PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	A study protocol for a phase II randomised, double-blind, placebo-controlled trial of sodium selenate as a disease-modifying treatment for behavioural variant frontotemporal dementia
AUTHORS	Vivash, Lucy; Malpas, Charles; Churilov, Leonid; Walterfang, Mark; Brodtmann, Amy; Piguet, Olivier; Ahmed, Rebekah M; Bush, Ashley; Hovens, Christopher M; Kalincik, T; Darby, David; Velakoulis, Dennis; O'Brien, Terence

VERSION 1 – REVIEW

REVIEWER	Per M Roos Institute of Environmental Medicine Karolinska Institutet Sweden
REVIEW RETURNED	13-May-2020

GENERAL COMMENTS	bmjopen-2020-040100 This study protocol describes conditions for a study of high dose selenium administrated to 120 participants with behavioural variant frontotemporal dementia. The authors have vast experience with similar studies and the background information is sufficient to motivate the study. Study outcome is important and valid also towards other neurodegenerative diseases. An extensive cognitive testing battery will be used and cerebrospinal fluid biomarkers measured. The topic is of interest and the protocol merits publication in bmjopen. Some additional comments may be important though, details below. Background/Aims/Methods • Participants and intervention: 120 participants will be enrolled in the study. Following screening, participants will be randomised (1:1) to blinded treatment with either sodium selenate (15 mg three times a day) or matching placebo. The number of participants is sufficient to create reliable results with the variables chosen. Yet the effects of selenium exposure on the brain volume of healthy individuals compared to placebo also has to be taken into consideration i.e. a control group. • showed that these benefits were restricted to the administration of the single selenium species, sodium selenate, and were only beneficial in a therapeutic setting when administered in supranutritional doses [17,

	23]. This may be true for sodium selenate however the potential neurotoxicity of other selenium species should also be discussed in this context (i.e. selenite vs ALS ref Vinceti) for balance. • Selenium levels in patients' serum and CSF were higher in the treatment group, which is evidence of penetration of the agent across the blood-brain barrier and into the CNS [23]. Selenium does not easily travel the BBB and only a few percent can be found inside of the barrier. Here the actual difference between serum and CSF in the referred study should be stated for clarification and for validation of this study protocol. • taken any of the following: NMDA receptor antagonists, oral and/or injectable steroids, digoxin, phenobarbitone or warfarin; commencement or titration of other medications known to have an effect on mood or cognition within the 4 weeks prior to screening, including anticholinergics, hypnotics, sedatives, anxiolytics, antidepressants, antiepileptics, antipsychotics, memory-enhancing drugs, nutraceuticals, and other supplements which contain selenium. In proper order. How is selenium exposure from potable water controlled for? What is the baseline selenium concentration in water in this region and how does it vary between participants in this study? • The initial dose will be two capsules (10 mg) three times a day Most individuals will react in one way or the other to a selenium dose of 30mg each day. How is the blinding towards placebo maintained in this situation? This should be clarified and discussed in this section of the protocol. • CSF sampling will take place at baseline Details of sampling technique should be given (needles, type of vials, cleaning procedures, storage routines). Is this ultraclean sampling?

REVIEWER	Sidharth Mehan Dr. Sidharth Mehan Ph.D., M.Pharm, Associate Professor, Neuropharmacology Division, Department of Pharmacology, ISF College of Pharmacy, Moga-142001, Punjab, India Institute Website: https://isfcp.org/i/ Mail: sidh.mehan@gmail.com, sidharthmehan@isfcp.org Phone: +91-8059889909, +91-9461322911 ORCID: 0000-0003-0034-835X
REVIEW RETURNED	22-May-2020

GENERAL COMMENTS	Dear author, After critical analysis of this manuscript, the clinical justification and

	significance of this research, their future perspectives and biochemical markers are not significantly established, perform as well as written in introduction, research methodology and discussion part.

REVIEWER	Dr J McCleery 1. Cochrane Dementia and Cognitive Improvement Group, Radcliffe Department of Medicine, University of Oxford 2. Oxford Health NHS Foundation Trust, UK
REVIEW RETURNED	18-Aug-2020

GENERAL COMMENTS	This is a valuable study in an area with a great need for therapeutic advances. It would be helpful to clarify to what extent you expect to be able to enrich the study sample for tau pathology through exclusion on the basis of family history with genetic testing, and whether the expected proportion of patients with non-tauopathy remaining in the sample has been taken into account in the sample size calculation.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. The number of participants is sufficient to create reliable results with the variables chosen. Yet the effects of selenium exposure on the brain volume of healthy individuals compared to placebo also has to be taken into consideration i.e. a control group.

We recognise the reasoning behind the suggestion. However, long-term administration of sodium selenate to healthy volunteers for the purposes of measuring brain volume would be difficult to justify ethically, to interpret scientifically and beyond the scope of this study (which is to test the hypothesis that sodium selenate treatment has a disease modifying effect in patients with bvFTD).

2. “Showed that these benefits were restricted to the administration of the single selenium species, sodium selenate, and were only beneficial in a therapeutic setting when administered in supranutritional doses [17, 23].”

This may be true for sodium selenate however the potential neurotoxicity of other selenium species should also be discussed in this context (i.e. selenite vs ALS ref Vinceti) for balance.

Our previous clinical trials determined that selenate at the supranutritional dose we propose in this protocol paper was well tolerated (Corcoran et al., 2010, Malpas et al., 2016, Vivash et al., under review). These are to our knowledge, the longest-term studies of treatment with selenate at these doses.
The metabolism of selenium species in mammals is complex and dozens of different organic and inorganic intermediates all with varying half-lives have been reported in the literature following oral administration. We do not think it is appropriate or warranted to comment on the toxicity or metabolism or other potential forms of selenium in this study, since it is outside of the scope of our protocol.

3. “Selenium levels in patients’ serum and CSF were higher in the treatment group, which is evidence of penetration of the agent across the blood-brain barrier and into the CNS [23].”

Selenium does not easily travel the BBB and only a few percent can be found inside of the barrier. Here the actual difference between serum and CSF in the referred study should be stated for clarification and for validation of this study protocol.

The reviewer is right to highlight the relatively low CNS penetrance of selenium and sodium selenate. Selenium levels in serum increased from 145ug/L at baseline to 858ug/L after 24 weeks of treatment (10mg tds), CSF levels increased from 1.4ug/L at baseline to 20ug/L post-treatment [23].
This has been added to the manuscript (page 5):
"In patients treated with 10mg tds of sodium selenate, serum selenium levels increased from 145.4±28.8 µg/L at baseline to 858.3±446.1 µg/L at week 24, and CSF selenium levels increased from 1.4 ± 0.5 µg/L to 20.2 ± 9.1 µg/L, no change in serum or CSF levels was observed in the placebo-treated patients [23]."

4. "Taken any of the following: NMDA receptor antagonists, oral and/or injectable steroids, digoxin, phenobarbitone or warfarin; commencement or titration of other medications known to have an effect on mood or cognition within the 4 weeks prior to screening, including anticholinergics, hypnotics, sedatives, anxiolytics, antidepressants, antiepileptics, antipsychotics, memory-enhancing drugs, nutraceuticals, and other supplements which contain selenium."

In proper order. How is selenium exposure from potable water controlled for? What is the baseline selenium concentration in water in this region and how does it vary between participants in this study?

Potable water is not controlled for. Selenium concentrations are consistently low in the regions the study will be conducted (<0.001 ug/L in Melbourne, and <0.0002 mg/L in Sydney; www.melbournewater.com.au, www.sydneywater.com.au), which is many orders of magnitude lower that the treatment does being tested in this trial.

5. "The initial dose will be two capsules (10 mg) three times a day"

Most individuals will react in one way or the other to a selenium dose of 30mg each day. How is the blinding towards placebo maintained in this situation? This should be clarified and discussed in this section of the protocol.

Respectfully we disagree with the reviewer. In our Phase 2a RCT in Alzheimer's Disease 50% of placebo-treated and 70% of sodium selenate-treated patients experienced treatment emergent adverse events over 24 weeks (Malpas et al., 2016), indicating the presence of adverse events does not compromise the blind, with only two patients withdrawing from the trial because of adverse effects.

Further in the open label extension (up to 23 months of treatment with sodium selenate) 68% of patients experienced treatment emergent solicited adverse events, with the most common adverse event occurring in 32% of patients (Vivash et al., under review). Similarly, in our current open-label phase 1b in bvFTD, 2 patients (17%) have not reported a single adverse event over the course of 52 weeks of treatment, and the most common treatment-emergent adverse event has occurred in 7/12 patients (<60%).

The presence and frequency of adverse events has the potential to compromise the blind in all placebo-controlled blinded studies. To minimise the impact on our study our primary outcome (brain volume), and one of the secondary outcomes are biomarkers (CSF tau levels) which are independent of any potential bias that could be introduced should the blind be compromised by the presence of overt adverse events.

6. “CSF sampling will take place at baseline”

Details of sampling technique should be given (needles, type of vials, cleaning procedures, storage routines). Is this ultraclean sampling?

No, we will not be performing ultraclean sampling. Details of sampling technique have been added to the manuscript (page 10):

“Atraumatic needles (20G) will be used for sampling. CSF (~20 mL) will be collected in polypropylene tubes (10 mL) cooled on ice. Samples will be kept on ice until processing, aliquoted in to 500 µL polypropylene aliquots and stored at -80 ºC.”

Reviewer 2

After critical analysis of this manuscript, the clinical justification and significance of this research, their future perspectives and biochemical markers are not significantly established, perform as well as written in introduction, research methodology and discussion part.

We are unclear exactly what the reviewer means by this comment.
bvFTD is a rare, progressive and incurable disease for which there is no available treatment other than symptomatic relief of behavioural disturbances. There is significant need for a disease modifying treatment, which will be trialled here. In addition, the data on biomarkers generated from this trial has great potential to benefit diagnosis and future clinical trials in bvFTD beyond our trial. The trial of sodium selenate for bvFTD is well justified based on strong scientific rationale supported by a large body of pre-clinical and clinical evidence. This is supported by the fact that the trial was funded by the Australian Government Medical Research Futures Fund (MRFF) after a highly competitive peer-review process.

Reviewer 3

This is a valuable study in an area with a great need for therapeutic advances. It would be helpful to clarify to what extent you expect to be able to enrich the study sample for tau pathology through exclusion on the basis of family history with genetic testing, and whether the expected proportion of patients with non-tauopathy remaining in the sample has been taken into account in the sample size calculation.

The presence of non-tau pathology is a potential confounder in this study. In our open-labelled phase 1b study approx. 20% of potential patients were pre-screened out of the study based on C9Orf72 expansion and GRN mutations. Similarly interim analyses of our phase 1b suggest 2/12 patients (17%) have non-tau pathology – based on CSF biomarkers (and in one instance confirmed by presence of C9Orf72 expansion). Combining these relatively low levels of non-tau pathology in our

previous cohort with our investigators' significant experience as clinician researchers in FTLDs we anticipate a ~30% presence of non-tau pathology in our cohort, which will allow for adequate power to detect a difference between groups. Should the proportion of non-tau pathology be higher that anticipated, this could affect power and the outcome of the trial. The trial may fail, but exploratory analyses may identify characteristics of treatment response or likely underlying pathology which may enable "precision medicine" in future trials.

The following has been clarified on page 10:

"As a proportion of participants will have a non-tau-based pathology (estimated ~30% based on previous experience), therefore we anticipate a sample size of 120 will allow for adequate power."