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

<table>
<thead>
<tr>
<th><strong>Full title of trial</strong></th>
<th>The Pre-eclampsia Intervention with Esomeprazole (PIE) trial: a double blind randomised, placebo-controlled trial to treat early onset pre-eclampsia.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short title</strong></td>
<td>PIE trial</td>
</tr>
</tbody>
</table>
| **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                                                                                                                                                                                         |
| **Principal investigator** | Dr Catherine Anne Cluver                                                                                                           |
| **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                                                                                                                      |
| **Funders**              | Kilvington Trust  
Medical Foundation for Mothers and Babies  
Sheila Hanbury Foundation                                                                                                           |
CONTACT DETAILS

Principal Investigator for clinical trial
Dr Catherine Cluver
Tygerberg Hospital
University of Stellenbosch
Address: 11 Bath Street, Gardens, Cape Town, South Africa 8001
Tel: +27823210298
Fax: +27219322455
Email: cathycluver@hotmail.com

External Supervisors/ Co-Investigators/ Promoters
Prof Stephen Tong
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: stong@unimelb.edu.au

Prof Susan Walker
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: spwalker@unimelb.edu.au

Internal Supervisor/ Co-Investigator/ Co-promoter
Prof Gerhardus Theron
Tygerberg Hospital
University of Stellenbosch
Tel: +27 21 938 9209
Email: gbth@sun.ac.za

Prof Ben Mol
The Robinson Institute, School of Paediatrics and Reproductive Health
University of Adelaide
Phone: +61 434122170
Email: ben.mol@adelaide.edu.au
Prof David Hall  
University of Stellenbosch  
Email: drh@sun.ac.za

Principal investigators for Laboratory Studies  
Prof Stephen Tong  
Mercy Hospital for Women  
University of Melbourne  
Tel: +613 8458 4380  
Email: stong@unimelb.edu.au

Dr Natalie Hannan  
Mercy Hospital for Women  
University of Melbourne  
Tel: +613 8458 4380  
Email: nhannan@unimelb.edu.au

Trial Design and Statistics  
Dr Richard Hiscock  
Mercy Hospital for Women  
Email: richardjhiscock@gmail.com

Laboratory Coordination  
South Africa:  
Dr Catherine Cluver  
Tygerberg Hospital  
University of Stellenbosch  
Tel: +27823210298  
Email: cathycluver@hotmail.com

Australia:  
Prof Stephen Tong  
Mercy Hospital for Women  
University of Melbourne  
Tel: +613 8458 4380  
Email: stong@unimelb.edu.au
TRIAL COMMITTEES

Trial Steering Committee

Chair:
Prof Ben Mol
Professor of Obstetrics and Gynaecology
The Robinson Institute, School of Paediatrics and Reproductive Health
University of Adelaide
Phone: +61 434122170
Email: ben.mol@adelaide.edu.au

Members:
Dr Catherine Cluver
Tygerberg Hospital
University of Stellenbosch
Tel: +27823210298
Email: cathycluver@hotmail.com

Prof Stephen Tong
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: stong@unimelb.edu.au

Prof Susan Walker
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: spwalker@unimelb.edu.au

Data Monitoring Committee

Chair: Dr Jim Holberton

Members
Dr Jim Holberton
Consultant Paediatrician
Mercy Hospital for Women
Email: JHolberton@mercy.com.au
Tel: +61 3845 84723
Fax: +61 3945 95389

Dr Jonathan Morris
Professor Obstetrics and Gynaecology
Kolling Institute of Medical Research
University of Sydney
Tel +61 2 9926 4500
Email: jonathan.morris@sydney.edu.au
Website: http://sydney.edu.au/medicine/northern

Statistician: Dr Lisa Yelland
NHMRC Postdoctoral Research Fellow
Women’s & Children’s Health Research Institute and School of Population Health
The University of Adelaide
Email: lisa.yelland@adelaide.edu.au
Phone: 8313 3215 / 8313 1428
Fax: 8223 4075
Website: http://www.adelaide.edu.au/directory/lisa.yelland
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

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

2. BACKGROUND
   2.1 Introduction
   2.2 Pathogenesis of pre-eclampsia
   2.3 Biological role of proton pump inhibitors as possible therapy
      2.3.1 Proton Pump Inhibitors upregulate a key placental protective enzyme: heme-oxygenase-1
      2.3.2 Proton Pump Inhibitors block the release of sFlt-1 and soluble endoglin
      2.3.3 Proton pump inhibitors decrease endothelial dysfunction of blood vessels
      2.3.4 Animal models
   2.4 Proton pump inhibitors
      2.4.1 Safety data in pregnancy
         2.4.1.1 Animal studies
         2.4.1.2 Human studies
      2.4.2 Esomeprazole drug information
         2.4.2.1 Dosage
         2.4.2.2 Contra-indications
         2.4.2.3 Warnings and precautions
         2.4.2.4 Adverse reactions
         2.4.2.5 Drug interactions
         2.4.2.6 Mechanism of action
         2.4.2.7 Pharmacokinetics
   2.5 Summary

3. AIMS AND OBJECTIVES
   3.1 Specific Aims and Outcomes
   3.2 Hypothesis

4. STUDY DESIGN
   4.1 Type of study
   4.2 Study population
   4.3 Intervention
   4.4 Sample size calculation
   4.5 Randomisation and allocation concealment

5. INCLUSION AND EXCLUSION CRITERIA
   5.1 Definitions
   5.2 Inclusion criteria
   5.3 Exclusion criteria
6. OUTCOMES
   6.1 Primary outcome
   6.2 Secondary outcomes
7. DESIGN AND CONDUCT
   7.1 Identification and enrolment of participants
      7.1.1 Identification
      7.1.2 Consent
      7.1.3 Recruitment
   7.2 Study treatment
      7.2.1 Trial drug
      7.2.2 Packaging, formulation and supply of treatment
      7.2.3 Route of administration, dosage regimen and treatment period
      7.2.4 Resupply of treatment
   7.3 Expectant management for pre-eclampsia
      Routine management
   7.4 Clinical follow-up
      7.4.1 Baseline clinical information
   7.5 Sample collection
      7.5.1 Blood samples
      7.5.2 Urine samples
      7.5.3 Placental samples
      7.5.4 Cord blood samples
   7.6 Withdrawal from the study
8. DATA MANAGEMENT AND STATISTICAL ANALYSIS
   8.1 Data management
   8.2 Data recording
   8.3 Handling of missing data
   8.4 Data processing
   8.5 Analysis plan
   8.6 Statistical methods
9. SAFTEY AND MONITORING PROCEDURES
   9.1 Adverse effect
   9.2 Serious adverse effect
   9.3 Expected serious adverse effect
   9.4 Unblinding
   9.5 Reasons to end the trial prematurely
10. DURATION OF THE PROJECT
11. PROJECT MANAGEMENT
   11.1 Quality control procedures
   11.2 Trial committees
11.3 Preparation for the trial
11.4 Conduct of the trial

12. ETHICAL ASPECTS
12.1 Confidentiality
12.2 Compensation for participation

13. POTENTIAL RISKS AND BENEFITS OF THE STUDY

14. RESOURCES AND STRENGTHS OF THE STUDY
14.1 Strengths
14.2 Limitations

15. PUBLICATION OF RESULTS

16. APPENDICES

17. REFERENCE LIST

APPENDIX A: PATIENT INFORMATION LEAFLET
APPENDIX B: CONSENT FORM
APPENDIX C: TRIAL SCHEMA FLOW CHART
APPENDIX D: BUDGET
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.

Esoemprazole:
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\(^1\). 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 (i.e., 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, which cause maternal endothelial dysfunction (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 antioxidant 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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.

<table>
<thead>
<tr>
<th>Figure 1: Proton Pump Inhibitors upregulate HO-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five different proton pump inhibitors were added at increasing doses (5,20,50,100 \textmu M concentrations) to endothelial cells in vitro.</td>
</tr>
<tr>
<td>There is a potent dose dependent increase in HO-1 caused by all five proton pump inhibitors.</td>
</tr>
<tr>
<td>Lans: Lansoprazole</td>
</tr>
<tr>
<td>Ome: Omeprazole</td>
</tr>
<tr>
<td>Pan: Pantoprazole</td>
</tr>
<tr>
<td>Rab: Rabeprazole</td>
</tr>
<tr>
<td>Eso: Esomeprazole</td>
</tr>
<tr>
<td>COPP: Our +ve control</td>
</tr>
</tbody>
</table>

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

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\textsuperscript{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\textsuperscript{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\textsuperscript{14}. sFlt-1 administered \textit{in vivo} to pregnant rats can induce hypertension and proteinuria\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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).
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).
Pravastatin entered clinical trials on the strength of preclinical data generated from rodent animal models\textsuperscript{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. 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). 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. VCAM-1 and endothelin-1 are widely accepted as markers of endothelial dysfunction.

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).
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. 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 implantation.
invasion of maternal spiral arteries given normal invasion is already shallow\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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. 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.

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. 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.

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. 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\textsuperscript{11}.

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: \textcolor{blue}{\texttt{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\textsuperscript{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.

\subsection*{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.

\subsection*{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.

\subsection*{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.

PIE trial, Version 2.4, 22/08/2014
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>
<thead>
<tr>
<th>Detectable difference (in days)</th>
<th>Group size, per arm</th>
<th>Group size x 1.15*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>116</td>
<td>134</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>27</td>
</tr>
</tbody>
</table>

* 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\(^45\).


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.

<table>
<thead>
<tr>
<th>Strata 1</th>
<th>Gestational age (GA)</th>
<th>EFW if GA is unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strata 1</td>
<td>26+0 up to and including 28+6 weeks</td>
<td>Less than and including 1100gm</td>
</tr>
<tr>
<td>Strata 2</td>
<td>29+0 up to and including 31+6</td>
<td>Greater than 1100gm</td>
</tr>
</tbody>
</table>

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. 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

<table>
<thead>
<tr>
<th>Pre-existing (chronic) hypertension</th>
<th>Gestational hypertension</th>
<th>Preeclampsia</th>
<th>Other hypertensive effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>* With comorbid condition(s)</td>
<td>* With comorbid condition(s)</td>
<td></td>
<td>Elevated BP may be due to environmental stimuli or the pain of labour, for example</td>
</tr>
<tr>
<td>* With evidence of preeclampsia</td>
<td></td>
<td></td>
<td>BP that is elevated in the office (sBP &gt; 140 mmHg or dBP &gt; 90 mmHg) but is consistently normal outside of the office (&lt;135/85 mmHg) by ABPM or HBPM</td>
</tr>
<tr>
<td>Comments</td>
<td></td>
<td></td>
<td>BP that is consistently normal in the office (sBP &lt; 140 mmHg or dBP &lt; 90 mmHg) but is elevated outside of the office (&gt; 135/85 mmHg) by ABPM or repeated HBPM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>* These may occur in women whose BP is elevated at &lt;20 or ≥20 weeks who are suspected of having pre-existing or gestational hypertension/preeclampsia, respectively.</td>
</tr>
</tbody>
</table>

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

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

* 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 MacGillivray47.

**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 x 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](http://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 plasma 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 1cm³ 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\textsuperscript{49}. These procedures have been used in previous multicentre trials and proven to be efficient and compliant with the GCP principles and data management\textsuperscript{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)\(^5\). 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 \(\leq 1\%\), 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 facilities 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


