An adjunctive antidepressant nutraceutical combination in treating major depression: Study protocol, and clinical considerations

Jerome Sarris a,b,*, Con Stough b, Chad Bousman b,c,d,e, Jenifer Murphy a, Karen Savage a,b, Deidre J. Smith a, Ranjit Menon a, Suneel Chamoli f, Georgina Oliver a, Michael Berk c, Gerard J. Byrne f, Chee Ng a, David Mischoulon g

a The University of Melbourne, Department of Psychiatry, The Melbourne Clinic, Australia
b Swinburne University of Technology, Centre for Human Psychopharmacology, Australia
c The University of Melbourne, Department of Psychiatry, Parkville, Australia
d Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, Australia
e The University of Melbourne, Department of General Practice, Parkville, VIC, Australia
f The University of Queensland, Discipline of Psychiatry, Australia
g Depression Clinical and Research Program, Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Article history:
Available online 2 April 2015

Keywords:
S-Adenosyl methionine
SAMe
Omega-3
Antidepressant
Nutraceutical
Nutrient
Depression
RCT
Protocol

ABSTRACT

Current treatment for major depressive disorder (MDD), a prevalent and disabling mental illness, is inadequate, with two-thirds of people treated with first-line antidepressants not achieving remission. MDD is for many a chronic condition, often requiring multiple treatment attempts, thus development of additional interventions is urgently required. An emerging approach to improve non-response to antidepressants is the use of adjunctive nutraceuticals. The pathophysiology of MDD is considered to involve a range of abnormalities (monoamine impairment, neuro-endocrinological changes, reduced brain-derived neurotrophic factor, and cytokine alterations). By targeting an array of these key neurobiological pathways via specific nutraceuticals (S-adenosyl methionine; [SAMe], 5-HTP [active tryptophan], folinic acid [active folic acid], omega-3 fatty acids, and zinc), there is the potential to provide a more comprehensive therapeutic biological approach to treat depression. We are currently conducting a National Health and Medical Research Council funded study in Australia (APP1048222). The clinical trial is phase II/III, multi-site, 3-arm, 8-week, randomised, double-blind, placebo-controlled study using SAMe + folic acid versus a combination nutraceutical (SAMe, 5-HTP, folinic acid, omega-3, and zinc) or matching placebo in 300 currently depressed participants with diagnosed MDD who are non-responsive to current antidepressants (ANZCTR, protocol number: 12613001300763). The results may provide evidence for a novel adjunctive neurobiological approach for treating depression.

1. Introduction

Major depressive disorder (MDD) is a prevalent and highly disabling mental illness, causing marked occupational and social impairment and reduced quality of life [1]. The National Health Survey conducted by the Australia Bureau of Statistics in 2001 estimated that 4.7% of Australians had taken an antidepressant medication for their mental wellbeing within the prior two weeks [2]. Further to this, depressed mood is for many a chronic condition, often requiring multiple treatment trials [3]. Complicating this, is that efficacy of established treatments are currently modest at best. This is evidenced by the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, a multi-site, prospective, randomised, multi-step clinical trial comparing a series of adjunctive or alternative treatments for patients who did not respond to the selective serotonin reuptake inhibitor (SSRI) citalopram [4]. Results confirmed that only a minority of people with MDD achieve remission via initial treatment with an SSRI. Switching, combining or augmenting produced benefits to some initial non-responders, however one third of people with MDD did not achieve complete remission, even after multiple treatment strategies. Furthermore, as revealed in a highly publicised
2010 meta-analysis by Fournier [5], there is only a small clinical effect size between antidepressants and placebo (d = 0.20) in mildly ill patients. As current treatment is inadequate, development of additional interventions is urgently required. An emerging approach to improve non-response to antidepressants including SSRI
ts is the use of adjunctive nutraceuticals.

The pathophysiology of MDD is considered to involve a range of abnormalities such as monoamine impairment, neuro-endocrine-logical changes, reduced brain-derived neurotrophic factor (BDNF), and cytokine alterations (see Fig. 1) [6]. Use of adjunctive nutraceuticals may improve the clinical effect of antidepressants by addressing several key neurobiological mechanisms underpinning the disorder. These nutraceuticals include: S-adenosyl methionine (SAMe), 5-hydroxytryptophan (5-HP
t), eicosapentaenoic acid (EPA), zinc, and folic acid (either folic acid or 5-MTHF) (Fig. 2).

1.1. S-Adenosyl methionine (SAMe)

SAMe is an endogenous sulphur-containing compound that is a critical neurochemical component involved in the one-carbon cycle responsible for the methylation of neurotransmitters that regulate mood [7,8]. SAMe may improve depressed mood via enhanced methylation of catecholamines and increased serotonin turnover, reuptake inhibition of norepinephrine, enhanced dopa-mimetic activity, decreased prolactin secretion, and increased phosphatidylcholine conversion [9]. Animal depression models have also shown SAMe to restore the levels of putrescine in the nucleus accumbens [10]; this polyamine being shown to have antidepressant effects [11].

A recent site-based reanalysis of 144 patients from a failed 12-week 3-arm double-blind RCT [12] using SAMe monotherapy (1600 mg/day) versus SSRI escitalopram (20 mg) and placebo in adults with diagnosed MDD found a significant difference between SAMe from baseline to week 12 (p = 0.039) versus placebo [13]. At the week 12 endpoint, remission rates on the Hamilton Depression Rating Scale (HAM-D < 7) were 34% for SAMe, 23% for escitalopram and 6% for placebo, significantly in favour of SAMe (p = 0.014). SAMe was found to be superior to placebo from week 1, and to escitalopram during weeks 2, 4, and 6. These results need to be interpreted with caution, however, in view of the failed parent study. In addition, a 6-week double-blind RCT by Papakostas et al. [14] involving 73 MDD patients non-responsive to SSRIs found response (HAM-D ≥ 50% reduction) and remission rates were significantly higher for patients treated with adjunctive SAMe (36.1% and 25.8%, respectively) than adjunctive placebo (17.6% vs. 11.7%, respectively).

1.2. 5-Hydroxytryptophan (5-HPt)

5-HPt is an essential monoamine precursor that is derived from L-tryptophan, and is required for the synthesis of serotonin [15]. 5-HPt and tryptophan have been studied as an antidepressant [16]. Eight controlled adjunctive studies using L-tryptophan or 5-HPt with antidepressants provide positive finding of augmentation effects in increasing the antidepressant response with phenelzine sulphate, clomipramine, tranylcypromine, and fluox
tine. A systematic review and meta-analysis [16] on two studies meeting criteria (pooled n = 64) suggest that 5-HPt and L-tryptophan monotherapy are more effective than placebo at alleviating depression (OR = 4.1, 95% CI = 1.3,13.2).

1.3. Omega-3 fatty acids

Omega-3 fatty acids have a critical role in neural function and great potential for treating depression, especially if an inflammatory causation is present [17,18]. The antidepressant activity of omega-3 fatty acids appears to occur via modulation of norepinephrine, dopamine and serotonin re-uptake, degradation, synthesis and receptor binding; anti-inflammatory effects; and the enhancement of cell membrane fluidity [19].

A meta-analysis by Martins [20] found that eicosapentaenoic acid (EPA) preparations, or those with higher EPA to docosahexaenoic acid (DHA) ratios, potentially have a stronger antidepressant effect than DHA alone. The meta-analytic comparison between DHA and EPA found that DHA monotherapy was not significant, whereas studies using supplements containing >50% EPA had a significant antidepressant effect (p = 0.0050). While not all monotherapy studies are supportive of omega-3 fatty acids for depression, it appears that strong evidence exists for adjunctive use with SSRIs [21]. A 12-month double-blind RCT used 460 mg EPA and 380 mg DHA in patients (n = 2081) with post-myocardial infarction [22]. While no effects from EPA/DHA supplementation over placebo were revealed on depressive symptoms (Beck Depression Inventory II), in a sub-sample taking conventional antidepressants, a significant antidepressant effect
was demonstrated. An 8-week double-blind RCT by Gertsik et al. [23] used 900 mg of EPA and 200 mg of DHA (or placebo) adjunctively with citalopram in the treatment of MDD. The combination therapy demonstrated significantly greater improvement in HAM-D scores at study endpoint ($p = 0.008$).

### 1.4. Zinc

The mineral zinc is a divalent cation that is one of the most prevalent trace elements in the amygdala, hippocampus, and neocortex, and is involved with hippocampal neurogenesis via up-regulation of BDNF, while also modifying N-methyl-D-aspartate (NMDA) and glutamate activity [24, 25]. A meta-analysis comparing peripheral blood zinc concentrations between depressed and non-depressed participants included 17 studies which revealed zinc concentrations were approximately $-1.85$ micromol/L lower in depressed participants compared to control subjects (CI 95%: $-2.51$ to $-1.19$ micromol/L, $p < 0.00001$) [26]. Greater depression severity was associated with greater relative zinc deficiency.

There is emerging evidence for zinc in improving depressed mood. A review by Lai et al. [27] aimed to synthesise results from all published RCTs on the efficacy of zinc supplementation for reducing or preventing depressive symptoms. They found four studies that met inclusion criteria. Two key 12-week RCTs that examined the effects of zinc (25 mg/d) monotherapy supplementation as an adjunct to antidepressants found that zinc significantly lowered depressive symptom scores of depressed patients compared to control subjects (CI 95%: $-2.51$ to $-1.19$ micromol/L, $p < 0.00001$) [26]. Greater depression severity was associated with greater relative zinc deficiency.

There is emerging evidence for zinc in improving depressed mood. A review by Lai et al. [27] aimed to synthesise results from all published RCTs on the efficacy of zinc supplementation for reducing or preventing depressive symptoms. They found four studies that met inclusion criteria. Two key 12-week RCTs that examined the effects of zinc (25 mg/d) monotherapy supplementation as an adjunct to antidepressants found that zinc significantly lowered depressive symptom scores of depressed patients compared to control subjects (CI 95%: $-2.51$ to $-1.19$ micromol/L, $p < 0.00001$) [26]. Greater depression severity was associated with greater relative zinc deficiency.

There is emerging evidence for zinc in improving depressed mood. A review by Lai et al. [27] aimed to synthesise results from all published RCTs on the efficacy of zinc supplementation for reducing or preventing depressive symptoms. They found four studies that met inclusion criteria. Two key 12-week RCTs that examined the effects of zinc (25 mg/d) monotherapy supplementation as an adjunct to antidepressants found that zinc significantly lowered depressive symptom scores of depressed patients compared to control subjects (CI 95%: $-2.51$ to $-1.19$ micromol/L, $p < 0.00001$) [26]. Greater depression severity was associated with greater relative zinc deficiency.

There is emerging evidence for zinc in improving depressed mood. A review by Lai et al. [27] aimed to synthesise results from all published RCTs on the efficacy of zinc supplementation for reducing or preventing depressive symptoms. They found four studies that met inclusion criteria. Two key 12-week RCTs that examined the effects of zinc (25 mg/d) monotherapy supplementation as an adjunct to antidepressants found that zinc significantly lowered depressive symptom scores of depressed patients compared to control subjects (CI 95%: $-2.51$ to $-1.19$ micromol/L, $p < 0.00001$) [26]. Greater depression severity was associated with greater relative zinc deficiency.

### 1.5. Folic acid (folinic acid)

Folate (nutraceutical form is folic acid) is involved with methylation pathways in the one-carbon cycle, and is responsible for the metabolism and synthesis of various monoamines, and most notably is involved with synthesis of SAMe from homocysteine [30]. Several studies have assessed the antidepressant effect of folic acid with concomitant antidepressant use, with most yielding positive results in enhancing either antidepressant response rates, or increasing the onset of response. For example, a study conducted by Coppen and Bailey [31] used 500 mcg of folic acid or placebo adjunctively with 20 mg fluoxetine in 127 subjects with a HAM-D score of $>20$. There was a statistically significant reduction in depression scores after 10 weeks for women in the fluoxetine plus folic acid condition compared with fluoxetine plus placebo ($p < 0.001$; Cohen’s $d = 0.73$). A recent study by Papa-kostas et al. [32] supported the 5-MTHF form (Deplin<sup>®</sup>) at 15 mg/day as an adjunctive treatment for MDD. The activated forms folic acid or 5-methyltetrahydrofolate (5-MTHF) are recommended as these are not affected by 5-MTHF reductase polymorphisms [33].

### 1.6. Benefits of studying a combination antidepressant nutraceutical

As the majority of MDD sufferers treated with first-line antidepressants do not achieve remission, safe and effective adjunctive treatments that improve therapeutic response to antidepressants are of potential benefit. In view of the theoretical foundations and favourable data of the antidepressant effect of nutraceuticals such as SAMe, 5-HTP, EPA, zinc and folic acid, they may serve as potential treatments for enhancing response of SSRIs in patients with clinical depression. Given this rationale, we are currently conducting an adjunctive double-blind RCT to confirm the efficacy of SAMe and a combination nutraceutical for the treatment of non-responsive antidepressant-medicated participants currently experiencing major depression.

### 2. Trial objectives

The objective of this trial is to measure the effectiveness and safety of SAMe versus a combination nutraceutical (CN) as adjunctive treatments to standard antidepressant medication for MDD. We will also measure the relationship between treatment response and changes in levels of key biomarkers [brain-derived neurotrophic factor (BDNF), zinc, folate, B12, fatty acids and...
homocysteine), and evaluate whether treatment response is modified by single nucleotide polymorphisms (SNPs) in specific genes involving methylation and monoamine pathways.

3. Hypotheses

The primary hypothesis:

(1) Adjunctive SAMe will be superior to adjunctive placebo in the treatment of MDD, while the adjunctive combination nutraceutical (CN), will be superior to both SAMe and placebo, as assessed by change in Montgomery–Asberg Depression Scale (MADRS) scores after the 8-week treatment phase

Secondary hypotheses:

(2) Self-rated depressed mood on the Beck Depression Inventory (BDI-II), and general health questionnaire (Short Form Survey 12), will also be significantly improved by both interventions over placebo

(3) SAMe and the CN will produce a significant increase in serum BDNF, and a significant decrease in homocysteine compared to placebo

(4) Changes in outcome rating scales with a lessening of depressive symptoms will correlate with increases in serum BDNF, and a lowering of serum homocysteine in both groups, but not the placebo

(5) A greater therapeutic effect will occur in males compared to females (as reflected in unpublished data [34]).

4. Trial design

4.1. Study design and plan

The design of the study is a phase II/III, multi-site, 3-arm, 8-week, randomised, double-blind, placebo-controlled trial using SAMe, a combination nutraceutical (SAMe + 5-HTP, folic acid in folinic form, EPA and zinc) or matching placebo in 300 currently depressed participants with MDD who are non-responsive to their current antidepressant medication (SSRI, SNRI, NaRI, tetracyclic or tetracyclic or 5-HT2c antagonist). The trial sites are at The Melbourne Clinic (The University of Melbourne), Richmond, Melbourne, Australia; and 5-HT2c antagonist). The trial sites are at The Melbourne Clinic (The University of Melbourne), Richmond, Melbourne, Australia; and the Royal Brisbane and Women’s Hospital (The University of Queensland), Herston, Brisbane, Australia. Recruitment is currently occurring from September 2013 and is planned to occur until September 2016.

Participants will be required to attend five visits at the study site at week 0 (baseline), 2, 4, 6 and 8, as well as a safety assessment over the phone at week 1. At the baseline visit, participants will be asked to complete consent forms, screening assessments, and mood and anxiety questionnaires. All eligible participants will be randomly allocated to a treatment arm, and corresponding treatment will be provided. All subsequent visits will follow the same outline of the baseline session excluding consent forms and screening assessments and including a safety assessment. In addition, participants will be required to provide a blood sample at baseline and prior to the week 8 visit. To compensate for their time and travel expenses, participants will be given $50 at the week 2 visit and another $50 at the final week 8 visit. On completion they will also be given two months’ supply of SAMe tablets. This study has ethical clearance (TMC_HREC 220, UQ_MREC 2013000199), and is registered on ANZCTR (protocol number: 12613001300763). The study is funded by an Australian National Health and Medical Research Council project grant (APP1048222), and is co-sponsored by Bioceuticals.

It should be noted that we are also conducting a monotherapy version of the study for participants not taking antidepressant medication, presenting with mild-moderate depression (MADRS 14–25). The design of the study is nearly identical (ANZCTR protocol number: 12613001299796, TMC_HREC 232, UQ_MREC_2014000702), however this paper focuses on the main adjunctive study protocol.

4.2. Outcome measures

- SAFER 2.0 criteria (for assessing suitability for study entry)
- MINI_version 6.0 (for assessing diagnosis)
- Mood measured via Montgomery–Asberg Depression Rating Scale (MADRS) [clinician-assessed primary outcome] and Beck Depression Inventory (BDI-II)
- Anxiety measured using the Hamilton Anxiety Rating Scale (HAMA)
- Health-related quality of life measured using the Short Form Survey-12 (SF-12)
- Sleep measured using the Leeds Sleep Evaluation Questionnaire (LSEQ)

Table 1

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Alternative ID</th>
<th>Gene symbol</th>
<th>Gene name</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>Val158Met</td>
<td>CDMT</td>
<td>Catechol-o-methyltransferase</td>
</tr>
<tr>
<td>rs1801133</td>
<td>G677T</td>
<td>MTHFR</td>
<td>Methylene tetrahydrofolate reductase</td>
</tr>
<tr>
<td>rs1801131</td>
<td>A1298C</td>
<td>MTHFR</td>
<td>Methylene tetrahydrofolate reductase</td>
</tr>
<tr>
<td>rs1799913</td>
<td>A779C</td>
<td>TPH1</td>
<td>Tryptophan hydroxylase 1</td>
</tr>
<tr>
<td>rs1800532</td>
<td>A218C</td>
<td>TPH1</td>
<td>Tryptophan hydroxylase 1</td>
</tr>
<tr>
<td>rs25531</td>
<td>–</td>
<td>SLC6A4</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td>rs13107325</td>
<td>–</td>
<td>SLC39A8</td>
<td>Zinc transporter</td>
</tr>
<tr>
<td>rs10764176</td>
<td>–</td>
<td>SLC39A12</td>
<td>Zinc transporter</td>
</tr>
<tr>
<td>rs174561</td>
<td>–</td>
<td>FADS1</td>
<td>Fatty acid desaturase 1</td>
</tr>
<tr>
<td>rs174549</td>
<td>–</td>
<td>FADS2</td>
<td>Fatty acid desaturase 2</td>
</tr>
<tr>
<td>rs164575</td>
<td>–</td>
<td>FADS2</td>
<td>Fatty acid desaturase 2</td>
</tr>
<tr>
<td>rs234706</td>
<td>C699T</td>
<td>CBS</td>
<td>Cystathionine b-synthase</td>
</tr>
<tr>
<td>rs3733890</td>
<td>716G4A</td>
<td>BHMT</td>
<td>Beteine homocysteine methyltransferase</td>
</tr>
<tr>
<td>rs1805087</td>
<td>Asp919Gly</td>
<td>MTR</td>
<td>Methionine synthase</td>
</tr>
<tr>
<td>rs1801198</td>
<td>Pro259Arg</td>
<td>TCN2</td>
<td>Transcobalamin-II</td>
</tr>
<tr>
<td>rs1045642</td>
<td>C435T</td>
<td>ARB81</td>
<td>P-glycoprotein</td>
</tr>
</tbody>
</table>

Other polymorphisms

- 5HTTLPR
- STin2 VNTR
- 845ins68

<table>
<thead>
<tr>
<th>SNP</th>
<th>Alternative ID</th>
<th>Gene symbol</th>
<th>Gene name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SLC6A4</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLC6A4</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CBS</td>
<td>Cystathionine b-synthase</td>
</tr>
</tbody>
</table>
• Sexual dysfunction as measured by the Arizona Sexual Experience Scale (ASEX)
• Psychomotor symptoms of depression measured using the CORE Assessment of Psychomotor Change
• Global rating of illness severity and improvement measured using the Clinical Global Impression (CGI) severity (CGI-S) and improvement (CGI-I) scales
• SAFTEE (for assessment of adverse reactions)
• Single nucleotide polymorphisms (SNPs) in specific genes involved in methylation and monoamine pathways measured via blood sample at baseline (Table 1)
• Levels of brain-derived neurotrophic factor (BDNF), zinc, folate, B12, fatty acids and homocysteine measured by obtaining blood samples

Inclusion criteria:

• Aged 18–70 years
• Currently taking an SSRI, SNRI, NaRI, tetracyclic or 5-HT2c antagonist, (or bupropion) for a minimum of four weeks, and on a stable dose for a minimum of two weeks.
• Fulfils the DSM-IV and DSM-V diagnostic criteria for Major Depressive Disorder (for DSM-IV-TR diagnosis this is based on the Mini International Neuropsychiatric Interview 6.0 [MINI 6.0]. We will switch to DSM-V sole diagnosis and will use the corresponding version of the MINI when available)
• Presents with moderate to severe depression (MADRS ≥ 18) at time of study entry
• Meets SAFER 2.0 criteria for participation in a clinical trial
• Fluent in written and spoken English
• Has the capacity to consent to the study and follow its procedures

Exclusion criteria:

• Currently taking MAOIs (reversible or non-reversible) or tricyclic antidepressant
• Current use of specified nutraceuticals, e.g. St John’s wort, SAMe, 5-HTP at any dose or folic acid >500 mcg per day, omega-3 >180 mg EPA per day, zinc >10 mg per day (in such cases a one week washout can be employed before rescreening and potential inclusion)
• Presents with suicidal ideation (>3 on MADRS suicidal thoughts domain) at the time of study entry
• Three or more failed trials of pharmacotherapy or somatic therapy (e.g. electroconvulsive therapy) for the current major depressive episode
• Meets DSM-IV diagnostic criteria for bipolar disorder I/II or presence of psychosis on structured interview (MINI 6.0)
• A primary clinical diagnosis of a substance/alcohol use disorder within the last 12 months on structured interview (MINI 6.0)
• Recently commenced psychotherapy (>4 weeks of stable treatment acceptable)

• Taking warfarin or phenytoin
• Known or suspected clinically unstable systemic medical disorder (including cancer, organ failure, or serious cardio/ cerebrovascular disease)
• Pregnancy or breastfeeding
• Not currently using medically approved contraception (including abstinence) if female and of childbearing age
• Allergy or intolerance to seafood or taking nutraceuticals

4.3. Treatment interventions

Participants are randomly assigned via computerised number generation (3 x 3 blocks) to Group A (SAMe), Group B (combination nutraceutical) or Group C (placebo). An independent researcher conducted the randomisation, while trial researchers and investigators are blinded as to which groups the participants will be assigned to (as are the participants themselves).

All participants are required to take 2 tablets and 2 capsules twice per day for 8 weeks.

Group A (SAMe)¹
• SAMe (800 mg/day)
• Folinic acid (500 mcg/day)
• Co-factor vitamin B12 (200 mcg/day) to assist in the conversion of homocysteine to SAMe [plus inactive placebo capsules]

Group B (combination nutraceutical)¹
• SAMe (800 mg/day)
• Folinic acid (500 mcg/day)
• Co-factor vitamin B12 (200 mcg/day) to assist in the conversion of homocysteine to SAMe
• PLUS
• Omega-3 fatty acid concentrate (EPA-esters 1000 mg/day, DHA-esters 656 mg/day)
• 5-HTP (200 mg/day)
• Zinc picolinate (30 mg elemental/day)
• Co-factors vitamin B6 (100 mg/day), vitamin C (60 mg/day), and magnesium (amino acid chelate, elemental 40 mg/day) to assist in the conversion of 5-HTP to serotonin
• Cofactor vitamin E (40 IU/day) to stabilise the omega 3

Group C (matching placebo)
• SAMe placebo matching tablets and CN placebo matching capsules (1% fish oil is added to the placebo capsules to assist with blinding)

4.4. Statistical analyses

Analysis of data will be conducted with blinding to group allocations. Linear mixed models will be used to determine differences in MADRS depressive symptom severity over the study period by group allocation. The mixed models approach enables use of all repeated measurements, accounts for clustering of participants within recruitment sites, and provides unbiased estimates in the presence of missing data. Potential covariates will be assessed for their association with group allocation and MADRS depressive symptom severity using chi-square, Fisher's

¹ The SAMe is provided in tablet form (blister pack sealed, and stored in refrigeration at 4–8 °C). The Combination Formula is provided in soft-gel capsules and stored at room temperature 15–25 °C.
enhancing antidepressant response, and improving depression in the public being offered an "evidence-based" approach to future judicious clinical application of these interventions. A biological modifying factors affecting response may allow for safe, and effective treatment option. Further, understanding the combination nutraceutical formula offers a potentially affordable, to contemporary antidepressants. SAMe and an enhanced SAMe cannot benefits in ameliorating depression in people non-responsive to current reductive approach to depression treatment may also be suboptimal, with several neurobiological pathways implicated in the disorder; yet medicinal approaches (and clinical trials) usually focus on modulating just one or two neurochemicals. While it may not be clear as to which nutrient components may be responsible for any found effect, it should be noted that each component in our presently studied formula has underpinning antidepressant evidence. Additionally, it is important to utilise co-factors involved in the metabolism of the key nutrients e.g. folic acid for SAMe in the one-carbon cycle, and zinc, magnesium, vitamin C, and B6 for 5-HTP conversion to serotonin [36]. Our design has taken this into account and has incorporated these co-factors into the formulation (Fig. 3). While it may be argued that a statistically significant effect between treatments and placebo is the goal of any study, the real goal of our study is to provide “clinically” significant from the CN group for antidepressant activity beyond that of what would be expected as occurring from the individual components alone e.g. SAMe. This would equate to an estimated differential reduction over placebo of over 6 points on the MADRS, being beyond the normal significant reduction of 3–4 points on the MADRS found on average in studies (sample sizes 80–150). In other words, if little difference occurred between SAMe and the CN, then there would be little point in broadly advising its use due to increased cost above SAMe or other individual evidence-based components such as EPA. Further analysis of the data however may still find individual characteristics for those who respond to the CN formula. In respect to general safety application of the combination formulation, all the major key nutrients are being used in therapeutic doses and all have good safety profiles. Regardless, SAMe use should be monitored for the unlikely event of switching in bipolar disorder; 5-HTP use while potentially enhancing serotonin levels should be observed closely if used in concert with antidepressants due to serotonin syndrome (of more potential concern in higher doses); Zinc should not be used beyond a therapeutic dose due to potentially unbalancing mineral.

One potential deficit of common nutraceutical research is the use of isolated nutrients as opposed to multi-component formulas. While it is appreciated that modern scientific paradigm mandates the study of isolated interventions to avoid the confound of multiple interventions providing no understanding of which components are effective, this approach may not be beneficial in studying nutrient-based nutraceuticals. Nutrients commonly work in concert [35] and as described above, a range of nutraceuticals modulate several key pathways involved with the pathogenesis of depression. As discussed, the current reductive approach to depression treatment may also be suboptimal, with several neurobiological pathways implicated in the disorder; yet medicinal approaches (and clinical trials) usually focus on modulating just one or two neurochemicals. While it may not be clear as to which nutrient components may be responsible for any found effect, it should be noted that each component in our presently studied formula has underpinning antidepressant evidence. Additionally, it is important to utilise co-factors involved in the metabolism of the key nutrients e.g. folic acid for SAMe in the one-carbon cycle, and zinc, magnesium, vitamin C, and B6 for 5-HTP conversion to serotonin [36]. Our design has taken this into account and has incorporated these co-factors into the formulation (Fig. 3). While it may be argued that a statistically significant effect between treatments and placebo is the goal of any study, the real goal of our study is to provide “clinically” significant from the CN group for antidepressant activity beyond that of what would be expected as occurring from the individual components alone e.g. SAMe. This would equate to an estimated differential reduction over placebo of over 6 points on the MADRS, being beyond the normal significant reduction of 3–4 points on the MADRS found on average in studies (sample sizes 80–150). In other words, if little difference occurred between SAMe and the CN, then there would be little point in broadly advising its use due to increased cost above SAMe or other individual evidence-based components such as EPA. Further analysis of the data however may still find individual characteristics for those who respond to the CN formula.

In respect to general safety application of the combination formulation, all the major key nutrients are being used in therapeutic doses and all have good safety profiles. Regardless, SAMe use should be monitored for the unlikely event of switching in bipolar disorder; 5-HTP use while potentially enhancing serotonin levels should be observed closely if used in concert with antidepressants due to serotonin syndrome (of more potential concern in higher doses); Zinc should not be used beyond a therapeutic dose due to potentially unbalancing mineral.

4.5. Power analysis

We aim to recruit a sample size of 300 participants (100 participants in each arm), with a projected loss of 15–20% based on our previous RCTs. The study is powered to detect a potential small to moderate difference between the SAMe and CN groups (using all data via intention-to-treat analysis). Based on a two tailed analysis with $\alpha = 0.05$, $\beta = 0.80$, and a critical $F_{2,298}$ of 3.02, 300 participants are required to detect a difference in MADRS scale score between SAMe, CN and placebo groups (Cohen’s $d$ effect size of 0.36 or greater). The sample size of 100 per arm is sufficiently powered to provide statistical difference between the SAMe and placebo groups.

5. Discussion

The nutraceuticals studied have the potential to show significant benefits in ameliorating depression in people non-responsive to contemporary antidepressants. SAMe and an enhanced SAMe combination nutraceutical formula offers a potentially affordable, safe, and effective treatment option. Further, understanding the biological modifying factors affecting response may allow for future judicious clinical application of these interventions. A positive finding will have the significant impact of clinicians and the public being offered an “evidence-based” approach to enhancing antidepressant response, and improving depression treatment.

4.5. Power analysis

We aim to recruit a sample size of 300 participants (100 participants in each arm), with a projected loss of 15–20% based on our previous RCTs. The study is powered to detect a potential small to moderate difference between the SAMe and CN groups (using all data via intention-to-treat analysis). Based on a two tailed analysis with $\alpha = 0.05$, $\beta = 0.80$, and a critical $F_{2,298}$ of 3.02, 300 participants are required to detect a difference in MADRS scale score between SAMe, CN and placebo groups (Cohen’s $d$ effect size of 0.36 or greater). The sample size of 100 per arm is sufficiently powered to provide statistical difference between the SAMe and placebo groups.

5. Discussion

The nutraceuticals studied have the potential to show significant benefits in ameliorating depression in people non-responsive to contemporary antidepressants. SAMe and an enhanced SAMe combination nutraceutical formula offers a potentially affordable, safe, and effective treatment option. Further, understanding the biological modifying factors affecting response may allow for future judicious clinical application of these interventions. A positive finding will have the significant impact of clinicians and the public being offered an “evidence-based” approach to enhancing antidepressant response, and improving depression treatment.
levels; Folic acid is recommended to be used in an active form e.g. 5-MTHF or folinic acid; Omega-3 fatty acids should present with little safety issues, however higher doses may interact with antioxidants. In our study, participants are rigorously monitored for any adverse effects, and these will be documented in the future published results.

In summary, it is intended that our study, positive results may encourage the study of more complex nutraceutical formulations for other medical disorders.

Conflicts of interest

J S has received honoraria, research support, royalties, or consultancy or travel grant funding from Integra Health, Blackmores, Bioceuticals, Taki Mai, Pepsico, HealthEd, Soho-Flordis, Pfizer, Elsevier, the Society for Medicinal Plant and Natural Product Research, CR Roper Fellowship, The NHMRC. DM has received research support from the Bowman Family Foundation, Fish-erWallace, Ganedden, Nordic Naturals, Methylation Sciences, Inc. (MSI), and PharmoRx Therapeutics. He has received honoraria for consulting, speaking, and writing from the Massachusetts General Hospital Psychiatry Academy. He has received royalties from Lippincott Williams & Wilkins for published book “Natural Medications for Psychiatric Disorders: Considering the Alternat-ives.” CS has received research grants from Government: Australian Research Council; NHMRC: NDLERF; NIDA: VDLERF; ARC; Vicpolice; Vicroads; SFI; Barry Cellebaut: Blackmores; Flordis; Zeller; Clover Corporation; Horphag; GSK; Bayer; Pharmalink; Siemens; Securatek; Efamol; Cognis; Integria; Medvet, Cooper Nutrition, and paid strategic consulting advice for SFI, Blackmores, Bayer; ARU, Carlton FC, GSK India. No other specific conflicts identified.

Acknowledgments

This grant is funded by a National Health and Medical Research Council project grant (APP1048222), and is co-sponsored by Bioceuticals. Dr Jerome Sarris is supported by a CR Roper Fellowship. Michael Berk is supported by a NHMRC Senior Principal Research Fellowship (1095660). Chad Bosman is supported by a Ronald Phillip Griffith Fellowship.

References