receive on two separate occasions a placebo dose and a tadalafil 20mg stat dose which appear identical in size, shape, weight and colour.

The patient pack numbers on the Pharmacy shelf correlates directly with the next available pack number on the blinded randomisation list held in the Pharmacy site file. Each patient pack contains two bottles, labelled as Bottle A and Bottle B. The randomisation list will be confidential to the trial statistician and will be summarised as treatment arm A and B, and not by Tadalafil and Placebo.

Measurement of Regional Cerebral Blood Flow

Whole brain perfusion will be determined by pseudo-continuous arterial spin labelling (ASL) [20] in a 3T MRI scanner (Philips). A total 20 min pseudo-continuous ASL acquisition time will be used to provide adequate signal-to-noise for CBF quantification in white matter. Other

image data acquired: an M_0 image, to enable quantification of CBF; high resolution 3D T1-weighted images for identification of grey and white matter regions of interest (including deep grey matter structures) for ASL analysis [20] and to map the ASL data to a standard brain atlas; Fluid Attenuated Inversion Recovery (FLAIR) for delineation of white matter hyperintensities (WMH); susceptibility weighted imaging for detection of micro-haemorrhages. These will provide participant-specific WMH load and location of WMH. Total scanning time is under 60 minutes per MRI session.

Cognitive Testing

Scores derived from the TOPF and MoCA instruments are recorded at the screening visit. These are included in the analyses as baseline data. They are not used as Inclusion or Exclusion criteria.

At the two dosing visits, the neuropsychological tests used are: Reaction Time (RTI) subtest of Cambridge Cognition CANTAB; Speed of Information Processing (SoIP) subtest of Brain Injury Rehabilitation Trust Memory and Information Processing Battery (BMIPB); Digit Span (DS) Forwards and Backwards subtest of Repeatable Battery for the Assessment of Neuropsychological Status (RBANS); Semantic Fluency subtest of RBANS.

Biochemical analyses

A blood sample is taken at the end of Visits 1 and 2 for haematocrit and full blood count. Plasma samples are stored at -80 ^{o}C for subsequent analysis of plasma tadalafil concentration.

Details of the Intervention

Each participant pack contains 1 bottle containing a single tadalafil 20mg capsule and 1 identical bottle containing a single matched placebo capsule. At each visit participants undergo cognitive tests and the first MRI scanning session of the day. Participants are then observed to swallow the appropriate investigational medicinal product (IMP) capsule, and receive a standard light lunch (450-750 kcal and 500 ml fluid). They undergo an equivalent, parallel version of the cognitive tests and the second MRI session of the day 3-5 h later. All participants are given a 24 hour emergency contact card with: study title, details of IMP, participant trial number, investigator's contact details and out-of-hours contact details (see Figure 2).

***** **Figure 2 near here**

All involved in the study (researchers, radiologists, pharmacists and participants) are blinded to treatment allocation for the duration of the study. Emergency un-blinding will take place in circumstances such as serious adverse events (SAE). Any SAE and safety endpoints will be reported in line with Clinical Trial regulations SI2004/1031 and Sponsor's procedures. We do not anticipate any serious adverse reactions to the medication since tadalafil is widely-used clinically and well-tolerated. The start point for SAE monitoring is the first intervention visit, ending 5 days after the second visit (based on a drug elimination period of 6 half-lives for the study medication, using a 20 hour half-life for tadalafil).

Power Calculation

From previous ASL studies of regional CBF we estimate baseline perfusion of 30 (±10) ml/100g/min (mean± SD) in subcortical white matter and 70 (±15) ml/100g/min in deep grey nuclei [21, 22]. To detect a treatment effect of 15 % (mean paired difference) with statistical

power of 90% a sample size of N=24 is required in deep grey matter nuclei and N=54 in subcortical white matter. We aim to recruit a target cohort of N=54.

Statistical Analysis

Baseline characteristics (age, sex, ethnic group, baseline BP, Modified Rankin Score, NIHSS, TOPF, MoCA) will be summarised as mean (SD) or median (Q1, Q3) for continuous variables, depending on distribution, and as number (percent) for categorical variables.

Changes in outcome variables will be calculated for each participant at each visit as (post-dose value) minus (pre-dose value). Data will be analysed using a linear mixed effects regression model with fixed effects for treatment (drug vs. placebo), visit (Visit 1, 2), treatment sequence and baseline response; and a random effect for participant nested within treatment sequence. Carry-over will be investigated by the treatment-by-visit interaction. If statistically significant, data from each visit will be analysed separately within linear regression models adjusting for treatment and pre-dose value. Clinical variables and other possible confounders (e.g. blood pressure at the time of the scan) will be included in the linear mixed effects models as adjustment variables. These will be pre-specified in the Statistical Analysis Plan.

All analyses will be intention-to-treat and no adjustment will be made for missing data. Statistical analyses will be performed using SAS® v9.3 for Windows or later. A p-value of >0.05 indicates the absence of a statistically significant effect.

Data Monitoring

Monitoring is performed by the Sponsor Clinical Trials Monitor in accordance with an agreed Risk-based monitoring plan. Case Report Form entries are verified against the source

documents and the participant medical notes. All data are entered directly from Case Report Forms to the PASTIS Access database by the PASTIS research team. Data transfer from the Case Report Form will be double-checked and where corrections are required these will carry a full audit trail and justification. Trial data storage conforms to St George's institutional Information Governance policies. Trial data, evidence of monitoring and system audits will be made available for inspection by the Sponsor and regulatory authorities as required.

Discussion

This randomised double-blind crossover phase II study will test whether tadalafil (20 mg) increases CBF in older people with SVD. Tadalafil was chosen over other PDE5i (such as sildenafil or vardenafil) owing to the documented brain penetration [12, 19] and longer plasma half-life of tadalafil [16, 17]. In the present trial we are simply testing for acute changes in response to a single dose of tadalafil. For this purpose a crossover design appeared optimal. In the event that a positive outcome is detected in the present study, it appears likely that a subsequent study testing tadalafil over a longer dosing period will be required. This will be needed to explore whether any tadalafil-mediated actions are maintained on chronic dosing and to test for any additional adverse reactions in participants who are likely to be taking concomitant stroke medications.

ASL was chosen to quantify regional CBF as it does not require injected radioisotopes or gadolinium compounds as tracers [20-22]. This MRI-based approach also enables acquisition of high resolution 3D T1-weighted images, T2-weighted FLAIR images and susceptibility weighted imaging. The neuropsychological tests that are used were chosen because each has four parallel versions of the test, to be applied at each screening point (Figure 1). The cognitive tests used measure processing speed, attention and executive function, which are

affected in SVD, as well as working memory and semantic fluency. Nevertheless it may be difficult to detect cognitive changes in such short term follow up as is employed here. The cognitive data obtained from this trial may be of value in assessing sample size and feasibility for any subsequent trial of tadalafil in cognitive function.

The trial commenced on 4th September 2015 and 25 participants have completed to date (8th November 2016). In addition to the European Union Clinical Trials Register (EudraCT number 2015-001235-20, date of registration: 13th May 2015) the trial has been registered on ClinicalTrials.gov (NCT02450253, date of registration: 18th May 2015). No serious adverse events have so far been observed. Inadvertent un-blinding due to the erectile effects of tadalafil has not occurred so far as we are aware. Spontaneous penile erection has been reported in a modest fraction (11%) of subjects taking 20 mg tadalafil [16, 17]. PASTIS is the first Phase II clinical trial of a selective PDE5 inhibitor in older people with symptomatic SVD. Outcomes are expected in late 2017 and may inform a larger trial for re-purposing of tadalafil in SVD and VCI.

Trial Status

The PASTIS trial is ongoing at the time of manuscript submission. Patient recruitment has not been completed.

List of Abbreviations

ASL: arterial spin labelling. CBF: cerebral blood flow. IMP: investigational medicinal product. PDE5i: phosphodiesterase-5 inhibitor. SAE: serious adverse event. SVD: small vessel disease. VCI: vascular cognitive impairment.

Declarations

Ethics approval and consent to participate

The PASTIS study received NHS National Research Ethics Service approval on 6th May 2015 (London-Brent Research Ethics Committee Ref 15/LO/0714). PASTIS has received MHRA authorisation (Ref 16745/0222/001-0001; 5th June 2015) and St George's University of London R&D approval (protocol number 14.0189; 4th Sept 2015). Informed consent to participate is obtained from all participants in the study. The trial opened for recruitment on 4th September 2015 and the first participant was enrolled on 14th September 2015. The PASTIS trial is sponsored by: Joint Research and Enterprise Office, St George's University of London, Cranmer Terrace, London SW17 0RE. A representative of the sponsor (DR) has made input to trial design and has contributed to this report.

Consent for publication -- Not Applicable

Availability of data and material -- Not Applicable

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

JDI, TRB, FAH, DR, SB, JBM, BM, UK, ACP, CK, ER, AHH contributed to study design. MMHP, ST, NC performed data collection. MMHP, TRB, CEH carried out data analysis. MMHP, NC drafted the manuscript. All authors contributed to revising the manuscript. AHH prepared the final manuscript. All authors read and approved the final manuscript.

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Reference List

1. O'Brien JT, Thomas A. Vascular dementia. Lancet 2015; 386:1698-706.

2. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 2010; 9:689-701.

3. Esiri MM, Wilcock GK, Morris JH. Neuropathological assessment of the lesions of significance in vascular dementia. J Neurol Neurosurg Psychiatry 1997; 63:749-53.

4. Ighodaro ET, Abner EL, Fardo DW, Lin AL, Katsumata Y, Schmitt FA, Kryscio RJ, Jicha GA, Neltner JH, Monsell SE, Kukull WA, Moser DK, Appiah F, Bachstetter AD, Van Eldik LJ, Nelson PT. Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals. J Cereb Blood Flow Metab 2017; 37:201-16.

5. 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; 69:48-53.

6. O'Sullivan M, Lythgoe DJ, Pereira AC, Summers PE, Jarosz JM, Williams SC, Markus HS. Patterns of cerebral blood flow reduction in patients with ischemic leukoaraiosis. Neurology 2002; 59:321-6.

7. Schuff N, Matsumoto S, Kmiecik J, Studholme C, Du A, Ezekiel F, Miller BL, Kramer JH, Jagust WJ, Chui HC, Weiner MW. Cerebral blood flow in ischemic vascular

dementia and Alzheimer's disease, measured by arterial spin-labeling magnetic resonance imaging. Alzheimers Dement 2009; 5:454-62.

8. Yao H, Sadoshima S, Ibayashi S, Kuwabara Y, Ichiya Y, Fujishima M. Leukoaraiosis and dementia in hypertensive patients. Stroke 1992; 23:1673-7.

9. Bernbaum M, Menon BK, Fick G, Smith EE, Goyal M, Frayne R, Coutts SB. Reduced blood flow in normal white matter predicts development of leukoaraiosis. J Cereb Blood Flow Metab 2015; 35:1610-5.

10. Arba F, Mair G, Carpenter T, Sakka E, Sandercock PA, Lindley RI, Inzitari D, Wardlaw JM. Cerebral White Matter Hypoperfusion Increases with Small-Vessel Disease Burden. Data From the Third International Stroke Trial. J Stroke Cerebrovasc Dis 2017.

11. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. Annu Rev Biochem 2007; 76:481-511.

12. Garcia-Osta A, Cuadrado-Tejedor M, Garcia-Barroso C, Oyarzabal J, Franco R. Phosphodiesterases as therapeutic targets for Alzheimer's disease. ACS Chem Neurosci 2012; 3:832-44.

13. Maurice DH, Ke H, Ahmad F, Wang Y, Chung J, Manganiello VC. Advances in targeting cyclic nucleotide phosphodiesterases. Nat Rev Drug Discov 2014; 13:290-314.

14. Lakics V, Karran EH, Boess FG. Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. Neuropharmacology 2010; 59:367-74.

15. Kruuse C, Khurana TS, Rybalkin SD, Birk S, Engel U, Edvinsson L, Olesen J. Phosphodiesterase 5 and effects of sildenafil on cerebral arteries of man and guinea pig. Eur J Pharmacol 2005; 521:105-14.

16. Forgue ST, Patterson BE, Bedding AW, Payne CD, Phillips DL, Wrishko RE, Mitchell MI. Tadalafil pharmacokinetics in healthy subjects. Br J Clin Pharmacol 2006; 61:280-8.

17. Forgue ST, Phillips DL, Bedding AW, Payne CD, Jewell H, Patterson BE, Wrishko RE, Mitchell MI. Effects of gender, age, diabetes mellitus and renal and hepatic impairment on tadalafil pharmacokinetics. Br J Clin Pharmacol 2007; 63:24-35.

18. Kloner RA, Jackson G, Hutter AM, Mittleman MA, Chan M, Warner MR, Costigan TM, Vail GM. Cardiovascular safety update of Tadalafil: retrospective analysis of data from placebo-controlled and open-label clinical trials of Tadalafil with as needed, three times-per-week or once-a-day dosing. Am J Cardiol 2006; 97:1778-84.

19. Garcia-Barroso C, Ricobaraza A, Pascual-Lucas M, Unceta N, Rico AJ, Goicolea MA, Salles J, Lanciego JL, Oyarzabal J, Franco R, Cuadrado-Tejedor M, Garcia-Osta

A. Tadalafil crosses the blood-brain barrier and reverses cognitive dysfunction in a mouse model of AD. Neuropharmacology 2013; 64:114-23.

20. Alsop DC, Detre JA, Golay X, Gunther M, Hendrikse J, Hernandez-Garcia L, Lu H, MacIntosh BJ, Parkes LM, Smits M, van Osch MJ, Wang DJ, Wong EC, Zaharchuk G. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med 2015; 73:102-16.

21. D'haeseleer M, Beelen R, Fierens Y, Cambron M, Vanbinst AM, Verborgh C, Demey J, De KJ. Cerebral hypoperfusion in multiple sclerosis is reversible and mediated by endothelin-1. Proc Natl Acad Sci U S A 2013; 110:5654-8.

22. Colloby SJ, Firbank MJ, He J, Thomas AJ, Vasudev A, Parry SW, O'Brien JT. Regional cerebral blood flow in late-life depression: arterial spin labelling magnetic resonance study. Br J Psychiatry 2012; 200:150-5.

Figure 1

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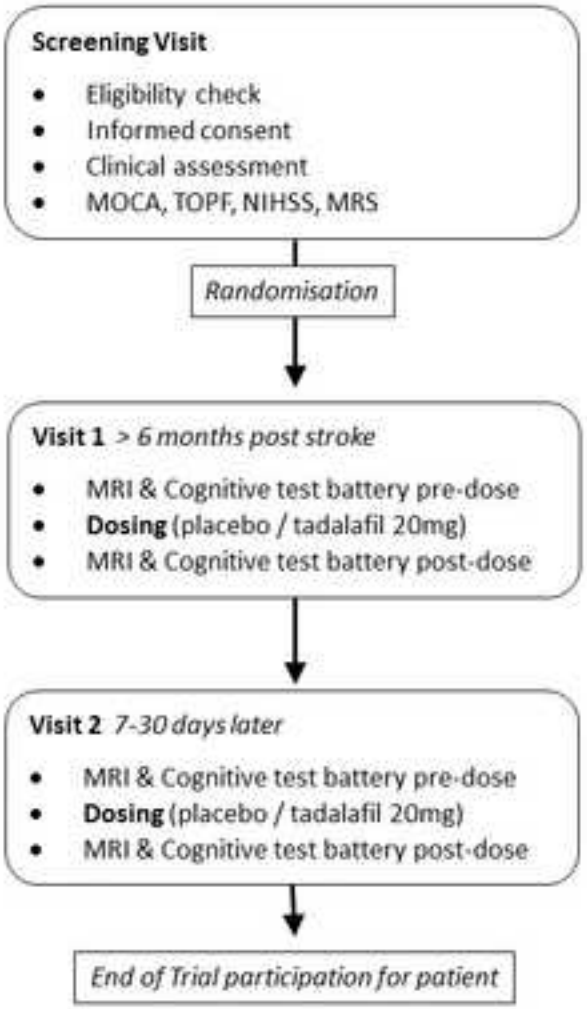

Figure 2

Click here to download Figure Fig 2 PASTIS_24 hour card.pdf

This person is taking part in **PASTIS** a Randomised, placebo controlled, Cross-over Trial of Tadalafil 20mg Stat dose in SVD

PASTIS_14.0189

Patient ID/Pack#

Tadalafil 20 mg PO Stat or matched Placebo PO Stat

PASTIS is coordinated by the Neurology Department, St George's Hospital & sponsored by St George's University of London

Please carry this card while you are participating in the study and show it to any other doctor who may be treating you.

Patient Name .....

In case of any medical problems or, if further information is required, please contact:

PI: **Dr Jeremy Isaacs at St George's Hospital**
Tel: **020 8725 4630**

Out of hours contact: **020 8672 1255**
and request the 'on call pharmacist'

Version 1.0_2nd April 2015 REC reference: 15/LO/0714

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Demographics	x						
Screening	x						
Modified Rankin Score	x						
MRI		x		x	x		x
Neuropsychological Test Batteries see Appendix 3	x	x		x	x		x
Dispensing/Administration of IMP			x			x	
Concomitant Medication	x	x			x		
Measure blood pressure	x	x		x	x		x
FBC*		x		x	x		x
plasma samples for Tadalafil drug levels				x			x

*Sample for full blood count (FBC) to be taken immediately following 1st scan and 2nd scan on both visits.