BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023314
Article Type:	Protocol
Date Submitted by the Author:	31-Mar-2018
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Keywords:	premenstrual syndrome, equol, isoflavone, dietary supplement, RCT

Effectiveness of natural S-equol supplement for premenstrual symptoms: protocol of a randomised, double-blind, placebo-controlled trial

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Word count (excluding title page, abstract, references, figures and tables): 2369 words

ABSTRACT

Introduction Premenstrual syndrome (PMS) comprises a range of mood, behavioural, and physical symptoms and impairs many women's quality of life. Isoflavones are expected to stabilise the natural fluctuation of the oestrogen cycle through their selective oestrogen receptor modulator-like activities that alleviate PMS symptoms. Equol, a metabolite of a soy isoflavone converted from daidzein by specific gut bacteria, has a greater bioavailability compared with other soy isoflavones. We aim to examine the effect of natural S-equol supplements on premenstrual symptoms.

Methods and analysis This study will enrol 124 women (aged 20 to 45 years) who have PMS symptoms and are non-equol producers in a double-blind, randomised, placebo-controlled trial, in which they will receive natural S-equol supplement (equol 10 mg a day) or placebo, orally, twice daily, for three menstrual cycles. The primary outcome measure (Daily Record of Severity of Problems total score) will be assessed during intervention cycles.

Ethics and dissemination The institutional review board at Kindai University approved the study. The findings of this trial will be submitted to an international peer reviewed journal. Abstracts will be submitted to national and international conferences.

Trial registration number UMIN: 000036335

Strength and limitations of this study

- This is the first study to assess the efficacy of equol supplement to alleviate PMS symptoms.
- A key strength of this study is that we will collect data from PMS women with strict criteria using Daily Record of Severity of Problems.

- It remains unclear whether equol 10 mg is a necessary and sufficient dose for alleviating PMS symptoms, and a dose finding study will be needed in the future.

Keywords: premenstrual syndrome; equol; isoflavone; dietary supplement; RCT

INTRODUCTION

Premenstrual syndrome

Premenstrual syndrome (PMS) comprises a range of mood, behavioural, and physical symptoms that are limited to the premenstrual phase.[1] Epidemiologic surveys have shown that the prevalence of premenstrual PMS is high (80–90%).[2] In approximately 5% of women, the symptoms are so severe that they interfere with personal or social relationships.[3] Such a severe form of PMS is defined as premenstrual dysphoric disorder (PMDD) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; American Psychiatric Association 2013).[4] The precise pathophysiology of PMS and PMDD remains unknown, but several possible causes have been suggested including hormonal changes, serotonergic dysfunction, stress, and diet.[5] Among these factors, dietary changes or supplements might be recommended as non-pharmacological medical approaches without serious adverse effects.[6]

Isoflavones and Equol

The consumption of isoflavones, which are predominantly derived from soybeans, reduces the risk of oestrogen-related diseases such as menopausal symptoms, breast cancer, osteoporosis, and cardiovascular disease.[7-9] Isoflavones act as selective oestrogen receptor (ER) modulators (SERMs), as ER agonists in low-oestrogen conditions, and as ER antagonists in high-oestrogen conditions.[10] Equol is a metabolite of a soy isoflavone, which is converted from daidzein by specific gut bacteria.[11] Only 30–60% of people can produce equol from daidzein after eating soy.[12] Equol is more bioavailable than other soy isoflavones and equol producers are believed to benefit from the consumption of soy isoflavones.[13] It is plausible that

isoflavones may stabilise the natural fluctuation of the oestrogen cycle through their SERM-like activities to alleviate PMS symptoms.[14] A previous report demonstrated that soy isoflavone supplementation had beneficial effects for the relief of PMS symptoms.[15]

Equol production status and PMS

Recently, we reported the relationship between PMS and equol production status and showed that equol non-production was a significant risk factor for PMS. Dietary interventions, such as supplementation with equol might be a safe treatment strategy for PMS. A natural S-equol supplement was reported to be effective for treating menopause symptoms without any serious adverse effects.[16]

TRIAL OBJECTIVES AND HYPOTHESIS

The aim of this study is to examine the effect of natural S-equol supplement on premenstrual symptoms. We hypothesise that equol is effective for alleviating premenstrual symptoms in equol non-producers.

METHODS AND ANALYSIS

Trial design

This study is designed as a double-blind placebo-controlled randomised trial with a ratio of 1:1, in which a stratified randomisation is conducted with adjustment factors of DRSP total score (≥50 or <50) and age (≥35 or <35 years) at baseline. The trial was registered at the University Hospital Medical Information Network (UMIN) center (ID

UMIN-000036335) and any important changes in the protocol will be reflected there. Table 1 shows the trial schedule.

Settings and participants

Recruitment will be by advertisement in local free papers and by posters displayed in Kindai University Hospital. The start of the recruitment is planned for May 2018 and it will be finished within 2 years.

Eligibility criteria

Eligible women for the trial must fulfil all of the following criteria:

- Age 20 to 45 years;
- Presence of PMS symptoms and fulfil the 'moderate-to-severe PMS' or 'PMDD' criteria according to the Premenstrual Symptoms Questionnaire (PSQ);[17]
- Regular menstrual cycles (25–38 days);
- Non-equol producers;
- No oral conceptive pill use for 4 weeks before study entry and no concurrent therapy for PMS;
- Provide written informed consent.

Study subjects will be excluded for the following reasons:

- Allergy to soybean or soybean products;
- Presence of neuropsychiatric disorders;
- Presence of very severe interference with work, usual activities, or relationships by PMS symptoms;
- Serious hepatic disease, heart disease, kidney disease, or malignant disease.

To meet criteria for randomisation, study subjects need to fulfil 2 consecutive menstrual cycles of PMS criteria as measured by the Daily Record of Severity of Problems (DRSP) [18] immediately before the 3 double-blind placebo or active treatment cycles.

PMS severity criteria include:

- A postmenstrual phase (Day 6 to 10) daily average ≤2.5 for each symptom;
- A premenstrual phase (Day −5 to −1 before menstruation) daily average ≥3.0 for three distinct items;
- A premenstrual phase daily average worsened by at least 50% compared with the corresponding postmenstrual phase daily average for three distinct items;
- A score ≥3 on functional impairment items for at least one day during the premenstrual phase.

Interventions

The intervention will be the administration of natural S-equol supplement (SE5-OH) tablet containing 2.5 mg S-equol produced from the fermentation of soy germ by *Lactococcus* 20-92.[16] The placebo tablets will contain lactose in place of SE5-OH and the appearance will be same as those of the active product. The study products (SE5-OH and placebo) will be manufactured as tablets and supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) free of charge. Otsuka Pharmaceutical Co., Ltd. will have no role in the conception, study planning, data analysis, or interpretation of the data.

Study procedure

Figure 1 shows the study flow and Table 1 shows the study time schedule. The study will include screening cycles (S-1 and S-2) and intervention cycles (I-1, I-2 and I-3). Subjects will receive oral and written information regarding the study. Written informed consent will be obtained by the physicians involved in the study.

Screening cycles

The equol-production status of subjects will be determined by soy-challenge test. Participants will be instructed to eat soy food containing approximately 50 mg isoflavones twice a day, and then first morning urine samples will be collected the next morning. These samples will be transported by mail to the Saga Nutraceuticals Research Institute of Otsuka Pharmaceutical Co., Ltd. Then, the equol concentration will be measured by high-performance liquid chromatography (HPLC) using the modified method of Lundh.[19] The detection limit of equol in this assay is 0.85 nmol/ml. Those subjects who have detectable urine equol will be classified as equol producers.

Only the non-equol producers will be selected and then they will be required to check their premenstrual symptoms by DRSP for 2 consecutive menstrual cycles. DRSP comprises 21 items of emotional and physical PMS symptoms and 3 items of functional impairment for social and life activity, which are rated for their severity on a six-point scale from 1 (not at all) to 6 (extreme).[18]

Intervention cycles

Eligible subjects will be randomly assigned to receive SE5-OH at a dose of 10 mg S-equol or placebo, orally, twice daily, receiving 4 tablets (2×2 tablets) on Day 7 (±2) of the menstruation cycle and continued for three menstruation cycles. They will be

required to check their PMS symptoms by DRSP every day.

At the start and the end of the intervention, on Day 7 (±2) of the I-1 and C-1 cycles, subjects will be required to collect their saliva samples themselves using a Saliva Collection Aid (Salimetrics, Carlsbad, CA, USA). At that time, they will be also required to check their PMS symptoms by PSQ.

After the intervention, they will be checked at a final assessment during the close-up cycle (C-1). The Clinical Global Impressions-Improvement (CGI-I) scale will be checked by a physician, and then the study subjects' involvement in the trial will cease.

Outcome measure

The primary outcome measure consists of 21 symptom items of DRSP. The average of the premenstrual phase (Day −5 to −1 before menstruation) for 21 items will be summed to generate the DRSP total score. The primary efficacy variable is the difference between the DRSP total score from the screening cycle (S-2) and the DRSP total score from the last intervention cycle (I-3).

Secondary outcome measurements are the percentage difference of the DRSP total score between S-2 and I-3, the difference between the salivary BDNF from baseline to the end of treatment, and the ratio of mild PMS in the PSQ and CGI-I scale at the end of treatment.

Saliva will be stored at −20°C until assayed. BDNF will be detected by sandwich ELISA according to the manufacturer's instructions (CYT306; Millipore Corp, Bedford, MA, USA) as described previously [20]. All samples will be tested in duplicate and the mean value will be determined.

CGI-I scale is a single-item measure, which is consists of a 7 point scale (1=very

much improved to 7=very much worse). We will define the response group as a score of 1 or 2, corresponding to very much improved or much improved.

Sample size calculation

A pilot study of 10 participants was conducted to estimate the sample size. The result of this open label pilot study showed that the change from baseline in the mean (± standard deviation [SD]) DRSP total score of SE5-OH treatment was −14.2 (± 15.4). As this pilot study included low DRSP total score cases, we expect that SE5-OH will achieve much better improvement of DRSP total score in this study. According to a previous report studying the effect of calcium for the treatment of PMS by double blind randomised placebo controlled trial,[21] the mean change in the DRSP total score of placebo treatment from baseline was −7.6. Accordingly, we assumed that the SE5-OH and placebo groups will show a mean decrease in DRSP total score from baseline of 16 and 8, respectively, with an SD of 15 for each group. Under these assumptions, the required sample size was calculated as 114 (57 in each group) with a significance level of 5% (two-sided) and power of 80%, where the Student's *t*-test was applied. Accounting for the dropout of a few subjects, we set the target sample size as 124 (62 in each group).

Randomisation

A randomisation list will be generated using a computer program by the Data Coordinating Center of Osaka University Hospital (Osaka, Japan). This procedure will be performed by a physician not involved in the study.

Blinding

The active product and placebo will be packaged in identical bottles and sealed. These are similar in look and taste. All participants and investigators will be blinded to the assigned treatment throughout the study.

Data collection and management

All subjects will be assigned a study identification number. Case report forms (CRFs) will be paper forms. Data will be entered and stored in an electronic database, which is managed by the Data Coordinating Center of Osaka University Hospital. All CRFs and study data will be stored in a locker within Kindai University.

Monitoring

An independent Data and Safety Monitoring Board (DSMB) will be set up prior to the start of the study. The DSMB will review data after the first participant, and then after the recruitment of 25%, 50% and 75% of participants to check the study progress and all adverse events.

Statistical analysis

Statistical analysis will be performed with the statistical software SAS 9.4 (SAS Institute, Cary, North Carolina, USA).

Baseline characteristics will be summarised for each group. Continuous variables will be presented using the mean, median, SD, and range. Categorical variables will be presented as the percentage.

To compare the primary outcomes (difference of DRSP total score between S-2 and I-3) between the SE5-OH group and placebo group, the difference between the mean

differences in these two groups will be determined with the 95% CI. Furthermore, the p-value will be determined using the Student's *t*-test, where the significance level is 5% (two-sided). The secondary outcomes (percentage difference of DRSP total score and salivary BDNF) will also be compared using the same summary measures. PSQ and CGI-I will be summarised as the difference of effective proportions with the 95% CI, where the p-value will be determined using Fisher's exact test.

Safety assessment

All subjects will be required to report any adverse event during the trial. All serious adverse events will be immediately reported to the project leader, ethics committee, all participating investigators and the manufacturer of the study products.

ETHICS AND DISSEMINATION

The trial protocol has been approved by the Ethics Committee of Kindai University (approval number 29-202). Any modification in protocol will be presented to the ethics committee. The results will be submitted to a peer-reviewed journal. Abstracts will be submitted to relevant national and international conferences. This study will follow the standards set by the guidelines of the Consolidated Standards of Reporting Trials (CONSORT).

Acknowledgement

We thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflict of interest

TT received lecture fees from Otsuka Pharmaceutical Co., Ltd., the manufacturer of SE5-OH.

Funding

This work was supported, in part, by a grant from Research, AMED Research Project for Improving Quality in Healthcare and Collecting Scientific Evidence on Integrative Medicine, Grant Number 17933369, Tokyo, Japan.

Author Contribution

TT, MS and YC contributed to the drafting of the manuscript. TT was the main contributor to the study design and conception. All authors agreed with the integrity of the study and gave their approval.

Data Sharing

No additional data are available.

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Figure legend

Figure 1. Flow chart of the study diagram.

Fig.1

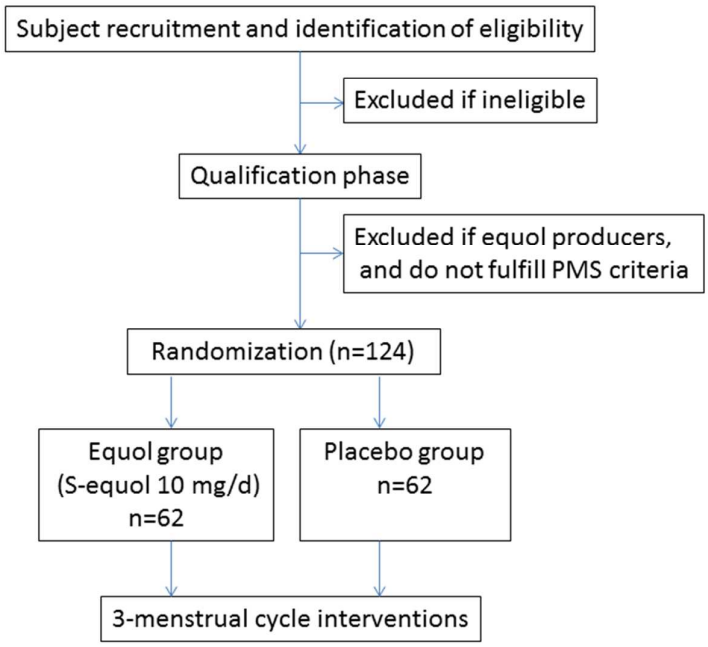

Table 1 Schedule of enrolment, interventions and assessments

	Enrolment	Screening cycles		Allocation	Intervention cycles			Close-out cycle
		S-1	S-2		I-1	I-2	I-3	C-1
Time point		CD1			CD7±2			CD7±2
Enrolment:								
Eligibility screen	+							
Informed consent	+							
Soy challenge test	+							
Allocation				+				
Interventions:								
SE5-OH					←	—	→	
Placebo					←	—	→	
Assessments								
DRSP		←	—	—	—	—	→	
PSQ					+		+	
Salivary BDNF					+		+	
CGI-I scale								+
Adverse events					←	—	→	+
Return of non-used study products								+

CD, cycle day; SE5-OH, natural S-equol supplement; DRSP, Daily Record of Severity of Problems; PSQ, Premenstrual Symptoms Questionnaire; BDNF, brain derived neurotrophic factor; CGI-I, Clinical Global Impressions-Improvement

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	16

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12,13
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	12

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	6
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset,	

		and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023314.R1
Article Type:	Protocol
Date Submitted by the Author:	31-May-2018
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Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Mental health, Obstetrics and gynaecology
Keywords:	premenstrual syndrome, equol, isoflavone, dietary supplement, RCT

Effectiveness of natural S-equol supplement for premenstrual symptoms: protocol of a randomised, double-blind, placebo-controlled trial

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Word count (excluding title page, abstract, references, figures and tables): 2395 words

ABSTRACT

Introduction Premenstrual syndrome (PMS) comprises a range of mood, behavioural, and physical symptoms and impairs many women's quality of life. Isoflavones are expected to stabilise the natural fluctuation of the oestrogen cycle through their selective oestrogen receptor modulator-like activities that alleviate PMS symptoms. Equol, a metabolite of a soy isoflavone converted from daidzein by specific gut bacteria, has a greater bioavailability compared with other soy isoflavones. We aim to examine the effect of natural S-equol supplements on premenstrual symptoms.

Methods and analysis This study will enrol 124 women (aged 20 to 45 years) who have PMS symptoms and are non-equol producers in a double-blind, parallel, randomised, placebo-controlled trial, in which they will receive natural S-equol supplement (equol 10 mg a day) or placebo, orally, twice daily, for three menstrual cycles. The primary outcome measure (Daily Record of Severity of Problems total score) will be assessed during intervention cycles. To compare the primary outcomes between the S-equol group and placebo group, the mean differences in Daily Record of Severity of Problems total score between the two groups will be determined. The p-values will be determined using the Student's *t*-test, where the significance level is 5% (two-sided).

Ethics and dissemination The institutional review board at Kindai University approved the study. The findings of this trial will be submitted to an international peer reviewed journal. Abstracts will be submitted to national and international conferences.

Trial registration number UMIN000031815.

Strength and limitations of this study

- This is the first study to assess the efficacy of equol supplement to alleviate PMS symptoms.
- A key strength of this study is that we will collect data from PMS women with strict criteria using Daily Record of Severity of Problems.
- It remains unclear whether equol 10 mg is a necessary and sufficient dose for alleviating PMS symptoms, and a dose finding study will be needed in the future.

Keywords: premenstrual syndrome; equol; isoflavone; dietary supplement; RCT

INTRODUCTION

Premenstrual syndrome

Premenstrual syndrome (PMS) comprises a range of mood, behavioural, and physical symptoms that are limited to the premenstrual phase.[1] Epidemiologic surveys have shown that the prevalence of premenstrual PMS is high (80–90%).[2] In approximately 5% of women, the symptoms are so severe that they interfere with personal or social relationships.[3] Such a severe form of PMS is defined as premenstrual dysphoric disorder (PMDD) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; American Psychiatric Association 2013).[4] The precise pathophysiology of PMS and PMDD remains unknown, but several possible causes have been suggested including hormonal changes, serotonergic dysfunction, stress, and diet.[5] Among these factors, dietary changes or supplements might be recommended as non-pharmacological medical approaches as was concluded in a review on the treatment and management modalities for PMDD.[6]

Isoflavones and Equol

The consumption of isoflavones, which are predominantly derived from soybeans, could reduce the risk of oestrogen-related diseases such as menopausal symptoms, breast cancer, osteoporosis, and cardiovascular disease.[7-9] Isoflavones act as selective oestrogen receptor (ER) modulators (SERMs), as ER agonists in low-oestrogen conditions, and as ER antagonists in high-oestrogen conditions.[10] Equol is a metabolite of a soy isoflavone, which is converted from daidzein by specific gut bacteria.[11] Only 30–60% of people can produce equol from daidzein after eating soy.[12] Equol is more bioavailable than other soy isoflavones and equol producers are

believed to benefit from the consumption of soy isoflavones.[13] It is plausible that isoflavones may stabilise the natural fluctuation of the oestrogen cycle through their SERM-like activities to alleviate PMS symptoms.[14] A previous report demonstrated that soy isoflavone supplementation had beneficial effects for the relief of specific PMS symptoms such as cramp and swelling.[15]

Equol production status and PMS

Recently, we reported the relationship between PMS and equol production status and showed that equol non-production was a significant risk factor for PMS.[16] Dietary interventions, such as supplementation with equol might be a safe treatment strategy for PMS. A natural S-equol supplement was reported to be effective for treating menopause symptoms without any serious adverse effects.[17]

TRIAL OBJECTIVES AND HYPOTHESIS

The aim of this study is to examine the effect of natural S-equol supplement on premenstrual symptoms. We hypothesise that equol is more effective than placebo in alleviating premenstrual symptoms in equol non-producers.

METHODS AND ANALYSIS

Trial design

This study is designed as a double-blind placebo-controlled parallel randomised trial with a ratio of 1:1, in which a stratified randomisation is conducted with adjustment factors of DRSP total score (≥50 or <50) and age (≥35 or <35 years) at baseline. The trial was registered at the University Hospital Medical Information Network (UMIN)

centre (ID UMIN000031815) and any important changes in the protocol will be reflected there. Table 1 shows the trial schedule.

Table 1 Schedule of enrolment, interventions and assessments

	Enrolment	Screening cycles		Allocation	Intervention cycles			Close-out cycle
		S-1	S-2		I-1	I-2	I-3	C-1
Time point		CD1			CD7±2			CD7±2
Enrolment:								
Eligibility screen	+							
Informed consent	+							
Soy challenge test	+							
Allocation				+				
Interventions:								
SE5-OH					←	→		
Placebo					←	→		
Assessments								
DRSP		←	→					
PSQ					+			+
Salivary BDNF					+			+
CGI-I scale								+
Adverse events					←	→		+
Return of non-used study products								+

CD, cycle day; SE5-OH, natural S-equol supplement; DRSP, Daily Record of Severity of Problems; PSQ, Premenstrual Symptoms Questionnaire; BDNF, brain derived neurotrophic factor; CGI-I, Clinical Global Impressions-Improvement

Settings and participants

This trial will be conducted at the Research Institute of Traditional Asian Medicine, Kindai University Hospital. Recruitment will be by advertisement in local free papers and by posters displayed in Kindai University Hospital. The start of the recruitment is planned for May 2018 and it will be finished within 2 years.

Potential participants will receive oral and written information regarding the study by a

doctor involved in this study.

Eligibility criteria

Eligible women for the trial must fulfil all of the following criteria:

- Age 20 to 45 years;
- Presence of PMS symptoms and fulfil the 'moderate-to-severe PMS' or 'PMDD' criteria according to the Premenstrual Symptoms Questionnaire (PSQ);[18]
- Regular menstrual cycles (25–38 days);
- Non-equol producers (the equol-production status of subjects will be determined by soy-challenge test as described in the Study procedure);
- No oral conceptive pill use for 4 weeks before study entry;
- No concurrent therapy for PMS, including antidepressants, herbs, supplements, for 4 weeks before study entry;
- Provide written informed consent.

Study subjects will be excluded for the following reasons:

- Allergy to soybean or soybean products;
- Presence of neuropsychiatric disorders;
- Presence of very severe interference with work, usual activities, or relationships by PMS symptoms;
- Serious hepatic disease, heart disease, kidney disease, or malignant disease.

To meet criteria for randomisation, study subjects need to fulfil 2 consecutive menstrual cycles of PMS criteria as measured by the Daily Record of Severity of Problems (DRSP) [19] immediately before the 3 double-blind placebo or active

treatment cycles.

PMS severity criteria include:

- A postmenstrual phase (Day 6 to 10) daily average ≤2.5 for each symptom;
- A premenstrual phase (Day −5 to −1 before menstruation) daily average ≥3.0 for three distinct items;
- A premenstrual phase daily average worsened by at least 50% compared with the corresponding postmenstrual phase daily average for three distinct items;
- A score ≥3 on functional impairment items for at least one day during the premenstrual phase.

Interventions

The intervention will be the administration of natural S-equol supplement (SE5-OH) tablet containing 2.5 mg S-equol produced from the fermentation of soy germ by *Lactococcus* 20-92.[17] The placebo tablets will contain lactose in place of SE5-OH and the appearance will be same as those of the active product. The study products (SE5-OH and placebo) will be manufactured as tablets and supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) free of charge. These tablets will be administered by a doctor involved in this study.

Study procedure

Figure 1 shows the study flow and Table 1 shows the study time schedule. The study will include screening cycles (S-1 and S-2) and intervention cycles (I-1, I-2 and I-3). Subjects will receive oral and written information regarding the study. Written informed consent will be obtained by the physicians involved in the study.

Screening cycles

The equol-production status of subjects will be determined by soy-challenge test. Participants will be instructed to eat soy food containing approximately 50 mg isoflavones twice a day, and then first morning urine samples will be collected the next morning. These samples will be transported by mail to the Saga Nutraceuticals Research Institute of Otsuka Pharmaceutical Co., Ltd. Then, the equol concentration will be measured by high-performance liquid chromatography (HPLC) using the modified method of Lundh.[20] The detection limit of equol in this assay is 0.85 nmol/ml. Those subjects who have detectable urine equol will be classified as equol producers.

Only the non-equol producers will be selected and then they will be required to check their premenstrual symptoms by DRSP for 2 consecutive menstrual cycles. DRSP comprises 21 items of emotional and physical PMS symptoms and 3 items of functional impairment for social and life activity, which are rated for their severity on a six-point scale from 1 (not at all) to 6 (extreme).[19]

Intervention cycles

Eligible subjects will be randomly assigned to receive SE5-OH at a dose of 10 mg S-equol or placebo, orally, twice daily, receiving 4 tablets (2×2 tablets) from Day 7 (±2) onwards to the start of the next menstruation, during three menstruation cycles continuously. They will be required to check their PMS symptoms by DRSP every day.

At the start and the end of the intervention, on Day 7 (±2) of the I-1 and C-1 cycles, subjects will be required to collect their saliva samples themselves using a Saliva Collection Aid (Salimetrics, Carlsbad, CA, USA). At that time, they will be also

required to check their PMS symptoms by PSQ.

After the intervention, they will be checked at a final assessment during the close-up cycle (C-1). The Clinical Global Impressions-Improvement (CGI-I) scale will be checked by a physician, and then the study subjects' involvement in the trial will cease.

Outcome measure

The primary outcome measure consists of 21 symptom items of DRSP. The average of the premenstrual phase (Day −5 to −1 before menstruation) for 21 items will be summed to generate the DRSP total score. The primary efficacy variable is the difference between the DRSP total score from the screening cycle (S-2) and the DRSP total score from the last intervention cycle (I-3).

Secondary outcome measurements are the percentage difference of the DRSP total score between S-2 and I-3, the difference between the salivary BDNF from baseline to the end of treatment, and the ratio of mild PMS in the PSQ and CGI-I scale at the end of treatment.

Saliva will be stored at −20°C until assayed. BDNF will be detected by sandwich ELISA according to the manufacturer's instructions (CYT306; Millipore Corp, Bedford, MA, USA) as described previously [21]. All samples will be tested in duplicate and the mean value will be determined.

CGI-I scale is a single-item measure, which is consists of a 7 point scale (1=very much improved to 7=very much worse). We will define the response group as a score of 1 or 2, corresponding to very much improved or much improved.

Sample size calculation

A pilot study of 10 participants was conducted to estimate the sample size. The result of this open label pilot study showed that the change from baseline in the mean (± standard deviation [SD]) DRSP total score of SE5-OH treatment was −14.2 (± 15.4). As this pilot study included low DRSP total score cases, we expect that SE5-OH will achieve much better improvement of DRSP total score in this study. According to a previous report studying the effect of calcium for the treatment of PMS by double blind randomised placebo controlled trial,[22] the mean change in the DRSP total score of placebo treatment from baseline was −7.6. Accordingly, we assumed that the SE5-OH and placebo groups will show a mean decrease in DRSP total score from baseline of 16 and 8, respectively, with an SD of 15 for each group. Under these assumptions, the required sample size was calculated as 114 (57 in each group) with a significance level of 5% (two-sided) and power of 80%, where the Student's *t*-test was applied. Accounting for the dropout of a few subjects, we set the target sample size as 124 (62 in each group).

Randomisation

A randomisation list will be generated using a computer program by the Data Coordinating Center of Osaka University Hospital (Osaka, Japan). This procedure will be performed by a physician not involved in the study.

Blinding

The active product and placebo will be packaged in identical bottles and sealed. These are similar in look and taste. All participants and investigators will be blinded to the assigned treatment throughout the study.

Data collection and management

All subjects will be assigned a study identification number. Case report forms (CRFs) will be paper forms. Data will be entered and stored in an electronic database, which is managed by the Data Coordinating Center of Osaka University Hospital. All CRFs and study data will be stored in a locker within Kindai University.

Monitoring

An independent Data and Safety Monitoring Board (DSMB) will be set up prior to the start of the study. The DSMB will review data after the first participant, and then after the recruitment of 25%, 50% and 75% of participants to check the study progress and all adverse events.

Statistical analysis

All efficacy variables will be analysed for the full analysis set, which is defined as all randomized subjects who have at least 1 day DRSP total score measurement at S-2 and I-3, without major protocol violations. The safety analysis set will consist of subjects who receive the study tablets at least once and who undergo safety assessment.

Statistical analysis will be performed with the statistical software SAS 9.4 (SAS Institute, Cary, North Carolina, USA).

Baseline characteristics will be summarised for each group. Continuous variables will be presented using the mean, median, SD, and range. Categorical variables will be presented as the percentage.

To compare the primary outcomes (difference of DRSP total score between S-2 and I-3) between the SE5-OH group and placebo group, the difference between the mean

differences in these two groups will be determined with the 95% CI. Furthermore, the p-value will be determined using the Student's *t*-test, where the significance level is 5% (two-sided). The secondary outcomes (percentage difference of DRSP total score and salivary BDNF) will also be compared using the same summary measures. PSQ and CGI-I will be summarised as the difference of effective proportions with the 95% CI, where the p-value will be determined using Fisher's exact test.

Safety assessment

All subjects will be required to report any adverse event during the trial. All serious adverse events will be immediately reported to the project leader, ethics committee, all participating investigators and the manufacturer of the study products.

Patient and Public Involvement

The research question regarding the effect of natural S-equol supplements on premenstrual symptoms was first proposed by a patient who failed the standard treatment. Patients were not involved in the planning of this study. The results will be disseminated to study participants via the website of our research institute.

ETHICS AND DISSEMINATION

The trial protocol has been approved by the Ethics Committee of Kindai University (approval number 29-202). Any modification in protocol will be presented to the ethics committee. The results will be submitted to a peer-reviewed journal. Abstracts will be submitted to relevant national and international conferences. This study will follow the standards set by the guidelines of the Consolidated Standards of Reporting Trials

(CONSORT).

Acknowledgement

We thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflict of interest

TT received lecture fees from Otsuka Pharmaceutical Co., Ltd., the manufacturer of SE5-OH. Otsuka Pharmaceutical Co., Ltd. will have no role in the conception, study planning, data analysis, or interpretation of the data.

Funding

This work was supported, in part, by a grant from Research, AMED Research Project for Improving Quality in Healthcare and Collecting Scientific Evidence on Integrative Medicine, Grant Number 17933369, Tokyo, Japan.

Author Contribution

TT, MS and YC contributed to the drafting of the manuscript. TT was the main contributor to the study design and conception. All authors agreed with the integrity of the study and gave their approval.

Data Sharing

No additional data are available.

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Figure legend

Figure 1. Flow chart of the study diagram.

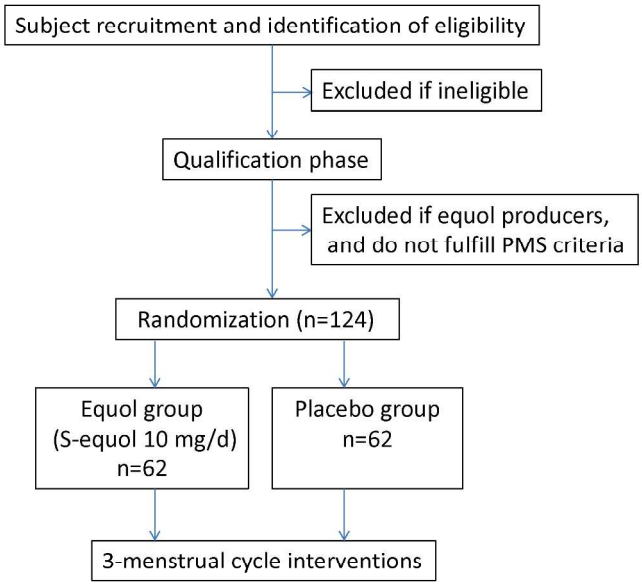

Fig. 1

254x190mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	16

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12,13
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	12

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	6
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset,	

		and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	