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Prolong: a double blind randomised placebo-controlled trial of broccoli sprout extract in women with early onset preeclampsia.

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Complete List of Authors:	Cox, Annie; Monash University School of Clinical Sciences at Monash Health, Obstetrics and Gynaecology ; Hudson Institute of Medical Research, Obstetrics and Gynaecology Marshall, Sarah; Monash University School of Clinical Sciences at Monash Health, Obstetrics and Gynaecology Palmer, Kirsten; Monash University School of Clinical Sciences at Monash Health, Obstetrics and Gynaecology Wallace, Euan; Monash University, Obstetrics and Gynaecology
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Title: Prolong: a double blind randomized placebo-controlled trial of broccoli sprout extract in women with early onset preeclampsia.

Authors: Annie G. Cox[1], Sarah A. Marshall[1], Kirsten R. Palmer[1,2], Euan M. Wallace[1]

Institutions: [1]The Ritchie Centre, Department of Obstetrics and Gynaecology, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia. [2]Monash Health, Clayton, Victoria, Australia

Emails: AGC: annie.cox@ monash.edu, SAM: sarah.marshall@monash.edu, KRP: kirsten.palmer@monash.edu, EMW: euan.wallace@monash.edu.

Corresponding author:

Professor Euan Wallace, Department of Obstetrics and Gynecology, Monash University, Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, T: +61 (3) 95945145 F: +61 (3) 95945003

Sponsor: Monash Health

Contact information: Dr Kirsten Palmer

Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, T: +61 (3) 95945145 F: +61 (3) 95945003

Abstract

Introduction: Preeclampsia complicates about 5% of pregnancies. It remains a leading cause of maternal and perinatal morbidity and mortality. Recent insights into the role of excessive oxidative stress in the underlying placental and maternal vascular dysfunction of preeclampsia have offered opportunities for new adjuvant therapies. One such therapy is a broccoli sprout extract rich in the organosulphur antioxidant sulforaphane. Sulforaphane reduces oxidative stress and placental secretion of the anti-angiogenic factors that contribute to the vascular dysfunction in preeclampsia. We propose a phase III clinical trial of broccoli sprout extract as an adjuvant therapy. We will assess the effects of a broccoli sprout supplement in women with early onset (<34 weeks) preeclampsia on (i) the interval between enrolment and delivery, recorded in days, (ii) biomarkers of placental and endothelial function, and (iii) maternal and fetal outcomes.

Methods and analysis: A double blind, placebo controlled randomised clinical trial will be conducted at Monash Health, Melbourne, Australia. A cohort of 90 pregnant women (45 in each arm) diagnosed with early onset preeclampsia will be recruited. Preeclampsia will be defined in accordance with Society for Obstetric Medicine of Australia and New Zealand (SOMANZ) guidelines. After admission to hospital, consenting women will be randomised to receive an oral dose of either a broccoli sprout extract, containing 24mg of activated sulforaphane, or an identical placebo, twice daily until delivery. Maternal blood will be collected throughout the trial for the measurement of biomarkers of preeclampsia, including soluble fms-like tyrosine kinase-1 (sFlt1), placental growth factor (PlGF), soluble endoglin (sEng) and activin A, as well as circulating sulforaphane metabolites. Maternal and perinatal outcomes will be monitored throughout the trial. All clinical care decisions, including the timing

of delivery, will be made by the treating team blinded to treatment allocation. Participation in this trial will not affect routine care. At delivery maternal and cord blood, and placental cotelydons will be collected. Adipose tissue will be collected from women giving birth by caesarean section to provide maternal blood vessels for vascular studies.

Ethics and dissemination: Ethical approval has been provided by Monash Health HREC: RES-18-0000-109A. Data will be published in peer-reviewed journals and presented at conferences, both nationally and internationally. All patient information will be de-identified for the purpose of publication.

Discussion: This is the first clinical trial to assess broccoli sprout as an adjuvant therapy for early onset preeclampsia. If successful in safely prolonging pregnancy, this trial will inform the design of future, larger efficacy trials addressing the effect of broccoli sprout extract on perinatal outcomes.

Strengths and limitations

Strengths

- Study design as a randomised, placebo controlled trial.
- Intervention is a naturally occurring nutritional supplement with excellent safety profile.
- Participants will likely be inpatients for the duration of the trial.

Limitations

- Study is not powered for secondary outcomes.
- Trial requires consumption of numerous capsules by participants (total six capsules daily).

Trial registry: Australian and New Zealand Clinical Trial Registry

ANZCTR registration number: ACTRN12618000216213, registered 9th February 2018.

ANZCTR registration URL:

https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12618000216213

Keywords: Preeclampsia, broccoli sprout, sulforaphane, antioxidant, clinical trial.

Background

Preeclampsia is defined as new onset hypertension after 20 weeks gestation with associated maternal organ dysfunction and/or fetal growth restriction[1]. It complicates 5-8% of pregnancies and is a leading cause of maternal and perinatal morbidity and mortality worldwide[1]. Even in high resource settings the risk of neonatal mortality is five fold greater in those born to a mother with preeclampsia compared to those born to a normotensive mother. This increased mortality is largely due to associated fetal growth restriction and the need for premature delivery. Indeed, preeclampsia is the leading cause of iatrogenic premature delivery, implicated in 20% of all premature births[1]. Unfortunately, the incidence of preeclampsia has not changed over the last century and, beyond controlling maternal blood pressure, we continue to lack effective targeted therapies for this serious disorder[1, 2].

Though much remains unknown about the pathological progression of preeclampsia, it is broadly accepted that a placenta, chronically injured by ischaemic-reperfusion insult, releases excessive vasoactive and inflammatory factors into the maternal circulation. In turn, these factors induce systemic maternal endothelial dysfunction[3]. The resulting vasoconstriction and increased vessel permeability cause hypertension, oedema, renal endotheliosis and secondary organ ischaemic injury. For the past fifty years the pharmacological management of preeclampsia has aimed solely to correct the maternal hypertension, allowing safer continuation of the pregnancy in the interests of improving fetal maturity. While the focus on controlling hypertension has improved maternal and perinatal outcomes it has neglected the underlying pathological processes of the disease and limited the potential gains in mitigating fetal risk, particularly in the setting of early onset disease[1]. Seeking to prolong the pregnancy further by targeting the oxidative stress-induced endothelial dysfunction is

an additional approach worth exploring.

In particular, inducers of the nuclear factor E2-like related factor 2 (Nrf2) antioxidant pathway offer an attractive approach. Inducing Nrf2 would be expected to have anti-inflammatory and antioxidant effects in both the placenta and in the maternal vasculature. Nuclear factor E2-related factor 2 is an endogenous inducer of cellular antioxidants[4, 5]. Under physiological conditions, bioavailable levels of Nrf2 are regulated by cytosolic binding to kelch-like ECH-associated protein 1 (KEAP-1), preventing rapid proteasome degradation[5]. Exposure to oxidative stress induces cysteine modifications to KEAP-1, loss of binding to Nrf2 and translocation of Nrf2 to the nucleus[4]. Within the nucleus, by combining with small maf-proteins in the promoter region of antioxidant "safeguarding" genes, Nrf2 stimulates antioxidant response elements resulting in the transcription of mRNA for a number of cellular antioxidants and phase two enzymes[4]. Numerous studies have shown therapeutic benefits from Nrf2 stimulation both in maintaining endothelial health and in treating vasculopathies[6].

The Nrf2 inducer sulforaphane is a naturally occurring organosulphur abundant in broccoli sprout extract[7-9] that has attracted attention in cardiovascular and cancer medicine[7, 8]. It stabilises Nrf2 by impairing ubiquitination and increasing Nrf2 phosphorylation, thereby preventing proteasomal degradation and causing cytosolic accumulation[5]. Sulforaphane also induces cytosolic transcription and nuclear translocation of Nrf2. As such, sulforaphane uses the Nrf2 pathways to enhance production of phase two and antioxidant enzymes, improving cellular resilience to oxidative stress[4, 10].

Rationale

Preeclampsia remains a leading cause of maternal and perinatal morbidity and mortality worldwide[1]. While the introduction of antihypertensives 60 years ago represented a major advance in the care of women with preeclampsia, further progress has all but stalled. Future benefits in maternal and/or perinatal outcomes are likely to come from improved screening and prevention[11] or from more effective treatment, beyond simply managing maternal hypertension[12]. In particular, therapies that target the maternal endothelial dysfunction that underlies the hypertension offer promise in further improving maternal and perinatal outcomes. The antioxidant and anti-inflammatory sulforaphane may be one such therapy. Preliminary data from our group supports a role for sulforaphane in reducing placental production of the anti-angiogenic factors soluble fms-like tyrosine kinas 1 (sFlt-1) and activin A. We have further shown that sulforaphane improves endothelial cell health and function after activation with tumour necrosis factor alpha (TNF-α) and serum from preeclamptic women. Whether sulforaphane has beneficial *in vivo* effects on placental and/or endothelial function in women with early onset preeclampsia remains unexplored. We aim to examine this possibility in our clinical trial, *Prolong*.

Aims

The overarching aim of this trial is to assess the utility of a commercial broccoli sprout extract (BroccoMax®) as an adjuvant therapy in the management of women with early onset (<34 weeks) preeclampsia.

Aim 1. To assess whether broccoli sprout extract can safely prolong the interval between enrolment and delivery (recorded in days) in women with early onset (<34 weeks) preeclampsia.

Aim 2. To assess the effects of a broccoli sprout supplement on production of

maternal circulating biomarkers of placental and endothelial health in women with early onset (<34 weeks) preeclampsia.

Aim 3. To assess effects of a broccoli sprout extract on maternal and perinatal outcomes (safety and tolerance) in women with early onset (<34 weeks) preeclampsia.

Methods and analysis

Study design

Double-blind, randomised, placebo controlled trial (Figure 1).

Sample size

A sample size calculation was performed based on the results of a trial of melatonin as an adjuvant therapy in women with early onset preeclampsia[13, 14]. In that trial, melatonin prolonged the enrolment-to-delivery interval by 6 days, from a mean (SD) of 10.4 (8.3) to 16.4 (11)[14]. Using these data we calculated that 42 women in each treatment group (1:1 ratio) would be sufficient to detect a 6 day difference in mean (two sided comparison) enrolment-to-delivery interval with 80% power. To allow for a 5% attrition rate, we elected a sample size of 45 in each arm, equating to a total of 90 participants.

Trial sites

Women will be recruited from Monash Medical Centre and Jessie McPherson Private Hospital, Clayton, Victoria, Australia. Both sites are Level 6 maternity services, as per Victorian government Maternity Capability Framework[15].

Participant inclusion criteria

A woman will be eligible for inclusion in the trial only if the following criteria are

met:

- aged 18-45,
- singleton pregnancy,
- diagnosis of preeclampsia, as defined by the SOMANZ guidelines[16],
- gestation between 24^{+0} and 33^{+6} weeks,
- live fetus
- able to safely continue pregnancy for at least 48 hours, as determined by the treating obstetrician,
- no known significant fetal anomaly,
- able to give written, informed consent.

Participant exclusion criteria

A woman will not be eligible for inclusion in this trial if any of the following criteria apply:

- eclampsia,
- current use of broccoli sprout extract supplement,
- contraindications to use of broccoli sprout extract supplement (eg, intolerance of broccoli sprout),
- unknown gestation,
- unwillingness or inability to follow the procedures outlined in the Participant Information and Consent Form,
- mentally, cognitively or legally incapacitated or ineligible to provide informed consent,
- co-recruitment/participation in another clinical trial where there is a pharmaceutical, herbal or nutritional intervention (such trial interventions would

also include complementary and alternative medicines).

Participant recruitment

Potential participants will be identified from the antenatal clinic, Pregnancy Assessment Unit, in-patient wards, and labour wards at Monash Medical Centre by the research team. Following discussion with the attending clinical team caring for the woman, eligible women will be approached by a member of the research team who has no involvement in the provision of patient care and provided with the Participant Information and Consent Form for the trial. The research team member will then provide a verbal explanation of the trial, including a description of the trial processes, the voluntary nature of the trial and that a decision to participate, or not, will not affect her normal clinical care. No trial related procedures will be performed on any individual without their prior written, informed consent.

Women who provide written and informed consent to participate will be randomised to receive either broccoli sprout extract (BroccoMax®, Jarrow Formulas, Los Angelos, CA) or an identical placebo (Jarrow Formulas). Allocation will be determined by a computer-generated sequence. After recruitment, each participant will be provided with a unique code so as to maintain participant confidentiality.

Randomisation

A randomisation sequence will be generated by a perinatal statistician not involved in the clinical trial, using a computer-generated code. Because gestation will affect interval between the possible prolongation of pregnancy, randomisation will be stratified within three gestation brackets: 24^{+0}–27^{+6}, 28^{+0}–31^{+6} and 32^{+0}–33^{+6}. Randomisation will be done through block sequence to ensure equivalent sample sizes

are allocated to each treatment group (BroccoMax® or placebo)[17].

The randomisation sequence will be provided to the pharmacist who will allocate capsules (BroccoMax® or placebo) to each participant and will dispense the allocated intervention into bottles accordingly. The pharmacist will maintain a record of participant trial identification number and treatment group.

Intervention

Each participant will take three Broccomax® capsules, each containing 8 mg of activated sulforaphane (total of 24mg), twice daily (BD), or three identical placebo capsules twice daily (BD). Participants and the research team will be blinded to group allocation. Capsules (BroccoMax® or placebo) will be dispensed by the pharmacy in individualised bottles containing sufficient capsules for five days, with additional capsules (amount known only by the research team), and provided to the midwives in charge of ward care. Dosing will be recorded on the patient drug chart and administered as per hospital protocol.

Where participants are discharged home they will record taking the capsules in a Patient Self Administration Diary and return the capsule bottle, including any residual capsules, after 5 days, or sooner if delivered earlier. After delivery, residual capsules will be collected and discarded; they will not be reissued to a participant.

Outcomes

Primary outcome

The interval between enrolment and delivery, recorded in days.

Secondary outcomes

- Preeclampsia severity, as assessed by: escalation of antihypertensive therapy, systolic and diastolic blood pressures, severe renal involvement (serum or plasma creatinine >90umol/L, oliguria <80mL/4hr), haematological involvement (haemolysis[1], platelets $<10^4$/uL, disseminated intravascular coagulation) liver transanimases >500IU.
- Indication for delivery.
- Mode of delivery.
- Composite maternal outcome, including maternal death, eclampsia, HELLP syndrome[2], pulmonary oedema[3], thromboembolic event (significant deep vein thrombosis or pulmonary embolus), placental abruption[4], major postpartum haemorrhage[5], severe renal impairment[6], liver haematoma or rupture.
- Composite fetal outcomes determined by Doppler ultrasound studies (uterine, umbilical, and middle cerebral artery and ductus venosus), abnormal amniotic fluid index, abnormal biophysical profile, abnormal fetal heart rate (by clinician assessment of CTG) and intrauterine fetal death.
- Composite neonatal outcomes, including stillbirth, neonatal death before hospital discharge, 5 minute APGAR score <7, umbilical lactate >5.0 at birth, admission to the neonatal intensive care unit, diagnosis of respiratory distress

[1] schistocytes or red cell fragments on blood film, raised bilirubin, raised lactate dehydrogenase >600IU/L, decreased haptoglobin

[2] Haemolysis (lactate dehydrogenase >/= 600u/L, platelet count < 100 x 10^9/L, aspartate aminotransferase > 60u/L, hemolysis on peripheral blood smear or a raised haptoglobin level.

[3] Clinical signs and symptoms warranting treatment in the presence of oxygen saturations < 90%

[4] Retroplacental clot of > 15% of maternal surface

[5] > 1000mL of blood loss

[6] creatinine >125umol/L or need for dialysis,

syndrome, bronchopulmonary dysplasia[7], sepsis, necrotising enterocolitis, intraventricular haemorrhage (grade III or IV), stage 4 or 5 retinopathy of prematurity, as determined by the treating clinician.

- Other neonatal outcomes that will be assessed independently include; birth weight < 5th percentile and gestation at delivery, admission to NICU, length of stay in NICU.
- Maternal biochemical (anti-angiogenic) markers including sFlt-1, soluble endoglin (sEng), placental growth factor (PlGF) and activin A.
- Placental biochemical markers.
- Maternal blood vessel function.
- Safety and tolerance of broccoli sprout extract.

Sample collection and storage

Samples will be collected at a number of time points (Table 1). All blood (10mL for serum and plasma and 5mL of cord blood) and urine samples (50mL) will be centrifuged at 4 °C and stored on-site at -80 °C. Placental cotyledons will be removed, washed free of blood and either fixed in 10% buffered formalin or frozen in RNAlater (Sigma-Aldrich) until analysis. Adipose tissue will be used within 24 hours for wire myography experiments to assess vascular reactivity. All biomarker investigations will be performed using enzyme linked immunosorbent assay (ELISA) and run in triplicates. Information regarding participant demographics, blood pressure, fetal biometry and results from routine investigations will be collected from patient records. All information will be de-identified and stored on password-protected devices within the institution. Only the research team will have access to the dataset.

[7] Need for oxygen after 28 days of life

Proposed analysis

As this is a superiority trial, participant data will be analysed using intention to treat. All continuous measures will be assessed for normality of distribution and compared using non-parametric or parametric testing where appropriate. Continuous data will described using mean (SD) if normally distributed and median (interquartile range; IQR) when the distribution is skewed. Differences in maternal and pregnancy characteristics will be compared between treatment arms using the appropriate standard statistical techniques to assess the randomisation. Differences in primary and secondary outcomes will be determined using intention to treat analysis. The primary outcome measure of the interval between enrolment and delivery will be presented as a mean time to delivery (days). Differences in time-to-delivery will also be determined using a cox-proportional hazards analysis. Adjustment for any significant differences in baseline characteristics between treatment groups will be performed if appropriate.

Biomarker values will be compared using (linear) mixed regression to account for repeated measures. Mean value will be shown over time. If there is a non-constant interaction between time and the outcome of interest, we will include this parameter in the model and investigate biochemical samples at specific pregnancy time points.

In the initial analysis, correction will only be made for baseline characteristics. Where appropriate, adjustment will be made using regression using a multivariate model.

Adverse events

Though unlikely, there may be unexpected adverse reactions associated with broccoli sprout supplements when used in pregnancy. To date, clinical studies have not demonstrated any serious adverse reactions to broccoli sprout supplements. However, metabolic changes during pregnancy may alter the pharmacological properties in unanticipated ways. A senior obstetrician will monitor participants for the duration of their inpatient admission. The investigator will be contactable by phone at all times. Adverse event (AE) assessment and reporting will be undertaken in line with the requirements of the Sponsor, Monash Health and the National Health Medical Research Council (NHMRC) [18]. All observed or volunteered AE and serious AE (SAE) will be recorded and reported in detail in participant medical records, to the Monash Health Human Ethics Committee and the Sponsor, Monash Health within 24 hours.

Written summaries of the trial status will be submitted to the sponsor, annually, or more frequently, if requested. All participant information and trial records will be securely stored to allow retrieval for audit or review purposes.

Data Safety Monitoring Board (DSMB) reporting

A data safety monitoring board (DSMB) has been established to ensure the safe continuation of this trial by reviewing data on the following:

1) Maternal admission to Intensive Care Unit or Coronary Care Unit.

2) Apgar score <7 at 5 minutes of age requiring active resuscitation (± subsequent admission to the Neonatal Intensive Care Unit).

3) Fetal surveillance outcomes (Doppler studies, CTG, biophysical profile).

4) Maternal or perinatal death.

5) All SAE/AEs submitted to the Sponsor, Monash Health.

The DSMB may request unbinding and will advocate for cessation or re-evaluation of the trial if either arm has a statistically significant or a 50% above baseline increase in any of these outcomes.

Trial discontinuation or modification

The trial will prematurely, permanently, or temporarily cease recruitment if the investigator, or the Sponsor believes that there are important issues pertaining to maternal and/or fetal welfare. Given the progressive nature of preeclampsia, worsening disease will not be considered an indication for discontinuation.

The trial will conclude when:

- 90 participants have been studied, delivered and discharged from Monash Health.
- Data collection and entry is complete and database lock has occurred.
- All data analysis has been performed.
- All necessary reporting has been completed.

There will be no allowance for modification of the trial intervention or protocol after recruitment has commenced unless directed by the DSMB or the HREC.

Un-blinding

Un-blinding in the trial may occur in the following circumstances:

- To make clinical treatment decisions or when an unexpected serious AE occurs and the intervention must be made known. This is called emergency un-blinding.
- During an unmasked analysis in accordance with the trial analysis plan.
- At the request of the Data Safety Monitoring Board.
- At the conclusion of the trial to determine the effect of the intervention.

When all participants (n=90) have completed the trial, all data entry and processing are complete and the database has been locked, the CPI will contact the Clinical Trials Pharmacy and request that un-blinding take place, prior to statistical analysis.

Ethics and dissemination

This trial will be conducted in compliance with all stipulations of this protocol, the conditions of Monash Health HREC approval, and all other relevant local national and international guidelines. Any amendments to the trial conduct, except those necessary to remove an apparent, immediate hazard to the participant, will be submitted, in writing to the Monash Health HREC, for their review and approval, before they are implemented

Data will be published in peer-reviewed journals and presented at conferences, both nationally and internationally. All patient information will be de-identified for the purpose of publication.

Patients and public involvement

Patients were not involved in the design of this trial, establishing the research question or development of recruitment procedures. Participants will be provided with the opportunity to receive the study findings ahead of publication or presentation at

learned meetings.

Discussion

Prolong is a pragmatic superiority trial designed to increase the interval between enrolment and delivery for women with preeclampsia. Here we propose the use of a novel antioxidant to target the oxidative stress underlying preeclampsia. Through this trial, we aim to add to the collective knowledge about novel therapeutics for preeclampsia and, if successful, ultimately establish a new medical intervention that improves outcomes for women with preeclampsia and their babies.

If effective, we believe that adjuvant use of a broccoli sprout extract, or a similar sulforaphane source, will significantly reduce the serious disease burden attributed to preeclampsia. Cheaply and simply reducing the morbidity and mortality associated with disease for both mother and child will have application in both high and low resource settings. However, sample size limitations are inevitable in a phase III trial and we acknowledge that there is a risk of under power and type II error. Therefore, this study was designed to power for only our primary outcome. Future investigation with larger populations and further assessment of short and long-term infant outcomes will be necessary. Similarly, the single centre nature of this trial and subsequent issues in population bias are a limitation of this study that will be addressed in future investigations. Larger trials of the efficacy and clinical application of broccoli sprout extract will be necessary if Prolong produces positive results. We hope that this initial trial will provide sufficient evidence to support and inform future such trials.

Trial status

Current protocol version: 3.0 March 25th 2018.

Date of anticipated enrollment of first participant: June 2019.

Approximate date of recruitment conclusion: May 2022.

List of abbreviations

AE	Adverse event
DSMB	Data safety monitoring board
IQR	Interquartile range
NHMRC	The National Health Medical Research Council
Nrf2	Nuclear related ECH-like related factor 2
PlGF	Placental growth factor
SAE	Serious adverse event
SD	Standard deviation
sEng	Soluble endoglin
sFlt-1	Soluble fms-like tyrosine kinase 1
SOMANZ	Society of Obstetric Medicine of Australia and New Zealand
TNF-α	Tumour necrosis factor alpha

Declarations

Ethics approval and consent to participate: The Monash Health Ethics Committee approved this trial (RES-18-0000-109A) on 2nd March 2018. All participants will

provide written, informed consent before enrolment into this trial.

Consent for publication: Not Applicable.

Availability of data and material: Not applicable. No data are presented in this protocol as this trial is ongoing.

Competing interests: The authors declare that they have no competing interests.

Funding: This project is funded by a NHMRC Program grant to EMW, ID: 111 3902. The funding body had no role in trial design or the writing of the manuscript.

Authors' contributions: All authors were involved in the design of the trial protocol, AGC wrote the manuscript, all authors drafted and have read and approved the final manuscript.

Sponsor: The Sponsor, Monash Health, has no part in the design or running of the clinical trial, nor will they be involved in publication.

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Figure 1. Flow chart indicating participant recruitment, enrollment and sample collection.

Potential participants will be identified from the labour ward and clinic and will be screened for eligibility by the research team. Eligible women will be approached for consent to participate. Where a woman is not eligible or declines to participate, no change will be made to her routine care and she will not be approached again. Consenting participants will be randomised to receive either broccoli sprout extract or placebo a which will be written on the participant drug chart and given as per hospital protocol. Samples will be collected throughout the participant stay in hospital. Initial samples will include maternal blood pressure, maternal bloods (10mL for serum and plasma) and maternal urine (50mL). At 48, 96 hours then weekly until delivery, maternal bloods and urine will be collected. Immediately prior to labour maternal blood will be collected. After delivery, placentae will be collected along with cord blood (5mL). Adipose tissue will be an optional addition for women undergoing caesarean section. Maternal urine sample will also be collected.

Figure 2. Timeline for sample collection.

After eligibility screening by the research team, eligible participants will be consented within 24 hours. Consenting participants will be randomised to receive either broccoli sprout extract or placebo a which will be written on the participant drug chart and given as per hospital protocol. This will be classified as time point 0. Samples will be collected throughout the participant stay in hospital at the beginning of treatment, 48

and 96 hours later and then weekly until and including delivery.

Figure 1. Flow chart indicating participant recruitment, enrollment and sample collection

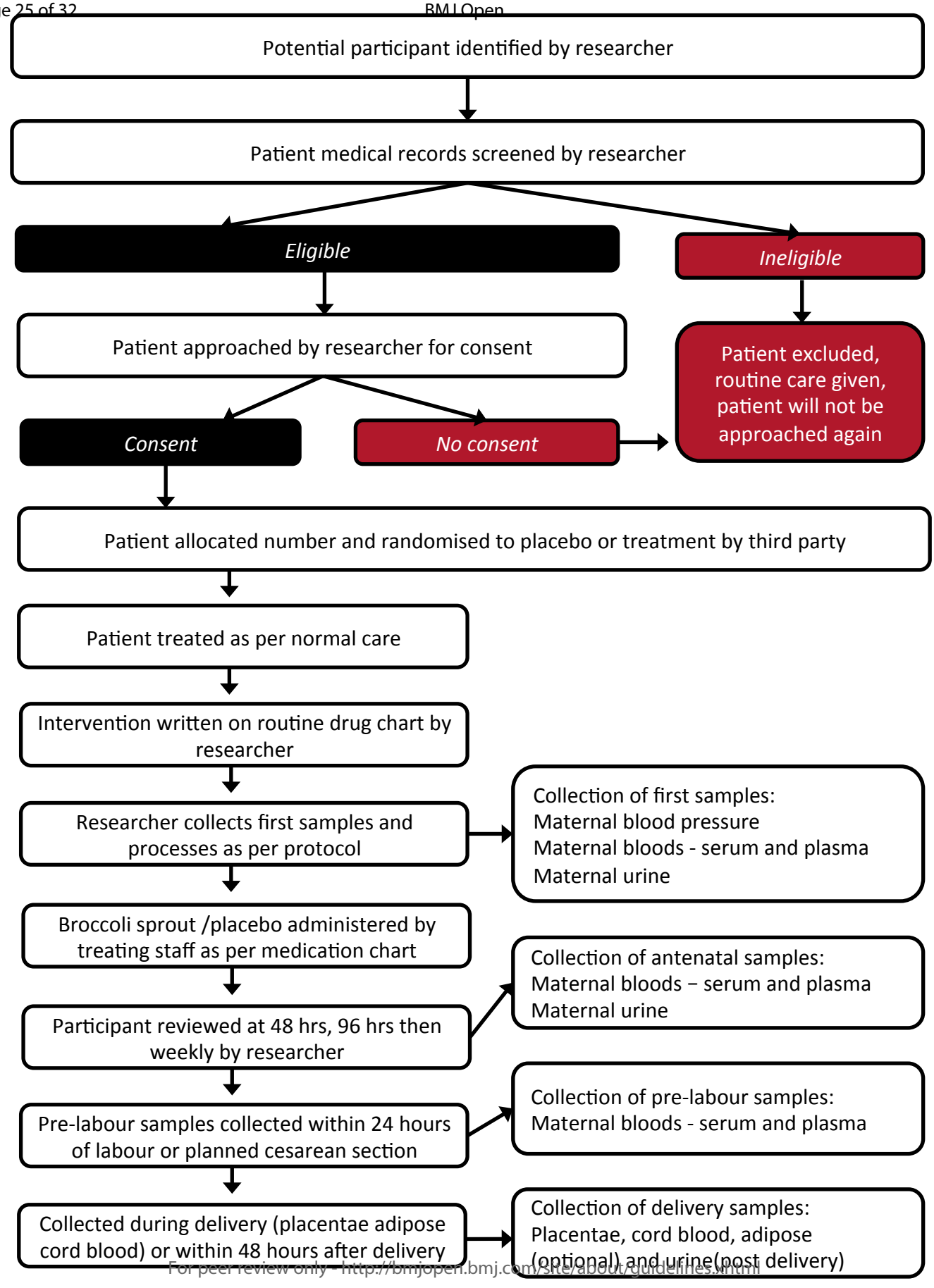

Figure. 2. Timeline for sample collection

	Enrolment	Intervention	Antenatal				
TIMEPOINT	***-24 hours***	**0**	***48 hrs***	***96 hrs***	***Weekly***	***Before delivery***	***After delivery***
	ENROLMENT						
Eligibility screen	X						
Informed consent	X						
Allocation	X						
	INTERVENTION						
Placebo		←	—	—	—	→	
Broccoli Sprout extract		←	—	—	—	→	
	ASSESSMENT						
Blood pressure		X	X	X	X	X	X
Blood sample		X	X	X	X	X	
Urine sample		X	X	X	X		X
Placental sample							X
Adipose tissue							X
Umbilical cord blood							X

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
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	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	18
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	20

	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)	15-16
Introduction			
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	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7-8
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, noninferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
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	8
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)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
	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)	16-17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9

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	_11-13 (timeline table 1)_
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)	___Table1.___
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	___8_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10_____ _

Methods: Assignment of interventions (for controlled trials)

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	____10-11__
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____10_____ _
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____10-11_
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____10-11____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____16-17____

Methods: Data collection, management, and analysis

Data collection methods	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	13-14, Table 1
	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	N/A
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	14
Statistical methods	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
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	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)	14
Methods: Monitoring			
Data monitoring	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	15-16
	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	16-17
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	14-15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14-15

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
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)	17
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19-20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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	N/A
Dissemination policy	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	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__Appendix 2-4_
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	____13_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Prolong: a double-blind randomised placebo-controlled trial of broccoli sprout extract in women with early onset preeclampsia. A clinical trial protocol.

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Title: Prolong: a double-blind randomised placebo-controlled trial of broccoli sprout extract in women with early onset preeclampsia. A clinical trial protocol.

Authors: Annie G. Cox[1], Sarah A. Marshall[1], Kirsten R. Palmer[1,2], Euan M. Wallace[1]

Institutions: [1]The Ritchie Centre, Department of Obstetrics and Gynaecology, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia. [2]Monash Health, Clayton, Victoria, Australia

Emails: AGC: annie.cox@monash.edu, SAM: sarah.marshall@monash.edu, KRP: kirsten.palmer@monash.edu, EMW: euan.wallace@monash.edu.

Corresponding author:

Professor Euan Wallace, Department of Obstetrics and Gynaecology, Monash University, Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, T: +61 (3) 95945145 F: +61 (3) 95945003

Sponsor: Monash Health

Contact information: Dr Kirsten Palmer

Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, T: +61 (3) 95945145 F: +61 (3) 95945003

Abstract

Introduction: Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality. There is a need for adjuvant, targeted therapies to improve outcomes. Broccoli sprout extract, rich in the antioxidant sulforaphane, reduces oxidative stress and placental secretion of the anti-angiogenic factors that contribute to vascular dysfunction in preeclampsia. We propose a phase III trial investigating broccoli sprout extract. We will assess broccoli sprout extract in women with early onset (<34 weeks) preeclampsia, investigating (i) the interval between enrolment and delivery (days), (ii) biomarkers of placental and endothelial function, and (iii) maternal and fetal outcomes.

Methods: A double blind, placebo controlled randomised trial will be conducted at Monash Health, Melbourne, Australia. One hundred and eighty women (45 each arm of each stratum) with early onset preeclampsia (defined as per Society for Obstetric Medicine of Australia and New Zealand (SOMANZ) guidelines) will be recruited. Consenting women will be randomised to receive an oral dose of either broccoli sprout extract (24mg of activated sulforaphane) or identical placebo, twice daily until delivery. Maternal blood will be collected antenatally for measurement of biomarkers of preeclampsia, including soluble fms-like tyrosine kinase-1 (sFlt1), placental growth factor (PlGF), soluble endoglin (sEng) and activin A, as well as circulating sulforaphane metabolites. Maternal and perinatal outcomes will be monitored throughout. All clinical care decisions, including the timing of delivery, will be made by the treating team, blinded to treatment allocation. Participation in this trial will not affect routine care. At delivery, maternal and cord blood and placentae will be collected to measure sulforaphane metabolites and sFlt-1, PlGF, sEng and activin A.

Ethics and dissemination: Approval to conduct the trial has been granted by Monash Health Human Research and Ethics Committee (RES-18-0000-109A). De-identified

data will be published in peer-reviewed journals and presented at learned society conferences, both nationally and internationally.

Discussion: This trial is the first to assess broccoli sprout as an adjuvant therapy for preeclampsia. If successful in safely prolonging pregnancy, this trial will inform the design of larger trials addressing the effect of broccoli sprout extract on perinatal outcomes.

Article summary

Strengths

- Study design is a double blind, randomised, placebo controlled trial.
- Intervention is a naturally occurring nutritional supplement with an excellent safety profile.

Limitations

- Sample size not adequate for secondary outcomes.
- Study participants restricted to women with early-onset preeclampsia

Trial registry: Australian and New Zealand Clinical Trial Registry

ANZCTR registration number: ACTRN12618000216213, registered 9th February 2018.

ANZCTR registration URL:

https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12618000216213

Keywords: Preeclampsia, broccoli sprout, sulforaphane, antioxidant, clinical trial.

Word count: 3362

Introduction

Preeclampsia is defined as new onset hypertension after 20 weeks gestation with associated maternal organ dysfunction and/or fetal growth restriction[1]. It complicates 5-8% of pregnancies and is a leading cause of maternal and perinatal morbidity and mortality worldwide[1]. Even in high resource settings the risk of neonatal mortality is fivefold greater in those born to a mother with preeclampsia compared to those born to a normotensive mother. This increased mortality is largely due to associated fetal growth restriction and the need for premature delivery. Indeed, preeclampsia is the leading cause of iatrogenic premature delivery, implicated in 20% of all premature births[1]. Unfortunately, the incidence of preeclampsia has not changed over the last century and, beyond controlling maternal blood pressure, we continue to lack effective targeted therapies for this serious disorder[1, 2].

Though much remains unknown about the pathological progression of preeclampsia, it is broadly accepted that a placenta, chronically injured by ischaemic-reperfusion insult, releases excessive vasoactive and inflammatory factors into the maternal circulation. In turn, these factors induce systemic maternal endothelial dysfunction[3]. The resulting vasoconstriction and increased vessel permeability cause hypertension, oedema, renal endotheliosis and secondary organ ischaemic injury. For the past fifty years the pharmacological management of preeclampsia has aimed solely to correct the maternal hypertension, allowing safer continuation of the pregnancy in the interests of improving fetal maturity. While the focus on controlling hypertension has improved maternal and perinatal outcomes it has neglected the underlying pathological processes of the disease and limited the potential gains in mitigating fetal risk, particularly in the setting of early onset disease[1]. Seeking to prolong the pregnancy further by targeting the oxidative stress-induced endothelial dysfunction is an additional approach worth exploring.

In particular, inducers of the nuclear factor E2-like related factor 2 (Nrf2) antioxidant pathway offer an attractive approach. Inducing Nrf2 would be expected to have anti-inflammatory and antioxidant effects in both the placenta and in the maternal vasculature. Nuclear factor E2-related factor 2 is an endogenous inducer of cellular antioxidants[4, 5]. Under physiological conditions, bioavailable levels of Nrf2 are regulated by cytosolic binding to kelch-like ECH-associated protein 1 (KEAP-1), preventing rapid proteasome degradation[5]. Exposure to oxidative stress induces cysteine modifications to KEAP-1, loss of binding to Nrf2 and translocation of Nrf2 to the nucleus[4]. Within the nucleus, by combining with small maf-proteins in the promoter region of antioxidant "safeguarding" genes, Nrf2 stimulates antioxidant response elements resulting in the transcription of mRNA for a number of cellular antioxidants and phase two enzymes[4]. Numerous studies have shown therapeutic benefits from Nrf2 stimulation both in maintaining endothelial health and in treating vasculopathies[6].

The Nrf2 inducer sulforaphane is a naturally occurring organosulphur abundant in broccoli sprout extract[7-9] that has attracted attention in cardiovascular and cancer medicine[7, 8]. It stabilises Nrf2 by impairing ubiquitination and increasing Nrf2 phosphorylation, thereby preventing proteasomal degradation and causing cytosolic accumulation[5]. Sulforaphane also induces cytosolic transcription and nuclear translocation of Nrf2. As such, sulforaphane uses the Nrf2 pathways to enhance production of phase two and antioxidant enzymes, improving cellular resilience to oxidative stress[4, 10].

Rationale

Preeclampsia remains a leading cause of maternal and perinatal morbidity and mortality

worldwide[1]. While the introduction of antihypertensives 60 years ago represented a major advance in the care of women with preeclampsia, further progress has all but stalled. Future benefits in maternal and/or perinatal outcomes are likely to come from improved screening and prevention[11] or from more effective treatment, beyond simply managing maternal hypertension[12, 13]. In particular, therapies that target the maternal endothelial dysfunction that underlies the hypertension offer promise in further improving maternal and perinatal outcomes. The antioxidant and anti-inflammatory sulforaphane may be one such therapy. Preliminary data from our group supports a role for sulforaphane in reducing placental production of the anti-angiogenic factors soluble fms-like tyrosine kinas 1 (sFlt-1) and activin A. We have further shown that sulforaphane improves endothelial cell health and function after activation with tumour necrosis factor alpha (TNF-α) and serum from preeclamptic women. Whether sulforaphane has beneficial *in vivo* effects on placental and/or endothelial function in women with early onset preeclampsia remains unexplored. We aim to examine this possibility in our clinical trial, *Prolong*.

Aims and hypothesis

We hypothesise that administration of Broccomax® will significantly increase duration of pregnancy, specifically the interval between diagnosis of preeclampsia and delivery.

The overarching aim of this trial is to assess the utility of a commercial broccoli sprout extract (BroccoMax®) as an adjuvant therapy in the management of women with early onset (<34 weeks) preeclampsia.

Aim 1. To assess whether broccoli sprout extract can safely prolong the interval between enrolment and delivery (recorded in days) in women with early onset (<34 weeks) preeclampsia.

Aim 2. To assess the effects of a broccoli sprout supplement on production of maternal

circulating biomarkers of placental and endothelial health in women with early onset (<34 weeks) preeclampsia.

Aim 3. To assess effects of a broccoli sprout extract on maternal and perinatal outcomes (safety and tolerance) in women with early onset (<34 weeks) preeclampsia.

Methods and analysis

Study design

Double blind, randomised, placebo controlled superiority trial (Figure 1).

Sample size

The size effect on the primary outcome was based on the results of a trial of melatonin as an adjuvant therapy in women with early onset preeclampsia[14, 15]. In that trial, melatonin prolonged the enrolment-to-delivery interval by 6 days, from a mean (SD) of 10.4 (8.3) to 16.4 (11)[15]. Using these data we calculated that 42 women in each treatment group (1:1 ratio) would be sufficient to detect a 6 day difference in mean (two sided comparison) enrolment-to-delivery interval with 80% power. To allow for a 5% attrition rate, we elected a sample size of 45 in each arm, equating to a total of 90 participants. Randomisation for this study will be stratified within two gestation brackets: 24^{+0}–30^{+0}, 30^{+0}–33^{+6}. Because the power analysis was performed based on a study with a single stratum, we elected to have 90 participants in each stratum, requiring a total of 180 participants. This study is powered on the primary outcome of interval between enrolment and delivery, rather than secondary outcomes.

Trial sites

Women will be recruited from Monash Medical Centre and Jessie McPherson Private Hospital, Clayton, Victoria, Australia. Both sites are Level 6 maternity services, as per Victorian government Maternity Capability Framework[16].

Participant inclusion criteria

A woman will be eligible for inclusion in the trial only if the following criteria are met:

- aged 18-45,
- singleton pregnancy,
- diagnosis of preeclampsia, as defined by the SOMANZ guidelines[17],
- gestation between 24^{+0} and 33^{+6} weeks,
- live fetus
- able to safely continue pregnancy for at least 48 hours, as determined by the treating obstetrician,
- no known significant fetal anomaly,
- able to give written, informed consent.

Participant exclusion criteria

A woman will not be eligible for inclusion in this trial if any of the following criteria apply:

- eclampsia,
- current use of broccoli sprout extract supplement,
- contraindications to use of broccoli sprout extract supplement (eg, intolerance of broccoli sprout),
- unknown gestation,
- unwillingness or inability to follow the procedures outlined in the Participant Information and Consent Form,
- mentally, cognitively or legally incapacitated or ineligible to provide informed consent,

- co-recruitment/participation in another clinical trial where there is a pharmaceutical, herbal or nutritional intervention (such trial interventions would also include complementary and alternative medicines).

Participant recruitment

Potential participants will be identified by the research team from the antenatal clinic, Pregnancy Assessment Unit, in-patient wards, and birth suite at Monash Medical Centre. Following discussion with the attending clinical team caring for the woman, eligible women will be approached by a member of the research team who has no involvement in the provision of patient care and provided with the Participant Information and Consent Form for the trial. The research team member will provide a verbal explanation of the trial, including a description of the trial processes, the voluntary nature of the trial, and that a decision to participate, or not, will not affect normal clinical care. No trial related procedures will be performed on any individual without their prior written, informed consent.

Women who provide written and informed consent to participate will be randomised to receive either broccoli sprout extract (BroccoMax®, Jarrow Formulas, Los Angeles, CA) or an identical placebo (Jarrow Formulas). Allocation will be determined by a computer-generated sequence. After recruitment, each participant will be provided with a unique code so as to maintain participant confidentiality.

Randomisation

A randomisation sequence will be generated by a perinatal statistician not involved in the clinical trial, using a computer-generated code. Because the gestation at diagnosis of preeclampsia may influence the duration of the interval between diagnosis and

delivery, randomisation will be stratified within two gestation brackets: 24^{+0}–30^{+0}, 30^{+0}–33^{+6}. Randomisation will be done through block sequence to ensure equivalent sample sizes are allocated to each treatment group (BroccoMax® or placebo)[18].

The randomisation sequence will be provided to the pharmacist who will allocate capsules (BroccoMax® or placebo) to each participant and will dispense the allocated intervention into bottles accordingly. The pharmacist will maintain a record of participant trial identification number and treatment group.

Intervention

Each participant will take three Broccomax® capsules, each containing 8 mg of activated sulforaphane (total of 24mg), twice daily (BD), or three identical placebo capsules twice daily (BD). Both participants and the research team will be blinded to group allocation. Capsules (BroccoMax® or placebo) will be dispensed by the pharmacy in individualised bottles containing sufficient capsules for five days, with additional capsules (amount known only by the research team), and provided to the midwives in charge of ward care. Dosing will be recorded on the patient drug chart and administered as per hospital protocol.

Where participants are discharged home they will record taking the capsules in a Patient Self Administration Diary and return the capsule bottle, including any residual capsules, after 5 days, or sooner if delivered earlier. After delivery, residual capsules will be collected and discarded; they will not be reissued to a participant.

Outcomes

Primary outcome

The interval between enrolment and delivery, recorded in days.

Secondary outcomes

The secondary outcomes will be collected principally as measures of safety and tolerability.

1. Preeclampsia severity, as assessed by: escalation of antihypertensive therapy, systolic and diastolic blood pressures, severe renal involvement (serum or plasma creatinine >90umol/L, oliguria <80mL/4hr), haematological involvement (haemolysis[1], platelets $<10^4$/uL, disseminated intravascular coagulation) liver transanimases >500IU.
2. Indication for delivery (maternal or fetal compromise).
3. Mode of labour and birth (prelabour caesarean section, intrapartum caesarean section, induced or spontaneous labour, spontaneous vaginal birth, assisted vaginal birth).
4. Composite maternal outcome including maternal death, eclampsia, HELLP syndrome[2], pulmonary oedema[3], thromboembolic event (significant deep vein thrombosis or pulmonary embolus), placental abruption[4], major postpartum haemorrhage[5], severe renal impairment[6], liver haematoma or rupture.

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[1] schistocytes or red cell fragments on blood film, raised bilirubin, raised lactate dehydrogenase >600IU/L, decreased haptoglobin

[2] Haemolysis (lactate dehydrogenase >/= 600u/L, platelet count < 100 x 10^9/L, aspartate aminotransferase > 60u/L, hemolysis on peripheral blood smear or a raised haptoglobin level.

[3] Clinical signs and symptoms warranting treatment in the presence of oxygen saturations < 90%

[4] Retroplacental clot of > 15% of maternal surface

5. Intrauterine fetal death (stillbirth).
6. Changes in fetal surveillance (fetal Doppler studies – umbilical or middle cerebral artery PI or abnormal ductus venosus – amniotic fluid volume <5cm, abnormal fetal heart rate on CTG).
7. Birth weight < 5th percentile.
8. Gestation at birth.
9. Composite neonatal outcomes, including neonatal death before hospital discharge, 5 minute APGAR score <7, umbilical lactate >5.0 at birth, admission to the neonatal intensive care unit, diagnosis of respiratory distress syndrome, bronchopulmonary dysplasia[7], sepsis, necrotising enterocolitis, intraventricular haemorrhage (grade III or IV), stage 4 or 5 retinopathy of prematurity, as determined by the treating clinician.
10. Duration of NICU care (days).
11. Maternal serum and placental angiogenic markers sFlt-1, soluble endoglin, placental growth factor and activin A.
12. Maternal TSH and free and total T3/T4 (measured at baseline and after delivery).

Maternal demographics will be sourced from patient medical records. These will include maternal BMI, smoking status, drug and alcohol use, age, parity, maternal comorbidities (thyroid dysfunction, diabetes (gestational Type I or Type II)), and maternal medications.

Additional covariates will include baseline sulforaphane and circulating sFlt-1 and

[5] > 1000mL of blood loss
[6] creatinine >125umol/L or need for dialysis,
[7] Need for oxygen after 28 days of life

PlGF levels. Adjustment will be made in statistical modelling for any significant difference in these covariates between treatment arms.

Sample collection and storage

Samples will be collected at a number of time points (Figure 2). All blood (10mL for serum and plasma and 5mL of cord blood) and urine samples (50mL) will be centrifuged at 4 °C and stored on-site at -80 °C. Placental cotyledons will be removed, washed free of blood and either fixed in 10% buffered formalin or frozen in RNAlater (Sigma-Aldrich) until analysis. All biomarker investigations will be performed using enzyme linked immunosorbent assay (ELISA) and run in triplicates**.** Sulforaphane and its metabolites will be measured in plasma by liquid chromatography mass spectrometry (LC-MS) using an established in-house methodology.

Information regarding participant demographics, blood pressure, fetal biometry and results from routine investigations will be collected from patient records. All information will be de-identified and stored on password-protected devices within the institution. Only the research team will have access to the dataset.

Proposed analysis

This is a superiority trial. Participant data will be analysed using intention to treat. All continuous measures will be assessed for normality of distribution. Differences in the primary outcome, time from enrolment to delivery in days, and secondary outcomes (safety data) will be compared between the two treatment groups. Continuous variables will be compared with a t-test (normally distributed variables) or Mann-Whitney U (non-normally distributed data). Categorical data will be assessed using a chi^2. If possibly, non-parametric data will be transformed to allow parametric comparisons. The interaction between gestation at diagnosis and treatment group will also be assessed

and regression approaches (using either an interaction term or gestation as a covariate) will be used to assess the relationship between treatment arm and time to delivery after assessing assumptions. Survival analysis will also be performed (after assessing assumptions) to account for censoring and survival/failure will be graphed with Kaplan Meir curves. Linear mixed models regression will be used to compare differences in maternal angiogenic markers, TSH and T3/T4 over time between the two treatment groups. If there is a non-constant interaction between time and the outcome of interest, we will include this parameter in the model and investigate biochemical samples at specific pregnancy time points.

In the initial analysis, correction will only be made for baseline characteristics. Where appropriate, adjustment will be made using regression using a multivariate model.
A p value <0.05(two tailed) will be considered statistically significant.

Adverse events

While not expected, there may be unexpected adverse reactions associated with broccoli sprout supplements when used in pregnancy. To date, clinical studies have not demonstrated any serious adverse reactions to broccoli sprout supplements. However, metabolic changes during pregnancy may alter the pharmacological properties in unanticipated ways. A senior obstetrician on the treating team will monitor participants for the duration of their inpatient admission. The investigator will be contactable by phone at all times. Adverse event (AE) assessment and reporting will be undertaken in line with the requirements of the Sponsor, Monash Health and the National Health Medical Research Council (NHMRC)[19]. All observed or volunteered AE and serious AE (SAE) will be recorded and reported in detail in participant medical records, to the

Monash Health Human Ethics Committee and the Sponsor, Monash Health within 24 hours.

Written summaries of the trial status will be submitted to the sponsor, annually, or more frequently, if requested. All participant information and trial records will be securely stored to allow retrieval for audit or review purposes.

Data Safety Monitoring Board (DSMB) reporting

A data safety monitoring board (DSMB) has been established to ensure the safe continuation of this trial by reviewing data on the following:

1) Maternal admission to Intensive Care Unit or Coronary Care Unit.

2) Apgar score <7 at 5 minutes of age requiring active resuscitation (± subsequent admission to the Neonatal Intensive Care Unit).

3) Fetal surveillance outcomes (Doppler studies, CTG, biophysical profile).

4) Maternal or perinatal death.

5) All SAE/AEs submitted to the Sponsor, Monash Health.

The DSMB may request unbinding and will advocate for cessation or re-evaluation of the trial if either arm has a statistically significant or a 50% above baseline increase in any of these outcomes.

Trial discontinuation or modification

The trial will prematurely, permanently, or temporarily cease recruitment if the investigator, or the Sponsor believes that there are important issues pertaining to maternal and/or fetal welfare. Given the progressive nature of preeclampsia, worsening disease will not be considered an indication for discontinuation.

The trial will conclude when:

- All participants (n=180) have been studied, delivered and discharged from Monash Health.
- Data collection and entry is complete and database lock has occurred.
- All data analysis has been performed.
- All necessary reporting has been completed.

There will be no allowance for modification of the trial intervention or protocol after recruitment has commenced unless directed by the DSMB or the HREC.

Un-blinding

Un-blinding in the trial may occur in the following circumstances:

- To make clinical treatment decisions or when an unexpected serious AE occurs and the intervention must be made known. This is called emergency un-blinding.
- During an unmasked analysis in accordance with the trial analysis plan.
- At the request of the Data Safety Monitoring Board.
- At the conclusion of the trial to determine the effect of the intervention.

When all participants (n=180) have completed the trial, all data entry and processing are complete and the database has been locked, the CPI will contact the Clinical Trials Pharmacy and request that un-blinding take place, prior to statistical analysis.

Ethics and dissemination

This trial will be conducted in compliance with all stipulations of this protocol, the conditions of Monash Health HREC approval, and all other relevant local national and

international guidelines. Any amendments to the trial conduct, except those necessary to remove an apparent, immediate hazard to the participant, will be submitted, in writing to the Monash Health HREC, for their review and approval, before they are implemented

Data will be published in peer-reviewed journals and presented at conferences, both nationally and internationally. All patient information will be de-identified for the purpose of publication.

Patients and public involvement

Patients were not involved in the design of this trial, establishing the research question or development of recruitment procedures. Participants will be provided with the opportunity to receive the study findings ahead of publication or presentation at learned meetings.

Discussion

Prolong is a pragmatic superiority trial designed to increase the interval between enrolment and delivery for women with preeclampsia. Here we propose the use of a novel antioxidant to target the oxidative stress underlying preeclampsia. Through this trial, we aim to add to the collective knowledge about novel therapeutics for preeclampsia and, if successful, ultimately establish a new medical intervention that improves outcomes for women with preeclampsia and their babies.

If effective, we believe that adjuvant use of a broccoli sprout extract, or a similar sulforaphane source, will significantly reduce the serious disease burden attributed to preeclampsia. Cheaply and simply reducing the morbidity and mortality associated with disease for both mother and child will have application in both high and low resource

settings. However, sample size limitations are inevitable in a phase III trial and we acknowledge that there is a risk of under power and type II error. Therefore, this study was designed to power for only our primary outcome. Future investigation with larger populations and further assessment of short and long-term infant outcomes will be necessary. Similarly, the single centre nature of this trial and subsequent issues in population bias are a limitation of this study that will be addressed in future investigations. Larger trials of the efficacy and clinical application of broccoli sprout extract will be necessary if Prolong produces positive results. We hope that this initial trial will provide sufficient evidence to support and inform future such trials.

Trial status

Current protocol version: 2.0 March 25th 2018.

Date of anticipated enrollment of first participant: June 2020.

Approximate date of recruitment conclusion: May 2025.

List of abbreviations

AE	Adverse event
DSMB	Data safety monitoring board
IQR	Interquartile range
NHMRC	The National Health Medical Research Council
Nrf2	Nuclear related ECH-like related factor 2
PlGF	Placental growth factor
SAE	Serious adverse event

SD	Standard deviation
sEng	Soluble endoglin
sFlt-1	Soluble fms-like tyrosine kinase 1
SOMANZ	Society of Obstetric Medicine of Australia and New Zealand
TNF-α	Tumour necrosis factor alpha

Declarations

Ethics approval and consent to participate: The Monash Health Ethics Committee approved this trial (RES-18-0000-109A) on 2nd March 2018. All participants will provide written, informed consent before enrolment into this trial.

Consent for publication: Not Applicable.

Availability of data and material: Not applicable. No data are presented in this protocol as this trial is ongoing.

Competing interests: The authors declare that they have no competing interests.

Funding: This work is funded by an NHMRC Program grant to EMW, ID: 111 3902. The funding body had no role in trial design or the writing of the manuscript.

Author contributions: The original concept for this study came from EMW. The trial design was established through discussions between EMW and AGC, with considerable input from SAM and KRP. The manuscript was written by AGC with drafting from SAM, KRP and EMW. All authors have read and approved the final manuscript.

Sponsor: The Sponsor, Monash Health, has no part in the design or running of the clinical trial, nor will they be involved in publication.

Acknowledgements: We would like to acknowledge Joanne Mockler whose input has

been paramount to the application for ethical approval of this trial. We thank the NHMRC for providing funding for this project.

Data sharing

Upon completion and publication of the trial results, de-identified trial data will be made available to others upon reasonable request. Such requests should be made to:

Professor Euan Wallace: euan.wallace@monash.edu

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Figure 1. Flow chart indicating participant recruitment, enrollment and sample collection.

Potential participants will be identified from the labour ward and clinic and will be screened for eligibility by the research team. Eligible women will be approached for

consent to participate. Where a woman is not eligible or declines to participate, no change will be made to her routine care and she will not be approached again. Consenting participants will be randomised to receive either broccoli sprout extract or placebo a which will be written on the participant drug chart and given as per hospital protocol. Samples will be collected throughout the participant stay in hospital. Initial samples will include maternal blood pressure, maternal bloods (10mL for serum and plasma) and maternal urine (50mL). At 48, 96 hours then weekly until delivery, maternal bloods and urine will be collected and blood pressure recorded. Immediately prior to labour maternal blood will be collected. After delivery, placentae will be collected along with cord blood (5mL). Maternal urine sample will also be collected.

Figure 2. Timeline for sample collection.

After eligibility screening by the research team, eligible participants will be consented within 24 hours. Consenting participants will be randomised to receive either broccoli sprout extract or placebo a which will be written on the participant drug chart and given as per hospital protocol. This will be classified as time point 0. Samples will be collected throughout the participant stay in hospital at the beginning of treatment, 48 and 96 hours later and then weekly until and including delivery.

Figure 1. Flow chart indicating participant recruitment, enrollment and sample collection

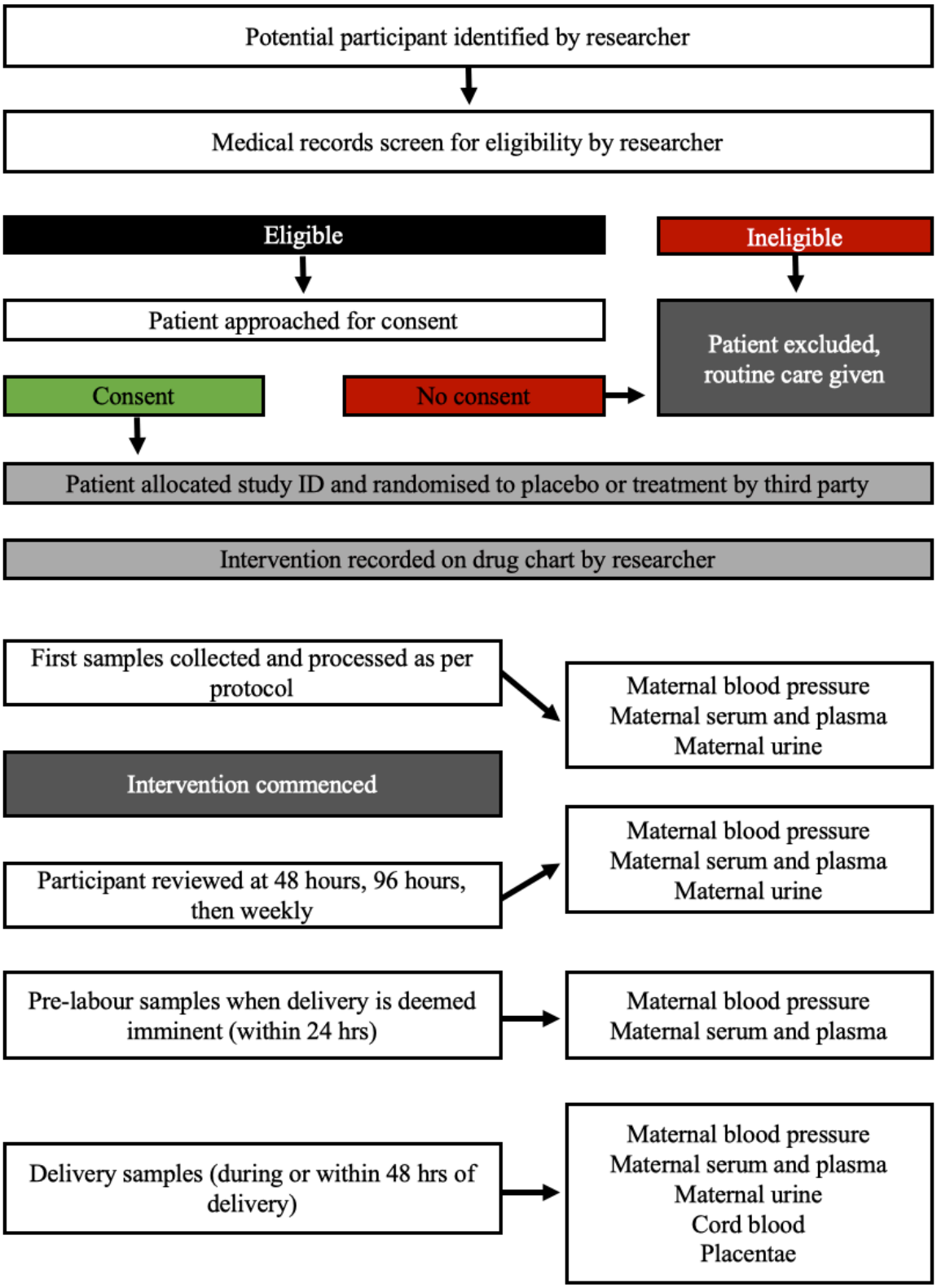

Figure. 2. Timeline for sample collection

	Enrolment	Intervention	Antenatal				
TIMEPOINT	***-24 hours***	**0**	***48 hrs***	***96 hrs***	***Weekly***	***Before delivery***	***After delivery***
	ENROLMENT						
Eligibility screen	X						
Informed consent	X						
Allocation	X						
	INTERVENTION						
Placebo		—	—	—	—	—	
Broccoli Sprout extract		—	—	—	—	—	
	ASSESSMENT						
Blood pressure		X	X	X	X	X	X
Blood sample		X	X	X	X	X	X
Urine sample		X	X	X	X		X
Placental sample							X
Umbilical cord blood							X

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
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	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	18
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	20

	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)	15-16
Introduction			
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	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7-8
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, noninferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
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	8
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)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
	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)	16-17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9

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	_11-13 (timeline table 1)_
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)	___Table1.___
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	___8_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10_____ _

Methods: Assignment of interventions (for controlled trials)

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	____10-11__
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____10_____ _
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____10-11_
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____10-11____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____16-17____

Methods: Data collection, management, and analysis

Data collection methods	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	13-14, Table 1
	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	N/A
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	14
Statistical methods	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
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	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)	14
Methods: Monitoring			
Data monitoring	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	15-16
	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	16-17
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	14-15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14-15

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
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)	17
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19-20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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	N/A
Dissemination policy	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	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__Appendix 2-4_
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	____13_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Prolong: a double-blind randomised placebo-controlled trial of broccoli sprout extract in women with early onset preeclampsia. A clinical trial protocol.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027493.R2
Article Type:	Protocol
Date Submitted by the Author:	20-Aug-2019
Complete List of Authors:	Cox, Annie; Monash University School of Clinical Sciences at Monash Health, Obstetrics and Gynaecology ; Hudson Institute of Medical Research, The Ritchie Centre Marshall, Sarah; Monash University School of Clinical Sciences at Monash Health, Obstetrics and Gynaecology Palmer, Kirsten; Monash University School of Clinical Sciences at Monash Health, Obstetrics and Gynaecology Wallace, Euan; Monash University, Obstetrics and Gynaecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Research methods, Evidence based practice
Keywords:	Maternal medicine < OBSTETRICS, Fetal medicine < OBSTETRICS, Clinical trials < THERAPEUTICS

Title: Prolong: a double-blind randomised placebo-controlled trial of broccoli sprout extract in women with early onset preeclampsia. A clinical trial protocol.

Authors: Annie G. Cox[1], Sarah A. Marshall[1], Kirsten R. Palmer[1,2], Euan M. Wallace[1]

Institutions: [1]The Ritchie Centre, Department of Obstetrics and Gynaecology, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia. [2]Monash Health, Clayton, Victoria, Australia

Emails: AGC: annie.cox@monash.edu, SAM: sarah.marshall@monash.edu, KRP: kirsten.palmer@monash.edu, EMW: euan.wallace@monash.edu.

Corresponding author:

Professor Euan Wallace, Department of Obstetrics and Gynaecology, Monash University, Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, T: +61 (3) 95945145 F: +61 (3) 95945003

Sponsor: Monash Health

Contact information: Dr Kirsten Palmer

Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, T: +61 (3) 95945145 F: +61 (3) 95945003

Abstract

Introduction: Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality. There is a need for adjuvant, targeted therapies to improve outcomes. Broccoli sprout extract, rich in the antioxidant sulforaphane, reduces oxidative stress and placental secretion of the anti-angiogenic factors that contribute to vascular dysfunction in preeclampsia. We propose a phase III trial investigating broccoli sprout extract. We will assess broccoli sprout extract in women with early onset (<34 weeks) preeclampsia, investigating (i) the interval between enrolment and delivery (days), (ii) biomarkers of placental and endothelial function, and (iii) maternal and fetal outcomes.

Methods: A double blind, placebo controlled randomised trial will be conducted at Monash Health, Melbourne, Australia. One hundred and eighty women (45 each arm of each stratum) with early onset preeclampsia (defined as per Society for Obstetric Medicine of Australia and New Zealand (SOMANZ) guidelines) will be recruited. Consenting women will be randomised to receive an oral dose of either broccoli sprout extract (24mg of activated sulforaphane) or identical placebo, twice daily until delivery. Maternal blood will be collected antenatally for measurement of biomarkers of preeclampsia, including soluble fms-like tyrosine kinase-1 (sFlt1), placental growth factor (PlGF), soluble endoglin (sEng) and activin A, as well as circulating sulforaphane metabolites. Maternal and perinatal outcomes will be monitored throughout. All clinical care decisions, including the timing of delivery, will be made by the treating team, blinded to treatment allocation. Participation in this trial will not affect routine care. At delivery, maternal and cord blood and placentae will be collected to measure sulforaphane metabolites and sFlt-1, PlGF, sEng and activin A.

Ethics and dissemination: Approval to conduct the trial has been granted by Monash Health Human Research and Ethics Committee (RES-18-0000-109A). De-identified

data will be published in peer-reviewed journals and presented at learned society conferences, both nationally and internationally.

Article summary

Strengths

- Study design is a double blind, randomised, placebo controlled trial.
- Intervention is a naturally occurring nutritional supplement with an excellent safety profile.

Limitations

- Sample size not adequate for secondary outcomes.
- Study participants restricted to women with early-onset preeclampsia

Trial registry: Australian and New Zealand Clinical Trial Registry

ANZCTR registration number: ACTRN12618000216213, registered 9th February 2018.

ANZCTR registration URL:

https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12618000216213

Keywords: Preeclampsia, broccoli sprout, sulforaphane, antioxidant, clinical trial.

Word count: 3362

Introduction

Preeclampsia is defined as new onset hypertension after 20 weeks gestation with associated maternal organ dysfunction and/or fetal growth restriction[1]. It complicates 5-8% of pregnancies and is a leading cause of maternal and perinatal morbidity and mortality worldwide[1]. Even in high resource settings the risk of neonatal mortality is fivefold greater in those born to a mother with preeclampsia compared to those born to a normotensive mother. This increased mortality is largely due to associated fetal growth restriction and the need for premature delivery. Indeed, preeclampsia is the leading cause of iatrogenic premature delivery, implicated in 20% of all premature births[1]. Unfortunately, the incidence of preeclampsia has not changed over the last century and, beyond controlling maternal blood pressure, we continue to lack effective targeted therapies for this serious disorder[1, 2].

Though much remains unknown about the pathological progression of preeclampsia, it is broadly accepted that a placenta, chronically injured by ischaemic-reperfusion insult, releases excessive vasoactive and inflammatory factors into the maternal circulation. In turn, these factors induce systemic maternal endothelial dysfunction[3]. The resulting vasoconstriction and increased vessel permeability cause hypertension, oedema, renal endotheliosis and secondary organ ischaemic injury. For the past fifty years the pharmacological management of preeclampsia has aimed solely to correct the maternal hypertension, allowing safer continuation of the pregnancy in the interests of improving fetal maturity. While the focus on controlling hypertension has improved maternal and perinatal outcomes it has neglected the underlying pathological processes of the disease and limited the potential gains in mitigating fetal risk, particularly in the setting of early onset disease[1]. Seeking to prolong the pregnancy further by targeting the oxidative stress-induced endothelial dysfunction is an additional approach worth exploring.

In particular, inducers of the nuclear factor E2-like related factor 2 (Nrf2) antioxidant pathway offer an attractive approach. Inducing Nrf2 would be expected to have anti-inflammatory and antioxidant effects in both the placenta and in the maternal vasculature. Nuclear factor E2-related factor 2 is an endogenous inducer of cellular antioxidants[4, 5]. Under physiological conditions, bioavailable levels of Nrf2 are regulated by cytosolic binding to kelch-like ECH-associated protein 1 (KEAP-1), preventing rapid proteasome degradation[5]. Exposure to oxidative stress induces cysteine modifications to KEAP-1, loss of binding to Nrf2 and translocation of Nrf2 to the nucleus[4]. Within the nucleus, by combining with small maf-proteins in the promoter region of antioxidant "safeguarding" genes, Nrf2 stimulates antioxidant response elements resulting in the transcription of mRNA for a number of cellular antioxidants and phase two enzymes[4]. Numerous studies have shown therapeutic benefits from Nrf2 stimulation both in maintaining endothelial health and in treating vasculopathies[6].

The Nrf2 inducer sulforaphane is a naturally occurring organosulphur abundant in broccoli sprout extract[7-9] that has attracted attention in cardiovascular and cancer medicine[7, 8]. It stabilises Nrf2 by impairing ubiquitination and increasing Nrf2 phosphorylation, thereby preventing proteasomal degradation and causing cytosolic accumulation[5]. Sulforaphane also induces cytosolic transcription and nuclear translocation of Nrf2. As such, sulforaphane uses the Nrf2 pathways to enhance production of phase two and antioxidant enzymes, improving cellular resilience to oxidative stress[4, 10].

Rationale

Preeclampsia remains a leading cause of maternal and perinatal morbidity and mortality

worldwide[1]. While the introduction of antihypertensives 60 years ago represented a major advance in the care of women with preeclampsia, further progress has all but stalled. Future benefits in maternal and/or perinatal outcomes are likely to come from improved screening and prevention[11] or from more effective treatment, beyond simply managing maternal hypertension[12, 13]. In particular, therapies that target the maternal endothelial dysfunction that underlies the hypertension offer promise in further improving maternal and perinatal outcomes. The antioxidant and anti-inflammatory sulforaphane may be one such therapy. Preliminary data from our group supports a role for sulforaphane in reducing placental production of the anti-angiogenic factors soluble fms-like tyrosine kinas 1 (sFlt-1) and activin A. We have further shown that sulforaphane improves endothelial cell health and function after activation with tumour necrosis factor alpha (TNF-α) and serum from preeclamptic women. Whether sulforaphane has beneficial *in vivo* effects on placental and/or endothelial function in women with early onset preeclampsia remains unexplored. We aim to examine this possibility in our clinical trial, *Prolong*.

Aims and hypothesis

We hypothesise that administration of Broccomax® will significantly increase duration of pregnancy, specifically the interval between diagnosis of preeclampsia and delivery.

The overarching aim of this trial is to assess the utility of a commercial broccoli sprout extract (BroccoMax®) as an adjuvant therapy in the management of women with early onset (<34 weeks) preeclampsia.

Aim 1. To assess whether broccoli sprout extract can safely prolong the interval between enrolment and delivery (recorded in days) in women with early onset (<34 weeks) preeclampsia.

Aim 2. To assess the effects of a broccoli sprout supplement on production of maternal

circulating biomarkers of placental and endothelial health in women with early onset (<34 weeks) preeclampsia.

Aim 3. To assess effects of a broccoli sprout extract on maternal and perinatal outcomes (safety and tolerance) in women with early onset (<34 weeks) preeclampsia.

Methods and analysis

Study design

Double blind, randomised, placebo controlled superiority trial (Figure 1).

Sample size

The size effect on the primary outcome was based on the results of a trial of melatonin as an adjuvant therapy in women with early onset preeclampsia[14, 15]. In that trial, melatonin prolonged the enrolment-to-delivery interval by 6 days, from a mean (SD) of 10.4 (8.3) to 16.4 (11)[15]. Using these data we calculated that 42 women in each treatment group (1:1 ratio) would be sufficient to detect a 6 day difference in mean (two sided comparison) enrolment-to-delivery interval with 80% power. To allow for a 5% attrition rate, we elected a sample size of 45 in each arm, equating to a total of 90 participants. Randomisation for this study will be stratified within two gestation brackets: 24^{+0}–30^{+0}, 30^{+0}–33^{+6}. Because the power analysis was performed based on a study with a single stratum, we elected to have 90 participants in each stratum, requiring a total of 180 participants. This study is powered on the primary outcome of interval between enrolment and delivery, rather than secondary outcomes.

Trial sites

Women will be recruited from Monash Medical Centre and Jessie McPherson Private Hospital, Clayton, Victoria, Australia. Both sites are Level 6 maternity services, as per Victorian government Maternity Capability Framework[16].

Participant inclusion criteria

A woman will be eligible for inclusion in the trial only if the following criteria are met:

- aged 18-45,
- singleton pregnancy,
- diagnosis of preeclampsia, as defined by the SOMANZ guidelines[17],
- gestation between 24^{+0} and 33^{+6} weeks,
- live fetus
- able to safely continue pregnancy for at least 48 hours, as determined by the treating obstetrician,
- no known significant fetal anomaly,
- able to give written, informed consent.

Participant exclusion criteria

A woman will not be eligible for inclusion in this trial if any of the following criteria apply:

- eclampsia,
- current use of broccoli sprout extract supplement,
- contraindications to use of broccoli sprout extract supplement (eg, intolerance of broccoli sprout),
- unknown gestation,
- unwillingness or inability to follow the procedures outlined in the Participant Information and Consent Form,
- mentally, cognitively or legally incapacitated or ineligible to provide informed consent,

- co-recruitment/participation in another clinical trial where there is a pharmaceutical, herbal or nutritional intervention (such trial interventions would also include complementary and alternative medicines).

Participant recruitment

Potential participants will be identified by the research team from the antenatal clinic, Pregnancy Assessment Unit, in-patient wards, and birth suite at Monash Medical Centre. Following discussion with the attending clinical team caring for the woman, eligible women will be approached by a member of the research team who has no involvement in the provision of patient care and provided with the Participant Information and Consent Form for the trial. The research team member will provide a verbal explanation of the trial, including a description of the trial processes, the voluntary nature of the trial, and that a decision to participate, or not, will not affect normal clinical care. No trial related procedures will be performed on any individual without their prior written, informed consent.

Women who provide written and informed consent to participate will be randomised to receive either broccoli sprout extract (BroccoMax®, Jarrow Formulas, Los Angeles, CA) or an identical placebo (Jarrow Formulas). Allocation will be determined by a computer-generated sequence. After recruitment, each participant will be provided with a unique code so as to maintain participant confidentiality.

Randomisation

A randomisation sequence will be generated by a perinatal statistician not involved in the clinical trial, using a computer-generated code. Because the gestation at diagnosis of preeclampsia may influence the duration of the interval between diagnosis and

delivery, randomisation will be stratified within two gestation brackets: 24^{+0}–30^{+0}, 30^{+0}–33^{+6}. Randomisation will be done through block sequence to ensure equivalent sample sizes are allocated to each treatment group (BroccoMax® or placebo)[18].

The randomisation sequence will be provided to the pharmacist who will allocate capsules (BroccoMax® or placebo) to each participant and will dispense the allocated intervention into bottles accordingly. The pharmacist will maintain a record of participant trial identification number and treatment group.

Intervention

Each participant will take three Broccomax® capsules, each containing 8 mg of activated sulforaphane (total of 24mg), twice daily (BD), or three identical placebo capsules twice daily (BD). Both participants and the research team will be blinded to group allocation. Capsules (BroccoMax® or placebo) will be dispensed by the pharmacy in individualised bottles containing sufficient capsules for five days, with additional capsules (amount known only by the research team), and provided to the midwives in charge of ward care. Dosing will be recorded on the patient drug chart and administered as per hospital protocol.

Where participants are discharged home they will record taking the capsules in a Patient Self Administration Diary and return the capsule bottle, including any residual capsules, after 5 days, or sooner if delivered earlier. After delivery, residual capsules will be collected and discarded; they will not be reissued to a participant.

Outcomes

Primary outcome

The interval between enrolment and delivery, recorded in days.

Secondary outcomes

The secondary outcomes will be collected principally as measures of safety and tolerability.

1. Preeclampsia severity, as assessed by: escalation of antihypertensive therapy, systolic and diastolic blood pressures, severe renal involvement (serum or plasma creatinine >90umol/L, oliguria <80mL/4hr), haematological involvement (haemolysis[1], platelets $<10^4$/uL, disseminated intravascular coagulation) liver transanimases >500IU.
2. Indication for delivery (maternal or fetal compromise).
3. Mode of labour and birth (prelabour caesarean section, intrapartum caesarean section, induced or spontaneous labour, spontaneous vaginal birth, assisted vaginal birth).
4. Composite maternal outcome including maternal death, eclampsia, HELLP syndrome[2], pulmonary oedema[3], thromboembolic event (significant deep vein thrombosis or pulmonary embolus), placental abruption[4], major postpartum haemorrhage[5], severe renal impairment[6], liver haematoma or rupture.

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[1] schistocytes or red cell fragments on blood film, raised bilirubin, raised lactate dehydrogenase >600IU/L, decreased haptoglobin

[2] Haemolysis (lactate dehydrogenase >/= 600u/L, platelet count < 100 x 10^9/L, aspartate aminotransferase > 60u/L, hemolysis on peripheral blood smear or a raised haptoglobin level.

[3] Clinical signs and symptoms warranting treatment in the presence of oxygen saturations < 90%

[4] Retroplacental clot of > 15% of maternal surface

5. Intrauterine fetal death (stillbirth).
6. Changes in fetal surveillance (fetal Doppler studies – umbilical or middle cerebral artery PI or abnormal ductus venosus – amniotic fluid volume <5cm, abnormal fetal heart rate on CTG).
7. Birth weight < 5th percentile.
8. Gestation at birth.
9. Composite neonatal outcomes, including neonatal death before hospital discharge, 5 minute APGAR score <7, umbilical lactate >5.0 at birth, admission to the neonatal intensive care unit, diagnosis of respiratory distress syndrome, bronchopulmonary dysplasia[7], sepsis, necrotising enterocolitis, intraventricular haemorrhage (grade III or IV), stage 4 or 5 retinopathy of prematurity, as determined by the treating clinician.
10. Duration of NICU care (days).
11. Maternal serum and placental angiogenic markers sFlt-1, soluble endoglin, placental growth factor and activin A.
12. Maternal TSH and free and total T3/T4 (measured at baseline and after delivery).

Maternal demographics will be sourced from patient medical records. These will include maternal BMI, smoking status, drug and alcohol use, age, parity, maternal comorbidities (thyroid dysfunction, diabetes (gestational Type I or Type II)), and maternal medications.

Additional covariates will include baseline sulforaphane and circulating sFlt-1 and

[5] > 1000mL of blood loss
[6] creatinine >125umol/L or need for dialysis,
[7] Need for oxygen after 28 days of life

PlGF levels. Adjustment will be made in statistical modelling for any significant difference in these covariates between treatment arms.

Sample collection and storage

Samples will be collected at a number of time points (Figure 2). All blood (10mL for serum and plasma and 5mL of cord blood) and urine samples (50mL) will be centrifuged at 4 °C and stored on-site at -80 °C. Placental cotyledons will be removed, washed free of blood and either fixed in 10% buffered formalin or frozen in RNAlater (Sigma-Aldrich) until analysis. All biomarker investigations will be performed using enzyme linked immunosorbent assay (ELISA) and run in triplicates**.** Sulforaphane and its metabolites will be measured in plasma by liquid chromatography mass spectrometry (LC-MS) using an established in-house methodology.

Information regarding participant demographics, blood pressure, fetal biometry and results from routine investigations will be collected from patient records. All information will be de-identified and stored on password-protected devices within the institution. Only the research team will have access to the dataset.

Proposed analysis

This is a superiority trial. Participant data will be analysed using intention to treat. All continuous measures will be assessed for normality of distribution. Differences in the primary outcome, time from enrolment to delivery in days, and secondary outcomes (safety data) will be compared between the two treatment groups. Continuous variables will be compared with a t-test (normally distributed variables) or Mann-Whitney U (non-normally distributed data). Categorical data will be assessed using a chi^2. If possibly, non-parametric data will be transformed to allow parametric comparisons. The interaction between gestation at diagnosis and treatment group will also be assessed

and regression approaches (using either an interaction term or gestation as a covariate) will be used to assess the relationship between treatment arm and time to delivery after assessing assumptions. Survival analysis will also be performed (after assessing assumptions) to account for censoring and survival/failure will be graphed with Kaplan Meir curves. Linear mixed models regression will be used to compare differences in maternal angiogenic markers, TSH and T3/T4 over time between the two treatment groups. If there is a non-constant interaction between time and the outcome of interest, we will include this parameter in the model and investigate biochemical samples at specific pregnancy time points.

In the initial analysis, correction will only be made for baseline characteristics. Where appropriate, adjustment will be made using regression using a multivariate model.
A p value <0.05(two tailed) will be considered statistically significant.

Adverse events

While not expected, there may be unexpected adverse reactions associated with broccoli sprout supplements when used in pregnancy. To date, clinical studies have not demonstrated any serious adverse reactions to broccoli sprout supplements. However, metabolic changes during pregnancy may alter the pharmacological properties in unanticipated ways. A senior obstetrician on the treating team will monitor participants for the duration of their inpatient admission. The investigator will be contactable by phone at all times. Adverse event (AE) assessment and reporting will be undertaken in line with the requirements of the Sponsor, Monash Health and the National Health Medical Research Council (NHMRC)[19]. All observed or volunteered AE and serious AE (SAE) will be recorded and reported in detail in participant medical records, to the

Monash Health Human Ethics Committee and the Sponsor, Monash Health within 24 hours.

Written summaries of the trial status will be submitted to the sponsor, annually, or more frequently, if requested. All participant information and trial records will be securely stored to allow retrieval for audit or review purposes.

Data Safety Monitoring Board (DSMB) reporting

A data safety monitoring board (DSMB) has been established to ensure the safe continuation of this trial by reviewing data on the following:

1) Maternal admission to Intensive Care Unit or Coronary Care Unit.

2) Apgar score <7 at 5 minutes of age requiring active resuscitation (± subsequent admission to the Neonatal Intensive Care Unit).

3) Fetal surveillance outcomes (Doppler studies, CTG, biophysical profile).

4) Maternal or perinatal death.

5) All SAE/AEs submitted to the Sponsor, Monash Health.

The DSMB may request unbinding and will advocate for cessation or re-evaluation of the trial if either arm has a statistically significant or a 50% above baseline increase in any of these outcomes.

Trial discontinuation or modification

The trial will prematurely, permanently, or temporarily cease recruitment if the investigator, or the Sponsor believes that there are important issues pertaining to maternal and/or fetal welfare. Given the progressive nature of preeclampsia, worsening disease will not be considered an indication for discontinuation.

The trial will conclude when:

- All participants (n=180) have been studied, delivered and discharged from Monash Health.
- Data collection and entry is complete and database lock has occurred.
- All data analysis has been performed.
- All necessary reporting has been completed.

There will be no allowance for modification of the trial intervention or protocol after recruitment has commenced unless directed by the DSMB or the HREC.

Un-blinding

Un-blinding in the trial may occur in the following circumstances:

- To make clinical treatment decisions or when an unexpected serious AE occurs and the intervention must be made known. This is called emergency un-blinding.
- During an unmasked analysis in accordance with the trial analysis plan.
- At the request of the Data Safety Monitoring Board.
- At the conclusion of the trial to determine the effect of the intervention.

When all participants (n=180) have completed the trial, all data entry and processing are complete and the database has been locked, the CPI will contact the Clinical Trials Pharmacy and request that un-blinding take place, prior to statistical analysis.

Ethics and dissemination

This trial will be conducted in compliance with all stipulations of this protocol, the conditions of Monash Health HREC approval, and all other relevant local national and

international guidelines. Any amendments to the trial conduct, except those necessary to remove an apparent, immediate hazard to the participant, will be submitted, in writing to the Monash Health HREC, for their review and approval, before they are implemented

Data will be published in peer-reviewed journals and presented at conferences, both nationally and internationally. All patient information will be de-identified for the purpose of publication.

Patients and public involvement

Patients were not involved in the design of this trial, establishing the research question or development of recruitment procedures. Participants will be provided with the opportunity to receive the study findings ahead of publication or presentation at learned meetings.

Discussion

Prolong is a pragmatic superiority trial designed to increase the interval between enrolment and delivery for women with preeclampsia. Here we propose the use of a novel antioxidant to target the oxidative stress underlying preeclampsia. Through this trial, we aim to add to the collective knowledge about novel therapeutics for preeclampsia and, if successful, ultimately establish a new medical intervention that improves outcomes for women with preeclampsia and their babies.

If effective, we believe that adjuvant use of a broccoli sprout extract, or a similar sulforaphane source, will significantly reduce the serious disease burden attributed to preeclampsia. Cheaply and simply reducing the morbidity and mortality associated with disease for both mother and child will have application in both high and low resource

settings. However, sample size limitations are inevitable in a phase III trial and we acknowledge that there is a risk of under power and type II error. Therefore, this study was designed to power for only our primary outcome. Future investigation with larger populations and further assessment of short and long-term infant outcomes will be necessary. Similarly, the single centre nature of this trial and subsequent issues in population bias are a limitation of this study that will be addressed in future investigations. Larger trials of the efficacy and clinical application of broccoli sprout extract will be necessary if Prolong produces positive results. We hope that this initial trial will provide sufficient evidence to support and inform future such trials.

Trial status

Current protocol version: 2.0 March 25th 2018.

Date of anticipated enrollment of first participant: June 2020.

Approximate date of recruitment conclusion: May 2025.

List of abbreviations

AE	Adverse event
DSMB	Data safety monitoring board
IQR	Interquartile range
NHMRC	The National Health Medical Research Council
Nrf2	Nuclear related ECH-like related factor 2
PlGF	Placental growth factor
SAE	Serious adverse event

SD	Standard deviation
sEng	Soluble endoglin
sFlt-1	Soluble fms-like tyrosine kinase 1
SOMANZ	Society of Obstetric Medicine of Australia and New Zealand
TNF-α	Tumour necrosis factor alpha

Declarations

Ethics approval and consent to participate: The Monash Health Ethics Committee approved this trial (RES-18-0000-109A) on 2nd March 2018. All participants will provide written, informed consent before enrolment into this trial.

Consent for publication: Not Applicable.

Availability of data and material: Not applicable. No data are presented in this protocol as this trial is ongoing.

Competing interests: The authors declare that they have no competing interests.

Funding: This work is funded by an NHMRC Program grant to EMW, ID: 111 3902. The funding body had no role in trial design or the writing of the manuscript.

Author contributions: The original concept for this study came from EMW. The trial design was established through discussions between EMW and AGC, with considerable input from SAM and KRP. The manuscript was written by AGC with drafting from SAM, KRP and EMW. All authors have read and approved the final manuscript.

Sponsor: The Sponsor, Monash Health, has no part in the design or running of the clinical trial, nor will they be involved in publication.

Acknowledgements: We would like to acknowledge Joanne Mockler whose input has

been paramount to the application for ethical approval of this trial. We thank the NHMRC for providing funding for this project.

Data sharing

Upon completion and publication of the trial results, de-identified trial data will be made available to others upon reasonable request. Such requests should be made to:

Professor Euan Wallace: euan.wallace@monash.edu

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Figure 1. Flow chart indicating participant recruitment, enrollment and sample collection.

Potential participants will be identified from the labour ward and clinic and will be screened for eligibility by the research team. Eligible women will be approached for

consent to participate. Where a woman is not eligible or declines to participate, no change will be made to her routine care and she will not be approached again. Consenting participants will be randomised to receive either broccoli sprout extract or placebo a which will be written on the participant drug chart and given as per hospital protocol. Samples will be collected throughout the participant stay in hospital. Initial samples will include maternal blood pressure, maternal bloods (10mL for serum and plasma) and maternal urine (50mL). At 48, 96 hours then weekly until delivery, maternal bloods and urine will be collected and blood pressure recorded. Immediately prior to labour maternal blood will be collected. After delivery, placentae will be collected along with cord blood (5mL). Maternal urine sample will also be collected.

Figure 2. Timeline for sample collection.

After eligibility screening by the research team, eligible participants will be consented within 24 hours. Consenting participants will be randomised to receive either broccoli sprout extract or placebo a which will be written on the participant drug chart and given as per hospital protocol. This will be classified as time point 0. Samples will be collected throughout the participant stay in hospital at the beginning of treatment, 48 and 96 hours later and then weekly until and including delivery.

Figure 1. Flow chart indicating participant recruitment, enrollment and sample collection

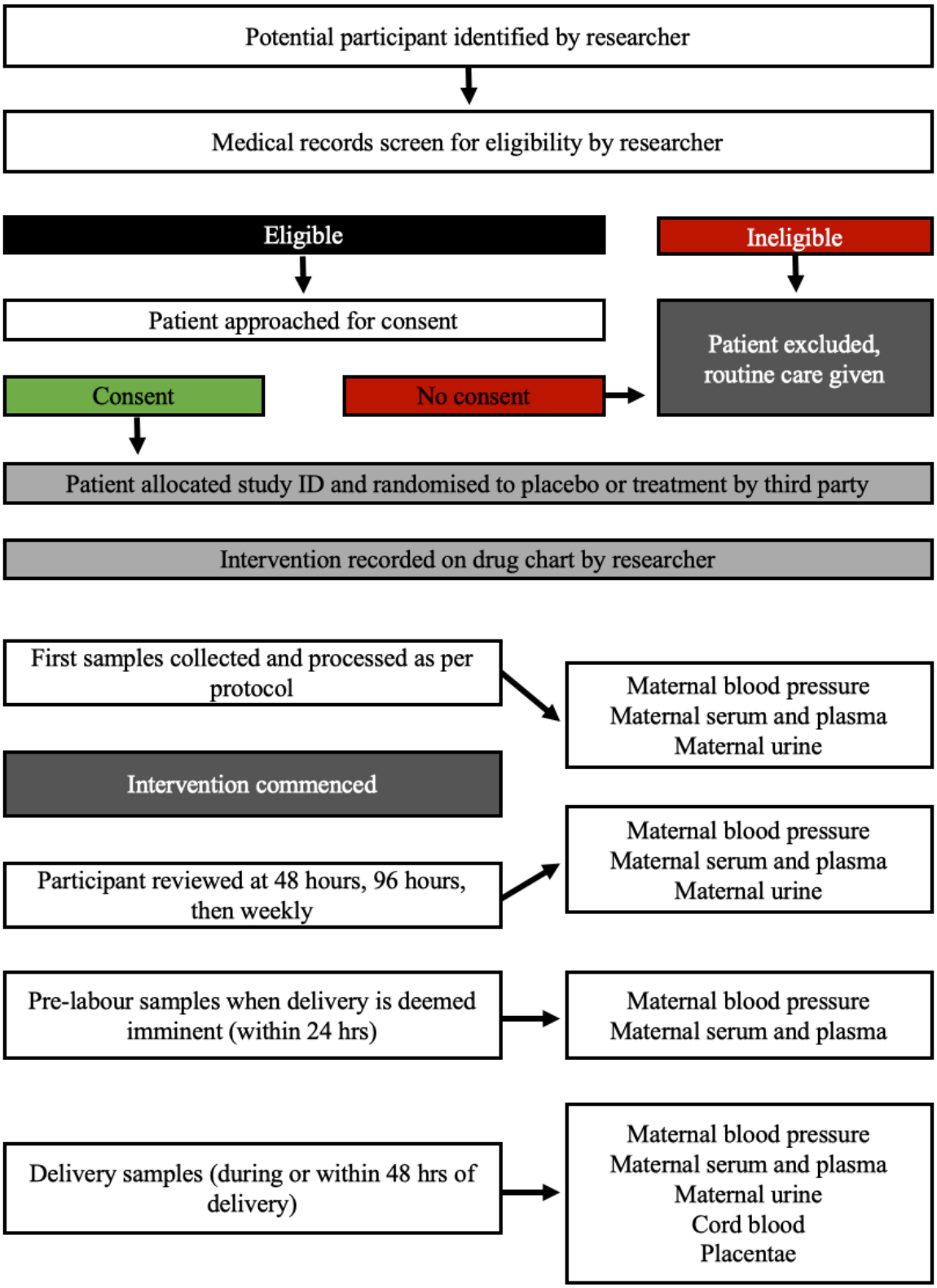

Figure. 2. Timeline for sample collection

	Enrolment	Intervention	Antenatal				
TIMEPOINT	***-24 hours***	**0**	***48 hrs***	***96 hrs***	***Weekly***	***Before delivery***	***After delivery***
	ENROLMENT						
Eligibility screen	X						
Informed consent	X						
Allocation	X						
	INTERVENTION						
Placebo		—	—	—	—	—	
Broccoli Sprout extract		—	—	—	—	—	
	ASSESSMENT						
Blood pressure		X	X	X	X	X	X
Blood sample		X	X	X	X	X	X
Urine sample		X	X	X	X		X
Placental sample							X
Umbilical cord blood							X

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
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	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	18
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	20

	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)	15-16
Introduction			
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	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7-8
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, noninferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
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	8
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)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
	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)	16-17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9

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	_11-13 (timeline table 1)_
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)	___Table1.___
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	___8_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10_____ _

Methods: Assignment of interventions (for controlled trials)

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	____10-11__
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____10_____ _
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____10-11_
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____10-11____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____16-17____

Methods: Data collection, management, and analysis

Data collection methods	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	13-14, Table 1
	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	N/A
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	14
Statistical methods	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
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
	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)	14
Methods: Monitoring			
Data monitoring	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	15-16
	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	16-17
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	14-15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14-15

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
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)	17
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19-20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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	N/A
Dissemination policy	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	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__Appendix 2-4_
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	____13_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.