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A double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): study protocol

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ABSTRACT

Introduction

Pre-eclampsia is a major complication of pregnancy, globally responsible for 60,000 maternal deaths per year, and far greater numbers of fetal losses. There is no definitive treatment other than delivery. A drug that can quench the disease process could be useful to treat preterm pre-eclampsia, as it could allow these pregnancies to safely continue to a gestation where fetal outcomes are significantly improved. We have generated preclinical data to show esomeprazole, a proton pump inhibitor widely used for gastric-reflux, has potent biological effects making it a promising candidate to treat early onset pre-eclampsia. We propose undertaking a phase II clinical trial to examine whether administering esomeprazole may be effective in treating women with preterm preeclampsia.

Methods and analysis

We propose undertaking a phase II, double blind, randomised controlled trial. We will recruit 120 women with early onset pre-eclampsia (gestational age of 26+0 to 31+6 weeks) who are being managed expectantly. They will be randomised to receive either esomeprazole or an identical placebo daily. The primary outcome will be to examine whether esomeprazole can prolong gestation for a further 5 days, compared to placebo. Secondary outcomes include maternal, fetal and neonatal composite and individual outcomes. Maternal outcomes include maternal death, eclampsia, pulmonary oedema, severe renal impairment, cerebral vascular events and liver haematoma or rupture. Neonatal outcomes include neonatal death within 6 weeks after the due date, intraventricular haemorrhage, necrotizing enterocolitis and bronchopulmonary dysplasia.

We will examine whether esomeprazole can decrease serum sFlt-1 and s-endoglin levels. Finally, we will examine the record the safety of esomeprazole and record adverse events.

Ethics and dissemination

This study has ethical approval (Protocol number M14/09/038 Federal Wide assurance Number 00001372, Institutional Review Board Number IRB0005239) and is registered with NHREC (Application ID 3649). Data will be presented at international conferences and published in peer-reviewed journals.

ARTICLE SUMMARY

Article focus

- Protocol of a phase II clinical trial to examine whether esomeprazole administered to women with preterm preeclampsia can safely prolong gestation, and improve fetal, maternal and neonatal outcomes.

Key Messages

- Pre-eclampsia is a severe complication of pregnancy for which there is no definitive treatment apart from delivery.
- For pre-eclampsia occurring at preterm gestations, a treatment that quenches the disease process could allow pregnancies to safely progress to a gestation where neonatal outcomes are improved.
- Our preclinical studies have suggested esomeprazole may be a candidate therapeutic for pre-eclampsia.

Strengths and limitation of this study

- This is a protocol for a randomised, double blind, placebo controlled clinical trial.
- This is the first trial to assess whether esomeprazole is a treatment option for pre-eclampsia.
- We plan to recruit 120 participants and we have designed this study to be sufficiently powered to identify a prolongation of pregnancy.
- It may be underpowered to show improvements in maternal and perinatal outcomes. Therefore, if the trial yields a positive result, a larger subsequent multi-centre study may be needed.

INTRODUCTION

Pre-eclampsia is one of the most serious complications of pregnancy, affecting 3-8 % of pregnancies worldwide and is a leading cause of maternal and fetal/neonatal morbidity (1-3). Pre-eclampsia is estimated to cause more than 60,000 maternal deaths annually (4). There is no treatment that can quench the disease progression and the only treatment option available to arrest the disease is delivery of the pregnancy (5). For pre-eclampsia occurring at preterm gestations, clinicians are often forced to deliver early on maternal indications to prevent major maternal morbidity, but in doing so, inflict severe prematurity on the fetus. In particular, fetuses delivered at less than 33 weeks' gestation are at significant risk of severe disability including cerebral palsy, stroke (intracerebral bleeding), retinopathy of prematurity, chronic lung disease and death (6,7).

If an affordable and safe treatment was available that could temporise the disease progression of pre-eclampsia, clinicians could safely delay delivery and gain gestation. This could save the lives of many infants and decrease the hospital burden caused by iatrogenic prematurity. Such a treatment would be in keeping with the United Nations Millennium Development Goals to reduce child mortality and improve maternal health (8).

The pre-eclamptic placenta releases anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation. These factors are responsible for causing widespread maternal endothelial dysfunction and organ injury seen in clinical disease (9). In addition, pre-eclampsia is strongly associated with placental and systemic oxidative stress.

Esomeprazole is a proton pump inhibitor widely used to treat women with gastric reflux in pregnancy. Large observational studies have not identified associations with adverse pregnancy outcomes, notably teratogenesis (10-12).

We have performed preclinical laboratory studies where we have identified esomeprazole as a promising candidate therapeutic for pre-eclampsia. We have found that esomeprazole potently decreases sFlt-1 and s-eng release from placental and endothelial cells/tissues (their two main sources), decreases endothelial dysfunction and up-regulates endogenous anti-oxidant defences in the placenta (manuscript reporting this preclinical data submitted, and recently presented at The International Society for the Study of Hypertension in Pregnancy XIX World Congress, New Orleans 2014) (13).

OBJECTIVES

The primary objective is to examine whether a single daily dose of 40 mg of esomeprazole can safely prolong gestation for an additional 5 days in women with early onset pre-eclampsia diagnosed 26+0 – 31+6 weeks who are being managed expectantly, compared to expectant management alone.

The secondary objectives are to determine whether esomeprazole can improve maternal, fetal and neonatal outcomes, and to determine whether esomeprazole can significantly decrease levels of circulating sFlt-1 and/or sEng. Furthermore, we will examine whether esomeprazole is safe and well tolerated in the mother and infant.

METHODS

The full protocol is included as supplementary information (Supplementary information 1).

Study design

Phase II hospital based, double blind, randomised, placebo-controlled trial.

Study population

Pregnant women diagnosed with early onset pre-eclampsia at a gestational age between 26+0 weeks to 31+6 weeks at Tygerberg hospital (Western Cape Provence of South Africa) will be invited to participate. To be enrolled, the treating team needs to have determined after their initial assessment that delivery is unlikely to be required within 48 hours.

Inclusion criteria:

We will recruit women with a singleton pregnancy diagnosed with pre-eclampsia, defined according to the criteria published by The International Society for the Study of Hypertension In Pregnancy (ISSHP) (16).

We will seek to recruit those with pregnancies at a gestational age between 26 + 0 weeks and 31 + 6 weeks, determined by either period dates (if the women is certain of her last menstrual period) or by an early, or mid trimester pregnancy ultrasound. If the gestational age is uncertain, we will recruit participants with an estimated fetal weight between 500 and 1800 grams, determined by ultrasound performed at presentation.

To be eligible for this study the treating clinicians need to have made an initial assessment and deemed that the patient is suitable for expectant management and does not require immediate delivery. A full list of the inclusion criteria is shown in table 1.

Exclusion criteria:

Exclusion criteria include women with established maternal or fetal compromise that necessitates delivery, the current use of a proton pump inhibitor, contraindications to the use of a proton pump inhibitor or the use of medications that interact with proton pump inhibitors. A full list of the exclusion criteria is shown in table 2.

Randomisation and allocation concealment

Randomisation will be done in an equal ratio of esomeprazole to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Both the researchers and participants will be blinded.

The gestational age at diagnosis is likely to affect allowable length of pregnancy prolongation. To ensure treatment group allocation is balanced for this potential variable, we will stratify randomisation into two strata based on gestational age. Strata 1 includes a gestational age of 26 + 0 up to and including 28 + 6 weeks (500 to 1200gm if gestation is unknown). Strata 2 includes a gestational age of 29 + 0 up to and including 31 + 6 weeks (1200 to 1800gm if gestation is unknown). Thus, randomization will include blocking within each gestational age stratum. We propose using blocks of 4 to 6 with the size and order randomly assigned.

Once the participants have been randomised the treatment pack with the same code will be allocated to the participant. All treatment packs will be identical and will contain either active tablets or placebo. The researchers will have no access to the randomisation list. This process will ensure that there is allocation concealment throughout the conduct of the trial.

Participant enrolment

Participants will be identified after they have been admitted to Tygerberg Hospital (tertiary referral centre) with a diagnosis of early onset pre-eclampsia for expectant management. An information leaflet will be given to all potential participants and informed consent will be obtained (Supplementary information 2 and 3). Each participant will be given an individual treatment pack containing either esomeprazole or placebo which will be produced by a contracted manufacturer IDT pharmaceuticals (http://en.idtaus.com.au). Labelling, storage and preparation will be done according to the requirements of the Medicines for Human Use (Clinical Trials) regulations.

Intervention

Participants will be randomised to daily administration of either active tablets containing 40 mg of esomeprazole or an identical placebo tablet orally once a day. Participants will remain under the care of the hospital treating team and the study will not alter or interfere with the care given routinely to women with early onset pre-eclampsia, including on when to deliver.

Routine expectant management for pre-eclampsia

Expectant management for early onset pre-eclampsia involves admission to hospital, and close maternal and fetal surveillance. Maternal surveillance includes four hourly blood pressure measurement, twice daily clinical assessment, daily urinalysis, and twice weekly assessments with blood tests (full blood count, renal function tests and hepatocellular enzymes if HELLP syndrome is suspected) and 24 hour urinary protein measurement on admission. Fetal surveillance includes six-hourly cardiotocography and ultrasound assessments every two weeks (or more frequently if clinically indicated) for Doppler velocimetry of the umbilical artery, middle cerebral artery, ductus venosus, amniotic fluid volume assessment and growth.

All participants will receive two doses of betamethasone 24 hours apart to reduce the risks of neonatal respiratory distress syndrome, intracranial haemorrhage and necrotising enterocolitis. A single repeat dose is usually given one week later. Most participants will be on antihypertensive treatment and the medication used will be documented. All women should already be receiving calcium, iron and folic acid supplementation.

Clinical care will be left up to the discretion of the clinical team. The indication for delivery will be a clinical decision. Indications for delivery may include failure to control blood pressure, the development of major maternal or fetal complications, or intrauterine fetal death. Expectant management will usually end at a gestation of 34 weeks.

Sample size and stratification according to gestation at recruitment

The reported duration that fetuses remained in utero after diagnosis of preterm preeclampsia was a mean of 11 days (standard deviation of 7 days) and a median 9 days (range of 1 to 47 days)(14). This data is derived from a descriptive study on expectant management of early onset pre-eclampsia at Tygerberg Hospital, where we propose to undertake this study (14). For 90% power, with a two-sided alpha set of 0.05, 43 patients are required in each group to identify a gain in gestation of 5 days. Given this data was skewed, the sample size has been multiplied by 1.15 to statistically correct for non-normality (15). This increases the number required to 50 per arm. An additional 10 per arm will be added to allow for drop-outs. Thus, a total of 120 participants will be recruited to provide sufficient power to examine our primary outcome. (Power calculation performed using PASS 12 software. Hintze, J. (2013). PASS 12. NCSS, LLC. Kaysville, Utah, USA)

Sample collection

Blood samples will be routinely collected twice a week. Two sets of specimens will be drawn, the routine pre-eclamptic monitoring samples and the trial samples. The routine blood samples include measurements of the haemoglobin, the platelet count and the urea and creatinine levels. These will be used by the managing clinicians to determine disease severity and may trigger delivery. The second set of samples will be the trial blood samples. These samples will be stored and will only be measured after delivery of the patient. These include measurements of sFlt, sEng, and endothelin 1. These results will not be made available to the managing clinicians and will not affect management. An additional blood sample will be taken 2 hours after taking the trial medication to determine the plasma esomeprazole levels.

Urine samples will be collected two times per week and sent for spot protein: creatinine ratios. 24-hour protein excretion is routinely measured only once on admission but for the purposes of this study we will repeat it weekly. Cord blood and placental samples will be collected at delivery.

Withdrawal from the study

All participants will be informed that they are free to withdraw from the study at any time, and that this will not affect their clinical care. In the event of a withdrawal, we will ask whether we can still collect clinical data and include them in our analyses.

Duration of the trial

It is anticipated that the study can be completed in approximately 4 years (2015 – 2018). This study has been registered with NHREC (South African Human Research Ethics Committee) and PACTR (Pan African Clinical Trials Registry).

Ethical approval and dissemination

This study has ethical approval (Protocol number M14/09/038 Federal Wide assurance Number 00001372, Institutional Review Board Number IRB0005239) and is registered with NHREC (Application ID 3649) and the Pan African Clinical Trials Registry. The South African Medical Control Council approval will also be obtained. Data will be resented at international conferences and published in peer-reviewed journals.

Confidentiality

Patient confidentiality will be protected according to the regulations set forth by Stellenbosch University's Human Research Ethics Committee or Institutional Review Board (IRB).

OUTCOMES

The primary outcome is to examine whether 40mg of daily esomeprazole can prolong a pregnancy complicated by preterm preeclampsia by five days. We believe that a further 5 days would have significant effects and could possibly improve perinatal outcome.

The secondary outcomes include maternal, fetal and neonatal mortality and morbidity, maternal serum biomarkers (including sFlt, sEng and endothelin 1) and placental samples (See tables 3 to 5 for more information regarding secondary outcomes). We will present a composite outcome of the important maternal outcomes which include maternal death, eclampsia, pulmonary oedema, severe renal impairment or the need for dialysis, cerebral vascular event and the development of a liver haematoma or rupture. Other maternal outcomes will be compared as individual outcomes as listed in table 3. We will present a composite outcome for fetal outcomes. This will include poor prognostic signs on ultrasound, significant changes on the cardiotocograph that necessitate delivery, intrauterine fetal demise and growth restriction (see

table 4). The composite outcome for neonatal outcomes will include neonatal death within 6 weeks after the due date, severe intraventricular haemorrhage, necrotizing enterocolitis or bronchopulmonary dysplasia. Individual neonatal outcomes will be compared as listed in table 4. We will measure weekly the biomarkers sFlt, sEng and endothelin 1 which are anti-angiogenic factors likely to play a role in the maternal endothelial dysfunction that is central to the pathophysiology of pre-eclampsia (ie it would be advantageous if treatments can decrease circulating levels of these circulating factors). Blood samples will be taken to determine the esomeprazole level in the maternal serum two hours after the first dose is given. The esomeprazole levels will also be determined in the cord blood to assess placental transfer. The placenta will be examined histologically, and we will quantify mRNA and protein expression of Heme Oxygenase-1 (HO-1) and sFlt-1 and sEng will be measured (see table 5). HO-1 is an endogenous anti-oxidant protein that may be beneficial in pre-eclampsia. We postulate placental HO-1 will be up-regulated by esomeprazole. mRNA will be quantified by quantitative PCR and protein will be quantified by western blot and densiometric analysis of the band obtained on the gel blot.

Data management and statistical analysis

Data will be collected prospectively. Data entry and checking will be continuous and queries will be followed vigorously to ensure clarification without delay.

The analyses will be on an intention-to-treat principle with comparisons made between proton pump inhibitors and placebo, for primary and secondary outcomes. Comparisons will be expressed as relative risks or mean differences with 95% confidence intervals.

The patient characteristics, by treatment group, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution. Significance level is set at 0.05 and all hypothesis testing will be two-sided.

The primary outcome will not be adjusted for multiple comparisons but the secondary outcomes will be adjusted for multiple comparisons using a small number of pre-specified outcomes. Two analyses are planned: 1) primary intention to treat analysis and 2) a treatment received analysis to examine response among those who actually took the tablets. The primary outcome (prolongation in days) between treatment groups will be tested using ANCOVA regression analysis with both treatment group and gestational strata as covariates.

Results will be presented as mean group difference with 95% confidence intervals (95%CI). Standard regression diagnostics and transformation of primary outcome, to achieve adequate distributions of residuals, will be performed if indicated. Survival analyses (i.e. time until delivery), using Cox proportional hazards regression and Kaplan-Meier survivorship curves will be used.

The secondary outcomes may be composite or single outcomes. Continuous variables will be compared using either T-test (for normally distributed variables) or Mann-Whitney U (non-normally distributed). Categorical values will be compared using chi-squared test. For the longitudinal data of plasma sFlt-1, sEng and endothelin 1 levels, we will 1) graph the data longitudinally 2) compare levels between the groups relative to gestational age 3) compare levels between groups relative to days after recruitment and 4) compare levels between groups at delivery. Finally, the placental expression of sFlt-1, sEng and HO-1 will be compared using simple statistics.

Adverse events

Reporting and handling of adverse events and serious adverse will be in accordance with the GCP guidelines (17).

Unblinding

Given the safety profile of esomeprazole in pregnancy has been well documented, we anticipate the need for unblinding will be very uncommon. However, we will have the following procedures in place should unblinding be required. Sheets with lists of 50 randomisation codes each will be sealed in individual, signed, numbered envelopes. If unblinding is requested, the relevant envelope will be opened by a person not involved in the trial in the presence of two witnesses, the group allocation read, and the list re-sealed in an envelope and signed. The randomisation envelopes will be accessible at all times to the principle investigator who will be contactable by mobile telephone. The principal investigator will co-ordinate this process.

Early termination of the trial

If for any reason there is a need to stop the trial prematurely this decision will be taken by the Data and Safety Monitoring committee.

DISCUSSION

Pre-eclampsia is a serious life-threatening condition for both the mother and fetus and is associated with severe maternal and perinatal morbidity. If a treatment were to be discovered, it would have a major impact on both maternal and perinatal health. An ideal drug would ameliorate the biological disease process of pre-eclampsia in the mother and placenta, reduce the risk of serious complications from developing and allow pregnancies that were complicated by the disease at an early gestation to gain gestation without putting the mother and fetus at significantly increased risk.

However, there is currently no treatment besides delivery of fetus and placenta. Furthermore, there have been very few candidate proposed treatments that have reached human trials. Thadhani et al proposed the use of apheresis (or dialysis) to reduce the sFlt-1 fraction in the blood as a means to treat pre-eclampsia (18). That was a case series of eight patients where three had serial apheresis treatments with a possible prolongation of the pregnancy as a result. However, this is perhaps too invasive a treatment to be widely used to treat pre-eclampsia and its usefulness still remains unproven. As a result of preclinical studies, mainly on animal models, the anti-cholesterol drug pravastatin is being evaluated as a possible treatment for pre-eclampsia (STAMP trial, UK based study) (19). We are not aware of other significant trials of orally available small molecules to treat pre-eclampsia.

It is known that the pre-eclamptic placenta releases anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation, causing widespread maternal endothelial dysfunction and organ injury (9). Pre-eclampsia is also associated with oxidative stress. A drug that can decrease sFlt-1 and sEng production, decrease endothelial dysfunction and oxidative stress may be a potential treatment for pre-eclampsia. We have

generated preclinical data suggesting esomeprazole may have such actions. On the basis of this preclinical data, we are now proposing to undertake this phase II randomized clinical trial. We propose recruiting 120 women at Tygerberg Hospital, in the Western Cape Provence of South Africa. There are two advantages to running the trial at this site. South Africa has a very high incidence of pre-eclampsia. Secondly, Tygerberg Hospital is a tertiary referral centre with a neonatal intensive care unit, a maternal critical care unit, an adult intensive care unit and an academic centre that actively contributes to the global scientific literature. Thus, we believe running this trial at Tygerberg Hospital represents a balance between obtaining sufficient number of cases of preterm pre-eclampsia, and offering modern obstetric and perinatal care, making the results potentially generalizable to both developed and developing countries.

Pravastatin is undergoing a randomised clinical trial in women with early onset preeclampsia (STAMP trial) and for women at risk of developing pre-eclampsia (19,20). However, pravastatin has been assigned to pregnancy category X by the Food and Drug Administration (FDA) categorisation system and database for prescribing medicines in pregnancy. The FDA classifies category X drugs in pregnancy as medications where studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits (21). Esomeprazole is classified as a Category C drug in pregnancy by the FDA. Category C is for drugs where animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in human, but potential benefits may warrant use of the drug in pregnant women despite potential risks(21). Esomeprazole may very well then be a safer option than pravastatin for treating pre-eclampsia.

Additional safety information on the use of proton pump inhibitors in pregnancy is also now available as they have been extensively used in pregnancy for the treatment of gastroesophageal conditions. Large population based cohorts and systematic reviews (including administration in the first trimester) have not found any adverse effects in pregnancy and specifically, these studies have not shown any increased risk for congenital abnormalities, spontaneous miscarriage or preterm delivery. The most recent systematic literature review which was published in 2009 included 1530 pregnancies exposed to proton pump inhibitors and had 133,410 non-exposed matched controls (10). The largest study to date, published in 2010, spanned a 13 year period and involved 840,968 pregnancies of which 5082 were exposed to proton pump inhibitors in the first trimester of pregnancy. This study concluded that exposure to proton pump inhibitors, even during the first trimester of pregnancy, was not associated with an increased risk of major birth defects (12). In 2012, a further large study involving 112 022 pregnancies of which 1 186 pregnancies that had been exposed to proton pump inhibitors confirmed there was no associated increase risk for congenital anomalies with proton pump inhibitor use. Importantly this study also found no increase in fetal growth restriction or adverse neonatal outcomes (including premature delivery and low Apgar scores) in pregnancies exposed to proton pump inhibitors in the first, second or third trimesters of pregnancies (11). Thus, esomeprazole is likely to be safe in pregnancy. Furthermore, we will also only be using treatment for a relatively short period in the late second and early third trimesters, well past the time of organogenesis.

We have powered our study to the primary outcome, which is to show esomeprazole can safely prolong gestation for five days. We believe such a gain of gestation in preterm pre-eclampsia is likely to result in significantly better neonatal outcomes. However, a limitation in this trial is that we have not specifically powered to detect improvements in maternal, fetal or neonatal outcomes (although these are planned secondary outcomes). The trial would need to be

significantly larger to detect such clinical improvements. Given esomeprazole has not been used to treat pre-eclampsia before, it seemed more pragmatic to firstly undertake the trial as planned. If this trial yields a positive result, a further phase III multi-centre randomised trial may be required to be sufficiently powered to demonstrate improvements in clinical outcomes.

A further potential novelty of this trial is that we will measure biomarkers that reflect disease severity. We will measure circulating levels of sFlt-1 and sEng in serial samples obtained from the mother. These anti-angiogenic factors are considered to play an important role in inciting maternal endothelial dysfunction and end-organ injury seen in pre-eclampsia. Their importance as biomarkers is highlighted by the fact that in the STAMP trial, the primary outcome is to examine whether pravastatin can decrease circulating sFlt-1 levels in the mother. Furthermore, we will measure expression of key molecules in the placental samples obtained at delivery.

Pre-eclampsia kills mothers, fetuses and neonates and is responsible for severe maternal and neonatal morbidity. This is especially the case in the developing world where there is a lack of resources, including staff, equipment and finances. Currently there is no treatment apart from delivery. If a treatment were to be discovered it would have dramatic effects on maternal and neonatal outcomes. Esomeprazole has shown potential as a therapeutic agent in preclinical work on pre-eclampsia. Further advantages of esomeprazole are that it is available in tablet form, is safe during pregnancy and is not expensive. This makes it an ideal candidate as a global therapeutic for pre-eclampsia. It is therefore imperative for this trial to be performed. If esomeprazole were proven to be effective at prolonging gestation in early onset pre-eclampsia it would be the first treatment option for this group of vulnerable mothers and could play an important role in decreasing the clinical burden of this dangerous condition.

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We would like to acknowledge Prof Jane Norman and Justus Hofmeyr for their contributions to the protocol development.

TABLES

Table 1: Inclusion criteria

A diagnosis of one of the following: Pre-eclampsia Gestational hypertension with evidence of preeclampsia Preexisting hypertension with evidence of pre-eclampsia Unclassified proteinuric hypertension
AND **all of the following is present:** Gestational age between 26 + 0 weeks and 31 + 6 weeks Estimated fetal weight by ultrasound between 500gm and 1800 gm (if gestation is not certain) Singleton pregnancy The managing clinicians have made the assessment to proceed with expectant management and that delivery is not expected within 48 hours The managing clinician and neonatologist believe that the fetus could potentially be delivered in a viable condition No suspicions of a major fetal anomaly or malformation. Patient will be admitted to hospital for expectant management and standardised care

Table 2: Exclusion criteria

Any of the following at the initial assessment:
Patient is unable or unwilling to give consent
Established fetal compromise that necessitates delivery
The presence of: Eclampsia
Severe hypertension
Cerebrovascular event
Posterior reversible encephalopathy syndrome (PRES)
Severe renal impairment
Pulmonary oedema
Left sided heart failure
Disseminated intravascular coagulation
Platelet count < $50x10^9$
Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome
Liver transaminases > 500IU/L
Liver haematoma or rupture
Severe ascites
Current use of a proton pump inhibitor
Contraindications or a hypersensitivity reaction to the use of a proton pump inhibitor
Current use of a drug that may be affected by a proton pump inhibitor:

Table 3: Maternal Secondary Outcomes

Maternal outcomes	
Composite outcome:	Maternal death
	Eclampsia
	Pulmonary oedema
	Severe renal impairment or dialysis
	Cerebral vascular event
	Liver haematoma or rupture
Individual outcomes:	Admission to a high care or intensive care unit
	Posterior reversible encephalopathy syndrome
	Left ventricular failure
	Serum creatinine > 125 µmol/l
	Proteinuria >3g/24h
	Severe hypertension
	Disseminated intravascular coagulation
	Platelet count less than $50x10^9$
	HELLP syndrome
	Liver transaminases >500IU/L
	Placental abruption
	Mode of delivery
	Use of antihypertensive agents
	Major postpartum haemorrhage
	Thromboembolic disease
	Moderate or severe ascites

Table 4: Fetal and neonatal outcomes

Fetal and neonatal outcomes
Fetal composite outcome: Reversed a-wave in the ductus venosus Significant changes in heart rate patterns Intrauterine fetal demise Fetal growth restriction Persistent reversed flow in the umbilical artery Redistribution in the middle cerebral artery
Neonatal composite outcome: Neonatal death within 6 weeks after the due date Grade III or IV intraventricular haemorrhage Necrotizing enterocolitis Bronchopulmonary dysplasia **Neonatal individual outcomes**: APGAR score < 7 at 5 minutes Umbilical artery pH < 7.05 Umbilical artery lactate Surfactant use Neonatal intensive care or special care unit admission Intubation and mechanical ventilation Continuous positive airway pressure (CPAP) support Grade III/IV hyaline membrane disease Length of oxygen treatment Hospital stay Incidence of retinopathy of prematurity Neonatal sepsis

Table 5: Biomarker outcomes

Maternal plasma: Maternal plasma sFlt1 sEng Placental Growth Factor (PGF) Endothelin 1 (these analytes will be measured in serial samples collected during expectant managements)
Placental samples: Heme Oxygenase-1 sFlt1 Vascular Endothelial Growth Factor Placental Growth Factor Hypoxia inducible factor-1 **α** (mRNA and protein levels will be measured in these placental samples taken at delivery)

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

Section/item	Item No	Description	
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	✓
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	✓
	2b	All items from the World Health Organization Trial Registration Data Set	✓
Protocol version	3	Date and version identifier	✓
Funding	4	Sources and types of financial, material, and other support	✓
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	✓
	5b	Name and contact information for the trial sponsor	✓
	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	✓
	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)	✓
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	✓
	6b	Explanation for choice of comparators	✓
Objectives	7	Specific objectives or hypotheses	✓
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)	✓

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	✓
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)	✓
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	✓
	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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	✓
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	✓
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	✓
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)	✓
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	✓
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	✓
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	✓

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	✓
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	✓
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	✓
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	✓
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	✓
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	✓
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	✓
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	✓
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	✓
	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)	✓
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	✓

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	✓
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	✓
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	✓
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	✓
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)	✓
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	✓
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	✓
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	✓
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	✓
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	✓
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	✓
Dissemination policy	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	✓
	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	✓
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	✓

*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

A double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): study protocol

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A double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): study protocol

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ABSTRACT

Introduction

Pre-eclampsia is a major complication of pregnancy, globally responsible for 60,000 maternal deaths per year, and far greater numbers of fetal losses. There is no definitive treatment other than delivery. A drug that can quench the disease process could be useful to treat early onset pre-eclampsia, as it could allow pregnancies to safely continue to a gestation where fetal outcomes are significantly improved. We have generated preclinical data to show esomeprazole, a proton pump inhibitor used for gastric-reflux, has potent biological effects that makes it a worthwhile therapeutic candidate. Esomeprazole potently decreases sFlt-1 and soluble endoglin secretion from placenta and endothelial cells, and has biological actions to mitigate endothelial dysfunction and oxidative stress.

Methods and analysis

We propose undertaking a phase II, double blind, randomised controlled clinical trial to examine whether administering 40mg esomeprazole daily may prolong gestation in women with early onset pre-eclampsia. We will recruit 120 women (gestational age of 26+0 to 31+6 weeks) who will be randomised to receive either esomeprazole or an identical placebo. The primary outcome will be the number of days from randomisation to delivery. Secondary outcomes include maternal, fetal and neonatal composite and individual outcomes. Maternal outcomes include maternal death, eclampsia, pulmonary oedema, severe renal impairment, cerebral vascular events and liver haematoma or rupture. Neonatal outcomes include neonatal death within 6 weeks after the due date, intraventricular haemorrhage, necrotizing enterocolitis and bronchopulmonary dysplasia.

We will examine whether esomeprazole can decrease serum sFlt-1 and s-endoglin levels and we will record the safety of esomeprazole in these pregnancies.

Ethics and dissemination

This study has ethical approval (Protocol version 2.4, M14/09/038, Federal Wide assurance Number 00001372, IRB0005239), and is registered with NHREC (ID 3649) and the Pan African Clinical Trial Registry (PACTR201504000771349). Data will be presented at international conferences and published in peer-reviewed journals.

ARTICLE SUMMARY

Article focus

- Protocol of a phase II clinical trial to examine whether esomeprazole administered to women with preterm pre-eclampsia can safely prolong gestation, and improve fetal, maternal and neonatal outcomes.

Key Messages

- Pre-eclampsia is a severe complication of pregnancy for which there is no definitive treatment apart from delivery.
- For pre-eclampsia occurring at preterm gestations, a treatment that quenches the disease process could allow pregnancies to safely progress to a gestation where neonatal outcomes are improved.
- Our preclinical studies have suggested esomeprazole may be a candidate therapeutic for pre-eclampsia.

Strengths and limitation of this study

- This is a protocol for a randomised, double blind, placebo controlled clinical trial.
- This is the first trial to assess whether esomeprazole is a treatment option for pre-eclampsia.
- We plan to recruit 120 participants and we have designed this study to be sufficiently powered to identify a prolongation of pregnancy.
- It may be underpowered to show improvements in maternal and perinatal outcomes. Therefore, if the trial yields a positive result, a larger subsequent multi-centre study may be needed.

MANUSCRIPT

INTRODUCTION

Pre-eclampsia is one of the most serious complications of pregnancy, affecting 3-8 % of pregnancies worldwide and is a leading cause of maternal and fetal/neonatal morbidity (1-3). Pre-eclampsia is estimated to cause more than 60,000 maternal deaths annually (4). There is no treatment that can quench the disease progression and the only treatment option available to arrest the disease is delivery of the pregnancy (5). For pre-eclampsia occurring at preterm gestations, clinicians are often forced to deliver early on maternal indications to prevent major maternal morbidity, but in doing so, inflict severe prematurity on the fetus. In particular, fetuses delivered at less than 33 weeks' gestation are at significant risk of severe disability including cerebral palsy, stroke (intracerebral bleeding), retinopathy of prematurity, chronic lung disease and death (6,7).

If an affordable and safe treatment was available that could temporise the disease progression of pre-eclampsia, clinicians could safely delay delivery and gain gestation to improve fetal outcome. This could save the lives of many infants and decrease the hospital burden caused by iatrogenic prematurity. Such a treatment would be in keeping with the United Nations Millennium Development Goals to reduce child mortality and improve maternal health (8).

The pre-eclamptic placenta releases anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation. These factors are responsible for causing widespread maternal endothelial dysfunction and organ injury seen in clinical disease (9). In addition, pre-eclampsia is strongly associated with placental and systemic oxidative stress.

Esomeprazole is a proton pump inhibitor widely used to treat women with gastric reflux in pregnancy. Large observational studies including administration during the first, second and third trimesters have not identified associations with adverse pregnancy outcomes, notably teratogenesis (10-12).

We have performed preclinical laboratory studies where we have identified esomeprazole as a promising candidate therapeutic for pre-eclampsia. Esomeprazole potently decreased sFlt-1 and soluble endoglin secretion from placenta and endothelial cells, has strong actions mitigating endothelial dysfunction and has anti-oxidant properties. (A manuscript reporting this preclinical data has been submitted elsewhere and this work was recently presented) (13).

OBJECTIVES

The primary objective is to examine whether a single daily dose of 40 mg of esomeprazole can safely prolong gestation in women with early onset pre-eclampsia diagnosed 26+0 – 31+6 weeks who are being managed expectantly, compared to expectant management alone.

The secondary objectives are to determine whether esomeprazole can improve maternal, fetal and neonatal outcomes, and to determine whether esomeprazole can significantly decrease levels of circulating sFlt-1 and/or sEng. Furthermore, we will examine whether esomeprazole is safe and well tolerated in the mother and infant.

METHODS

The full protocol is included as supplementary information (Supplementary information 1).

Study design

Phase II hospital based, double blind, randomised, placebo-controlled trial.

Study population

Pregnant women diagnosed with early onset pre-eclampsia at a gestational age between 26+0 weeks to 31+6 weeks at Tygerberg hospital (Western Cape Provence of South Africa) will be invited to participate. To be enrolled, the treating team needs to have determined after their initial assessment that delivery is unlikely to be required within 48 hours. A starting point of 26+0 weeks has been chosen as this would be the earliest gestation that Tygerberg Hospital would consider to be viable and are suitable to be offered expectant management.

Inclusion criteria:

We will recruit women with a singleton pregnancy diagnosed with pre-eclampsia, defined according to the criteria published by The International Society for the Study of Hypertension in Pregnancy (ISSHP) (14).

We will seek to recruit those with pregnancies at a gestational age between 26 + 0 weeks and 31 + 6 weeks, determined by either period dates (if the women is certain of her last menstrual period) or by an early, or mid trimester pregnancy ultrasound. If the gestational age is uncertain, we will recruit participants with an estimated fetal weight between 500 and 1800 grams, determined by ultrasound performed at presentation. At Tygerberg Hospital we would not consider a pregnancy to be viable under 500 grams and we would not offer expectant management if the fetal weight was above 1800 grams.

To be eligible for this study the treating clinicians need to have made an initial assessment and deemed that the patient is suitable for expectant management,that the fetus would benefit from expectant management and that immediate delivery is not required. A full list of the inclusion criteria is shown in table 1.

Exclusion criteria:

Exclusion criteria include women with established maternal or fetal compromise that necessitates delivery, the current use of a proton pump inhibitor, contraindications to the use of a proton pump inhibitor or the use of medications that may interact with proton pump inhibitors. A full list of the exclusion criteria is shown in table 2.

Randomisation and allocation concealment

Randomisation will be done in an equal ratio of esomeprazole to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Both the researchers and participants will be blinded.

The gestational age at diagnosis is likely to affect allowable length of pregnancy prolongation. To ensure treatment group allocation is balanced for this potential variable, we will stratify randomisation into two strata based on gestational age. Strata 1 includes a gestational age of 26 + 0 up to and including 28 + 6 weeks (500 to 1200gm if gestation is unknown). Strata 2 includes a gestational age of 29 + 0 up to and including 31 + 6 weeks (1200 to 1800gm if gestation is unknown). Thus, randomization will include blocking within each gestational age stratum. We propose using blocks of 4 to 6 with the size and order randomly assigned.

Once the participants have been randomised the treatment pack with the same code will be allocated to the participant. All treatment packs will be identical and will contain either active tablets or placebo. The researchers will have no access to the randomisation list. This process will ensure that there is allocation concealment throughout the conduct of the trial.

Participant enrolment

Participants will be identified after they have been admitted to Tygerberg Hospital (tertiary referral centre) with a diagnosis of early onset pre-eclampsia for expectant management. An information leaflet will be given to all potential participants and informed consent will be obtained (Supplementary information 2 and 3). Each participant will be given an individual treatment pack containing either esomeprazole or placebo which will be produced by a contracted manufacturer IDT pharmaceuticals (http://en.idtaus.com.au). Labelling, storage and preparation will be done according to the requirements of the Medicines for Human Use (Clinical Trials) regulations.

Intervention

Participants will be randomised to daily administration of either active tablets containing 40 mg of esomeprazole or an identical placebo tablet orally once a day. Participants will remain under the care of the hospital treating team and the study will not alter or interfere with the care given routinely to women with early onset pre-eclampsia, including on when to deliver.

Routine expectant management for pre-eclampsia

Expectant management for early onset pre-eclampsia involves admission to hospital, and close maternal and fetal surveillance. Maternal surveillance includes four hourly blood pressure measurement, twice daily clinical assessment, daily urinalysis, and twice weekly assessments with blood tests (full blood count, renal function tests and hepatocellular enzymes if HELLP syndrome is suspected) and 24 hour urinary protein measurement on admission. Fetal

surveillance includes ultrasound assessments to assess growth of the fetus, the amniotic fluid index and fetal wellbeing including Doppler velocimetry of the umbilical artery, the ductus venosus and the middle cerebral artery. If there are no signs of fetal growth restriction or fetal compromise the ultrasound is repeated two weekly to ensure there has been adequate trajectory of fetal growth. If there are signs of fetal growth restriction or fetal compromise the frequency of ultrasound surveillance will be increased. Six hourly cardiotocographs are performed to assess the ongoing fetal condition. We will follow the Tygerberg Hospital protocols to monitor preterm fetal growth restriction and delivery may occur on fetal grounds if required.

All participants will receive two doses of betamethasone 24 hours apart to reduce the risks of neonatal respiratory distress syndrome, intracranial haemorrhage and necrotising enterocolitis. A single repeat dose is usually given one week later. Most participants will be on antihypertensive treatment with the aim to stabalise the systolic blood pressure between 140 and 150 mmHg and the diastolic blood pressure between 90 and 100 mmHg. The medications used to treat the blood pressure will be documented. All women should already be receiving calcium, iron and folic acid supplementation.

Clinical care will be left up to the discretion of the clinical team. The indication for delivery will be a clinical decision. Indications for delivery may include failure to control blood pressure, the development of major maternal or fetal complications, or intrauterine fetal death. Expectant management will usually end at a gestation of 34 weeks.

Sample size and stratification according to gestation at recruitment

The reported duration that fetuses remain in utero after diagnosis of preterm pre-eclampsia is a mean of 11 days (standard deviation of 7 days) and a median 9 days (range of 1 to 47 days) (15). This data is derived from a descriptive study on expectant management of early onset pre-

eclampsia at Tygerberg Hospital, where we propose to undertake this study (15). For 90% power, with a two-sided alpha set of 0.05, 43 patients are required in each group to identify a gain in gestation of 5 days. Given this data was skewed, the sample size has been multiplied by 1.15 to statistically correct for non-normality (16). This increases the number required to 50 per arm. An additional 10 per arm will be added to allow for drop-outs. Thus, a total of 120 participants will be recruited to provide sufficient power to examine our primary outcome. (Power calculation performed using PASS 12 software. Hintze, J. (2013). PASS 12. NCSS, LLC. Kaysville, Utah, USA)

Sample collection

Blood samples will be routinely collected twice a week. Two sets of specimens will be drawn, the routine pre-eclamptic monitoring samples and the trial samples. The routine blood samples include measurements of the haemoglobin, the platelet count and the urea and creatinine levels. These will be used by the managing clinicians to determine disease severity and may trigger delivery. The second set of samples will be the trial blood samples. These samples will be stored and will only be measured after delivery of the patient. These include measurements of sFlt, sEng, and endothelin 1. These results will not be made available to the managing clinicians and will not affect management.

There is little data available on the pharmacokinetics of esomeprazole in pregnancy. In healthy males the plasma elimination half-life is approximately 1 to 1,5 hours and the peak plasma concentration occurs within 1 to 4 hours after dosing. We propose to perform pharmacokinetic testing on a subgroup to determine if there are differences in the pregnant population. Fifteen patients in each group will undergo pharmacokinetic testing so that blinding and allocation concealment is not affected. Blood will be drawn from an indwelling catheter in a forearm vein at 5 minutes before the medication is given (reference sample) and then at the following dosing

interval: 15, 30 and 45 minutes and then at 1,1,5,2,4,8 and 24 hours after the initial dose is given. The sampling will be repeated on day 5.

Urine samples will be collected two times per week and sent for spot protein: creatinine ratios. 24-hour protein excretion is routinely measured only once on admission but for the purposes of this study we will repeat it weekly. Cord blood and placental samples will be collected at delivery.

Withdrawal from the study

All participants will be informed that they are free to withdraw from the study at any time, and that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry.

Duration of the trial

It is anticipated that the study can be completed in approximately 4 years (2015 – 2018). This study has been registered with NHREC (South African Human Research Ethics Committee) and PACTR (Pan African Clinical Trials Registry).

Ethical approval and dissemination

This study has ethical approval (Protocol number M14/09/038 Federal Wide assurance Number 00001372, Institutional Review Board Number IRB0005239) is registered with NHREC (Application ID 3649) and the Pan African Clinical Trials Registry and has the South African Medical Control Council approval (MCC trial reference 20150309). Data will be presented at international conferences and published in peer-reviewed journals.

Confidentiality

Patient confidentiality will be protected according to the regulations set forth by Stellenbosch University's Human Research Ethics Committee or Institutional Review Board (IRB).

OUTCOMES

The primary outcome is to examine whether 40mg of daily esomeprazole can prolong a pregnancy complicated by early onset pre-eclampsia. We have powered the study for a further 5 day prolongation. This surrogate primary outcome marker has been chosen as it provides a sample size that is feasible and attainable for a phase II trial to test efficacy. Powering the trial to neonatal outcomes would require far greater numbers. If esomeprazole is proven to be effective this trial would then form the basis for a larger multicentre trial powered to detect meaningful improvements in neonatal outcomes.

The secondary outcomes include maternal, fetal and neonatal mortality and morbidity, maternal serum biomarkers (including sFlt, sEng and endothelin 1) and placental samples (See tables 3 to 5 for more information regarding secondary outcomes). We will present a composite outcome of the important maternal outcomes which include maternal death, eclampsia, pulmonary oedema, severe renal impairment or the need for dialysis, cerebral vascular event and the development of a liver haematoma or rupture. Other maternal outcomes will be compared as individual outcomes as listed in table 3. We will present a composite outcome for fetal outcomes. This will include poor prognostic signs on ultrasound, significant changes on the cardiotocograph that necessitate delivery, intrauterine fetal demise and growth restriction (see table 4). The composite outcome for neonatal outcomes will include neonatal death within 6 weeks after the due date, severe intraventricular haemorrhage, necrotizing enterocolitis or bronchopulmonary dysplasia. Individual neonatal outcomes will be compared as listed in table 4. We will measure weekly the biomarkers sFlt, sEng and endothelin 1 which are anti-

angiogenic factors likely to play a role in the maternal endothelial dysfunction that is central to the pathophysiology of pre-eclampsia (ie it would be advantageous if treatments can decrease circulating levels of these circulating factors). Esomeprazole levels will be tested on a selected subgroup to determine if the pharmacokinetics are altered in pregnancy. Esomeprazole levels will also be determined in the cord blood to assess placental transfer. The placenta will be examined histologically, and we will quantify mRNA and protein expression of Heme Oxygenase-1 (HO-1) and sFlt-1 and sEng will be measured (see table 5). HO-1 is an endogenous anti-oxidant protein that may be beneficial in pre-eclampsia. We postulate placental HO-1 will be up-regulated by esomeprazole. mRNA will be quantified by quantitative PCR and protein will be quantified by western blot and densiometric analysis of the band obtained on the gel blot.

Data management and statistical analysis

Data will be collected prospectively. Data entry and checking will be continuous and queries will be followed vigorously to ensure clarification without delay.

The analyses will be on an intention-to-treat principle with comparisons made between proton pump inhibitors and placebo, for primary and secondary outcomes. Comparisons will be expressed as relative risks or mean differences with 95% confidence intervals.

The patient characteristics, by treatment group, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution. Significance level is set at 0.05 and all hypothesis testing will be two-sided.

The primary outcome will not be adjusted for multiple comparisons but the secondary outcomes will be adjusted for multiple comparisons using a small number of pre-specified outcomes. Two analyses are planned: 1) primary intention to treat analysis and 2) a treatment received analysis

to examine response among those who actually took the tablets. The primary outcome (prolongation in days) between treatment groups will be tested using ANCOVA regression analysis with both treatment group and gestational strata as covariates. If a fetus does demise during expectant management we will give it a length of pregnancy prolongation of zero and will include it in the primary outcome analysis.

Results will be presented as mean group difference with 95% confidence intervals (95%CI). Standard regression diagnostics and transformation of primary outcome, to achieve adequate distributions of residuals, will be performed if indicated. Survival analyses (i.e. time until delivery), using Cox proportional hazards regression and Kaplan-Meier survivorship curves will be used.

The secondary outcomes may be composite or single outcomes. Continuous variables will be compared using either T-test (for normally distributed variables) or Mann-Whitney U (non-normally distributed). Categorical values will be compared using chi-squared test. For the longitudinal data of plasma sFlt-1, sEng and endothelin 1 levels, we will 1) graph the data longitudinally 2) compare levels between the groups relative to gestational age 3) compare levels between groups relative to days after recruitment and 4) compare levels between groups at delivery. Finally, the placental expression of sFlt-1, sEng and HO-1 will be compared using simple statistics.

Adverse events

Reporting and handling of adverse events and serious adverse will be in accordance with the GCP guidelines (17).

Unblinding

Given the safety profile of esomeprazole in pregnancy has been well documented, we anticipate the need for unblinding will be very uncommon. However, we will have the following procedures in place should unblinding be required. Sheets with lists of 50 randomisation codes each will be sealed in individual, signed, numbered envelopes. If unblinding is requested, the relevant envelope will be opened by a person not involved in the trial in the presence of two witnesses, the group allocation read, and the list re-sealed in an envelope and signed. The randomisation envelopes will be accessible at all times to the principle investigator who will be contactable by mobile telephone. The principal investigator will co-ordinate this process.

Early termination of the trial

If for any reason there is a need to stop the trial prematurely this decision will be taken by the Data and Safety Monitoring committee.

DISCUSSION

Pre-eclampsia is a serious life-threatening condition for both the mother and fetus and is associated with severe maternal and perinatal morbidity. If a treatment were to be discovered, it would have a major impact on both maternal and perinatal health. An ideal drug would ameliorate the biological disease process of pre-eclampsia in the mother and placenta, reduce the risk of serious complications from developing and allow pregnancies that were complicated by the disease at an early gestation to gain gestation without putting the mother and fetus at significantly increased risk.

However, there is currently no treatment besides delivery of fetus and placenta. Furthermore, there have been very few candidate proposed treatments that have reached human trials. Thadhani et al proposed the use of apheresis (or dialysis) to reduce the sFlt-1 fraction in the blood as a means to treat pre-eclampsia (18). That was a case series of eight patients where three had serial apheresis treatments with a possible prolongation of the pregnancy as a result. However, this is perhaps too invasive a treatment to be widely used to treat pre-eclampsia and its usefulness still remains unproven. As a result of preclinical studies, mainly on animal models, the anti-cholesterol drug pravastatin is being evaluated as a possible treatment for pre-eclampsia (STAMP trial, UK based study) (19). We are not aware of other significant trials of orally available small molecules to treat pre-eclampsia.

It is known that the pre-eclamptic placenta releases anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation, causing widespread maternal endothelial dysfunction and organ injury (9). Pre-eclampsia is also associated with oxidative stress. A drug that can decrease sFlt-1 and sEng production, decrease endothelial dysfunction and oxidative stress may be a potential treatment for pre-eclampsia. We have

generated preclinical data suggesting esomeprazole may have such actions. On the basis of this preclinical data, we are now proposing to undertake this phase II randomized clinical trial. We propose recruiting 120 women at Tygerberg Hospital, in the Western Cape Provence of South Africa. There are two advantages to running the trial at this site. South Africa has a very high incidence of pre-eclampsia. Secondly, Tygerberg Hospital is a tertiary referral centre with a neonatal intensive care unit, a maternal critical care unit, an adult intensive care unit and an academic centre that actively contributes to the global scientific literature. Thus, we believe running this trial at Tygerberg Hospital represents a balance between obtaining sufficient number of cases of preterm pre-eclampsia, and offering modern obstetric and perinatal care, making the results potentially generalizable to both developed and developing countries.

Pravastatin is undergoing a randomised clinical trial in women with early onset preeclampsia (STAMP trial) and for women at risk of developing pre-eclampsia (19,20). However, pravastatin has been assigned to pregnancy category X by the Food and Drug Administration (FDA) categorisation system and database for prescribing medicines in pregnancy. The FDA classifies category X drugs in pregnancy as medications where studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits (21). Esomeprazole is classified as a Category C drug in pregnancy by the FDA. Category C is for drugs where animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in human, but potential benefits may warrant use of the drug in pregnant women despite potential risks(21). Esomeprazole may very well then be a safer option than pravastatin for treating pre-eclampsia.

Additional safety information on the use of proton pump inhibitors in pregnancy is also now available as they have been extensively used in pregnancy for the treatment of gastroesophageal conditions. Large population based cohorts and systematic reviews (including administration in the first trimester) have not found any adverse effects in pregnancy and specifically, these studies have not shown any increased risk for congenital abnormalities, spontaneous miscarriage or preterm delivery. The most recent systematic literature review which was published in 2009 included 1530 pregnancies exposed to proton pump inhibitors and had 133,410 non-exposed matched controls (10). The largest study to date, published in 2010, spanned a 13 year period and involved 840,968 pregnancies of which 5082 were exposed to proton pump inhibitors in the first trimester of pregnancy. This study concluded that exposure to proton pump inhibitors, even during the first trimester of pregnancy, was not associated with an increased risk of major birth defects (12). In 2012, a further large study involving 112 022 pregnancies of which 1 186 pregnancies that had been exposed to proton pump inhibitors confirmed there was no associated increase risk for congenital anomalies with proton pump inhibitor use. Importantly this study also found no increase in fetal growth restriction or adverse neonatal outcomes (including premature delivery and low Apgar scores) in pregnancies exposed to proton pump inhibitors in the first, second or third trimesters of pregnancies (11). Thus, esomeprazole is likely to be safe in pregnancy. Furthermore, we will also only be using treatment for a relatively short period in the late second and early third trimesters, well past the time of organogenesis.

We have powered our study to the primary outcome, which is to show esomeprazole can safely prolong gestation for five days. We believe such a gain of gestation in preterm pre-eclampsia is likely to result in significantly better neonatal outcomes. However, a limitation in this trial is that we have not specifically powered to detect improvements in maternal, fetal or neonatal outcomes (although these are planned secondary outcomes). The trial would need to be

significantly larger to detect such clinical improvements. Given esomeprazole has not been used to treat pre-eclampsia before, it seemed more pragmatic to firstly undertake the trial as planned. If this trial yields a positive result, a further phase III multi-centre randomised trial may be required to be sufficiently powered to demonstrate improvements in clinical outcomes.

A further potential novelty of this trial is that we will measure biomarkers that reflect disease severity. We will measure circulating levels of sFlt-1 and sEng in serial samples obtained from the mother. These anti-angiogenic factors are considered to play an important role in inciting maternal endothelial dysfunction and end-organ injury seen in pre-eclampsia. Their importance as biomarkers is highlighted by the fact that in the STAMP trial, the primary outcome is to examine whether pravastatin can decrease circulating sFlt-1 levels in the mother. Furthermore, we will measure expression of key molecules in the placental samples obtained at delivery.

Pre-eclampsia kills mothers, fetuses and neonates and is responsible for severe maternal and neonatal morbidity. This is especially the case in the developing world where there is a lack of resources, including staff, equipment and finances. Currently there is no treatment apart from delivery. If a treatment were to be discovered it would have dramatic effects on maternal and neonatal outcomes. Esomeprazole has shown potential as a therapeutic agent in preclinical work on pre-eclampsia. Further advantages of esomeprazole are that it is available in tablet form, is safe during pregnancy and is not expensive. This makes it an ideal candidate as a global therapeutic for pre-eclampsia. It is therefore imperative for this trial to be performed. If esomeprazole were proven to be effective at prolonging gestation in early onset pre-eclampsia it would be the first treatment option for this group of vulnerable mothers and could play an important role in decreasing the clinical burden of this dangerous condition.

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CONTRIBUTORSHIP STATEMENT

CC, SW and ST developed and designed the trial.

SW and ST obtained funding for this trial

CC, SW and ST wrote the first draft of this manuscript.

RH helped with the statistical aspects of this protocol.

NH was involved in the design of the laboratory arm of this trial.

All authors were involved in revision of the manuscript.

All authors approved the final version to be submitted.

CC and ST responded to the reviewers comments.

COMPETING INTERESTS

The authors report no competing interests or conflict of interest.

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NHMRC provides salary support to ST and BWM.

DATA SHARING STATEMENT

The full protocol, patient information leaflet and consent forms are available as supplementary files. For further information please contact Dr Catherine Cluver by email: cathycluver@hotmail.com

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TABLES

Table 1: Inclusion criteria

A diagnosis of one of the following: Pre-eclampsia (14) Gestational hypertension with evidence of preeclampsia Preexisting hypertension with evidence of pre-eclampsia Unclassified proteinuric hypertension
AND **all of the following is present:** Gestational age between 26 + 0 weeks and 31 + 6 weeks Estimated fetal weight by ultrasound between 500gm and 1800 gm (if gestation is not certain) Singleton pregnancy The managing clinicians have made the assessment to proceed with expectant management and that delivery is not expected within 48 hours The managing clinician and neonatologist believe that the fetus could potentially be delivered in a viable condition No suspicions of a major fetal anomaly or malformation. Patient will be admitted to hospital for expectant management and standardised care

Table 2: Exclusion criteria

Any of the following at the initial assessment:
Patient is unable or unwilling to give consent
Established fetal compromise that necessitates delivery
The presence of: Eclampsia
Severe hypertension
Cerebrovascular event
Posterior reversible encephalopathy syndrome (PRES)
Severe renal impairment
Pulmonary oedema
Left sided heart failure
Disseminated intravascular coagulation
Platelet count < $50x10^9$
Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome
Liver transaminases > 500IU/L
Liver haematoma or rupture
Severe ascites
Current use of a proton pump inhibitor
Contraindications or a hypersensitivity reaction to the use of a proton pump inhibitor
Current use of a drug that may be affected by a proton pump inhibitor:

Table 3: Maternal Secondary Outcomes

Maternal outcomes	
Composite outcome:	Maternal death
	Eclampsia
	Pulmonary oedema
	Severe renal impairment or dialysis
	Cerebral vascular event
	Liver haematoma or rupture
Individual outcomes:	Admission to a high care or intensive care unit
	Posterior reversible encephalopathy syndrome
	Left ventricular failure
	Serum creatinine > 125 µmol/l
	Proteinuria >3g/24h
	Severe hypertension
	Disseminated intravascular coagulation
	Platelet count less than $50x10^9$
	HELLP syndrome
	Liver transaminases >500IU/L
	Placental abruption
	Mode of delivery
	Use of antihypertensive agents
	Major postpartum haemorrhage
	Thromboembolic disease
	Moderate or severe ascites

Table 4: Fetal and neonatal secondary outcomes

Fetal and neonatal outcomes
Fetal composite outcome: Reversed a-wave in the ductus venosus Significant changes in heart rate patterns Intrauterine fetal demise Fetal growth restriction Persistent reversed flow in the umbilical artery Redistribution in the middle cerebral artery
Neonatal composite outcome: Neonatal death within 6 weeks after the due date Grade III or IV intraventricular haemorrhage Necrotizing enterocolitis Bronchopulmonary dysplasia **Neonatal individual outcomes**: APGAR score < 7 at 5 minutes Umbilical artery pH < 7.05 Umbilical artery lactate Surfactant use Neonatal intensive care or special care unit admission Intubation and mechanical ventilation Continuous positive airway pressure (CPAP) support Grade III/IV hyaline membrane disease Length of oxygen treatment Hospital stay Incidence of retinopathy of prematurity Neonatal sepsis

Table 5: Secondary biomarker outcomes

Maternal plasma: Maternal plasma sFlt1 sEng Placental Growth Factor (PGF) Endothelin 1 (these analytes will be measured in serial samples collected during expectant managements)
Placental samples: Heme Oxygenase-1 sFlt1 Vascular Endothelial Growth Factor Placental Growth Factor Hypoxia inducible factor-1 **α** (mRNA and protein levels will be measured in these placental samples taken at delivery)

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The Pre-eclampsia Intervention with Esomeprazole (PIE) trial: a double blind randomised, placebo-controlled trial to treat early onset pre-eclampsia

Phase II study

CLINICAL TRIAL PROTOCOL

Full title of trial	The Pre-eclampsia Intervention with Esomeprazole (PIE) trial: a double blind randomised, placebo-controlled trial to treat early onset pre-eclampsia.
Short title	PIE trial
Version and date	22 August, 2014 Version 2.4
Ethics approval	Protocol number M14/09/038 Federal Wide Assurance Number 00001372 Institutional Review Board (IRB) Number: IRB0005239
NHREC Application ID	3649
Pan African Clinical Trial Registry ID	PACTR201504000771349
Trial medication	Esomeprazole
Phase of trial	Phase II
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Supervisors/ Co-investigators	Professor Stephen Tong (external) Professor Susan Walker (external) Prof Gerhardus Theron (internal) Prof Ben Mol Prof DR Hall Dr N Hannan Dr H Hiscock
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Protocol Versions

2.4

Signatures

The investigators have discussed this protocol. The investigators agree to perform this trial as set out by the protocol and will only deviate from the protocol in the case of a medical emergency or when the departure is mutually agreed upon in writing by all parties involved.

Principal investigator:
Date:

Abbreviations

CTG	Cardiotocograph
FDA	Food and Drug Administration
GA	Gestational age
HO-1	Heme oxygenase-1
ITT	Intention to treat
ISSHP	International Society for the Study of Hyperensive disorders in Pregnancy
KEAP-1	Kelch-like ECH-associated protein 1
mg	Milligrams
Nrf-2	Nuclear factor (erythroid-derived 2)-like 2
PIE	Pre-eclampsia Intervention with Esomeprazole
PPIs	Proton pump inhibitors
PRES	Posterior reversible encephalopathy syndrome
sEng	Soluble Endoglin
sFlt1	Soluble Fms Like Tyrosine Kinase -1
STAMP	STatins to AMeliorate early onset Pre-eclampsia
TNF	Tumour Necrosis Factor
TR	Treatment received
VCAM-1	Vascular Cell Adhesion Molecule -1

CONTENTS

1. Summary/ Synopsis

Title

The Pre-eclampsia Intervention with Esomeprazole (PIE) trial: a double blind randomised, placebo-controlled trial to treat early onset pre-eclampsia.

Short Introduction and literature overview

Pre-eclampsia is globally responsible for 60,000 maternal deaths per year, and far greater numbers of fetal losses. It is one of the leading causes of maternal mortality in South Africa and a major problem in developing countries. At present there is no treatment for pre-eclampsia apart from delivery which results in severe perinatal morbidity and mortality associated with prematurity. This is especially a problem in developing countries where there is a shortage of neonatal intensive care and high care beds.

Esomeprazole is a proton pump inhibitor widely used for the treatment of gastric acid-related disorders, such as peptic ulcer disease and gastro-oesophageal reflux. Recently preclinical data has been generated showing that the proton pump inhibitor esomeprazole has potent biological effects making it a lead candidate to treat early onset pre-eclampsia.

Esomeprazole:

1) ***decreases the release of soluble endoglin (sEng) and soluble Fms-like Tyrosine Kinase 1 (sFlt-1)*** from both primary placental tissue and primary endothelial cells in vitro. These are anti-angiogenic factors released from the placenta that are thought to play a central role in the pathogenesis of pre-eclampsia.
2) ***upregulates heme oxygenase-1***, a potent 'cytoprotective' molecule in the placenta.
3) **decreases endothelial dysfunction** in in vitro assays.

Esomeprazole is inexpensive and is available in most developing countries. If proven to work it could have a major impact on maternal and perinatal health in developing countries.

Research question

Can a single daily dose of esomeprazole, compared with placebo, prolong gestation for a further 5 days and improve biochemical markers in women with early onset pre-eclampsia managed with expectant management.

Aims

Primary aim

- To examine whether 40 mg of daily esomeprazole can safely further prolong gestation for 5 days in women with early onset pre-eclampsia diagnosed 26+0 –

31+6 weeks, compared to expectant management alone.

Secondary aims

- To determine whether a single daily dose of 40 mg esomeprazole improves 1) maternal, 2) fetal and 3) neonatal outcomes in early onset pre-eclampsia compared to placebo.
- To examine whether a single daily dose of 40 mg esomeprazole can significantly decrease circulating levels of sFlt-1 and/ or sEng in women with early onset pre-eclampsia, compared to placebo.
- To examine whether a single daily dose of 40 mg esomeprazole is safe and well tolerated in the mother, fetus and neonate compared with placebo.

Methods (Overview)

We will perform a double blind randomised controlled trial of 120 women with early onset pre-eclampsia. Informed consent will be obtained. Pregnant women between the ages of 18 and 50 years who present with early onset pre-eclampsia at a gestation of 26+0 to 31+6 weeks at Tygerberg Hospital who are considered stable enough to undergo expectant management will be randomised to either esomeprazole or placebo. They will receive either 40mg of daily esomeprazole or an identical placebo daily. The ongoing management and decision to deliver will be left to the discretion of the treating clinician who will be blinded to treatment group allocation. We will obtain clinical information, including maternal, fetal and neonatal outcomes, clinical investigation results of mother, fetus and neonate, and data on tolerability and safety. Blood samples and urine samples will be collected at enrolment and then twice weekly when routine blood are taken. At delivery we will collect a cord blood sample and placental tissue if consent has been given.

Timeline

4 years (2014 to 2017)

Ethical considerations

Pregnant women are a vulnerable population but early onset pre-eclampsia is a major cause of maternal, fetal and neonatal morbidity and mortality. Esomeprazole has been widely used in pregnancy and may be a treatment for this serious condition. For this reason we believe that it is important to conduct this trial.

Anticipated overall outcome

By the end of this phase II study, we will obtain evidence whether esomeprazole may be able to allow women diagnosed with early onset pre-eclampsia to safely gain gestation and whether it is a possible treatment option.

2.0 Background

2.1 Introduction

Pre-eclampsia is a major disease of pregnancy

Pre-eclampsia is one of the most serious complications of pregnancy affecting 3-8 % of pregnancies worldwide [1,2]. It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit[3]. In its most severe form, it affects the brain, causing seizures (eclampsia), cerebrovascular events and even death.

It is a leading cause of maternal and fetal/neonatal morbidity[4]. Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[5] and in South Africa hypertensive disorders of pregnancy are responsible for 14% of maternal deaths[6]. In the United States it is estimated that for every pre-eclampsia related death there are probably 50-100 other women who experience significant morbidity associated with pre-eclampsia[7].

There is no known treatment for pre-eclampsia apart from delivery

Despite considerable research the only treatment available is termination/delivery of the pregnancy[8]. This poses a difficult clinical dilemma for early onset pre-eclampsia. Clinicians are often forced to deliver early on maternal indications to prevent major maternal morbidity (ie severe maternal organ injury), as there are no treatments to arrest disease progression, but in doing so, inflict severe prematurity on the fetus. In particular, fetuses delivered at less than 33 weeks' gestation are at significant risk of severe disability including cerebral palsy, stroke (intracerebral bleeding), retinopathy of prematurity, chronic lung disease and death[9,10].

Currently, there are trials investigating the possible use of pravastatin to treat pre-eclampsia (STAMP trial, UK based study), and to prevent it (Pravastatin for the Prevention of Pre-eclampsia, run by the MFM network, a US based study). There are no other significant trials of orally available small molecules to treat pre-eclampsia that we are aware of.

Why is it important to find a treatment for pre-eclampsia?

If an affordable and safe treatment was available it could temporise the disease progression of pre-eclampsia thereby delaying delivery to gain gestation. This could save the lives of many infants and decrease the hospital burden caused by iatrogenic prematurity and is in keeping

with the United Nations Development goals to reduce child mortality and improve maternal health.

2.2 Pathogenesis of Pre-eclampsia

Oxidative stress, anti-angiogenic factors and endothelial dysfunction: key steps in the pathogenesis of pre-eclampsia

In normal pregnancy, the placenta implants and invades into the inner third of the myometrium. It remodels the maternal spiral arterioles, stripping them of the contractile smooth muscle and turning them into large non-contractile vessels. The maternal vascular system becomes a high capacitance (i.e. high volume) and low pressure system. This remodelling optimises the amount of maternal blood flow to the placental interface, maximising oxygen and nutrient exchange.

In early pregnancy, the pre-eclamptic placenta fails to correctly implant in the myometrium. There is shallow placental implantation leading to inadequate remodelling of the spiral arterioles. The maternal arterioles become a low capacitance and high pressure system. As a consequence, there is less exchange of oxygen and nutrients and the placenta is rendered chronically hypoxic for the remainder of the pregnancy. Chronic placental hypoxia may induce generalised vasoconstriction in the fetoplacental circulation with increased resistance to umbilical artery blood flow. In addition, the shallow implantation also results in chronic oxidative stress in the placenta[11].

There are other schools of thought that believe the primary insult during the first stage is predominantly oxidative stress and ischaemic/re-perfusion injury rather than hypoxia[12]. Whatever the mechanism of injury, most agree shallow placental implantation is an intrinsic key step to this first stage.

In the second half of the pre-eclamptic pregnancy the persistent oxidative stress and hypoxia provokes the release of soluble Fms Like Tyrosine Kinase -1 (sFlt1)[13] and soluble endoglin (sEng)[14] into the maternal circulation. These are anti-angiogenic factors, released in vastly elevated amounts in pre-eclampsia[13], which cause maternal endothelial dysfunction[8] (injury to maternal vessels) and the end-organ injury seen with clinical disease[3].

Thus, key aspects in the pathophysiology of pre-eclampsia are 1) placental oxidative stress (and hypoxia) 2) placental release of the anti-angiogenic factors sFlt1 and soluble endoglin and 3)

maternal endothelial dysfunction. A drug that can counter these pathological steps could be a strategy to treat pre-eclampsia.

The greatest benefit for a potential therapeutic to treat pre-eclampsia would be one that could be administered to pregnancies diagnosed with early onset pre-eclampsia before 32-33 weeks. It is possible that such a therapeutic could significantly quench the disease process and stabilise the maternal condition. If so, it could allow the pregnancy to safely continue to a gestation where the risks to the fetus are much diminished (e.g. >34 weeks gestation). This could diminish the morbidity rates of many neonates, particularly in developing countries where babies of less than 34 weeks gestation are very vulnerable.

2.3 Biological role of proton pump inhibitors as possible therapy

The proton pump inhibitor esomeprazole: an unexpected drug candidate for pre-eclampsia.

The Translational Obstetrics Group at Melbourne University has generated strong preclinical evidence suggesting esomeprazole may have potent actions giving it significant potential as a treatment for pre-eclampsia (unpublished data).

Esomeprazole counters three key steps in pre-eclampsia pathogenesis, by:

1) **Up-regulating heme oxygenase-1 (HO-1),** a key cytoprotective enzyme with potent **anti-oxidant** actions in cells.

2) **Strongly decreasing the release of antiangiogenic factors sFlt-1 and sEng.**

3) Quenching **endothelial dysfunction.**

2.3.1 Proton pump inhibitors up regulate a key placental protective enzyme: heme-oxygenase-1

Seminal work during the 1970-80s revealed oxidative stress (present both in placenta and maternal vessels) is a key ingredient in the pathogenesis of pre-eclampsia[15]. Heme-oxygenase-1 (HO-1) is a key cellular protection enzyme. It mobilises a number of anti-oxidant defenses and switches on cytoprotective genes[16]. It has been proposed that decreased HO-1 is important in the pathogenesis of pre-eclampsia and leads to increased oxidative stress seen in the disease[17-20]. Many researchers have thus speculated that identifying a drug that up-regulates HO-1 could

be an effective strategy to treat pre-eclampsia[21]. HO-1 has therefore emerged as a key molecule of interest in the field.

The Translational Obstetrics Group has found that proton pump inhibitors (PPIs) potently upregulate HO-1 expression (ie a class effect common to all proton pump inhibitors) See Figure 1.

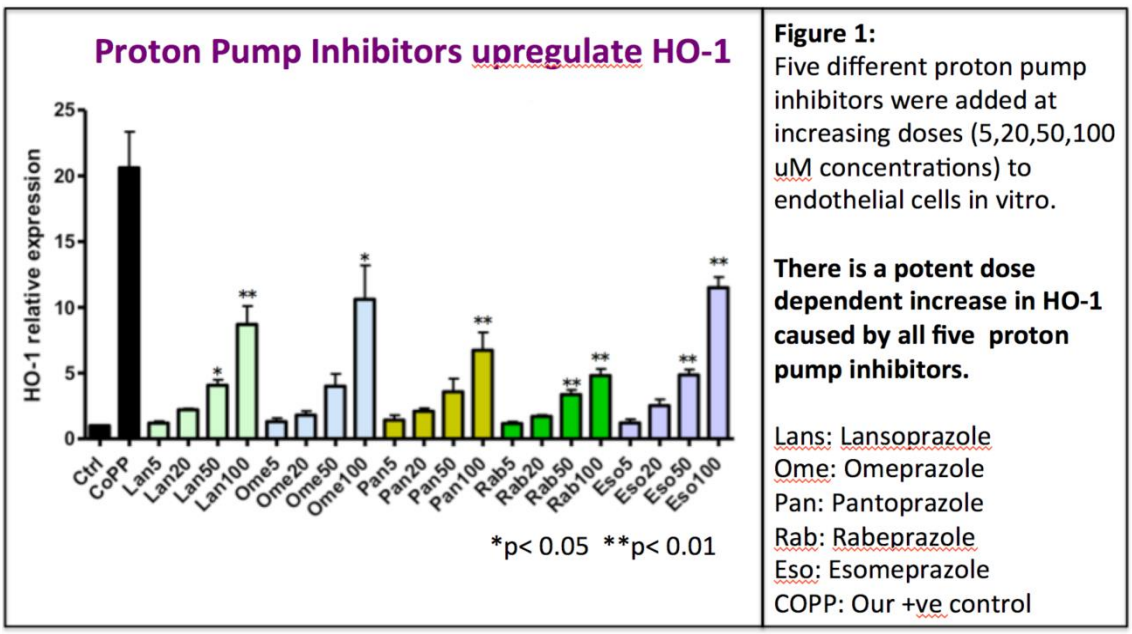

Figure 1: Proton Pump Inhibitors upregulate HO-1

Specifically, esomeprazole was found to have extremely potent effects in inducing HO-1. Esomeprazole induced HO-1 by **11.5 fold** in endothelial (primary HUVEC) cells and **3.9 fold** in purified primary trophoblast cells. See Figure 2.

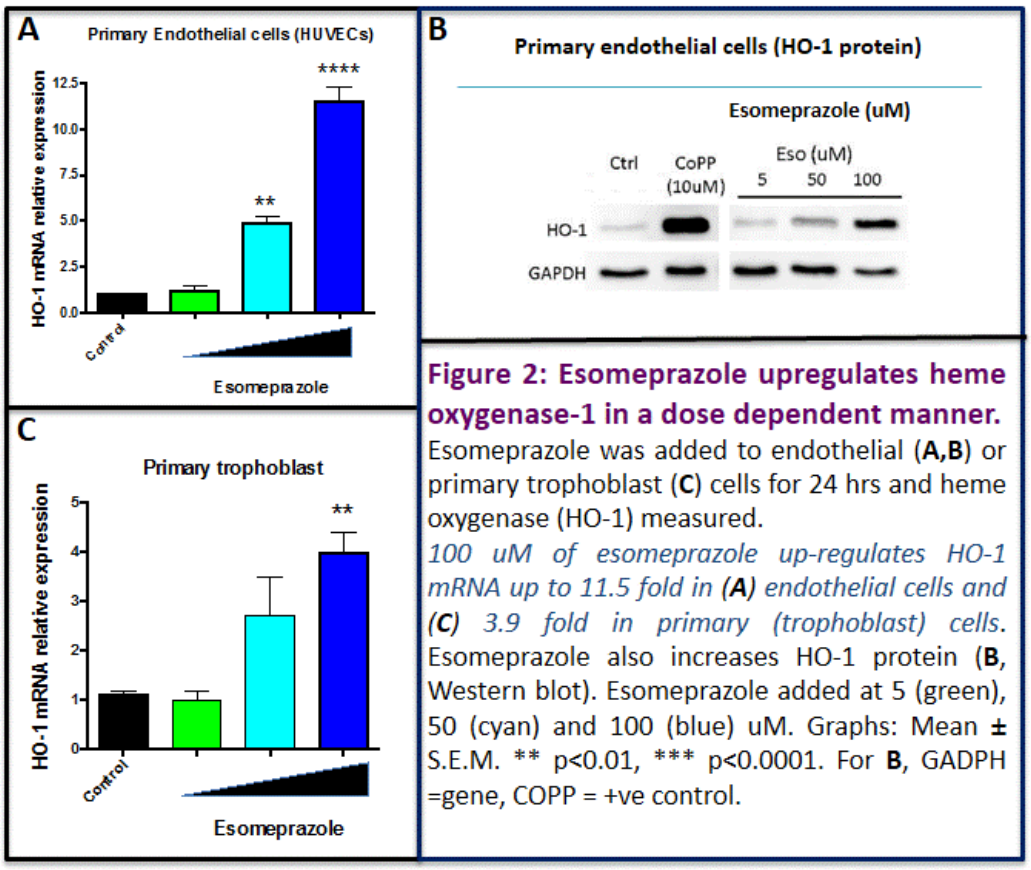

Figure 2: Esomeprazole upregulates heme oxygenase-1 in a dose dependent manner.
Esomeprazole was added to endothelial (**A,B**) or primary trophoblast (**C**) cells for 24 hrs and heme oxygenase (HO-1) measured.
*100 uM of esomeprazole up-regulates HO-1 mRNA up to 11.5 fold in (**A**) endothelial cells and (**C**) 3.9 fold in primary (trophoblast) cells.*
Esomeprazole also increases HO-1 protein (**B**, Western blot). Esomeprazole added at 5 (green), 50 (cyan) and 100 (blue) uM. Graphs: Mean ± S.E.M. ** p<0.01, *** p<0.0001. For **B**, GADPH =gene, COPP = +ve control.

Figure 2: Esomeprazole upregulates heme oxygenase-1 in a dose dependent manner

Thus, esomeprazole potently induces the anti-oxidant enzyme HO-1 in both primary human endothelial and trophoblast (placental) cells. This makes it an exciting candidate drug therapy for pre-eclampsia.

Our current hypothesis is that proton pump inhibitors up-regulate HO-1 by increasing the levels of nuclear factor erythroid-derived 2-like 2 (NRF-2). NRF-2 is a transcription factor upstream of HO-1. Once activated, NRF-2 enters the nucleus and activates a host of genes that have anti-oxidant actions, especially HO-1, that up-regulate genes in the oxidative phosphorylation pathway (beneficial to counteract hypoxia). The Translational Obstetrics Group has shown that PPIs induce NRF-2 translocation into the nucleus (data not shown).

NRF-2 is usually bound by Kelch-like ECH-associated protein 1 (KEAP-1) and targeted for degradation. KEAP-1 is a molecular sensor of oxidative stress and also senses other molecules including drugs. While not yet proven in the laboratory, our hypothesis is that KEAP-1 senses PPIs which induce a structural change in KEAP-1. In the new confirmation state, KEAP-1 is less able to bind and degrade NRF-2. NRF-2, free from KEAP-1, is then able to enter the cells and up-regulate anti-oxidant genes, including HO-1.

2.3.2 Proton pump inhibitors block the release of sFLt-1 and soluble endoglin (sEng)

A major advance in the field of pre-eclampsia was made with the identification of sFlt-1 and sEng as the likely 'toxins' released from the placenta causing the severe maternal organ end-injury seen in pre-eclampsia[20]. Their effect is probably mediated via anti-angiogenic mechanisms.

The evidence implicating sFlt-1 and sEng as central to the pathogenesis of pre-eclampsia is compelling[22]. Serum sFlt-1 and sEng are increased in women with pre-eclampsia many weeks preceding clinical disease and there is a dose dependent relationship between serum levels and disease severity[14]. sFlt-1 administered *in vivo* to pregnant rats can induce hypertension and proteinuria[23]. Impressively, co-administration of both sFlt-1 and sEng in pregnant rats recapitulates the entire spectrum of end-organ injury seen in severe pre-eclampsia[24]. sFlt-1 and sEng are, by far and away, the most studied molecules in the field of pre-eclampsia.

Blocking sFlt-1 and sEng release is therefore a potential therapeutic strategy to treat pre-eclampsia. sFlt-1 and sEng are present in the serum of normal pregnancies, and increase with advancing gestation[14]. Thus, an effective therapeutic may only need to decrease levels and it may not be necessary to completely abolish production altogether.

The acceptance of the strategy to reduce levels of these anti-angiogenic factors as a means to treat pre-eclampsia is highlighted by the design of the 'Statins to Ameliorate early onset Pre-eclampsia' (STAMP) trial[21]. This UK based trial which has been approved by the University of Birmingham seeks to examine the potential of using pravastatin to treat early onset pre-eclampsia, and is the only significant randomised trial of an oral agent to treat early onset pre-eclampsia that we are aware of. The primary outcome of this trial is to show a significant reduction in serum sFlt-1.

The Translational Obstetrics Group has generated preclinical data showing esomeprazole induces marked decreases in sFlt-1 and sEng in both primary endothelial and trophoblast cells (see figure 3). The reductions are extremely potent: the highest dose of esomeprazole of 100 uM decreased sFlt-1 in primary trophoblast by >50% (Fig 3B) and reduced sEng by >90% (Fig 3D).

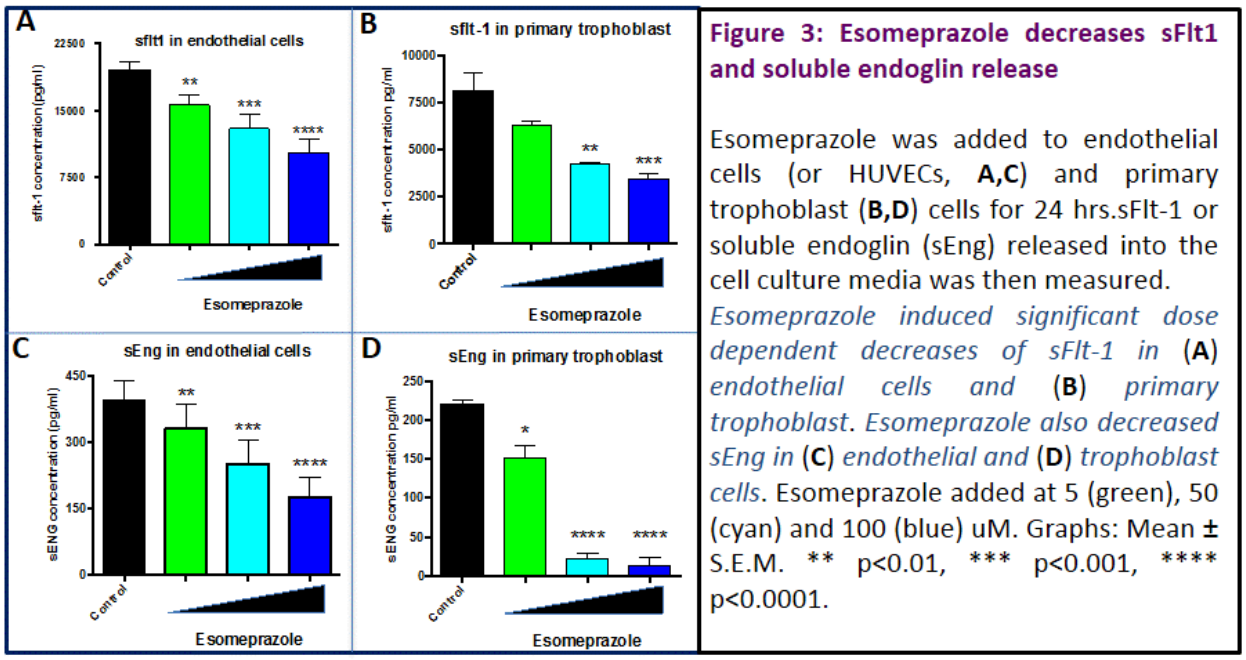

Figure 3: Esomeprazole decreases sFlt1 and soluble endoglin release

Esomeprazole was added to endothelial cells (or HUVECs, **A,C**) and primary trophoblast (**B,D**) cells for 24 hrs.sFlt-1 or soluble endoglin (sEng) released into the cell culture media was then measured. *Esomeprazole induced significant dose dependent decreases of sFlt-1 in* (**A**) *endothelial cells and* (**B**) *primary trophoblast. Esomeprazole also decreased sEng in* (**C**) *endothelial and* (**D**) *trophoblast cells*. Esomeprazole added at 5 (green), 50 (cyan) and 100 (blue) uM. Graphs: Mean ± S.E.M. ** p<0.01, *** p<0.001, **** p<0.0001.

Figure 3: Esomeprazole decreases sFlt1 and soluble endoglin release

To confirm the choice of esomeprazole as the best candidate in the proton pump inhibitor group it was tested against 4 other proton pump inhibitors. The effect on sFlt-1 and soluble endoglin was measured (see figure 4). Esomeprazole again had the most potent dose dependent effect (data still to be published).

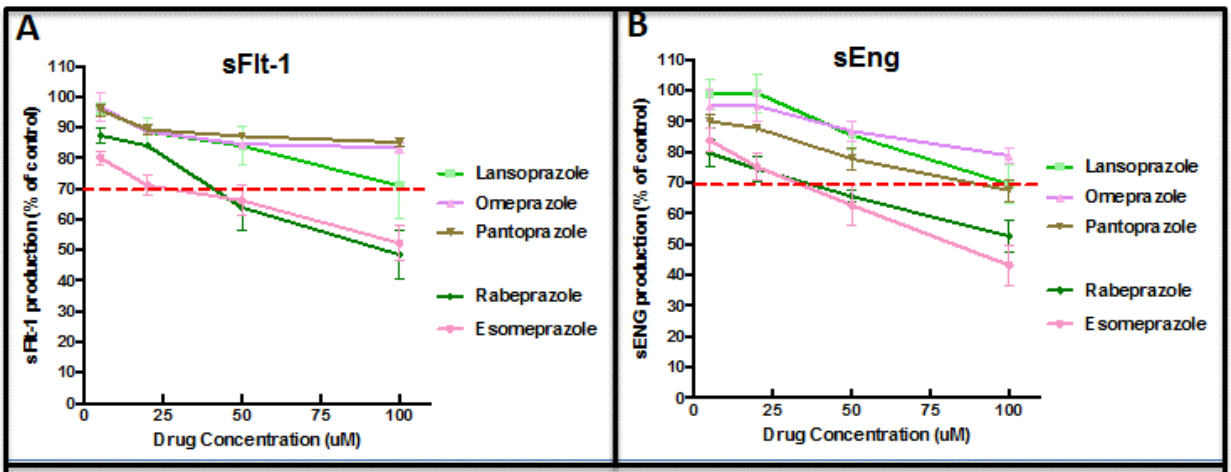

Figure 4: A comparison of five proton pump inhibitors shows esomeprazole is the most potent in reducing sFlt-1 and sEng.

The graphs show sFlt (A) and soluble endoglin (sEng B) levels with different drug concentrations of five different proton pump inhibitors. The red line shows the IC-30, drug concentrations required to decrease either molecule by 30% of control levels.

The graph shows rabeprezole and esomeprazole are the most potent proton pump inhibitors to decrease sFlt-1 and sEng of the five screened.

Experiment done in HUVEC cells, and is the same experimental protocol as the results presented in figure 3A and C.

Figure 4: A comparison of five proton pump inhibitors shows esomeprazole is the most potent in reducing sFlt-1 and sEng.

Pravastatin entered clinical trials on the strength of preclinical data generated from rodent animal models[25-27]. Surprisingly, there has been a lack of published data reporting whether it can reduce sFlt1 and sEng production from human blood vessels and placenta. The Translational Obstetrics Group examined pravastatin using the same assays done for esomeprazole (ie Figure 3). At the same doses used to test the proton pump inhibitors (5, 50, 100 uM), pravastatin had *no effect* on either sFlt-1 or sEng production. Dose response experiments showed that when the pravastatin dose was increased to 2000 UM (20 times the top dose of esomeprazole) sFlt-1 only showed a 30% drop in level and there was actually a dose dependent increase of sEng. Therefore, in functional assays using primary human tissues, esomeprazole appears to considerably outperform pravastatin in potency and desired effect.

Currently, we are not aware of any other candidate drugs in the literature that induce such potent reductions in these anti-angiogenic factors and that decreases both of these anti-angiogenic factors as the proton pump inhibitor group does.

2.3.3 Proton pump inhibitors decrease endothelial dysfunction of blood vessels

A hallmark of pre-eclampsia is endothelial dysfunction, a form of maternal blood vessel injury[20]. It is the likely reason that raised blood pressure is one of the most predictable clinical responses in an illness that is notorious for its varied clinical presentations.

When endothelial dysfunction occurs, the blood vessels express adhesion proteins on the cell surface, principally Vascular Cell Adhesion Molecule -1 (VCAM-1)[28]. VCAM-1 directly binds to leukocytes, causing leukocyte adhesion to the vascular endothelium. With dysfunction, endothelial cells also release endothelin-1, a potent vasoconstrictor, into the circulation. Levels of endothelin-1 are increased with pre-eclampsia[29]. VCAM-1 and endothelin-1 are widely accepted as markers of endothelial dysfunction[28].

The Translational Obstetrics Group has recently found that esomeprazole potently blocks up-regulation of VCAM-1 and endothelin-1 induced by Tumour Necrosis Factor-α (TNF-α), an inflammatory molecule involved in endothelial dysfunction (see Figure 5). The effects were very potent: at the top dose of esomeprazole, VCAM-1 mRNA is undetectable and mRNA of endothelin 1 is reduced by >80% (data still to be published).

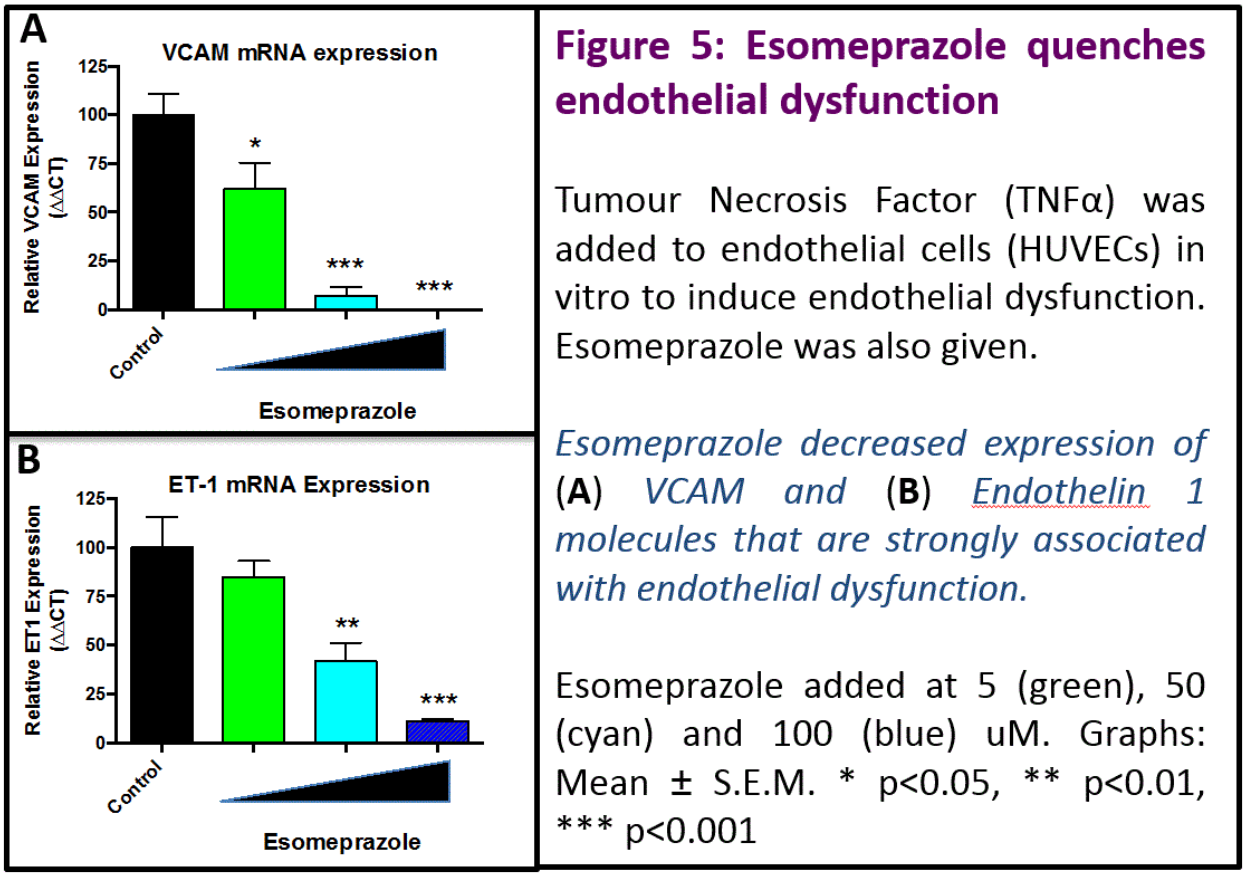

Figure 5: Esomeprazole quenches endothelial dysfunction

Thus, esomeprazole may be able to powerfully quench endothelial dysfunction, a hallmark of pre-eclampsia.

The preclinical evidence presented in this application has been derived from functional studies using primary human tissues, not immortalized cells. *Thus, generated from primary human tissues, this data represents a high level of preclinical evidence*. Secondly, all data presented were derived from the mean of at least three biological replicates (ie each experiment was repeated at least three times). The preclinical data is therefore robust.

2.3.4 Animal Models for pre-eclampsia

Unfortunately pre-eclampsia is a disease that is seen only in humans and there are no convincing animal models of pre-eclampsia.

There are a number of animal models that have been proposed but differences in placentation among mammals make the search for a model that satisfies the criteria of pre-eclampsia a challenge[30]. For instance, for cloven-hoofed mammals such as sheep, normal placental implantation is very shallow. This severely limits their utility to model the poor placental

invasion of maternal spiral arteries given normal invasion is already shallow[31]. In non-primate humans like monkeys and baboons the full spectrum of pre-eclampsia has never been reported. Placental implantation of guinea pigs is generally deeper and therefore more similar to humans, but these animals also do not exhibit the signs and symptoms of pre-eclampsia[30,32]. There are some mouse models of pre-eclampsia where the anti-angiogenic factors are inserted into the mice by plasmids and the protein levels artificially over-expressed. While they can recapitulate increased anti-angiogenic factor production, hypertension and even maternal end-organ injury, they are a little less convincing as they do not model shallow placental implantation that is a key pathophysiological step in pre-eclampsia.

Furthermore, we propose esopremazole acts by decreasing oxidative stress, decreasing release of anti-angiogenic factors and decreasing endothelial dysfunction. There is no animal model of preeclampsia that has all three elements of oxidative stress, elevated release of anti-angiogenic factors and endothelial dysfunction.

In summary, studies on pre-eclampsia treatment are limited by the fact that there are no accurate animal models. This makes it extremely difficult to test new medications and doses as initial trials need to be performed on humans to assess applicable doses efficacy.

2.4 Proton Pump inhibitors

2.4.1 Safety data in pregnancy

Proton pump inhibitors have been commonly used in pregnancy to treat gastroesophageal reflux disorders and more serious gastrointestinal complications like Helicobacter pylori-infection, peptic and duodenal ulcers and Zollinger-Ellison syndrome[35].

2.4.1.1 Animal studies:

Reproductive studies have been performed on rats and rabbits, with doses greater than 57 times and 35 times respectively of the human dose of 40mg[36]. These doses have revealed no evidence of impaired fertility or teratogenicity to the fetus. Changes in bone morphology were observed in offspring of rats dosed through most of pregnancy and lactation at doses equal or greater than 33.6 times an oral human dose of 40mg. (Nexium product information). These changes were reversible after birth.

2.4.1.2 Human studies

Esomeprazole has recently been reclassified as a Category C drug in pregnancy from a Pregnancy Category B drug by the Food and Drug Administration (FDA). Category B is for medications where studies in animals have not shown a risk to the fetus but where adequate data in humans are not available[37]. Category C is for drugs where animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in human, but potential benefits may warrant use of the drug in pregnant women despite potential risks[38].

The reason for changing the classification of esomeprazole was based on the above animal studies where bone changes were noted. In human pregnancies doses equal to 33.6 times the oral human dose of 40mg are never used. There have been no reports of changes in bone morphology in newborns exposed to esomeprazole during pregnancy (Nexium product information).

The Australian categorisation system and database for prescribing medicines in pregnancy classifies esomeprazole as a category B3 drug in pregnancy. A category B3 drug is one that has been taken by a limited number of pregnant women and women of childbearing age, without an increase in the frequency of malformation or other direct or indirect harmful effects on the human fetus having been observed and where animal studies have shown evidence of an increased occurrence of fetal damage, the significance of which is considered uncertain in humans. (http://www.tga.gov.au/hp/medicines-pregnancy.htm)

One nested case control analysis which suggested a possible link with proton pump inhibitors and septal cardiac birth defects. The authors suggested that this result be confirmed with larger observational studies[39]. Larger observational studies have not proven this to be true.

Large population based cohort studies and systematic reviews (including administration during the first trimester) did not find adverse effects in pregnancy. Specifically, these studies did not find an increased risk of congenital defects or any adverse neonatal outcomes. The most recent systematic literature review published in 2009 included 1530 pregnancies exposed to proton pump inhibitors and had 133,410 non-exposed matched controls. There was no increase in major congenital abnormalities, no increased risk of spontaneous miscarriages or preterm delivery[35].

The largest study to date, published in 2010, spanned a 13 year period and involved 840,968 pregnancies of which 5082 were exposed to proton pump inhibitors in the first trimester of pregnancy. This study concluded that exposure to proton pump inhibitors, even during the first trimester of pregnancy, was not associated with an increased risk of major birth defects[40]. In 2012, a further large study involving 112 022 pregnancies of which 1 186 pregnancies had been exposed to proton pump inhibitors confirmed there was no associated increase risk for

congenital anomalies with proton pump inhibitor use. Importantly this study also found no increase in fetal growth restriction or adverse neonatal outcomes (including premature delivery and low Apgar scores) in pregnancies exposed to proton pump inhibitors in the first, second or third trimesters of pregnancies[41].

Pravastatin has been assigned to pregnancy category X by the FDA and the Australian categorisation system and database for prescribing medicines in pregnancy. The Australian system classifies drugs that have a high risk of causing permanent damage to the fetus as category X. They feel that these drugs should not be used in pregnancy or when there is a possibility of pregnancy. The FDA classifies category X drugs in pregnancy as medications where studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits. Despite this, pravastatin has been approved for trials in women with early onset preeclampsia (STAMP trial) and for women at risk of developing preeclampsia. In both of these studies the risk to the mother of early onset preeclampsia was felt to warrant trials with a category X drug.

We are proposing a similar trial design for the same medical condition with a drug where the classification systems state that use may be of benefit in certain clinical situations. Esomeprazole and other proton pump inhibitors have been used in thousands of human pregnancies no increase in fetal growth restriction or adverse neonatal outcomes (including premature delivery and low Apgar scores) in the first, second or third trimesters of pregnancies. We will only be using treatment for a relatively short period in the late second and early third trimesters.

2.4.2 Esomeprazole drug information

Product information on esomeprazole can be found at the following link: *www1.astrazeneca-us.com/pi/Nexium.pdf*

2.4.2.1 Dosage

Esomeprazole is available as delayed-release tablet sachet or capsule for oral administration in two strengths: 20mg and 40mg. The standard dosage for the treatment of gastroesophageal reflux disease is 20 to 40mg once daily. We will be giving our participants a single daily dose of 40mg.

There is currently no pharmacokinetic data available on pregnant women using esomeprazole as they are were excluded from the original phase 1 trials.

We have decided to use the standard dose of 40mg daily (rather than 20mg) after reviewing the available pharmacokinetic data on esomeprazole[42,43]. 40mg provides a longer steady state and better suppression of symptoms. Pregnancy is associated with haemodilution and for this reason 40mg may already result in lower levels than in non-pregnant women. As such, we would be concerned 20mg could be less efficacious given the haemodilution seen in pregnancy.

Notably, 40mg is a dose that has been used in pregnancy in the late second and third trimester. This dose appears adequate in providing substantial symptomatic relief from reflux implying it results in adequate maternal circulation drug levels and an efficacious therapeutic response. Critically, large cohort studies (equivalent to post-marketing surveillance data done in large populations after the release of any drug) did not find this drug to be associated with adverse effects among pregnant women.

We do not feel comfortable using higher dosages as there is no published data examining the safety of higher doses have in pregnancy. Furthermore, given there are no adequate animal models of pre-eclampsia, we cannot use in vivo animal models to guide us in deciding whether an alternative dose other than 40mg is more appropriate.

If esomeprazole was proven to be a treatment option for pre-eclampsia, then we have the option then of instigating further larger multicentre trials to test different doses. Currently it is not pragmatically viable for us to do a multiarm study examining different doses given the numbers needed would be prohibitively large.

2.4.2.2 Contraindications

Esomeprazole is contraindicated in patients who have a known hypersensitivity to proton pump inhibitors. For this reason a previous hypersensitivity reaction to proton pump inhibitors will be an exclusion criterion for the study.

2.4.2.3 Warnings and precautions

There may be a small risk of clostridium difficile associated diarrhoea in severely ill patients. Our participants will be closely monitored for any effects of the medication and if significant diarrhoea develops the trial nurse will urgently notify the attending clinician and will inform them of the potential risk of clostridium difficile associated diarrhoea.

2.4.2.4 Adverse reactions

The most common adverse reactions in adults are headache, diarrhoea, nausea, flatulence, abdominal pain, constipation and a dry mouth. Caution will be exercised as some of these symptoms overlap with those of pre-eclampsia. All participants will be given an information sheet about all the possible effects of esomeprazole.

2.4.2.5 Drug interactions

Antiretroviral drugs

Decreased serum levels of the antiretroviral drugs atazanavir and nelfinavir have been reported with the use of omeprazole. This could lead to antiretroviral drug resistance and concomitant use is therefore not recommended. Increased serum levels have been reported with the concomitant use of omeprazole with saquinavir. A decrease in the dose of saquinavir may be indicated if used together. For these reasons, women using the above antiretroviral medication will be ineligible for our study.

Drugs for which gastric pH can affect bioavailability

Esomeprazole inhibits gastric acid secretion so the absorption of drugs such as digoxin may increase with concomitant use and the absorption of drugs such as ketoconazole and iron salts may be decreased.

Women using digoxin and ketoconazole will be excluded from the study. Most pregnant women with anaemia at Tygerberg Hospital are treated with iron salts. We will therefore monitor haemoglobin levels to ensure anaemia is not developing due to a lack of iron absorption potentially caused by esomeprazole.

Effects on hepatic metabolism

Esomeprazole is metabolised by the CYP 2C19 and CYP 3A4 pathways in the liver.
It can potentially interfere with the CYP 2C19 pathway and may result in a decreased clearance of diazepam, estimated to be a 45% decrease. If a mother is using diazepam she will not be eligible for the study.

Clopidogrel is an antiplatelet agent that is partially metabolised to its active agent by the CYP2C19 pathway. Therefore the concomitant use of esomeprazole with clopidogrel should be avoided as esomeprazole decreases the activity. Clopidogrel is rarely used in pregnancy. However, if a mother is on this treatment she will not be eligible for the study.

Drugs known to induce the CYP2C19 or CYP3A4 pathways like St John's Wort or rifampicin can substantially decrease esomeprazole concentrations and should not be used in combination with esomeprazole. The use of these medications will be an exclusion criterion.

Effects on renal impairment

Data is limited in patients with impaired renal function, but patients will be delivered before significantly elevated levels of serum creatinine are reached.

Other medications

The use of the following medications will be an exclusion criterion for this study since esomeprazole may affect their bioavailability:

- Warfarin
- Voriconazole
- Cilostazol
- Tacrolimus
- Erlotinib
- Methotrexate

These medications are not generally used in pregnancy.

2.4.2.6 Mechanism of action

Esomeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H+/K+ ATPase in the gastric parietal cell.

As discussed, esomeprazole appears to 1) up regulate HO-1 2) decrease production of sFlt-1 and sEng and 3) decrease markers of endothelial dysfunction in endothelial cells. We have yet to elucidate the exact molecular target that esomeprazole directly interacts with to elicit these changes. However, they are likely to be independent to its effects that cause proton pump inhibition.

As noted above, we postulate the up regulation of HO-1 may be mediated through a direct interaction of esomeprazole and KEAP-1. This releases NRF-2 which translocates to the nucleus and up regulates a raft of genes (including HO-1) involved in anti-oxidant defences.

2.4.2.7 Pharmacokinetics

Esomeprazole is 97% bound to plasma proteins and is metabolised by the liver. The metabolites lack antisecretory activity. The plasma elimination half-life is about one to one and a half hours. Less than 1% of the parent drug is excreted in the urine with 80% being excreted as inactive metabolites in the urine and the rest excreted as inactive metabolites in the faeces.

2.5 Summary

Currently, the only option for the treatment of pre-eclampsia is delivery. At extremely premature gestations this subjects the neonate to the risk of significant morbidity and mortality.

Preclinical data has uncovered potent biological actions suggesting esomeprazole may be a lead candidate therapeutic to treat pre-eclampsia**.** Treatment with a proton pump inhibitor in the late second trimester or early third trimester, which is well after organogenesis has occurred, for a relatively short period, is likely to have a negligible risk of causing fetal anomalies and adverse perinatal outcomes.

We have commenced a unique collaboration between Stellenbosch and Melbourne University that will allow us to test this hypothesis in Tygerberg Academic Hospital, South Africa. This population has a high prevalence of early onset pre-eclampsia. A trial of similar size in the developed world would require much larger numbers of participating sites.

3.0 Aims and Objectives

3.1 Specific Aims and Outcomes:

Primary aim:

1) To examine whether a single daily dose of 40 mg of esomeprazole can safely prolong gestation in women with early onset pre-eclampsia diagnosed 26+0 – 31+6 weeks who are being managed expectantly compared to expectant management alone.

Primary outcome:

1) Prolongation of gestation measured from the time of enrolment to the time of delivery, in hours and days.

Secondary aims:

2) To determine whether esomeprazole can improve 1) maternal 2) fetal and 3) neonatal outcomes in early onset pre-eclampsia being managed expectantly compared to expectant management alone.
3) To examine whether 40 mg of daily esomeprazole can significantly decrease levels of circulating sFlt-1 and/or sEng in women with early onset pre-eclampsia who are being managed expectantly compared to expectant management alone.
4) To examine whether 40 mg of daily esomeprazole is safe and well tolerated in the mother and infant, compared with placebo.

3.2 Hypothesis

Primary Hypothesis:

1) 40 mg of daily oral esomeprazole can safely prolong gestation in women with early onset pre-eclampsia diagnosed between 26+0 – 31+6 weeks, compared to expectant management alone.

Other hypotheses:

2) 40 mg of daily oral esomeprazole can improve maternal, fetal and neonatal outcomes, in women diagnosed with early onset preeclampsia, compared to expectant management alone.
3) 40 mg of daily oral esomeprazole can significantly decrease the circulating levels of sFlt1 and sEng in early onset preeclampsia being managed expectantly compared to expectant management alone.
4) 40 mg of daily oral esomeprazole is safe and well tolerated in the mother, fetus and infant.

4.0 Study Design

4.1 Type of study

Hospital based phase II placebo-controlled, double-blind parallel randomised control trial.

To classify the phase of this study is somewhat difficult given esomeprazole is already an approved drug. Historically pregnant women and children have been excluded from all phase 1 trials as they do not fall under the category of "healthy volunteers". This makes any trial in pregnant women more difficult. Furthermore, phase I trials are typical those where the primary outcome is safety. There already has been very large cohort studies to suggest 40mg of esomeprazole in pregnancy women is safe.

We have classified this trial as a phase 2 trial as we will be assessing efficacy and safety. We aim to determine whether esomeprazole is effective for the treatment of pre-eclampsia.

4.2 Study population

Pregnant women diagnosed with early onset pre-eclampsia at a gestational age between 26+0 weeks to 31+6 weeks who qualify for expectant management. All who consent and meet eligibility criteria will be enrolled in the study.

4.3 Intervention

Participants will be randomised to daily administration of either active tablets containing 40 mg of esomeprazole or an identical placebo tablet. The study will not alter or interfere with any treatment or care given routinely to women with early onset pre-eclampsia. A single dose of 40 mg will be given orally once a day.

4.4 Sample size calculations

The primary outcome will be to determine whether treatment with esomeprazole can result in a gain in length of gestation.

In the largest descriptive study on expectant management of early onset pre-eclampsia Hall, et al., described the length of gestation gained by such management before delivery[44].

Importantly, this retrospective study was performed at the same hospital where we plan to run this trial (Tygerberg Hospital) and clinical practice regarding expectant management is well organised, and has not significantly changed since that landmark publication. Thus, it provides valuable data with which to base a power calculation for the present study. The reported duration that fetuses remained in utero after diagnosis and admission of pregnancies complicated by early onset pre-eclampsia was a mean of 11 days (with a standard deviation of 7 days) and a median 9 days (range of 1 to 47 days)[44].

We believe an intervention that allows a gain in gestation of a further 5 days would be a clinically important difference and likely to have important beneficial implications for the fetus (although perinatal outcomes will not be a primary outcome in this phase II study).

For 90% power, with a two-sided alpha set of 0.05, 43 patients are required in each group (table 1) to identify a gain in gestation of 5 days. Given the data reported by Hall et al was skewed, we multiplied this by 1.15 to make a statistical allowance for non-normality[45]. This increases the numbers to 50 per arm (43x1.15). We will add an additional 10 per arm to allow for drop-outs, which equates to 60 per arm. *Thus, a total of 120 participants will be recruited to provide sufficient power to examine our primary outcome.*

Table 1: Sample size calculation

Detectable difference (in days)	Group size, per arm	Group size x 1.15*
3	116	134
4	66	76
5	43	50
6	30	35
7	23	27

* The reason for expanding the group size by 15% is that the data in the Hall paper is skewed and not normally distributed. The inflation of the sample size by 15% provides an adjustment that makes an allowance for non-normality[45].

(Power calculation Performed using PASS 12 software. Hintze, J. (2013). PASS 12. NCSS, LLC. Kaysville, Utah, USA)

The gestational age at diagnosis is likely to affect allowable length of pregnancy prolongation. For instance, those diagnosed at 31+6 will be delivered at 34 weeks (should they reach that gestation), as it is unit policy to deliver at that gestation. Thus, such patients will have a maximum length of pregnancy prolongation of 15 days. In contrast, those diagnosed at 28 could conceivably obtain many weeks of pregnancy prolongation (if the disease remains stabilised). Further it is possible that esomeprazole efficacy may vary with gestational age at diagnosis.

To ensure treatment group allocation is balanced for this potential variable, we will stratify randomisation into strata based on gestational age.

	Gestational age (GA)	**EFW if GA is unknown**
Strata 1	26 +0 up to and including 28+6 weeks	Less than and including 1100gm
Strata 2	29+0 up to and including 31+6	Greater than 1100gm

Stratified randomisation will ensure that the number of participants in each gestational age strata is balanced.

4.5 Randomisation and allocation concealment

Randomisation will be done in an equal ratio of esomeprazole to placebo. An online, web-based sequence generator system will be used to avoid chance imbalances in the stratification process. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will both be blinded.

Randomization will include blocking within each stratum. Blocking ensures that the control and treatment group numbers remain close both within the strata and overall. We propose using blocks of 4 to 6 with the size and order randomly assigned. Its use of stratified randomization ensures balance between treatment and control groups for gestational age however its use induces within stratum induces correlation. We will take this into account in the analysis by treating strata as a covariates in regression analysis or as levels in Mantel-Haenszel pooled treatment effects.

Once the participants have been randomised the treatment pack with the same code will be allocated to the participant. All treatment packs will be identical and will contain either active tablets or placebo. The treatment packs will be prepared by the manufacturer contracted to produce the trial medication. The researchers will have no access to the randomisation list. This process will ensure that there is allocation concealment throughout the conduct of the trial. The allocation will only be released once the trial is completed.

5.0 Inclusion and exclusion criteria

5.1 Definitions:

There are many classification systems for the hypertensive disorders of pregnancy. The International Society for the Study of Hypertensive Disorders in Pregnancy (ISSHP) has recently published a new classification system for hypertensive disorders in pregnancy[46]. We will use this classification system in this study.

The following definitions are used in this classification system:

Hypertension in pregnancy: office or in hospital systolic blood pressure greater than or equal to 140 mmHg and/or a diastolic blood pressure greater than or equal to 90 mmHg on the average of at least two measurements, taken at least 15 minutes apart, using the same arm.

Severe Hypertension: systolic blood pressure greater than or equal to 160 mmHg or a diastolic blood pressure greater than or equal to 110 mmHg on the average of at least two measurements, taken at least 15 minutes apart, using the same arm.

Pre-existing (chronic) hypertension: hypertension that pre-dates the pregnancy or appears before 20 weeks gestation.

Gestational Hypertension: hypertension that appears at or after 20 weeks of gestation.

Pre-eclampsia: gestational hypertension and new proteinuria or one or more adverse conditions or one or more serious complications (see table 3 for definitions of adverse conditions and serious complications).

Adverse condition: consists of maternal symptoms, signs, abnormal laboratory results and abnormal fetal monitoring that may herald the development of severe maternal or fetal complications.

Significant proteinuria: greater than or equal to 0,3 g/d in a complete 24-hour urine collection or a spot (random) urine sample with greater than or equal to 30 mg/mmol urinary creatinine.

Severe pre-eclampsia: pre-eclampsia associated with a severe complication that warrants delivery regardless of gestational age.

Table 2: ISSHP classification of hypertensive disorders in pregnancy[46]

Classification of the HDP.

	Comments
Pre-existing (chronic) hypertension	This is defined as hypertension that was present either pre-pregnancy or that develops at <20^0 weeks gestation
• With comorbid condition(s)	Comorbid conditions (e.g., pre-gestational type I or II diabetes mellitus or kidney disease) warrant tighter BP control outside of pregnancy because of their association with heightened cardiovascular risk
• With evidence of preeclampsia	This is also known as 'superimposed preeclampsia' and is defined by the development of one or more of the following at ⩾ 20 weeks: • Resistant hypertension, *or* • New or worsening proteinuria, *or* • One/more adverse condition(s) *or* • One/more severe complication(s) Severe preeclampsia is defined as preeclampsia with one or more severe complication(s)
Gestational hypertension	This is defined as hypertension that develops for the first time at ⩾ 20^0 weeks' gestation
• With comorbid condition(s)	Comorbid conditions (e.g., pregestational type I or II diabetes mellitus or kidney disease) warrant tighter BP control outside of pregnancy because of their association with heightened cardiovascular risk
• With evidence of preeclampsia	Evidence of preeclampsia may appear many weeks after the onset of gestational hypertension. Preeclampsia is defined by gestational hypertension and one or more of the following: • New proteinuria, *or* • One/more adverse condition(s) *or* • One/more severe complication(s) Severe preeclampsia is defined as preeclampsia with one or more severe complication(s)
Preeclampsia	Preeclampsia may arise *de novo*. It is defined by gestational hypertension and one or more of the following: • New proteinuria, *or* • One/more adverse condition(s) *or* • One/more severe complication(s) Severe preeclampsia is defined as preeclampsia with one or more severe complications[v]
'Other hypertensive effects'*	
Transient hypertensive effect	Elevated BP may be due to environmental stimuli or the pain of labour, for example
White coat hypertensive effect	BP that is elevated in the office (sBP ⩾ 140 mmHg or dBP ⩾ 90 mmHg) but is consistently normal outside of the office (<135/85 mmHg) by ABPM or HBPM
Masked hypertensive effect	BP that is consistently normal in the office (sBP < 140 mmHg or dBP < 90 mmHg) but is elevated outside of the office (⩾135/85 mmHg) by ABPM or repeated HBPM

ABPM, ambulatory BP monitoring; BP, blood pressure; HBPM, home BP monitoring.
* These may occur in women whose BP is elevated at <20^0 or ⩾20^0 weeks who are suspected of having pre-existing or gestational hypertension/preeclampsia, respectively.

Table 3: ISSHP classification of adverse conditions and severe complications of preeclampsia[39]

Adverse conditions and severe complications of preeclampsia.

Organ system affected	Adverse conditions (that increase the risk of severe complications)	Severe complications (that warrant delivery)
CNS	○ Headache/visual symptoms	○ Eclampsia ○ PRES ○ Cortical blindness or retinal detachment ○ Glasgow coma scale < 13 ○ Stroke, TIA, or RIND
Cardiorespiratory	○ Chest pain/dyspnoea ○ Oxygen saturation < 97%	○ Uncontrolled severe hypertension (over a period of 12hr despite use of three antihypertensive agents), ○ Oxygen saturation < 90%, need for ⩾ 50% oxygen for > 1hr, intubation (other than for Caesarean section), pulmonary oedema ○ Positive inotropic support ○ Myocardial ischaemia or infarction
Haematological	○ Elevated WBC count ○ Elevated INR or aPTT ○ Low platelet count	○ Platelet count < 50x10^9/L ○ Transfusion of any blood product
Renal	○ Elevated serum creatinine ○ Elevated serum uric acid	○ Acute kidney injury (creatinine > 150 μM with no prior renal disease) ○ New indication for dialysis
Hepatic	○ Nausea or vomiting ○ RUQ or epigastric pain ○ Elevated serum AST, ALT, LDH, or bilirubin ○ Low plasma albumin	○ Hepatic dysfunction (INR > 2 in absence of DIC or warfarin) ○ Hepatic haematoma or rupture
Feto-placental	○ Non-reassuring FHR ○ IUGR ○ Oligohydramnios ○ Absent or reversed end-diastolic flow by Doppler velocimetry	○ Abruption with evidence of maternal or fetal compromise ○ Reverse ductus venosus A wave ○ Stillbirth

AST, aspartate aminotransferase; ALT, alanine aminotransferase; DIC, disseminated intravascular coagulation; FHR, fetal heart rate; LDH, lactate dehydrogenase; PRES, posterior reversible leukoencephalopathy syndrome; RIND, reversible neurological deficit < 48hr; RUQ, right upper quadrant; TIA, transient ischaemic attack.

A number of patients seen at Tygerberg Hospital only book after a gestational age of 20 weeks. The ISSHP classification systems does not have a class for these patients. We will classify these patients according to the classification of Davey and MacGillivray[47].

Unclassified proteinuric hypertension:
Hypertension and proteinuria diagnosed in a patient who is seen for the first time after 20 weeks of gestation.

We will then contact these patients 6 weeks postpartum to determine whether their blood pressure has normalized or not and will then retrospectively reclassify them as either as pre-eclampsia or chronic hypertension with superimposed pre-eclampsia.

5.2 Inclusion criteria

A diagnosis of pre-eclampsia, gestational hypertension with evidence of preeclampsia, preexisting hypertension with evidence of pre-eclampsia or unclassified proteinuric hypertension has been made by the attending clinician who is of the opinion that the patient and fetus would benefit from expectant management.

AND all of the following is present:

- Gestational age between 26 + 0 weeks and 31 + 6 weeks
- Estimated fetal weight by ultrasound between 500gm and 1800 gm (if gestation is not certain)
- Singleton pregnancy
- The managing clinicians have made the assessment to proceed with expectant management and that delivery is not expected within 48 hours
- The managing clinician and neonatologist believe that the fetus could potentially be delivered in a viable condition

ALSO:

- No suspicions of a major fetal anomaly or malformation. A major fetal anomaly is defined as anomalies or malformations that create significant medical problems for the patient or that require specific surgical or medical management. Major anomalies or malformations are not considered a variation of the normal spectrum.
- The mother must be able to understand the information provided, with the use of an interpreter if needed
- The mother must be able to give informed consent
- Patient will be admitted to hospital for expectant management and standardised care

5.3 Exclusion criteria

- Patient is unable or unwilling to give consent
- Established fetal compromise that necessitates delivery. This will be decided by the clinical team before expectant management is offered to the patient.
- The presence of any of the following at presentation:
  - Eclampsia defined as the new onset of grand mal seizure activity and/or an unexplained coma during pregnancy with signs or symptoms of pre-eclampsia[48].
  - Severe hypertension defined as a systolic blood pressure greater than or equal to 160 mmHg or diastolic blood pressure greater than or equal to 110 mmHg that cannot be controlled with antihypertensive medication within 48 hours of admission.
  - Cerebrovascular event defined as an ischaemic or haemorrhagic stroke associated with clinical symptoms and definitive signs on imaging.
  - Posterior reversible encephalopathy syndrome (PRES) associated with pre-eclampsia defined on imaging as reversible vasogenic oedema, usually in the occipital or parietal lobes.
  - Severe renal impairment with a creatinine level of greater or equal to 125 µmol/l or a need for dialysis.
  - Signs of left ventricular failure which include pulmonary oedema requiring treatment or oxygen saturations of less than 90% caused by left sided heart failure.
  - Disseminated intravascular coagulation defined as an INR greater than 2
  - Platelet count at presentation less than $50x10^9$ (platelet aggregation excluded)
  - Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100×10^9/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase > 600 µ/L or haemolysis on a peripheral blood smear or a raised haptoglobin level)
  - Liver transaminases greater than or equal to 500IU/L
  - Liver haematoma or rupture
  - Fetal distress on cardiotocography
  - Severe ascites on ultrasound as defined by the sonographer
- Contra-indications for expectant management of pre-eclampsia
- Current use of a proton pump inhibitor
- Contraindications to the use of a proton pump inhibitor
  - Previous hypersensitivity reaction to a proton pump inhibitor
- Current use of a drug that may be affected by a proton pump inhibitor: warfarin, ketoconazole, voriconazole, atazanavir, nelfinavir, saquinavir, digoxin, St John's Wort, rifampin, cilostazol, diazepam, tacrolimus, erlotinib, methotrexate and clopidogrel.

6.0 Outcomes

6.1 Primary outcome

Prolongation of gestation measured from the time of enrolment to the time of delivery, in hours and days.

6.2 Secondary outcomes

Maternal:

- (Composite outcome) The occurrence of any of the following serious maternal outcomes:
  - Maternal death
  - Eclampsia
  - Pulmonary oedema (oxygen saturation ≤90%, with clinical signs and symptoms requiring treatment)
  - Severe renal impairment or the need for dialysis
  - Cerebral vascular event
  - Liver haematoma or rupture

- Other maternal outcomes, where all of the following will be compared as individual outcomes:
  - Admission to a high care or intensive care unit
  - Posterior reversible encephalopathy syndrome (diagnosed on imaging)
  - Left ventricular failure (diagnosed on echocardiography)
  - Serum creatinine greater than or equal to 125 µmol/l
  - Proteinuria greater than or equal to 3g/24h
  - Hypertension with a systolic blood pressure greater than 160mmHg or a diastolic blood pressure greater than 110mmHg despite anti-hypertensive treatment
  - Disseminated intravascular coagulation
  - Platelet count less than $50x10^9$
  - Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100×10^9/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase greater than or equal to 600 µ/L or haemolysis on a peripheral blood smear or a raised haptoglobin level)
  - Liver transaminases >500IU/L
  - Placental abruption defined as a retroplacental clot covering more than 15% of the maternal surface or a diagnosis on histology

- Mode of delivery
- Use of antihypertensive agents (number of agents and daily dose at delivery)
- Major postpartum haemorrhage (defined as blood loss of more than 1000mls in 24 hours)
- Thromboembolic disease (defined as a deep-vein thrombosis , pulmonary embolism or both)
- Moderate or severe ascites noted on ultrasound or at delivery

Fetal:

(Composite outcome) The occurrence of any of the following serious fetal outcomes:

- Reversed a-wave in the ductus venosus on fetal ultrasound
- Significant changes in heart rate patterns on the non-stress test or cardiotocograph, as defined by the attending clinician, that necessitate delivery
- Intrauterine fetal demise
- Incidence of fetal growth restriction at birth as defined by growth charts based on the local population
- Persistent reversed flow in the umbilical artery confirmed on two fetal ultrasounds
- Redistribution in the middle cerebral artery

There is no standard classification system for the non-stress test. The attending clinician usually assess the heart rate patterns by looking at the baseline, assessing for baseline shifts, by assessing the variability, by looking at the presence or absence of accelerations and the presence of decelerations and by assessing for a sinusoidal pattern. The gestational age of the fetus is also taken into consideration when assessing the non-stress test. The Royal College of Obstetricians and Gynaecologists Evidence-based Clinical guideline Number 8 on electronic monitoring is routinely used at Tygerberg hospital for and will be used by the attending clinician to assess the cardiotocograph. (www.nice.org.uk/nicemedia/pdf/efmguidelinercog.pdf)

Neonatal:

Composite outcome) The occurrence of any of the following serious neonatal outcomes:

- Neonatal death within 6 weeks after the due date
- Grade III or IV intraventricular haemorrhage defined on imaging as enlarged ventricles associated with haemorrhage or when the haemorrhage extends into the cerebral tissue around the ventricles
- Necrotizing enterocolitis diagnosed on radiographic studies
- Bronchopulmonary dysplasia defined as needing oxygen at day 28 of life, either on a ventilator, by CPAP or via a nasal catheter and significant neonatal sepsis as defined by the attending paediatrician.

Other neonatal outcomes, where all of the following will be compared as individual outcomes:

- APGAR score of less than 7 at 5 minutes
- Umbilical artery pH below 7.05
- Umbilical artery lactate
- Surfactant use
- Neonatal intensive care admission or special care unit admission
- Intubation and mechanical ventilation or continuous positive airway pressure (CPAP) support
- Grade III/IV hyaline membrane disease
- Length of oxygen treatment
- Hospital stay
- Incidence of retinopathy of prematurity
- Early neonatal sepsis (Haematological infection diagnosed on blood culture with associated clinical findings or as defined by attending paediatrician)

Biomarkers:

We will ship the following samples from Tygerberg Hospital to The Mercy Hospital, where the following assays will be done (by researchers blinded to clinical groupings) in the Translational Obstetrics Laboratory at Melbourne University if consent has been given by the mother.

- Maternal plasma samples: sFlt1, sEng, endothelin 1 and esomeprazole levels.
- Placental samples: mRNA and protein expression of HO-1, sFlt1 and endothelin 1.
- Immunohistochemistry will be also performed for these same molecules.
- Umbilical cord plasma obtained delivery: esomeprazole levels.

7.0 Trial conduct

7.1 Identification and enrolment of participants

7.1.1 Identification

Potential participants will be identified after they have been admitted to Tygerberg Hospital (tertiary referral centre) with a diagnosis of early onset pre-eclampsia. The clinical management team will decide if the patient qualifies for expectant management and is suitable for admission as per the hospital protocol (potentially can be recruited), or delivery is likely to be imminent within 48 hours (exclusion criteria). The research midwife and principal investigator will be notified by the ward staff and/or clinical treating team.

Normal clinical care will be maintained throughout the study. The use of steroids and magnesium sulphate will be according to local protocols and will be decided upon by the managing clinician. The use of aspirin and calcium will be noted.

Inclusion and exclusion criteria will be assessed before approaching the patient.

7.1.2 Consent

Information about the trial will be given to the patient and a translator (working from a script) will be used if necessary. Information sheets and study details will be given to the patient and any questions about the study will be answered. A translator will be used so the patient will have the opportunity to go through the information in their own language. If they would like to be involved in the study they will be asked to provide written informed consent. The consent form will be made available on self-duplicating paper in order that a signed copy can be given to the participant, a copy can be put in the clinical notes and a copy kept by the study investigators. The consent forms will be translated into English, Afrikaans and Xhosa. Only the study investigators and research midwives will be able to take consent. The patient will be given as much time as they need to decide whether they would like to be involved. Once the participant has signed consent they will be enrolled in the study.

7.1.3 Recruitment

Once the eligibility criteria have been checked and the informed consent document has been signed we will obtain the following:

- Baseline clinical information regarding the pregnancy (maternal age, parity, obstetric history, antenatal history, medical history, drug history, allergies, smoking status, alcohol and drug intake)
- Baseline information
  - Degree of proteinuria (24 hour protein excretion)
  - Maternal assessment (renal function, liver function, blood count)
  - Fetal assessment with ultrasound (biometry, estimated fetal weight, Doppler and amniotic fluid index findings)

7.2 Study treatment

5.2.1 Trial drug

After randomisation an individual treatment pack will be allocated to the participant according to an online web-based randomisation process. The participant will be allocated an individual trial number. The participant's name and trial number will be written on the pack. The packs will be identical in shape, colour, weight and feel. Each pack will contain proton pump inhibitor tablets or identical placebo tablets.

7.2.2 Packaging, formulation and supply of the treatment

The trial drug and placebo will be produced by a contracted manufacturer IDT pharmaceuticals (http://en.idtaus.com.au). The trial drugs will be placed into containers and labelled randomly according to the computer generated randomisation list. Labelling, storage and preparation will be done according to the requirements of the Medicines for Human Use (Clinical Trials) regulations. The placebo will undergo a placebo analysis before the trial is started to confirm the contents.

7.2.3 Route of administration, dosage regimen and treatment period

Each participant will be given a treatment pack. The treatment pack will contain treatment for 21 days. A single capsule will be taken, once daily one hour before meals (if the patient is eating). On the first day of randomisation the first dose will be taken in the evening. Treatment will then be taken daily in the evening until the pregnancy has ended. Trial drugs will be kept in the packaging they are provided in and under no circumstances will it be used for other participants. The trial medication will be written up on the treatment chart and the chart will be signed by the nursing staff to confirm that the participant has taken the medication and to confirm compliance. The research midwife will monitor the treatment chart to assure compliance. As a second compliance check the patient will be asked return the empty packaging

to the research midwife who will record the number of empty tablet containers at the time of delivery of the patient.

7.2.4 Resupply of treatment

It is assumed that for most patients, delivery will occur within 21 days of randomisation. However in some cases delivery may occur after 21 days of randomisation. In these cases continuation trial drug packs will be available. In these cases the principal investigator will contact the randomisation office and ask for a number for a continuation pack. The continuation pack will be matched to the contents of the initial treatment pack and will contain a further 21 day supply of the treatment.

7.3 Expectant management for pre-eclampsia

7.3.1 Routine management

Expectant management for early onset pre-eclampsia involves admission to hospital, and close maternal and fetal surveillance.

Maternal surveillance includes four hourly blood pressure measurement, twice daily clinical assessment, daily urinalysis, and twice weekly assessments with blood tests (full blood count, renal function tests and hepatocellular enzymes if HELLP syndrome is suspected) and 24 hour urinary protein measurement on admission.

Fetal surveillance includes six-hourly cardiotocography and ultrasound assessments every two weeks (or more frequently if clinically indicated) for Doppler velocimetry of the umbilical artery, middle cerebral artery, ductus venosus, amniotic fluid volume assessment and growth.

All participants will receive two doses of betamethasone 24 hours apart to reduce the risks of neonatal respiratory distress syndrome, intracranial haemorrhage and necrotising enterocolitis. A single repeat dose is usually given one week later.

Most participants will be on antihypertensive treatment and the medication used will be documented. All women should already be receiving iron and folic acid supplementation.

Clinical care will be left up to the discretion of the clinical team. The indication for delivery will be a clinical decision. Indications for delivery may include failure to control blood pressure, the development of major maternal or fetal complications, or intrauterine fetal death. Expectant management will usually end at a gestation of 34 weeks.

7.4 Clinical Follow-up

7.4.1 Baseline clinical information

Baseline clinical information collected will include maternal age, gravidity and parity, gestation, obstetric history, antenatal history, medical and surgical history, medication history, allergies, smoking status, alcohol and drug intake, height, weight and body mass index. A photocopy of the antenatal notes will be made and stored with the data capture sheets.

Ongoing clinical data will be collected daily on data capture sheets. This will include clinical measurements, medications, haematological parameters, biochemical parameters, fetal ultrasound and fetal heart rate assessments. Patient folders will be reviewed after delivery to confirm that the data collected is complete and accurate. Once the patient has delivered the folder will be photocopied to assure that all the data is recorded.

7.5 Sample collection

7.5.1 Blood samples

Blood samples are taken as part of routine clinical care (twice a week). These include measurements of the haemoglobin, the platelet count and the urea and creatinine levels. If HELLP syndrome is suspected the hepatocellular enzymes are measured and a coagulation profile may be ordered. We will collect an extra 9 mls of blood each time routine bloods are taken to obtain plasma samples. These will be processed immediately and frozen at -80°C for later analysis of sFlt, sEng, and endothelin 1. In addition, all participants we undergo the following test once*:* we will precisely time blood sampling 2 hours after taking the trial medication where we will measure plasma esomeprazole levels.

Tubes will be labelled with the participant's trial number and folder number. The samples will be spun, and split into two aliquots. One aliquot will be used to measure sFlt-1 and sEng. The laboratory doing the measurement of sFlt-1 and s-Eng will be confirmed closer to the start of the trial. The other aliquot will be stored in a -80 degree freezer and sent to Melbourne University for analysis and will be analysed in the Translational Obstetrics Laboratory.

We plan to measure the following in the blood samples:

- ***Biomarkers of endothelial injury***: endothelin 1 and inflammatory cytokines

- ***Antiangiogenic factors associated with pre-eclampsia:*** sFlt1 and soluble endoglin
- ***Esomeprazole levels in the blood:*** These will be collected at 2 hours after the initial dose. The plasma elimination half life of esomeprazole is approximately 1 to 1,5 hours. Single 40mg oral doses generally give rise to peak plasm esomeprazole concentrations of 0,5-1,0 mg/l within 1 to 4 hours
  The samples sent to Melbourne may be used to measure other relevant analytes associated with pre-eclampsia. No genetic testing will be performed on these samples.

The purpose of these blood analyses is to have more objective (and scientifically robust) evidence of maternal disease regression than the clinical and routine laboratory indicators described above. We hypothesise these biomarkers (particularly sFlt1 and soluble endoglin) will be significantly lower among the group given esomeprazole.

7.5.2 Urine samples

Urine samples will be collected 2 times per week and sent for spot protein: creatinine ratios. 24-hour protein excretion is routinely measured only once on admission. For the purposes of this study we will repeat it weekly. This will be performed at the Tygerberg Laboratory.

7.5.3 Cord Blood Samples

Cord Blood Samples will be collected at delivery and will be labelled with the participant's trial number and folder number. These samples will also be stored and sent to the University of Melbourne for further analyses where they will be analysed in the Translational Obstetrics Laboratory. We plan to measure esomeprazole levels in the cord blood obtained at the time of the delivery.

7.5.4 Placental samples

Placental samples will be collected at delivery from women who have consented for this to be performed. We will take three 1cm^3 full thickness biopsies of the placenta. Each sample will be frozen and stored in -80 degree freezer within a PIE Trial storage box. A further sample will be collected and fixed in formalin.

We plan to measure the following in the placental samples:

- **Expression of heme-oxygenase-1 (both mRNA and protein levels)**
- **Expression of anti-angiogenic factors in the placentas**

- Perform a microarray to examine the **mRNA expression of key anti-oxidant and hypoxia pathways**.
- **Antiangiogenic factors associated with pre-eclampsia:** sFlt1 and soluble Endoglin
- **Immunohistochemistry**

In addition, we plan to store remaining samples to measure other relevant analytes that may yet be discovered in relation to pre-eclampsia. We will not perform any genetic testing on these samples. These tests will be done in the Translational Laboratory at Melbourne University.

7.6 Withdrawal from the study

All participants will be informed that they are free to withdraw from the study at any time, and that this will not affect their clinical care. In the event of a withdrawal, we will ask whether we can still collect clinical data and include them in our analyses.

8.0 Data management and statistical analysis

8.1 Data management

Data will be collected prospectively by the researchers and data checking and entry of the completed data collection forms will be reviewed. Duplicate copies of original data collection forms will be used for quality control purposes.

8.2 Data recording

Data will be collected on data capture sheets will be transcribed onto a Microsoft Excel spreadsheet. Only the participant number will be entered onto the spreadsheets. Data cleaning will be performed on an ongoing basis and again at the end of the study.

8.3 Handling of missing data

If data is missing the original data extraction sheets will be reviewed and if needed the original patient notes will be reviewed.

8.4 Data processing

Data entry and checking will be continuous and queries will be followed vigorously to ensure clarification without delay.

8.5 Analysis plan

The analyses will be on an intention-to-treat principle with comparisons made between proton pump inhibitors and placebo, for primary and secondary outcomes. Comparisons will be expressed as relative risks or mean differences with 95% confidence intervals.

8.6 Statistical methods

The patient characteristics, by treatment group, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution. Significance level is set at 0.05 and all hypothesis testing will be two-sided.

The primary outcome will not be adjusted for multiple comparisons but the secondary outcomes will be adjusted for multiple comparisons using a small number of pre-specified outcomes.

Primary outcome

We plan to perform two analyses: 1) primary intention to treat analysis (ITT) and 2) a treatment received (TR) analysis to examine response among those who actually took the tablets. The primary outcome (prolongation in days) between treatment groups will be tested using ANCOVA regression analysis with both treatment group and gestational strata as covariates.

We will present results as mean group difference with 95% confidence intervals (95%CI). Standard regression diagnostics will be performed and transformation of primary outcome, to achieve adequate distributions of residuals, if indicated. We will also present survival analyses (ie time until delivery), using Cox proportional hazards regression and Kaplan-Meier survivorship curves.

Secondary Outcomes

For these composite outcomes or single outcomes, continuous variables will be compared using either T-test (for normally distributed variables) or Mann-Whitney U (non-normally distributed). Categorical values will be compared using chi-squared test. For the longitudinal data of plasma sFlt-1, sEng and endothelin 1 levels, we will 1) graph the data longitudinally 2) compare levels between the groups relative to gestational age 3) compare levels between groups relative to days after recruitment and 4) compare levels between groups at delivery.

The various maternal and fetal outcomes will be compared as either composite outcomes or as single variables, listed as per 6.2. Continuous variables will be compared using either T-test (for normally distributed variables) or Mann-Whitney U (non-normally distributed). Categorical values will be compared using chi-squared test.

The same approach will be used to compare circulating sFlt-1, sEng or other molecules/variables measured subsequently in the laboratory.

9.0 SAFETY MONITORING AND PROCEDURES

9.1 Adverse event

All Adverse events will be documented and reported. An adverse event includes

- Any unintentional, unfavourable clinical signs or symptoms. This includes complications of pre-eclampsia.
- Any new illness or disease or the deterioration of existing disease or illness.
- Any clinically relevant deterioration in any laboratory or clinical tests.

These events will be recorded on the daily data capture sheets.

The following are not considered adverse effects:

- A pre-existing condition (unless it worsens significantly in pregnancy over and above what may be expected with the concurrent diagnosis of pre-eclampsia).
- Diagnostic or therapeutic procedures such as surgery.

9.2 Serious adverse event

Serious adverse events include any of the following

- Maternal or fetal death.
- Threat to the life of the mother or baby.
- Event that results in a longer post-natal hospital stay.
- Event that results in a persistent or significant disability in the mother.
- Congenital or birth defect in the baby that is detected in the post-natal period and was not detected on ultrasound.

9.3 Expected serious adverse events

Certain serious adverse events will be expected as we are treating women with early onset pre-eclampsia with premature fetuses. We will expect to have serious adverse events related to the diagnosis of pre-eclampsia which include severe hypertension, intracranial haemorrhage, renal failure, abnormal hepatic function, disseminated intravascular coagulopathy, eclampsia, left ventricular failure, pulmonary oedema and haemorrhage. Complications of prematurity that may be expected include sepsis, necrotising enterocolitis, respiratory complications, seizures, hypoglycaemia and intra-ventricular haemorrhage.

All serious adverse events will be reported to the principal investigator (or other nominated clinician) as soon as they have been identified. Full details including the diagnosis (if possible), the duration, actions taken, treatment given, outcome, causality and whether the event is expected or unexpected will be reported. A serious adverse event document will be completed and this will be sent to the data monitoring committee as soon as physically possible. The report will be emailed to the committee.

If an event is considered potentially related to the trial medication (and not in keeping with pre-eclampsia) it will then be immediately reported to the data monitoring committee and the manufacturer. Reporting and handling of adverse events will be in accordance with the GCP guidelines[49]. These procedures have been used in previous multicentre trials and proven to be efficient and compliant with the GCP principles and data management[49].

9.4 Unblinding

The need for unblinding should be very uncommon as the trial intervention is rarely associated with severe side effects and it will not delay or prevent standard management of the patient. If, however, unblinding is needed for any reason the principal investigator will be informed and, if necessary, the treatment will be revealed. Sheets with lists of 50 randomisation codes each will be sealed in individual, signed, numbered envelopes. If unblinding is requested, the relevant envelope will be opened by a person not involved in the trial in the presence of two witnesses, the group allocation read, and the list re-sealed in an envelope and signed. The randomisation envelopes will be accessible at all times to the principle investigator who will be contactable by mobile telephone. The principal investigator will co-ordinate the above process.

9.5 Reasons to stop the trial

If for any reason there is a need to stop the trial prematurely this decision will be taken by the Data and Safety Monitoring committee.

10. Duration of the project

It is anticipated that the study can be completed in approximately 4 years (2015 – 2018). The duration of the study has been approximated using data from the study by Hall et al., who studied expectant management of pre-eclampsia in the same group of patients in the same hospital. They averaged 56 patients per year and the cohort was obtained from 1992 to 1997. Since then deliveries at Tygerberg hospital have increased substantially. In the past year at Tygerberg Hospital it is estimated that 146 women were expectantly managed with early onset pre-eclampsia. If we were able to randomise half of these women we would be able to recruit 73 per year. We would then be able to recruit 120 women over a two to three year period, but have decided to plan for a four year period in case the numbers are less than expected.

The recruitment will begin as soon as we have approval from Human Research Ethics and the South African Medicines Control Council and Tygerberg Hospital. This study has been registered with NHREC (South African Human Research Ethics Committee) and PACTR (Pan African Clinical Trials Registry). We anticipate that this process will require six to nine months.

11. Project management

The trial coordination and management will be done by the principal investigator (PI Cluver) in South Africa. The trial management will include coordination and execution of the following activities which require administrative and clinical research input.

11.1 Quality control procedures

Before recruitment

A trial pilot run on recruitment, trial procedures, data collection and sample collection will be done before the study starts.

During recruitment

1. Data checking and entry of the completed data collection forms will be continuous. All data will be double entered from the trial data sheets, cleaned and queries checked immediately. Data sheets and all other documents will be stored for future reference, audits and queries in a secure location.
2. Double-blinding with identical-looking placebos will avoid any biases at entry to the trial and during the monitoring of women and assessment of outcomes, with respect to the main comparison.
3. Randomisation will occur after informed consent has been given. If a treatment pack is not used for whatever reason, it will be retained unopened with the woman's name on it. The woman will remain in the trial and all data collected and reported on an 'intention to treat' basis. The used and unused packs will be kept in the centres until completion of the trial and any quality assurance checks thought necessary.
4. Good Clinical Practice (GCP) procedures (WHO 1995) will be followed.
5. A random sample of unused packs will be tested for content to ascertain whether the content matches coding.

After recruitment

Data will be analysed and reported on an intention-to-treat basis. The draft analysis plan will be finalised before recruitment starts. The trial report will include requirements laid out in the CONSORT statement (Begg et al 1996).

Follow-up procedures

All participants will be followed from enrolment until their discharge from hospital after delivery and all data will be recorded.

11.2 Trial committee

Data Monitoring Committee

The Data Monitoring Committee will have meetings four times per year to discuss the progress of the trial. They will be supplied with reports of progress every three months. They will be notified as per the protocol of all serious adverse effects. If there are serious concerns they will arrange an emergency meeting and they will be able to stop the trial at any time. Minutes of their meetings will be sent to the chair of the PIE Steering Committee for documentation and auditing purposes.

11.3 Preparation for the trial

Coordination activities

- Investigator meeting before recruitment: standardisation of trial procedures; reception, handling and storing of trial materials; recruitment rate
- Discussion of logistics of treatment administration
- Establish communication procedures
- Designation of Data Monitoring Committee
- Organization of initial and final collaborators meetings

Trial materials

- Preparation of data collection forms and consent forms
- Preparation of trial manuals
- Preparation of the boxes for the treatment packs and their contents

Data processing and system preparation

- Randomisation of subjects

- System set-up for data entry and validation
- System set-up for production of monitoring reports

Statistical issues

- Preparation of dummy tables
- Definition of monitoring reports to be produced

11.4 Conduct of the trial

Coordination activities

- Monitor trial progress
- Communication with the data monitoring committee

Data management and statistical analysis

- Data entry
- Data validation and production of queries
- Update of the master file using batches of new data or corrections coming from validation checks and/or answers to queries
- Monitoring reports: recruitment, adverse events, losses to follow-up, completeness of data for main outcomes

Administrative

- Assistance with the organization of trial-related meetings including travel arrangements
- Maintaining a mailing list of trial contacts (collaborators and data monitoring committee members)
- Posting, photocopying, faxing

12. Ethical aspects

12.1 Confidentiality

Participant confidentiality will be maintained throughout the course of the study. Only on-site study staff will have access to the data. In order to protect participant confidentiality, each participant will be assigned a unique Participant Identification number (Participant ID). Patient confidentiality will be protected according to the regulations set forth by Stellenbosch University's Human Research Ethics Committee or Institutional Review Board (IRB). Biological samples that are collected will only be identified with the folder number and participant's trial number. Laboratory staff will not have access to the personal data.

All personal data on paper format will be stored in a secure location and will be treated as strictly confidential. No data that could identify a participant will be released.

All data collected will be stored for a minimum of 5 years or longer as defined by the requirements of the Ethics Committee once the trial is completed.

12.2 Compensation for participation

There will be no compensation for participation in this trial and treatment will be no different to standard care.

13. Potential risks and benefits of the study

This study aims to find a treatment for early onset pre-eclampsia. If a treatment is found it could decrease both maternal and neonatal morbidity and mortality rates. If the drug was able to prolong gestation for women suffering from early onset pre-eclampsia it would enable them to deliver a healthier baby and would decrease the work load considerably for the neonatal services.

Proton pump inhibitors have been used in pregnancy and the chance of maternal or fetal effects is likely to be very small.

14. Resources and strengths of the study

14.1 Strengths of our study

1) Incidence of early onset pre-eclampsia is extremely high in South Africa: For reasons that are unknown the rates of early onset pre-eclampsia (and eclampsia) are higher in the developing world. Duley, for instance, reported the incidence of eclampsia in the developed world is 2-3/10,000, but 16-69/10,000 in the developing world (ie 8-30 fold increase)[50]. Audit data on the number of cases of early onset pre-eclampsia managed at Tygerberg Hospital suggests that this trial can be feasibly performed in just one hospital. In stark contrast with the incidence of early onset, pre-eclampsia in the developed world being ≤1%, a number of recruiting sites would be needed in first world countries. As an example the STAMP trial (assessing the treatment of early onset pre-eclampsia with pravastatin) aims to recruit 120 women in the UK. They have over 15 recruiting sites. Currently the two external supervisors (Prof Stephen Tong and Prof Susan Walker) are running a pilot trial in Australia on the use of pravastatin for the treatment of early onset pre-eclampsia. Pravastatin is a category D/X drug. In South Africa we will be using a class C drug.

2) Running the trial at Tygerberg Hospital will make the findings relevant globally: Tygerberg Hospital has many modern state of the art facilitates on par with tertiary referral hospitals in the developed world. We have a neonatal special care unit, a maternal high dependency unit and an academic centre that actively contributes to the global scientific literature. Thus, running this study here has the dual advantages of: 1) having large numbers of early onset pre-eclampsia seen only in the developing world and 2) a centre that practises modern obstetrics similar to that in the developed world.

3) It capitalises on a unique collaborative opportunity between Australia and South Africa: Dr Catherine Cluver is an academic clinician based at Tygerberg, South Africa. In 2013, she was the visiting maternal-fetal medicine Fellow at Mercy Hospital (where Prof Walker and Prof Stephen Tong are based). A close clinical and academic collaboration developed among the CIs who have worked together to generate this trial protocol.

14.2 Limitations of this study:

This study is powered to identify a prolongation of pregnancy of five days, and is underpowered to detect benefits such as a shorter (but still beneficial) prolongation of pregnancy. It is not powered to determine differences in significant maternal outcomes or neonatal benefits.

If esomeprazole does prove to be effective we would then proceed to a larger multicentre study which would be better powered to investigate these outcomes.

Furthermore, while this study may provide immense value in the developing world, it is possible any positive findings would need to be confirmed in the developed world before it is considered globally relevant.

15. Publication of results

We will publish the results of this trial (whether or not we prove our hypothesis) in publically accessible, peer reviewed journals. If we obtain a positive finding, it is possible the findings could be published in a high impact journal and could change clinical practice.

16. Appendices

Consent form
Patient information sheet
Trial schema flow chart
Study site description
Insurance certificate
Budget

17. References

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PATIENT INFORMATION LEAFLET: PIE TRIAL

SUMMARY

Name of principal investigator: Dr Cathy Cluver

Name of research midwife: ……..

Contact number: ……

Website: …….

What is pre-eclampsia?
Pre-eclampsia is a serious condition that is only found in pregnancy.
It is associated with high blood pressure and can affect different parts of your body like the kidneys.
At present there is no effective treatment for pre-eclampsia except for birth of your baby but premature birth may cause medical problems for the baby.
For this reason we will keep you in hospital to monitor your pregnancy and health so that we can try and safely prolong your pregnancy to prevent your baby from possibly suffering some of the complications of a premature birth. This is the normal care that all women with early pre-eclampsia have.

The trial:
You are being invited to participate in a clinical trial that may help with finding a treatment for pre-eclampsia. This trial is to determine whether esomeprazole (a medication that is commonly used for reflux) can treat pre-eclampsia. This treatment may or may not improve your condition and may or may not delay the need for your baby to be born prematurely. This medication is safe in pregnancy and has been used by many pregnant patients.

If you decide to take part in the trial you may be given esomeprazole tablets or you may be given a placebo (dummy) tablet. You, the doctors and the nurses will not know what treatment you are being given. There is a 1 in 2 chance that you will be given the placebo or the esomeprazole tablet. Both tablets will look identical. Everyone in the study will receive exactly the same treatment as patients that are not in the study. If you are involved in the study you may have extra ultrasounds and we may need to collect extra blood from you while pregnant and at delivery a sample of blood from the cord and part of the placenta when you deliver.

If you would like to be involved in the clinical trial it will be important to start the medication as soon as possible. If you have any further questions about the study you can ask us questions at any time and we can be contacted on the telephone numbers given above.

BACKGROUND INFORMATION ON PRE-ECLAMPSIA

What is pre-eclampsia?

Pre-eclampsia is a serious medical condition that affects only pregnant women. It is caused by products released from the placenta that cause the mother to then suffer from high blood pressure. It commonly also effects the kidneys and this is seen by measuring the amount of protein in the urine. Pre-eclampsia can affect other organs in the body and can cause liver problems, blood clotting problems, and in severe cases seizures and stroke.

How do we treat pre-eclampsia?

At present there is no known treatment for this condition apart from delivery of the baby and the placenta. Early delivery can be associated with medical problems for the baby. Babies that are born too early can suffer from breathing problems, problems with their intestines and can have bleeding in the brain. We know that every day in early pregnancy can make a difference to the babies' survival and for this reason we admit pregnant mothers with this condition early in pregnancy to the hospital. Many studies have shown that this is a safe form of management as long as mothers are monitored very closely in the hospital.

When will I have my baby?

When you reach 34 weeks we will consider delivering the baby as we know that most babies born at this age do well. If you develop any complications or if the baby is in distress we will deliver your baby before we reach 34 weeks.

What treatment will I receive?

During your stay in hospital we will be monitoring your blood pressure. We will be checking your urine everyday to see how much protein you are losing through the kidneys. Twice a week we will be doing blood tests to monitor for complications. A doctor will be seeing you every day to check you and your baby's health. Every week there will be a large number of doctors that come and do a ward round to make sure that you and your baby are well. Your baby will be monitored 4 times a day with a monitor on your tummy. Ultrasound examinations of your baby will be performed.

You will receive treatment to control your blood pressure if it is needed and we may prescribe some pregnancy vitamins and supplementations. If you loose a large amount of protein in your urine you may be started on a treatment to prevent blood clots.

INVITATION TO BE INVOLVED IN THE PIE TRIAL

You are being invited to participate in a research project to find out whether a drug called esomeprazole can be used to treat pre-eclampsia. The name of the study is the Pre-eclampsia Intervention with Esomeprazole (PIE) trial. It is important that you read all the information provided about the trial before you decide to take part and that you understand why we are doing the research and what you would need to do if you were involved in the study. If you have any questions about the trial you can ask your doctor, the midwives or any of the staff from the research project. You can talk to any of the other women who have decided to be involved or not involved in the study.

Why are we doing this study?

Pre-eclampsia is a dangerous condition in pregnancy. If we could find a treatment for pre-eclampsia we would possibly be able to save many pregnant mothers and babies. There is no known treatment for pre-eclampsia. Other researchers in England are doing studies with a drug called pravastatin but have not finished their study yet.

What is the purpose of this study?

Recent research has shown that there are substances produced by the placenta that can cause pre-eclampsia. Work done in a laboratory in Melbourne, Australia has shown that these substances may be reduced by the group of drugs called proton pump inhibitors. Esomeprazole is one of these drugs. The PIE trial is the first trial in the world that will look to see if esomeprazole can be used to treat pre-eclampsia.

Why have I been invited to be involved in this study?

You have been invited to be in this study as you have been diagnosed with pre-eclampsia. We would like to include 120 women with pre-eclampsia in this study.

Do I have to be in the study?

It is voluntary to be in the study and it is your choice to be involved or to not be involved. Your treatment will not be any different if you are not involved in the study. You can decide at any stage in the pregnancy to withdraw from the study and you will not have to give a reason for why you want to withdraw.

Will I need to do anything extra if I am in the study?

Once you have decided to be in the trial you will need to sign an informed consent document. This form will say that you want to be involved in the study and that you have read and understood the information we have given you about the trial.

You will then need to take one extra tablet in the evening before you go to bed until the baby is delivered. One of the research team will visit you every day to see how you are feeling and to collect information about your pregnancy. When your routine blood tests are done we will take an extra sample of blood for the study. On the first day that you take the medication we will need to take an extra sample of blood. We may need to do extra ultrasound examinations of your baby. Once your baby is born and the cord has been cut we will take a small amount of blood from the placenta. We will take a small sample from the placenta which will be sent for testing. We will follow you up after the delivery of the baby. The samples taken may be sent overseas for further testing to try to find a treatment for pre-eclampsia. Only tests related to finding a cure for pre-eclampsia will be performed on the samples taken.

Is esomeprazole treatment safe in pregnancy?

Esomeprazole is used in pregnancy to treat reflux and gastric ulcers. There have been no reports of fetal problems in humans or complications in human pregnancies caused by this medication or other medications in the same class of drug.

Are there any side effects of esomeprazole?

All drugs may have side effects. Side effects that have been associated with the use of esomeprazole include headache, diarrhea, abdominal pain, constipation and a dry mouth. There are certain drugs that cannot be used with esomeprazole. If you are taking one of these drugs you will not be asked to participate in the trial. We will provide you with a copy of the product information leaflet if you would like more information.

Will I receive the placebo treatment or the esomeprazole treatment?

We will not know until the study is completed whether you were taking the dummy/placebo tablet or the esomeprazole tablet. The tablets will look identical and the midwives, nurses and doctors will not know which tablet you are taking. The tablet packages will be the same and only the pharmacy organizing the tablets will know what is in each packet. Once the study is completed we will then find out what tablets you were taking.

What will happen to the blood samples and the samples taken from the placenta?

These samples will be stored and may be sent to a laboratory at Melbourne University, Australia. The laboratory staff may do tests to see if esomeprazole can be used to treat pre-eclampsia. Only tests related to pre-eclampsia will be done on the samples. Your samples will not be used for genetic testing.

Will my information be kept confidential?

All information collected in the study will be kept strictly confidential. Information collected will only be available to people directly involved in the study. Your

information will be given a study number and your name will not be used for identifying any of your samples. The data collected will be locked in a secure location and only people involved in the study will have access to this information. Your name will not appear on any presentations or publications relating to this study. Only your study number will be on the samples taken and none of the laboratory staff will have access to your name or contact details.

Are there any benefits of me for being involved with this study?

Only half of the women in this trial will be given esomeprazole. If you do receive this treatment you may or may not benefit from the effects of this drug and you may or may not have improvement in your pre-eclampsia. There are no other direct benefits for you being involved in this study. By being involved you may help us find a treatment for pre-eclampsia which could help many pregnant mothers in your situation in the future.

What are the disadvantages of being in the study?

You will need to take an extra tablet in the evenings and we may need to take a few extra samples of your blood for testing.

What will happen if there is any new information while I am involved in the study?

There will be an independent committee that will be reviewing the results of the trial on an ongoing basis. If there is any new information you will be informed about it and will then be able to decide if you would like to continue with the trial

What will happen with the results of this research project?

The results of this study will be published in medical journals and will be presented at medical conferences. Your private details will not be included in the articles or presentations

Who has developed this study and who has reviewed the study?

This study has been developed by a team of researchers from Melbourne University in Australia and Stellenbosch University, South Africa. We have had experts in the field of pre-eclampsia involved with the study. This study has been approved by the Research Ethics Committee at the University of Stellenbosch and by the South African Medical Research Council.

Who has paid for this study?

This study has been funded by grants from the Medical Research Foundation for Mothers and Babies, and University of Melbourne.

Who do I contact if I have a problem?

If you have any concerns or problems you will be able to speak to the researchers involved in the study at anytime. If you have a more serious concern there is a safety and adverse event committee that you will be able to contact.

This study has been approved by the **Health Research Ethics Committee (HREC) at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

You can contact Dr Cathy Cluver at telephone number 082 321 0298 if you have any further queries or encounter any problems.

You can contact the **Health Research Ethics Committee** at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided above). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar of Medicines
Medicines Control Council
Department of Health
Private Bag X828
PRETORIA
0001

Fax: (012) 395 9201
e-mail: mogobm@health.gov.za

What do I do if I do not want to continue with the study?

If you decide to not continue with the trial at any stage you may withdraw and it will not affect the care that you are receiving in any way. You will not be asked to give us a reason for why you want to withdraw from the study. We will ask you if it will be possible to collect information about your pregnancy and delivery and we will ask you if it is possible for us to use the samples that we have already collected.

Who do I speak to if I have questions about the study?

If you have any questions you can discuss these with the research team, your doctor, the midwives involved in your care or with any of the other participants in the trial.

Thank you for taking the time to read this information leaflet about the PIE trial.

We hope that you will consider being involved in our study.

Please keep this copy of the information leaflet. If you do decide to be involved in the study you will be given a copy of the consent form

The Pre-eclampsia Intervention with Esomeprazole (PIE) trial: a double blind randomised, placebo-controlled trial to treat early onset severe preeclampsia

INFORMED CONSENT FORM

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Dr Catherine Anne Cluver

RESEARCH MIDWIFE: Name: ……
Contact number: ……
Email: ……

ADDRESS: Department of Obstetrics and Gynaecology
Tygerberg Hospital and University of Stellenbosch

You are being invited to take part in a research project. Please take some time to read the patient information leaflet given to you which will explain the details of this project.

Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. You will be given as much time as you need to decide whether you would like to be involved in the study. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee (HREC) at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

You can contact Dr Cathy Cluver at telephone number 082 321 0298 if you have any further queries or encounter any problems.

You can contact the **Health Research Ethics Committee** at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I …… agree to take part in a research study entitled: The Pre-eclampsia Intervention with Esomeprazole (PIE) trial: a double blind randomised, placebo-controlled trial to treat early onset preeclampsia.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) …… on (*date*) ……..

…… ……

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* …… declare that:

- I explained the information in this document to ……
- I encouraged her to ask questions and took adequate time to answer them.
- I am satisfied that she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ..... on (*date*) .....

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ..... declare that:

- I assisted the investigator (*name*) ..... to explain the information in this document to (*name of participant*) ..... using the language medium of Afrikaans/Xhosa.
- We encouraged her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all her question satisfactorily answered.

Signed at (*place*) ..... on (*date*) .....

....

Signature of interpreter **Signature of witness**

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

Section/item	Item No	Description	Full Protocol page no.
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Cover
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Cover
	2b	All items from the World Health Organization Trial Registration Data Set	Cover
Protocol version	3	Date and version identifier	Cover
Funding	4	Sources and types of financial, material, and other support	Cover
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1- 2
	5b	Name and contact information for the trial sponsor	N/A
	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	N/A
	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)	3-4
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	12-20
	6b	Explanation for choice of comparators	31
Objectives	7	Specific objectives or hypotheses	38-40
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)	31

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	57 and Study Site addendum
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)	34-37
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	31
	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)	49-50
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	42-43
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	43
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	38-40
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)	43-46 Flow chart appendix
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	31-33
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	57
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	33
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	33
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	33, 41-42
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	33
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	50
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	47-48
	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	44-46
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	47-48
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	47-48
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	47-48
	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)	47-48

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	3-4
	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	53
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	49-50
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	53
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Cover
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)	52-53
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	41
	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	55
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	N/A
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	N/A
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	See insurance appendix

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	59
	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	See Appenxix
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	44-47

*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.