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# BMJ Open

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

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

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## INTRODUCTION

### **Premenstrual syndrome**

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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

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5 isoflavones may stabilise the natural fluctuation of the oestrogen cycle through their  
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7 SERM-like activities to alleviate PMS symptoms.[14] A previous report demonstrated  
8  
9 that soy isoflavone supplementation had beneficial effects for the relief of PMS  
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11 symptoms.[15]  
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### 13 14 15 16 **Equol production status and PMS**

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

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35 The aim of this study is to examine the effect of natural S-equol supplement on  
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37 premenstrual symptoms. We hypothesise that equol is effective for alleviating  
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39 premenstrual symptoms in equol non-producers.  
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### 44 **METHODS AND ANALYSIS**

#### 45 46 **Trial design**

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48 This study is designed as a double-blind placebo-controlled randomised trial with a ratio  
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50 of 1:1, in which a stratified randomisation is conducted with adjustment factors of  
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52 DRSP total score ( $\geq 50$  or  $< 50$ ) and age ( $\geq 35$  or  $< 35$  years) at baseline. The trial was  
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54 registered at the University Hospital Medical Information Network (UMIN) center (ID  
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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 contraceptive 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  $\leq 2.5$  for each symptom;
- A premenstrual phase (Day -5 to -1 before menstruation) daily average  $\geq 3.0$  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  $\geq 3$  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**

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5 Figure 1 shows the study flow and Table 1 shows the study time schedule. The study  
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7 will include screening cycles (S-1 and S-2) and intervention cycles (I-1, I-2 and I-3).  
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9 Subjects will receive oral and written information regarding the study. Written informed  
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11 consent will be obtained by the physicians involved in the study.  
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#### 14 15 16 Screening cycles

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18 The equol-production status of subjects will be determined by soy-challenge test.  
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20 Participants will be instructed to eat soy food containing approximately 50 mg  
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22 isoflavones twice a day, and then first morning urine samples will be collected the next  
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24 morning. These samples will be transported by mail to the Saga Nutraceuticals Research  
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26 Institute of Otsuka Pharmaceutical Co., Ltd. Then, the equol concentration will be  
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28 measured by high-performance liquid chromatography (HPLC) using the modified  
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30 method of Lundh.[19] The detection limit of equol in this assay is 0.85 nmol/ml. Those  
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32 subjects who have detectable urine equol will be classified as equol producers.  
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36 Only the non-equol producers will be selected and then they will be required to check  
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38 their premenstrual symptoms by DRSP for 2 consecutive menstrual cycles. DRSP  
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40 comprises 21 items of emotional and physical PMS symptoms and 3 items of functional  
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42 impairment for social and life activity, which are rated for their severity on a six-point  
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44 scale from 1 (not at all) to 6 (extreme).[18]  
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#### 48 49 Intervention cycles

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51 Eligible subjects will be randomly assigned to receive SE5-OH at a dose of 10 mg  
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53 S-equol or placebo, orally, twice daily, receiving 4 tablets (2×2 tablets) on Day 7 (±2)  
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55 of the menstruation cycle and continued for three menstruation cycles. They will be  
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5 required to check their PMS symptoms by DRSP every day.  
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7 At the start and the end of the intervention, on Day 7 ( $\pm 2$ ) of the I-1 and C-1 cycles,  
8 subjects will be required to collect their saliva samples themselves using a Saliva  
9 Collection Aid (Salimetrics, Carlsbad, CA, USA). At that time, they will be also  
10 required to check their PMS symptoms by PSQ.  
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15 After the intervention, they will be checked at a final assessment during the close-up  
16 cycle (C-1). The Clinical Global Impressions-Improvement (CGI-I) scale will be  
17 checked by a physician, and then the study subjects' involvement in the trial will cease.  
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### 23 24 **Outcome measure**

25 The primary outcome measure consists of 21 symptom items of DRSP. The average of  
26 the premenstrual phase (Day -5 to -1 before menstruation) for 21 items will be  
27 summed to generate the DRSP total score. The primary efficacy variable is the  
28 difference between the DRSP total score from the screening cycle (S-2) and the DRSP  
29 total score from the last intervention cycle (I-3).  
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37 Secondary outcome measurements are the percentage difference of the DRSP total  
38 score between S-2 and I-3, the difference between the salivary BDNF from baseline to  
39 the end of treatment, and the ratio of mild PMS in the PSQ and CGI-I scale at the end of  
40 treatment.  
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45 Saliva will be stored at  $-20^{\circ}\text{C}$  until assayed. BDNF will be detected by sandwich  
46 ELISA according to the manufacturer's instructions (CYT306; Millipore Corp, Bedford,  
47 MA, USA) as described previously [20]. All samples will be tested in duplicate and the  
48 mean value will be determined.  
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54 CGI-I scale is a single-item measure, which is consists of a 7 point scale (1=very  
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5 much improved to 7=very much worse). We will define the response group as a score of  
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7 1 or 2, corresponding to very much improved or much improved.  
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### 10 11 **Sample size calculation**

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13 A pilot study of 10 participants was conducted to estimate the sample size. The result of  
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15 this open label pilot study showed that the change from baseline in the mean ( $\pm$  standard  
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17 deviation [SD]) DRSP total score of SE5-OH treatment was  $-14.2 (\pm 15.4)$ . As this pilot  
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19 study included low DRSP total score cases, we expect that SE5-OH will achieve much  
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21 better improvement of DRSP total score in this study. According to a previous report  
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23 studying the effect of calcium for the treatment of PMS by double blind randomised  
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25 placebo controlled trial,[21] the mean change in the DRSP total score of placebo  
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27 treatment from baseline was  $-7.6$ . Accordingly, we assumed that the SE5-OH and  
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29 placebo groups will show a mean decrease in DRSP total score from baseline of 16 and  
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31 8, respectively, with an SD of 15 for each group. Under these assumptions, the required  
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33 sample size was calculated as 114 (57 in each group) with a significance level of 5%  
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35 (two-sided) and power of 80%, where the Student's *t*-test was applied. Accounting for  
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37 the dropout of a few subjects, we set the target sample size as 124 (62 in each group).  
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### 44 **Randomisation**

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

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5 The active product and placebo will be packaged in identical bottles and sealed. These  
6 are similar in look and taste. All participants and investigators will be blinded to the  
7 assigned treatment throughout the study.  
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### 11 12 13 **Data collection and management**

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

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

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42 Statistical analysis will be performed with the statistical software SAS 9.4 (SAS  
43 Institute, Cary, North Carolina, USA).  
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46 Baseline characteristics will be summarised for each group. Continuous variables will  
47 be presented using the mean, median, SD, and range. Categorical variables will be  
48 presented as the percentage.  
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52 To compare the primary outcomes (difference of DRSP total score between S-2 and  
53 I-3) between the SE5-OH group and placebo group, the difference between the mean  
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5 differences in these two groups will be determined with the 95% CI. Furthermore, the  
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7 p-value will be determined using the Student's *t*-test, where the significance level is 5%  
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9 (two-sided). The secondary outcomes (percentage difference of DRSP total score and  
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11 salivary BDNF) will also be compared using the same summary measures. PSQ and  
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13 CGI-I will be summarised as the difference of effective proportions with the 95% CI,  
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15 where the p-value will be determined using Fisher's exact test.  
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### 22 **Safety assessment**

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24 All subjects will be required to report any adverse event during the trial. All serious  
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26 adverse events will be immediately reported to the project leader, ethics committee, all  
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28 participating investigators and the manufacturer of the study products.  
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### 32 **ETHICS AND DISSEMINATION**

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

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52 We thank Edanz Group ([www.edanzediting.com/ac](http://www.edanzediting.com/ac)) for editing a draft of this  
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54 manuscript.  
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**Conflict of interest**

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

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**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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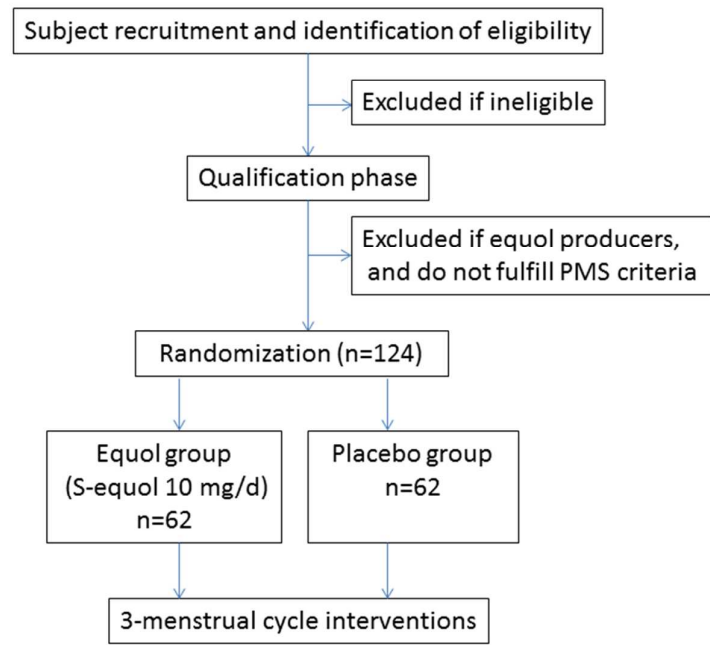
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5 **Figure legend**  
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7 Figure 1. Flow chart of the study diagram.  
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Fig.1



EW Only

Table 1 Schedule of enrolment, interventions and assessments

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

CD, cycle day; SES-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

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For peer review only

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# 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.

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

1	sponsor contact			
2	information			
3				
4	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	16
5	responsibilities:		collection, management, analysis, and interpretation of	
6	sponsor and funder		data; writing of the report; and the decision to submit the	
7			report for publication, including whether they will have	
8			ultimate authority over any of these activities	
9				
10				
11				
12	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating	12,13
13	responsibilities:		centre, steering committee, endpoint adjudication	
14	committees		committee, data management team, and other individuals or	
15			groups overseeing the trial, if applicable (see Item 21a for	
16			data monitoring committee)	
17				
18				
19				
20	Background and	<a href="#">#6a</a>	Description of research question and justification for	4
21	rationale		undertaking the trial, including summary of relevant studies	
22			(published and unpublished) examining benefits and harms	
23			for each intervention	
24				
25				
26				
27	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	4
28	rationale: choice of			
29	comparators			
30				
31				
32	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	6
33				
34				
35	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel	6
36			group, crossover, factorial, single group), allocation ratio,	
37			and framework (eg, superiority, equivalence, non-inferiority,	
38			exploratory)	
39				
40				
41				
42	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic,	6
43			academic hospital) and list of countries where data will be	
44			collected. Reference to where list of study sites can be	
45			obtained	
46				
47				
48	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable,	7
49			eligibility criteria for study centres and individuals who will	
50			perform the interventions (eg, surgeons, psychotherapists)	
51				
52				
53				
54	Interventions:	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow	8
55	description		replication, including how and when they will be	
56			administered	
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1	Interventions:	<a href="#">#11b</a>	Criteria for discontinuing or modifying allocated	
2	modifications		interventions for a given trial participant (eg, drug dose	
3			change in response to harms, participant request, or	
4			improving / worsening disease)	
5				
6				
7	Interventions:	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols,	11
8	adherence		and any procedures for monitoring adherence (eg, drug	
9			tablet return; laboratory tests)	
10				
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12				
13	Interventions:	<a href="#">#11d</a>	Relevant concomitant care and interventions that are	7
14	concomitant care		permitted or prohibited during the trial	
15				
16				
17	Outcomes	<a href="#">#12</a>	Primary, secondary, and other outcomes, including the	11
18			specific measurement variable (eg, systolic blood pressure),	
19			analysis metric (eg, change from baseline, final value, time	
20			to event), method of aggregation (eg, median, proportion),	
21			and time point for each outcome. Explanation of the clinical	
22			relevance of chosen efficacy and harm outcomes is strongly	
23			recommended	
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28	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any	Figure1
29			run-ins and washouts), assessments, and visits for	
30			participants. A schematic diagram is highly recommended	
31			(see Figure)	
32				
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34				
35	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study	12
36			objectives and how it was determined, including clinical and	
37			statistical assumptions supporting any sample size	
38			calculations	
39				
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41				
42	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment to	6
43			reach target sample size	
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46	Allocation: sequence	<a href="#">#16a</a>	Method of generating the allocation sequence (eg,	12
47	generation		computer-generated random numbers), and list of any	
48			factors for stratification. To reduce predictability of a random	
49			sequence, details of any planned restriction (eg, blocking)	
50			should be provided in a separate document that is	
51			unavailable to those who enrol participants or assign	
52			interventions	
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57	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence (eg,	12
58	concealment		central telephone; sequentially numbered, opaque, sealed	
59				



1	mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
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3				
4	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
5	implementation			
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9	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
10				
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14	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
15	emergency			
16	unblinding			
17				
18				
19				
20	Data collection plan	<a href="#">#18a</a>	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
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31	Data collection plan:	<a href="#">#18b</a>	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	
32	retention			
33				
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37				
38	Data management	<a href="#">#19</a>	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
39				
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46	Statistics: outcomes	<a href="#">#20a</a>	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
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51	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
52	analyses			
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55	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
56	population and			
57	missing data			
58				
59				

1	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC); summary	13
2	formal committee		of its role and reporting structure; statement of whether it is	
3			independent from the sponsor and competing interests; and	
4			reference to where further details about its charter can be	
5			found, if not in the protocol. Alternatively, an explanation of	
6			why a DMC is not needed	
7				
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10	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping guidelines,	
11	interim analysis		including who will have access to these interim results and	
12			make the final decision to terminate the trial	
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16	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and managing	15
17			solicited and spontaneously reported adverse events and	
18			other unintended effects of trial interventions or trial conduct	
19				
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21	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct, if any,	
22			and whether the process will be independent from	
23			investigators and the sponsor	
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27	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee / institutional	15
28	approval		review board (REC / IRB) approval	
29				
30				
31	Protocol	<a href="#">#25</a>	Plans for communicating important protocol modifications	6
32	amendments		(eg, changes to eligibility criteria, outcomes, analyses) to	
33			relevant parties (eg, investigators, REC / IRBs, trial	
34			participants, trial registries, journals, regulators)	
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37	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from potential	9
38			trial participants or authorised surrogates, and how (see	
39			Item 32)	
40				
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43	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use of	
44	ancillary studies		participant data and biological specimens in ancillary	
45			studies, if applicable	
46				
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48	Confidentiality	<a href="#">#27</a>	How personal information about potential and enrolled	13
49			participants will be collected, shared, and maintained in	
50			order to protect confidentiality before, during, and after the	
51			trial	
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55	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	15
56	interests		investigators for the overall trial and each study site	
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59	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset,	
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and disclosure of contractual agreements that limit such access for investigators

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4	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for
5	trial care		compensation to those who suffer harm from trial
6			participation
7			
8			
9	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial
10	trial results		results to participants, healthcare professionals, the public,
11			and other relevant groups (eg, via publication, reporting in
12			results databases, or other data sharing arrangements),
13			including any publication restrictions
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17	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of
18	authorship		professional writers
19			
20			
21	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol,
22	reproducible		participant-level dataset, and statistical code
23	research		
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27	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given
28	materials		to participants and authorised surrogates
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31	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of
32			biological specimens for genetic or molecular analysis in the
33			current trial and for future use in ancillary studies, if
34			applicable
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# BMJ Open

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

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<b>Primary Subject Heading</b>:	Complementary medicine
Secondary Subject Heading:	Mental health, Obstetrics and gynaecology
Keywords:	premenstrual syndrome, equol, isoflavone, dietary supplement, RCT

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Manuscripts

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5 1 **Effectiveness of natural S-equol supplement for premenstrual symptoms: protocol**  
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7 2 **of a randomised, double-blind, placebo-controlled trial**  
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9 3 Takashi Takeda, MD, PhD<sup>1\*</sup>, Masami Shiina, MD, PhD<sup>1</sup>, Yasutaka Chiba MD, PhD<sup>2</sup>  
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30 14 Word count (excluding title page, abstract, references, figures and tables): 2395 words  
31  
32 15

## 1 ABSTRACT

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

9 **Methods and analysis** This study will enrol 124 women (aged 20 to 45 years) who  
10 have PMS symptoms and are non-equol producers in a double-blind, parallel,  
11 randomised, placebo-controlled trial, in which they will receive natural S-equol  
12 supplement (equol 10 mg a day) or placebo, orally, twice daily, for three menstrual  
13 cycles. The primary outcome measure (Daily Record of Severity of Problems total  
14 score) will be assessed during intervention cycles. To compare the primary outcomes  
15 between the S-equol group and placebo group, the mean differences in Daily Record of  
16 Severity of Problems total score between the two groups will be determined. The  
17 p-values will be determined using the Student's *t*-test, where the significance level is  
18 5% (two-sided).

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

22 **Trial registration number** UMIN000031815.

23  
24 **Strength and limitations of this study**

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

7  
8  
9 Keywords: premenstrual syndrome; equol; isoflavone; dietary supplement; RCT  
10

## 1 INTRODUCTION

### 2 **Premenstrual syndrome**

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

### 16 **Isoflavones and Equol**

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



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5 1 believed to benefit from the consumption of soy isoflavones.[13] It is plausible that  
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7 2 isoflavones may stabilise the natural fluctuation of the oestrogen cycle through their  
8  
9 3 SERM-like activities to alleviate PMS symptoms.[14] A previous report demonstrated  
10  
11 4 that soy isoflavone supplementation had beneficial effects for the relief of specific PMS  
12  
13 5 symptoms such as cramp and swelling.[15]  
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17

### 18 7 **Equol production status and PMS**

19  
20 8 Recently, we reported the relationship between PMS and equol production status and  
21  
22 9 showed that equol non-production was a significant risk factor for PMS.[16] Dietary  
23  
24 10 interventions, such as supplementation with equol might be a safe treatment strategy for  
25  
26 11 PMS. A natural S-equol supplement was reported to be effective for treating menopause  
27  
28 12 symptoms without any serious adverse effects.[17]  
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### 33 14 **TRIAL OBJECTIVES AND HYPOTHESIS**

34  
35 15 The aim of this study is to examine the effect of natural S-equol supplement on  
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37 16 premenstrual symptoms. We hypothesise that equol is more effective than placebo in  
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39 17 alleviating premenstrual symptoms in equol non-producers.  
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### 44 19 **METHODS AND ANALYSIS**

#### 45 46 20 **Trial design**

47  
48 21 This study is designed as a double-blind placebo-controlled parallel randomised trial  
49  
50 22 with a ratio of 1:1, in which a stratified randomisation is conducted with adjustment  
51  
52 23 factors of DRSP total score ( $\geq 50$  or  $< 50$ ) and age ( $\geq 35$  or  $< 35$  years) at baseline. The  
53  
54 24 trial was registered at the University Hospital Medical Information Network (UMIN)  
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1 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

1 doctor involved in this study.

### 3 Eligibility criteria

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

- 5 ● Age 20 to 45 years;
- 6 ● Presence of PMS symptoms and fulfil the ‘moderate-to-severe PMS’ or ‘PMDD’  
7 criteria according to the Premenstrual Symptoms Questionnaire (PSQ);[18]
- 8 ● Regular menstrual cycles (25–38 days);
- 9 ● Non-equol producers (the equol-production status of subjects will be determined by  
10 soy-challenge test as described in the Study procedure);
- 11 ● No oral contraceptive pill use for 4 weeks before study entry;
- 12 ● No concurrent therapy for PMS, including antidepressants, herbs, supplements, for  
13 4 weeks before study entry;
- 14 ● Provide written informed consent.

15 Study subjects will be excluded for the following reasons:

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

21  
22 To meet criteria for randomisation, study subjects need to fulfil 2 consecutive  
23 menstrual cycles of PMS criteria as measured by the Daily Record of Severity of  
24 Problems (DRSP) [19] immediately before the 3 double-blind placebo or active

1 treatment cycles.

2 PMS severity criteria include:

- 3 ● A postmenstrual phase (Day 6 to 10) daily average  $\leq 2.5$  for each symptom;
- 4 ● A premenstrual phase (Day -5 to -1 before menstruation) daily average  $\geq 3.0$  for  
5 three distinct items;
- 6 ● A premenstrual phase daily average worsened by at least 50% compared with the  
7 corresponding postmenstrual phase daily average for three distinct items;
- 8 ● A score  $\geq 3$  on functional impairment items for at least one day during the  
9 premenstrual phase.

## 11 **Interventions**

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

## 20 **Study procedure**

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

1

## 2 Screening cycles

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

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

16

## 17 Intervention cycles

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

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

1 required to check their PMS symptoms by PSQ.

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

### 6 **Outcome measure**

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

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

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

20 CGI-I scale is a single-item measure, which is consists of a 7 point scale (1=very  
21 much improved to 7=very much worse). We will define the response group as a score of  
22 1 or 2, corresponding to very much improved or much improved.

### 24 **Sample size calculation**

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

### 15 **Randomisation**

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

### 20 **Blinding**

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

24

## **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



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

### 8 **Safety assessment**

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

### 13 **Patient and Public Involvement**

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

### 19 **ETHICS AND DISSEMINATION**

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

1 (CONSORT).

2

### 3 **Acknowledgement**

4 We thank Edanz Group ([www.edanzediting.com/ac](http://www.edanzediting.com/ac)) for editing a draft of this  
5 manuscript.

### 6 **Conflict of interest**

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

10

### 11 **Funding**

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

### 15 **Author Contribution**

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

### 19 **Data Sharing**

20 No additional data are available.

21

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- 1 **Figure legend**
- 2 Figure 1. Flow chart of the study diagram.
- 3

For peer review only

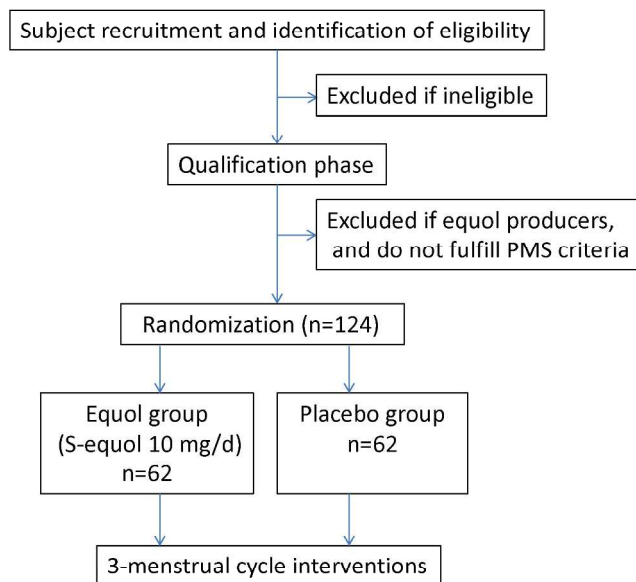


Fig. 1

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

1	sponsor contact			
2	information			
3				
4	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	16
5	responsibilities:		collection, management, analysis, and interpretation of	
6	sponsor and funder		data; writing of the report; and the decision to submit the	
7			report for publication, including whether they will have	
8			ultimate authority over any of these activities	
9				
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11				
12	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating	12,13
13	responsibilities:		centre, steering committee, endpoint adjudication	
14	committees		committee, data management team, and other individuals or	
15			groups overseeing the trial, if applicable (see Item 21a for	
16			data monitoring committee)	
17				
18				
19				
20	Background and	<a href="#">#6a</a>	Description of research question and justification for	4
21	rationale		undertaking the trial, including summary of relevant studies	
22			(published and unpublished) examining benefits and harms	
23			for each intervention	
24				
25				
26				
27	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	4
28	rationale: choice of			
29	comparators			
30				
31				
32	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	6
33				
34				
35	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel	6
36			group, crossover, factorial, single group), allocation ratio,	
37			and framework (eg, superiority, equivalence, non-inferiority,	
38			exploratory)	
39				
40				
41				
42	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic,	6
43			academic hospital) and list of countries where data will be	
44			collected. Reference to where list of study sites can be	
45			obtained	
46				
47				
48	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable,	7
49			eligibility criteria for study centres and individuals who will	
50			perform the interventions (eg, surgeons, psychotherapists)	
51				
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54	Interventions:	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow	8
55	description		replication, including how and when they will be	
56			administered	
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1	Interventions:	<a href="#">#11b</a>	Criteria for discontinuing or modifying allocated	
2	modifications		interventions for a given trial participant (eg, drug dose	
3			change in response to harms, participant request, or	
4			improving / worsening disease)	
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6				
7	Interventions:	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols,	11
8	adherence		and any procedures for monitoring adherence (eg, drug	
9			tablet return; laboratory tests)	
10				
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13	Interventions:	<a href="#">#11d</a>	Relevant concomitant care and interventions that are	7
14	concomitant care		permitted or prohibited during the trial	
15				
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17	Outcomes	<a href="#">#12</a>	Primary, secondary, and other outcomes, including the	11
18			specific measurement variable (eg, systolic blood pressure),	
19			analysis metric (eg, change from baseline, final value, time	
20			to event), method of aggregation (eg, median, proportion),	
21			and time point for each outcome. Explanation of the clinical	
22			relevance of chosen efficacy and harm outcomes is strongly	
23			recommended	
24				
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28	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any	Figure1
29			run-ins and washouts), assessments, and visits for	
30			participants. A schematic diagram is highly recommended	
31			(see Figure)	
32				
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34				
35	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study	12
36			objectives and how it was determined, including clinical and	
37			statistical assumptions supporting any sample size	
38			calculations	
39				
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42	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment to	6
43			reach target sample size	
44				
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46	Allocation: sequence	<a href="#">#16a</a>	Method of generating the allocation sequence (eg,	12
47	generation		computer-generated random numbers), and list of any	
48			factors for stratification. To reduce predictability of a random	
49			sequence, details of any planned restriction (eg, blocking)	
50			should be provided in a separate document that is	
51			unavailable to those who enrol participants or assign	
52			interventions	
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57	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence (eg,	12
58	concealment		central telephone; sequentially numbered, opaque, sealed	
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1	mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
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3				
4	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
5	implementation			
6				
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9	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
10				
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14	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
15	emergency			
16	unblinding			
17				
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20	Data collection plan	<a href="#">#18a</a>	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
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31	Data collection plan:	<a href="#">#18b</a>	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	
32	retention			
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38	Data management	<a href="#">#19</a>	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
39				
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46	Statistics: outcomes	<a href="#">#20a</a>	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
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51	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
52	analyses			
53				
54				
55	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
56	population and			
57	missing data			
58				
59				

1	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC); summary	13
2	formal committee		of its role and reporting structure; statement of whether it is	
3			independent from the sponsor and competing interests; and	
4			reference to where further details about its charter can be	
5			found, if not in the protocol. Alternatively, an explanation of	
6			why a DMC is not needed	
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10	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping guidelines,	
11	interim analysis		including who will have access to these interim results and	
12			make the final decision to terminate the trial	
13				
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16	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and managing	15
17			solicited and spontaneously reported adverse events and	
18			other unintended effects of trial interventions or trial conduct	
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21	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct, if any,	
22			and whether the process will be independent from	
23			investigators and the sponsor	
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27	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee / institutional	15
28	approval		review board (REC / IRB) approval	
29				
30				
31	Protocol	<a href="#">#25</a>	Plans for communicating important protocol modifications	6
32	amendments		(eg, changes to eligibility criteria, outcomes, analyses) to	
33			relevant parties (eg, investigators, REC / IRBs, trial	
34			participants, trial registries, journals, regulators)	
35				
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37	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from potential	9
38			trial participants or authorised surrogates, and how (see	
39			Item 32)	
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43	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use of	
44	ancillary studies		participant data and biological specimens in ancillary	
45			studies, if applicable	
46				
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48	Confidentiality	<a href="#">#27</a>	How personal information about potential and enrolled	13
49			participants will be collected, shared, and maintained in	
50			order to protect confidentiality before, during, and after the	
51			trial	
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55	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	15
56	interests		investigators for the overall trial and each study site	
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59	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset,	
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		and disclosure of contractual agreements that limit such access for investigators	
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4	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for
5	trial care		compensation to those who suffer harm from trial
6			participation
7			
8			
9	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial
10	trial results		results to participants, healthcare professionals, the public,
11			and other relevant groups (eg, via publication, reporting in
12			results databases, or other data sharing arrangements),
13			including any publication restrictions
14			
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17	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of
18	authorship		professional writers
19			
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21	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol,
22	reproducible		participant-level dataset, and statistical code
23	research		
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26			
27	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given
28	materials		to participants and authorised surrogates
29			
30			
31	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of
32			biological specimens for genetic or molecular analysis in the
33			current trial and for future use in ancillary studies, if
34			applicable
35			
36			

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