PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Trial of Remote Ischaemic Pre-Conditioning in Vascular Cognitive Impairment (TRIC-VCI): Protocol for a randomised controlled trial
AUTHORS	Ganesh, Aravind; Barber, Philip; Black, Sandra; Corbett, Dale; Field, Thalia; Frayne, Richard; Hachinski, Vladimir; Ismail, Zahinoor; Mai, Lauren; McCreary, Cheryl; Sahlas, Demetrios; Sharma, Mukul; Swartz, Rick; Smith, Eric E

VERSION 1 – REVIEW

REVIEWER	Lucette Cysique The University of New South Wales, Australia I am a visiting scholar at the University of Toronto (NeuroHIV research, Prof. Sean Rourke group)
REVIEW RETURNED	19-Jul-2020

GENERAL COMMENTS	This is an excellent and needed study as there are no treatment for CSVD I am worried that planning linear mixed-effect model on this sample size is not a correct statistical strategy. Linear mixed effect modelling requires large numbers. I am also worried that the success of RIC will be associated with the level of baseline cognitive impairment of the participant more than anything else. Is there a way to better involve a cognitively healthy caregiver? Also caregivers are likely to help at home, and that should be documented.

REVIEWER	Perminder Sachdev University of New South Wales Sydney, Australia
REVIEW RETURNED	20-Jul-2020

GENERAL COMMENTS	This is a phase 2a study of RIC to assess the tolerability and safety of 2 doses of the procedure. It is well-designed and has been written clearly. It is a really worthwhile are of investigation. The author may wish to clarify and/or elaborate on some aspects: • The rationale for the choice of these two doses – once a day vs twice a day – is not well described. The two durations being selected are also somewhat arbitrary. The literature is not very helpful in this regard, with studies ranging from intervention on one day to daily intervention for 180 days. • The decision to use 200 mm Hg as standard needs to be justified. Is it not enough to go above the systolic BP by about 20-25 mm Hg? Would going to 200 mm Hg in someone who has a systolic BP of say 120 mm not cause unnecessary discomfort and possibly local adverse effects?

	• Why did the authors decide not to investigate several different doses and modes of administration (unilateral/bilateral; upper/lower limb) etc in a tolerability study before deciding on the more definitive modalities? • The inclusion criteria are not well described. What comprises Mild vascular NCD for this study? Will amyloid PET be done to exclude early AD? How much WMH and/or lacunes will be needed as evidence of SVD? • Cortical rCBF is being measured. My concern is that the figures cited for rCBF reduction in SVD come from a small study (n=8), who had mild dementia and not mild NCD (Schuff et al). The sample for the biomarker component may therefore be an under-estimate. Moreover, rCBF using ASL will be measured in 5 different centres using different scanners. This will lead to a great degree of variability which the investigators may have under-estimated. • The study is of course not designed to determine efficacy. Without a sham control group, this is not possible. Consider that practice effects are quite strong over this time frame, both groups are likely to improve in their test performance, and separating them may be really difficult. • While PCASL is the best technique available, it still does not overcome the low SNR of ASL in comparison with exogenous contrast based techniques. It' sensitivity to change is therefore not very high, thereby its ability to separate the two groups.

VERSION 1 – AUTHOR RESPONSE

REVIEWER #1:

This is an excellent and needed study as there are no treatment for CSVD
Response: We thank the Reviewer for their encouraging comments.

1. I am worried that planning linear mixed-effect model on this sample size is not a correct statistical strategy. Linear mixed effect modelling requires large numbers.
Response: We appreciate the Reviewer's concern. Please note that the linear mixed models will be used only for secondary outcome measures. Prior methodological publications have noted that linear mixed modelling can provide helpful perspectives when analyzing small samples (Muth C, et al Educ Psychol Meas 2016;76:64-87) at times with lower standard error estimates and greater relative statistical power than generalized estimating equations (McNeish and Harring, Communications in Statistics - Simulation and Computation 2017;46:855-69. That being said, we have now noted in the analysis plan that in the event that the models do not converge in our analyses, we will compare the difference from baseline to 30- or 90-days in the two arms using the t-test or Wilcoxon rank-sum test as appropriate (page 14, last paragraph).

2. I am also worried that the success of RIC will be associated with the level of baseline cognitive impairment of the participant more than anything else.
Response: We agree that this could be the case. Although our study dataset is likely too small to test this hypothesis, we can adjust our analyses of each secondary clinical outcome of interest – MoCA, Trail-Making, COWAT, CERAD 10-item word list learning score, MBI checklist, and BADLS scores – for the participants' respective baseline score on that measure. We have now clarified this point in the section on statistical analysis (page 15 paragraph 3).

3. Is there a way to better involve a cognitively healthy caregiver? Also caregivers are likely to help at

home, and that should be documented.
Response: We agree that the perspective of the caregivers is critical for this type of study, particularly when it comes to assessing changes in cognition, behaviour, and daily activities. We have clarified this on Page 12 paragraph 2:
"Each study participant will also have an informant, ideally one who lives with them or is a caregiver, who will provide important collateral data about their cognitive and behavioural status (via the AD8 informant questionnaire, IQCODE, and the MBI checklist) and daily activities (via the Bristol Activities of Daily Living Scale longitudinally)."

REVIEWER #2:

This is a phase 2a study of RIC to assess the tolerability and safety of 2 doses of the procedure. It is well-designed and has been written clearly. It is a really worthwhile area of investigation.
Response: We thank the Reviewer for their encouraging comments.

The author may wish to clarify and/or elaborate on some aspects:
1. The rationale for the choice of these two doses – once a day vs twice a day – is not well described. The two durations being selected are also somewhat arbitrary. The literature is not very helpful in this regard, with studies ranging from intervention on one day to daily intervention for 180 days.
Response: We agree that the extant literature is of limited value in guiding the choice of the two doses used in this study. We have emphasized this point on page 7 paragraph 1 of the Introduction:
"As there are few human data to guide dose choices, the most comprehensive dose-finding data comes from an animal study[33] which found that more than one limb, more than four cycles, and more than 5-minutes of ischaemia conferred no additional reductions in infarct size in a mouse model of acute myocardial infarction."

2. The decision to use 200 mm Hg as standard needs to be justified. Is it not enough to go above the systolic BP by about 20-25 mm Hg? Would going to 200 mm Hg in someone who has a systolic BP of say 120 mm not cause unnecessary discomfort and possibly local adverse effects?
Response: We concede the Reviewer's point that a target of 200mmHg may be unnecessary in many patients to achieve occlusion. However, the same device with the same pressure settings was tolerated well by patients in a Danish study of acute stroke (Hougaard et al 2014). We have noted this in our discussion about the device (page 8 paragraph 3):
"Whereas the target inflation pressure of 200 mmHg is likely higher than what is needed to achieve occlusion in many patients, the same device with the same pressure settings was well tolerated by patients in a Danish study of acute stroke[27]".

3. Why did the authors decide not to investigate several different doses and modes of administration (unilateral/bilateral; upper/lower limb) etc in a tolerability study before deciding on the more definitive modalities?
Response: Unpublished communications from other investigators indicate that bilateral upper limb was not tolerable, despite the high adherence reported by the Chinese studies we have cited. This leaves us the option of using one limb once a day or twice a day. For practical reasons of cost and the available patient population, it is not feasible for us to test multiple different paradigms at this point. However, as part of the TRIC-VCI research program, we are conducting parallel animal studies that might shed light on dose response. If we obtain neutral effects in the next phase with sham control, we may have to consider whether a more intensive regimen would be needed. We have added a note about our plans to pursue parallel animal studies on page 18 last paragraph:
"The data will complement work by our basic science collaborators who will be conducting parallel animal studies to explore dose response relationships with various additional RIC regimens– which we are unable to do in our trial for practical reasons of cost and the available patient population."

4. The inclusion criteria are not well described. What comprises Mild vascular NCD for this study? Will amyloid PET be done to exclude early AD? How much WMH and/or lacunes will be needed as evidence of SVD?
Response: We have discussed the inclusion criteria only briefly in the main body of the text but have included full details in Table 1. We have now made it clearer in the body of the text (page 11 paragraph 3) that the full details and definitions of the inclusion criteria are available in Table 1.

As noted in Table 1 and more briefly on page 10, the following criteria will be used for mild or early major neurocognitive impairment:
o Objective evidence of cognitive impairment (MoCA ≤24 as noted in inclusion criteria, but not <13 as noted in the exclusion criteria)
o Concern on the part of the patient, caregiver, or clinician that there has been a decline from previous level of cognitive functioning (as supported by two or more positive responses by an informant on the AD8 questionnaire, or clinical judgement based on self-report by the participant or observations by the examiner)
o Currently independent with basic activities of daily living (BADLS response (a) for questions 2, 4, 5, 6, 7, 8, 9, and 14).
We have now explicitly stated that testing for biomarkers of Alzheimer's Disease will not be required for this study, with the acknowledgement that some participants may well have mixed dementia (page 12 paragraph 1):
"Whereas all patients will meet our inclusion criteria for demonstrating evidence of cSVD on CT/MRI, we will not require testing for biomarkers of Alzheimer's Disease in our study, with the understanding that some patients will have co-pathology or mixed dementia."
The vascular/cSVD contribution to cognitive impairment will be supported by the following criteria for the burden of WMH/infarcts:
o Beginning confluent WMH (ARWMC grade 2) on any slice on CT/MRI or
o Two or more supratentorial subcortical infarcts

5. Cortical rCBF is being measured. My concern is that the figures cited for rCBF reduction in SVD come from a small study (n=8), who had mild dementia and not mild NCD (Schuff et al). The sample for the biomarker component may therefore be an under-estimate. Moreover, rCBF using ASL will be measured in 5 different centres using different scanners. This will lead to a great degree of variability which the investigators may have under-estimated.
Response: We agree with the Reviewer that this is a relatively novel use of ASL, with attendant limitations in estimating treatment effects, and should be considered exploratory, as clarified on page 13 paragraph 1:
"Since this is a relatively novel use of ASL, and our estimate for CBF increase are based on a small study sample with mild dementia, our sample size estimation for the biomarker component must be interpreted with caution, and the CBF measure is best interpreted as an exploratory outcome."
To avoid relying solely on this metric, we are also collecting pilot data on a more traditional marker (WMH volume) and a more advanced marker that has been used in other multicentre studies of cSVD (DTI-based peak skeletonized mean diffusivity) – these are listed as secondary efficacy endpoints #1 and #2 in Table 2.

6. The study is of course not designed to determine efficacy. Without a sham control group, this is not possible. Consider that practice effects are quite strong over this time frame, both groups are likely to improve in their test performance, and separating them may be really difficult.
Response: We agree with the Reviewer's assessment, and have noted the absence of sham control in the introductory section on strengths and limitations (page 4):
"The use of intent-to-treat analysis, pre-specified primary and secondary outcomes, and candidate biomarkers for monitoring treatment response will improve upon previous small studies of remote

ischaemic pre-conditioning in cSVD; however, the lack of a non-treated or sham control group means that only within-patient changes can be analyzed."
Our decision about which dose to use for the next study phase will be based on the best tolerated dose of RIC and not the very preliminary estimates of cognitive differences that will be obtained from this trial.

7. While PCASL is the best technique available, it still does not overcome the low SNR of ASL in comparison with exogenous contrast based techniques. Its sensitivity to change is therefore not very high, thereby its ability to separate the two groups.
Response: We agree that the signal-to-noise ratio for ASL is low in comparison to contrast-based techniques. However, our current approach is more practical and avoids the risks of contrast exposure. Our review of the co-efficient of variation for ASL measurement suggests that it should be possible to detect changes over time with reasonable sensitivity (please see sample size calculations on page 15 paragraph 1 with the caveats as noted above about the exploratory nature). Our main motivation for implementing ASL in this phase of the trial program is to determine its suitability for the next phase, so we do not assume that it will work out. Based on the Reviewer's feedback, we will be adding an analysis plan to examine how well ASL performed in the study (page 14 paragraph 4):
"Since our main motivation for implementing ASL in this study is to determine its suitability as an outcome measure for a larger subsequent trial, we will also examine the variation in ASL measurements across sites and within-person variation at each site."

VERSION 2 – REVIEW

REVIEWER	Lucette Cysique UNSW Australia
REVIEW RETURNED	26-Aug-2020

GENERAL COMMENTS	The authors have adequately addressed my comments.

REVIEWER	Perminder Sachdev University of New South Wales
REVIEW RETURNED	18-Aug-2020

GENERAL COMMENTS	The authors have adequately addressed my concerns.