

**Pre-eclampsia Intervention 2 (PI2) Trial:**  
**a double blind randomised, placebo-controlled**  
**trial of metformin to treat early onset pre-**  
**eclampsia**

**Phase II study**

**CLINICAL TRIAL PROTOCOL**

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1.2

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Date: 30/01/2019

## Abbreviations

<b>CTG</b>	Cardiotocograph
<b>FDA</b>	Food and Drug Administration
<b>GA</b>	Gestational age
<b>HO-1</b>	Heme oxygenase-1
<b>ITT</b>	Intention to treat
<b>ISSHP</b>	International Society for the Study of Hypertensive disorders in Pregnancy
<b>KEAP-1</b>	Kelch-like ECH-associated protein 1
<b>mg</b>	Milligrams
<b>Nrf-2</b>	Nuclear factor (erythroid-derived 2)-like 2
<b>PI2</b>	Pre-eclampsia Intervention 2
<b>PPIs</b>	Proton pump inhibitors
<b>PRES</b>	Posterior reversible encephalopathy syndrome
<b>sEng</b>	Soluble Endoglin
<b>sFlt1</b>	Soluble Fms Like Tyrosine Kinase -1
<b>HIF 1<math>\alpha</math></b>	Hypoxic inducible factor 1 $\alpha$
<b>TNF</b>	Tumour Necrosis Factor
<b>TR</b>	Treatment received
<b>VCAM-1</b>	Vascular Cell Adhesion Molecule -1

# CONTENTS

Summary .....	12
1.1 Title.....	12
1.2 Short Introduction and literature overview.....	12
1.3 Research question.....	12
1.4 Aims.....	13
1.4.1 Primary aim .....	13
1.4.2 Secondary aims .....	13
1.5 Methods (Overview) .....	13
1.6 Timeline.....	13
1.7 Ethical considerations .....	13
1.8 Anticipated overall outcome.....	14
Background .....	15
2.1 Introduction .....	15
2.2 Pathogenesis of Pre-eclampsia .....	16
2.3 Biological role of metformin as possible therapy .....	16
2.3.1 Metformin inhibits hypoxic inducible factor 1 $\alpha$ (HIF 1 $\alpha$ ) .....	17
2.3.2 Metformin reduces sFlt-1 secretion from placental endothelial cells and placental tissue .....	17
2.3.3 Metformin reduces VCAM-1 expression on endothelial cells .....	21
2.3.4 Metformin induces vasodilation in maternal vessels and enhances angiogenic sprouting .....	22
2.4 Clinical trials showing metformin may decrease the incidence of pre-eclampsia .....	22
2.5 Metformin .....	22
2.5.1 Safety data in pregnancy.....	22
2.5.2 Metformin drug information .....	23
2.6 Summary .....	26
Aims and Objectives.....	27
3.1 Specific Aims and Outcomes:.....	27
Primary aim: .....	27



Primary outcome:.....	27
Secondary aims: .....	27
3.2 Hypothesis.....	27
Primary Hypothesis: .....	27
Other hypotheses:.....	28
Study Design.....	29
4.1 Type of study.....	29
4.2 Study population.....	29
4.3 Intervention.....	29
4.4 Sample size calculations.....	29
4.5 Randomisation and allocation concealment .....	31
Inclusion and exclusion criteria.....	33
5.1 Definitions: .....	33
5.2 Inclusion criteria.....	35
5.3 Exclusion criteria .....	35
Outcomes .....	38
6.1 Primary outcome.....	38
6.2 Secondary outcomes.....	38
6.2.1. Composite maternal outcome: .....	38
6.2.2. Composite neonatal outcome: .....	38
6.3 Exploratory outcomes.....	39
6.3.1 Exploratory maternal outcomes .....	39
6.3.2 Exploratory fetal outcomes.....	40
6.3.3 Exploratory neonatal outcomes.....	40
6.4.....	40
Biomarkers: .....	40
Trial conduct.....	42
7.1 Identification and enrolment of participants.....	42
7.1.1 Identification .....	42
7.1.2 Consent .....	42
7.1.3 Recruitment .....	42

7.2 Study treatment .....	43
7.2.1 Trial drug .....	43
7.2.2 Packaging, formulation and supply of the treatment.....	43
7.2.3 Route of administration, dosage regimen and treatment period .....	43
7.2.4 Resupply of treatment .....	44
7.2.5 Dosage.....	44
7.3 Expectant management for pre-eclampsia.....	44
7.3.1 Routine management .....	44
7.4 Clinical Follow-up .....	45
7.4.1 Baseline clinical information .....	45
7.5 Sample collection .....	45
7.5.1 Blood samples .....	45
Cord Blood Samples .....	46
Placental samples.....	46
7.6 Withdrawal from the study.....	47
Data management and statistical analysis.....	48
8.1 Data management.....	48
8.2 Data recording.....	48
8.3 Handling of missing data .....	48
8.4 Data processing .....	48
8.5 Analysis plan.....	48
8.6 Statistical methods.....	48
SAFETY MONITORING AND PROCEDURES .....	51
9.1 Adverse event .....	51
9.2 Serious adverse event .....	51
9.3 Expected serious adverse events .....	51
9.4 Unblinding .....	52
9.5 Reasons to stop the trial .....	52
Duration of the project .....	53
Project management.....	54
11.1 Quality control procedures .....	54
	10

Before recruitment .....	54
During recruitment .....	54
After recruitment .....	54
Follow-up procedures .....	55
11.2 Trial committee .....	55
Data Monitoring Committee .....	55
11.3 Preparation for the trial .....	55
Coordination activities .....	55
Trial materials.....	55
Data processing and system preparation .....	56
Statistical issues .....	56
11.4 Conduct of the trial .....	56
Coordination activities .....	56
Data management and statistical analysis.....	56
Administrative.....	56
Ethical aspects.....	57
12.1 Confidentiality.....	57
12.2 Compensation for participation .....	57
Potential risks and benefits of the study .....	58
Resources and strengths of the study.....	59
14.1 Strengths of our study.....	59
14.2 Limitations of this study:.....	59
Publication of results.....	61
Role of study funders .....	62
References.....	63

# Summary

## 1.1 Title

Pre-eclampsia Intervention 2 (PI2) Trial: a double blind randomised, placebo-controlled trial of metformin to treat early onset pre-eclampsia

## 1.2 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.

Metformin is widely used in pregnancy for the treatment of gestational diabetes. Recently, preclinical data has been generated that shows that metformin has potent biological effects making it a lead candidate to treat early onset pre-eclampsia.

Metformin:

- inhibits hypoxic inducible factor 1 $\alpha$  (HIF 1 $\alpha$ )
- reduces sFlt-1 and sEng secretion from primary endothelial cells and placental tissue
- reduces VCAM-1 expression on endothelial cells
- induces vasodilation in maternal vessels and enhances angiogenic sprouting

Clinical trials in pregnant mothers have shown that the use of metformin is associated with a trend to less hypertensive disorders of pregnancy but the trials were not powered for this outcome.

Metformin is inexpensive, off patent 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.

## 1.3 Research question

Can metformin, 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?

## **1.4 Aims**

### **1.4.1 Primary aim**

To examine whether 3 grams of metformin daily can safely further prolong gestation for 5 days in women with early onset pre-eclampsia diagnosed 26+0 – 31+6 weeks, compared to standard of care, expectant management alone.

### **1.4.2 Secondary aims**

To determine whether metformin improves 1) maternal, 2) fetal and 3) neonatal outcomes in early onset pre-eclampsia compared to placebo.

To examine whether metformin can significantly decrease circulating levels of sFlt-1 and/ or sEng in women with early onset pre-eclampsia, compared to placebo.

## **1.5 Methods (Overview)**

We will perform a double blind randomised controlled trial of 150 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 receive either metformin 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.

## **1.6 Timeline**

3 years (2018 to 2021)

## **1.7 Ethical considerations**

Pregnant women are a vulnerable population. Early onset pre-eclampsia is a major cause of maternal, fetal and neonatal morbidity and mortality. Metformin is widely used in pregnancy for the treatment of diabetes and is considered safe in pregnancy. Metformin may be a treatment for pre-eclampsia and for this reason we believe that it is ethical to conduct this trial.

## **1.8 Anticipated overall outcome**

This phase II study will provide insight in the question whether metformin may be able to allow women diagnosed with early onset pre-eclampsia to safely gain gestation.

# 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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually<sup>4,5</sup> and in South Africa hypertensive disorders of pregnancy are responsible for 14% of maternal deaths.<sup>6</sup> 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.<sup>7</sup>

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

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

We have completed a double blind randomised control trial at Tygerberg Hospital, the Pre-Eclampsia Intervention with Esomeprazole (PIE) trial, to evaluate whether esomeprazole can be used as a treatment for early onset pre-eclampsia.<sup>11</sup> This would be the second trial evaluating key therapeutics for this serious condition at Tygerberg Hospital.

### *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 to reduce child mortality and improve maternal health, which is and is one of the United Nations Development goals.

## 2.2 Pathogenesis of Pre-eclampsia

### *Hypoxia, 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. Consequently, 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.<sup>12,13</sup> 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 hypoxia provokes the release of soluble Fms Like Tyrosine Kinase -1 (sFlt1) and soluble endoglin (sEng) into the maternal circulation.<sup>14,15</sup> 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.<sup>2,9</sup>

Thus, the key aspects in the pathophysiology of pre-eclampsia are the placental release of the anti-angiogenic factors sFlt1 and soluble endoglin and 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 metformin as possible therapy

A recent publication in the American Journal of Obstetrics and Gynecology, Brownfoot et al., (ie our external collaborators in Melbourne) showed that metformin, which inhibits hypoxic



inducible factor 1 $\alpha$  (HIF 1 $\alpha$ ), reduced soluble fms-like tyrosine kinase 1 and soluble endoglin secretion from primary human tissues. Metformin was also shown to reduce endothelial dysfunction, it enhanced vasodilation in maternal omental arteries and induced angiogenesis.<sup>16</sup> These characteristics make it a potential therapeutic to treat pre-eclampsia. Possible biological explanations include the following:

- Metformin inhibits hypoxic inducible factor 1 $\alpha$  (HIF 1 $\alpha$ )
- Metformin reduces sFlt-1 and sENG secretion from primary endothelial cells and placental tissue
- Metformin reduces inflammatory molecule VCAM-1 expression on endothelial cells
- Metformin induces vasodilation in maternal vessels and enhances angiogenic sprouting

### **2.3.1 Metformin inhibits hypoxic inducible factor 1 $\alpha$ (HIF 1 $\alpha$ )**

HIF 1 $\alpha$  is upregulated in ischaemia and hypoxia and is known to facilitate the secretion of sFlt (a key pathogenic factor in pre-eclampsia, see below). By inhibiting HIF 1 $\alpha$  the secretion of sFlt is decreased which may improve clinical outcomes in pre-eclampsia.<sup>16</sup> Metformin is an ideal HIF 1 $\alpha$  inhibitor as it is safe in pregnancy. In contrast other HIF 1 $\alpha$  inhibitors (e.g YC-1 and ouabain) which are currently being trialed to treat pulmonary hyperplasia and cancer respectively, have not been used in pregnant patients and therefore do not have a safety profile in pregnancy.

### **2.3.2 Metformin reduces sFlt-1 secretion from placental endothelial cells and placental tissue**

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.<sup>17</sup> Their effect is probably mediated via anti-angiogenic mechanisms.

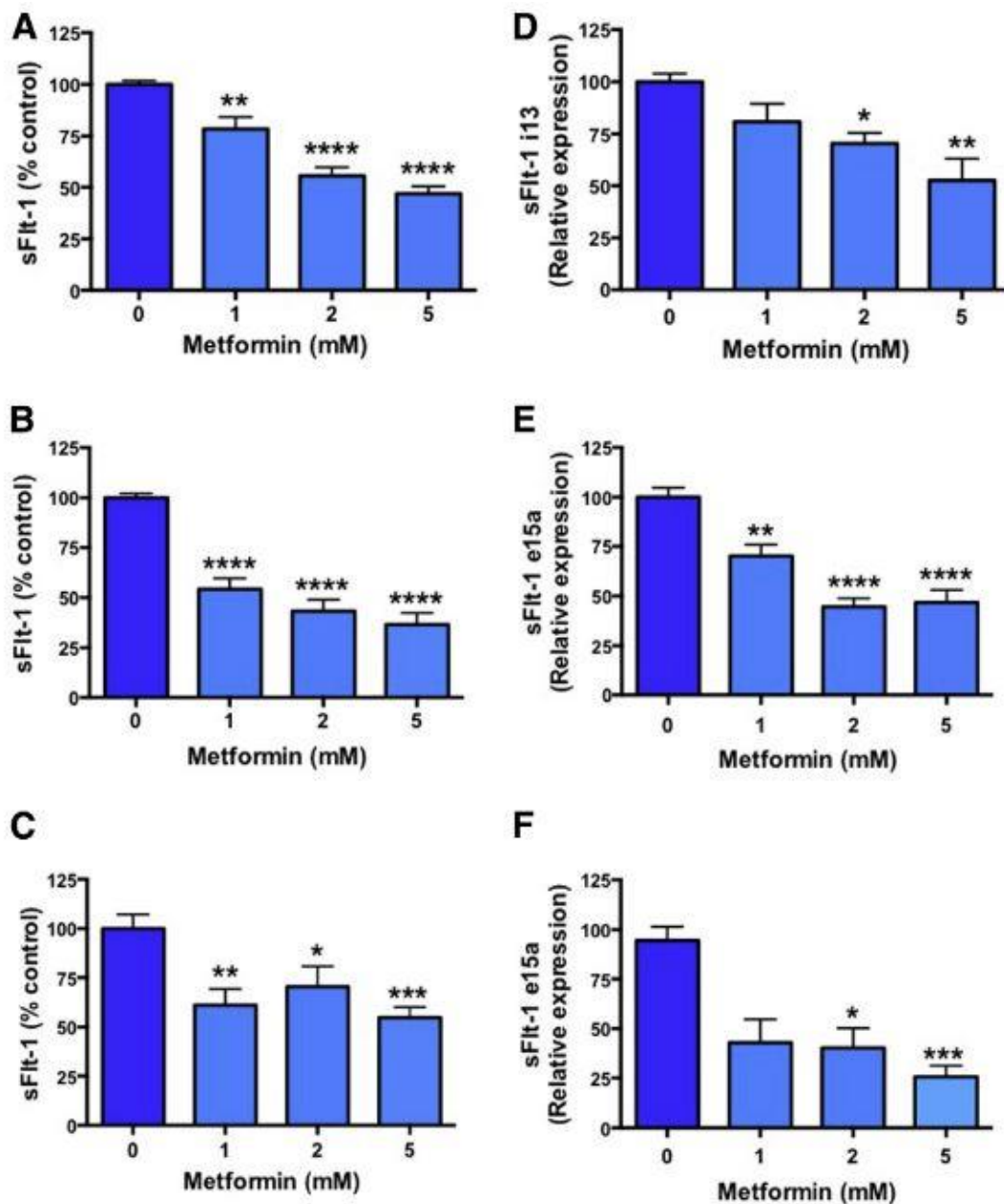
The evidence implicating sFlt-1 and sEng as central to the pathogenesis of pre-eclampsia is compelling.<sup>18</sup> Serum sFlt-1 and sEng are increased in women with pre-eclampsia many weeks preceding clinical disease and there is a dose dependent relationship between serum levels and disease severity.<sup>14</sup> sFlt-1 administered *in vivo* to pregnant rats induces hypertension and proteinuria.<sup>19</sup> 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.<sup>20</sup> 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.<sup>14</sup> 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 to treat pre-eclampsia is highlighted by the design of the 'Statins to Ameliorate early onset Pre-eclampsia' (STAMP) trial. This UK based trial, which was approved by the University of Birmingham examined the potential of using pravastatin to treat early onset pre-eclampsia. The primary outcome of this trial was to show a significant reduction in serum sFlt-1.

Brownfoot et al., has generated preclinical data showing that metformin induces marked decreases in sFlt-1 and sEng in both primary endothelial and trophoblast cells(see figure 3 and 4).<sup>16</sup>

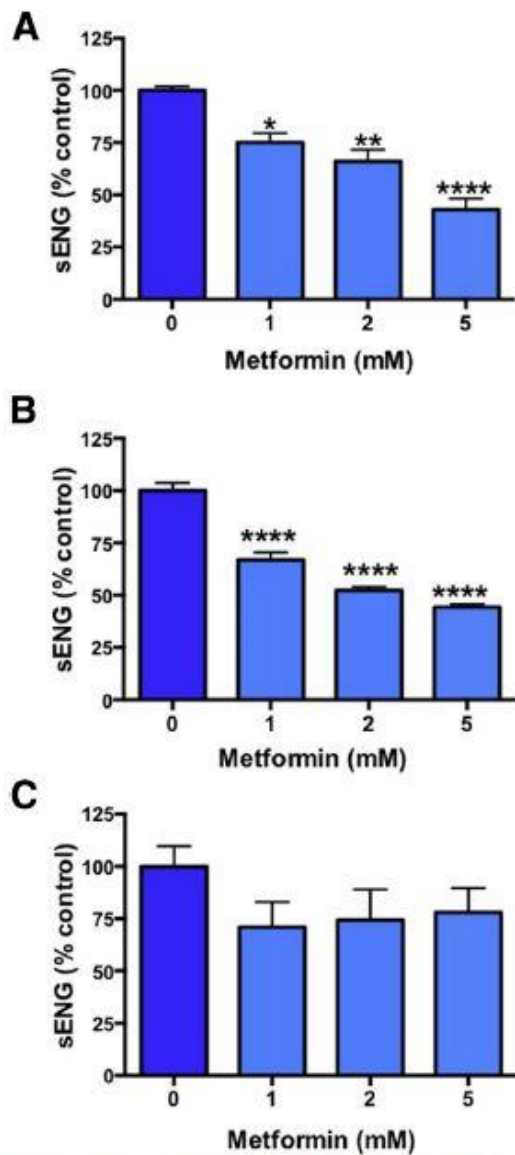
Figure 3: Effect of metformin on soluble fms-like tyrosine kinase 1 secretion and isoforms e15a and i13 expression in endothelial cells and placental tissue



Metformin (0, 1, 2, 5 mmol/L) dose-dependently reduced soluble fms-like tyrosine kinase 1 secretion from **A**, endothelial cells, **B**, villous cytotrophoblast cells, and **C**, preterm preeclamptic placental villous explants. Metformin reduced endothelial cell expression of **D**, *sFlt-1 i13* isoform, **E**, villous cytotrophoblast cells, and **F**, preterm preeclamptic placental villous explant messenger RNA expression of *sFlt-1 e15a*. The *single asterisk* indicates  $P < .05$ ; the *double asterisks* indicate  $P < .01$ ; the *triple asterisks* indicate  $P < .0001$ ; the *quadruple asterisks* indicate  $P < .00001$ . *sFlt-1*, soluble fms-like tyrosine kinase 1.

Brownfoot et al. Metformin decreases sFlt-1 and sENG, improves endothelial function, and is angiogenic. *Am J Obstet Gynecol* 2016.

Figure 4: Effect of metformin on soluble endoglin secretion from endothelial cells and placental tissue



Metformin (0, 1, 2, and 5 mmol/L) reduced soluble endoglin secretion from **A**, endothelial cells and **B**, villous cytotrophoblast cells. Metformin did not change soluble endoglin secretion from **C**, preterm preeclamptic placental villous explants. The *single asterisk* indicates  $P < .05$ ; the *double asterisks* indicate  $P < .01$ ; the *quadruple asterisks* indicate  $P < .00001$ .

sENG, soluble endoglin.

Brownfoot et al. Metformin decreases sFlt-1 and sENG, improves endothelial function, and is angiogenic. *Am J Obstet Gynecol* 2016.

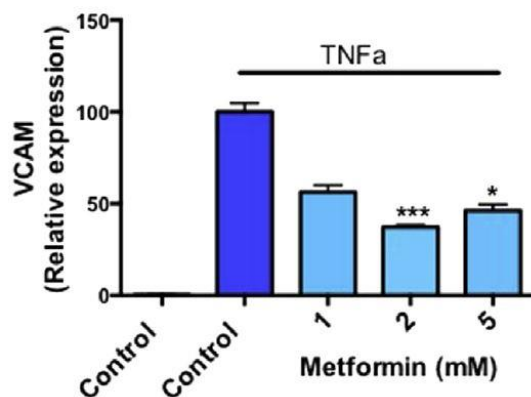
### 2.3.3 Metformin reduces VCAM-1 expression on endothelial cells

A hallmark of pre-eclampsia is endothelial dysfunction, a form of maternal blood vessel injury.<sup>17</sup> 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 is an adhesion molecule that is expressed on the luminal surface of blood vessels and can cause an inflammatory mesh and snare circulating blood cells by directly binding to leukocytes, causing leukocyte adhesion to the vascular endothelium. VCAM-1 is widely accepted as a marker of endothelial dysfunction.<sup>21</sup>

It has recently been discovered that metformin potently blocks up-regulation of VCAM-1 induced by Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), an inflammatory molecule involved in endothelial dysfunction and increased in the serum of women with preeclampsia (17) (see Figure 5).

Figure 5: Effect of metformin on endothelial cell vascular cell adhesion molecule 1 expression



Inflammatory cytokine tumor necrosis factor  $\alpha$  increased endothelial cell expression of vascular cell adhesion molecule 1 and was significantly reduced with increasing doses of metformin (0, 1, 2, and 5 mmol/L). The *single asterisk* indicates  $P < .05$ ; the *triple asterisks* indicate  $P < .0001$ .

TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; VCAM 1, vascular cell adhesion molecule 1.

Brownfoot et al. Metformin decreases sFlt-1 and sENG, improves endothelial function, and is angiogenic. *Am J Obstet Gynecol* 2016.

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

### **2.3.4 Metformin induces vasodilation in maternal vessels and enhances angiogenic sprouting**

Peripheral vasoconstriction and decreased arterial compliance are thought to be the causes for hypertension in pre-eclampsia. Metformin has been shown to reverse vasoconstriction in vessels in conditioned placental culture medium. Reduced angiogenesis is thought to contribute to placental hypoxia and to the development of pre-eclampsia. Metformin has been shown to rescue sFlt induced inhibition of angiogenic sprouting making it a possible prevention and treatment strategy for pre-eclampsia.<sup>16</sup>

## **2.4 Clinical trials showing metformin may decrease the incidence of pre-eclampsia**

There have been several randomised controlled trials assessing the effect of metformin in pregnancy. Preeclampsia and gestational hypertension have been secondary outcomes reported in each of these trials. The MIG trial assessed metformin compared with insulin to treat gestational diabetes mellitus. The maximum dose of metformin was 2500 mg. This trial showed a nonsignificant decrease in the incidence of both gestational hypertension (3,9% in the metformin arm versus 6,2% in the insulin arm) and pre-eclampsia (5,5% metformin arm versus 7% in the insulin arm) among those treated with metformin.<sup>22</sup> A second randomised trial which compared metformin to placebo in obese pregnant women without diabetes mellitus with the primary outcome of a reduction in neonatal birthweight showed a non-significant decrease in the incidence of pre-eclampsia (5,5% in the placebo group and 3,0% in the metformin group).<sup>23</sup> The EMPOWaR trial was a double blind randomised placebo controlled trial which looked at the maternal and fetal effects of metformin in obese women. They showed no decrease in the incidence of hypertensive disorders of pregnancy with the use of metformin.<sup>24</sup>

## **2.5 Metformin**

### **2.5.1 Safety data in pregnancy**

Metformin has been extensively used for the treatment of gestational diabetes in pregnancy and in the first trimester of pregnancy in women polycystic ovarian syndrome and is considered a category B drug in pregnancy 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.<sup>25</sup>

### **2.5.1.1 Animal studies:**

Reproductive studies have been performed in rats and rabbits, with doses up to 600mg/kg/day and have shown no teratogenic effects which represents dosages of 2-6 times the maximum human daily dose. Determination of fetal concentrations showed a partial placental barrier to metformin.<sup>26,27</sup>

### **2.5.1.2 Human studies**

A recent meta-analysis of nine controlled studies with women affected by polycystic ovarian syndrome determined that the rate of major birth defects in women exposed to metformin in the first trimester was not statistically increased compared with the disease-matched control group and concluded that there was no evidence that metformin was associated with an increased risk of major birth defects in women affected by polycystic ovarian syndrome.<sup>28</sup>

There are numerous studies of metformin for the treatment of diabetes in pregnancy which have shown no adverse fetal effects when used in the first, second and third trimesters of pregnancy.<sup>22,29-32</sup> The American College of Obstetricians and Gynaecologists, the American Diabetes Association and the South African Provincial Government Western Cape Diabetes in Pregnancy Guidelines recommend the use of Metformin for the treatment of Diabetes in pregnancy.<sup>33</sup>

## **2.5.2 Metformin drug information**

Product information on metformin can be found at the following link:  
[http://packageinserts.bms.com/pi/pi\\_glucoophage\\_xr.pdf](http://packageinserts.bms.com/pi/pi_glucoophage_xr.pdf)

### **2.5.2.1 Dosage**

Metformin is available as 500mg, 850mg or 1000mg metformin hydrochloride tablets. Extended release tablets are available as 500mg or 750mg tablets and contain the inactive ingredients sodium carboxymethyl cellulose, hypomellose and magnesium stearate.

Pharmacokinetic data on pregnant women using metformin in the third trimester of pregnancy show that the pharmacokinetics are similar to nonpregnant patients and that no dosage adjustment is required.<sup>32</sup>

### **2.5.2.2 Contraindications**

Metformin is contraindicated in

- Renal disease or renal dysfunction (suggested by serum creatinine levels  $\geq 123\mu\text{mol/L}$  or abnormal creatinine clearance) which may also result from conditions such as cardiovascular collapse (shock), acute myocardial infarction, and septicemia.

- Known hypersensitivity to metformin hydrochloride.
- Acute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma.

### **2.5.2.3 Warnings and precautions**

There is very small risk of lactic acidosis (0.03 cases/1000 patient years) and reported cases have been associated with renal insufficiency which is a contraindication for the use of metformin. Patients should be warned of the following nonspecific symptoms which include malaise, myalgia, respiratory distress, somnolence and nonspecific abdominal pain and should report these symptoms to their attending physician.

### **2.5.2.4 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 side-effects of metformin.

### **2.5.2.5 Drug interactions**

Furosemide—Pharmacokinetic parameters of both compounds are affected by coadministration. Furosemide increases the metformin plasma and blood C<sub>max</sub> by 22% and blood AUC by 15%, without any significant change in metformin renal clearance.

Nifedipine—A single-dose, metformin-nifedipine drug interaction study in normal healthy volunteers demonstrated that coadministration of nifedipine increased plasma metformin C<sub>max</sub> and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. T<sub>max</sub> and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine.

Cationic drugs—Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Such interaction between metformin and oral cimetidine have been observed in normal healthy volunteers in both single- and multiple-dose, metformin-cimetidine drug interaction studies, with a 60% increase in peak metformin plasma and whole blood concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics.



### **2.5.2.6 Mechanism of action**

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. It does not cause hypoglycaemia and hyperinsulinaemia.

As discussed above, the proposed mechanisms for the treatment of pre-eclampsia are the following:

- Metformin inhibits hypoxic inducible factor 1 $\alpha$  (HIF 1 $\alpha$ )
- Metformin reduces sFlt-1 and sENG secretion from primary endothelial cells and placental tissue
- Metformin reduces VCAM-1 expression on endothelial cells
- Metformin induces vasodilation in maternal vessels and enhances angiogenic sprouting.

### **2.5.2.7 Pharmacokinetics**

The absolute bioavailability of metformin 500mg tablet given under fasting conditions is approximately 50% to 60%. Studies using single oral doses of metformin 500mg to 1500mg, and 850 to 2550mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination.

Food decreases the extent of, and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak plasma concentration (C<sub>max</sub>), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (T<sub>max</sub>) following administration of a single 850mg tablet of metformin. C<sub>max</sub> is achieved with a median value of 7 hours and a range of 4 to 8 hours.

After repeated administration metformin did not accumulate in plasma.

Metformin is negligibly bound to plasma proteins. Metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing steady state plasma concentrations of metformin are reached within 24 to 48 hours and are usually <1  $\mu$ g/mL. During controlled clinical trials, maximum plasma levels did not exceed 5  $\mu$ g/mL, even at maximum doses.

Metformin is excreted unchanged in the urine and does not undergo hepatic metabolism or biliary excretion. Renal clearance is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the

elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

## **2.6 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 and clinical data has uncovered potent biological actions suggesting metformin may be a lead candidate therapeutic to treat pre-eclampsia. Treatment with metformin in pregnancy is considered safe as it is used extensively for the treatment of diabetes.

We have a unique collaboration between Stellenbosch and Melbourne University that allows us to test this hypothesis in Tygerberg Academic Hospital, South Africa. This population has a high prevalence of early onset pre-eclampsia. We have completed the Pre-eclampsia Intervention trial with Esomeprazole (PIE) and his trial shows that the collaboration is successful, efficient and cost effective..

## Aims and Objectives

### 3.1 Specific Aims and Outcomes:

#### Primary aim:

- 1) To examine whether metformin can prolong gestation in women with early onset pre-eclampsia diagnosed 26+0 – 31+6 weeks who are being managed expectantly as 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 metformin 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 metformin 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 metformin is safe and well tolerated in the mother and infant, compared with placebo.

### 3.2 Hypothesis

#### Primary Hypothesis:

- 1) Metformin can prolong gestation in women with early onset pre-eclampsia, compared to expectant management alone.

**Other hypotheses:**

- 2) Metformin improves maternal, fetal and neonatal outcomes, in women diagnosed with early onset preeclampsia, compared to expectant management alone.
- 3) Metformin decreases the circulating levels of sFlt1 and sEng in early onset preeclampsia being managed expectantly compared to expectant management alone.

## 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 metformin 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 typically those where the primary outcome is safety.

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

### 4.2 Study population

Tygerberg Hospital has extensive experience with the management of early onset pre-eclampsia (37). 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. Women who consent and meet eligibility criteria (see 5.2) will be enrolled in the study.

### 4.3 Intervention

Participants will be randomised to administration of either active tablets containing metformin or an identical placebo. Once they have been recruited they will start by taking one tablet twice a day. If they have no side effects after 24 to 48 hours they will increase to 2 tablets twice a day. If again they experience no side effects after 24 to 48 hours they will increase to 3 tablets twice a day. If they are not tolerating the tablets the dose can also be decreased. The maximum dose of metformin will be 1.5mg in the morning and evening. Treatment will continue until delivery. The study will not alter or interfere with any treatment or care given routinely to women with early onset pre-eclampsia.

### 4.4 Sample size calculations

The primary research question is does treatment with metformin result in a gain in the median length of gestation of greater than or equal to 5 days compared to the mother receiving placebo therapy. This magnitude of prolongation we believe to 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).

Given the focus on median prolongation and the skewed nature of the gestation prolongation (seen in the two trials listed below) we based sample size calculations on the Geometric Mean Ratio (GMR). Analysis was performed both using PASS 13 (Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA, [ncss.com/software/pass](http://ncss.com/software/pass)) and the Powercal program running in Stata v15 (Stata statistical software. 2017 Release 15. Stata Corp. College Station, TX: StataCorp LLC)<sup>34</sup>.

Power calculations settings were: 90% power, with two-sided alpha set at 0.05, control median prolongation of 8.3 days and clinically important prolongation of gestation of 5 days. The measure of variability used in this analysis is the Coefficient of Variation (CV) = (data SD)/ data Mean) in mothers treated with standard expectant management. We have two sources for gestational prolongation in pre-eclamptic mothers receiving expectant management after PE diagnosis both undertaken at the same hospital where we plan to run this trial (Tygerberg Hospital). The first was by Hall et al, who in the largest descriptive study on expectant management of early onset pre-eclampsia reported a mean gestation prolongation of 11 (SD 7) days equivalent to a CV = 0.64.<sup>35</sup> The second was the PIE trial, performed by Cluver et al, where patients randomized to the placebo arm had an overall mean prolongation of 13.1 (SD 12.2) days, a CV = 0.93. In this study mothers entering with greater than 29 week gestations the CV = 0.8.<sup>11</sup> We have chosen to use a CV = 1.0, slightly larger than the 0.93 found in our PIE study, to allow for adequate power if greater variability in prolongation times occurs in PI2.

The results of sample size requirements for a limited range of GMR and two CVs are presented in table 1.

Table 1: Sample size calculation

Detectable prolongation in median days*	Treated group Median	GMR	Number per group (CV = 1.0)	Number per group (CV = 0.93)	Number per group (CV = 0.80)
3.11	11.43	1.37	137	134	105
4	12.32	1.48	88	87	69
5	13.32	1.6	<b>65</b>	61	49

\*placebo group median = 8.32 days

Using a CV = 1.0, 65 patients are required per group. Allowing for 10 dropouts/group, we require 75 patients per arm..

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 metformin 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 2: Gestational age stratification**

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

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

## **4.5 Randomisation and allocation concealment**

Randomisation will be done in an equal ratio of metformin 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 correlation. We will take this into account in the analysis by treating strata as covariates in regression analysis.

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.



## 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) published a new classification system for hypertensive disorders in pregnancy in 2014.<sup>36</sup> 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<sup>36</sup>**

Classification of the HDP.

	Comments
<b>Pre-existing (chronic) hypertension</b>	This is defined as hypertension that was present either pre-pregnancy or that develops at <20 <sup>0</sup> weeks gestation
• With comorbid condition(s)	Comorbid conditions (e.g., pre-gestational type I or II diabetes mellitus or kidney disease) warrant tighter BP control outside of pregnancy because of their association with heightened cardiovascular risk
• With evidence of preeclampsia	This is also known as 'superimposed preeclampsia' and is defined by the development of one or more of the following at ≥ 20 weeks: <ul style="list-style-type: none"> <li>• Resistant hypertension, or</li> <li>• New or worsening proteinuria, or</li> <li>• One/more adverse condition(s) or</li> <li>• One/more severe complication(s)</li> </ul> Severe preeclampsia is defined as preeclampsia with one or more severe complication(s)
<b>Gestational hypertension</b>	This is defined as hypertension that develops for the first time at ≥ 20 <sup>0</sup> weeks' gestation
• With comorbid condition(s)	Comorbid conditions (e.g., pregestational type I or II diabetes mellitus or kidney disease) warrant tighter BP control outside of pregnancy because of their association with heightened cardiovascular risk
• With evidence of preeclampsia	Evidence of preeclampsia may appear many weeks after the onset of gestational hypertension. Preeclampsia is defined by gestational hypertension and one or more of the following: <ul style="list-style-type: none"> <li>• New proteinuria, or</li> <li>• One/more adverse condition(s) or</li> <li>• One/more severe complication(s)</li> </ul> Severe preeclampsia is defined as preeclampsia with one or more severe complication(s)
<b>Preeclampsia</b>	Preeclampsia may arise <i>de novo</i> . It is defined by gestational hypertension and one or more of the following: <ul style="list-style-type: none"> <li>• New proteinuria, or</li> <li>• One/more adverse condition(s) or</li> <li>• One/more severe complication(s)</li> </ul> Severe preeclampsia is defined as preeclampsia with one or more severe complications <sup>v</sup>
<b>'Other hypertensive effects'</b>	
Transient hypertensive effect	Elevated BP may be due to environmental stimuli or the pain of labour, for example
White coat hypertensive effect	BP that is elevated in the office (sBP ≥ 140 mmHg or dBP ≥ 90 mmHg) but is consistently normal outside of the office (<135/85 mmHg) by ABPM or HBPM
Masked hypertensive effect	BP that is consistently normal in the office (sBP < 140 mmHg or dBP < 90 mmHg) but is elevated outside of the office (≥ 135/85 mmHg) by ABPM or repeated HBPM

ABPM, ambulatory BP monitoring; BP, blood pressure; HBPM, home BP monitoring.

<sup>v</sup> These may occur in women whose BP is elevated at <20<sup>0</sup> or ≥20<sup>0</sup> weeks who are suspected of having pre-existing or gestational hypertension/preeclampsia, respectively.

**Table 3: ISSHP classification of adverse conditions and severe complications of preeclampsia<sup>36</sup>**

Adverse conditions and severe complications of preeclampsia.

Organ system affected	Adverse conditions (that increase the risk of severe complications)	Severe complications (that warrant delivery)
<b>CNS</b>	<ul style="list-style-type: none"> <li>○ Headache/visual symptoms</li> </ul>	<ul style="list-style-type: none"> <li>○ Eclampsia</li> <li>○ PRES</li> <li>○ Cortical blindness or retinal detachment</li> <li>○ Glasgow coma scale &lt; 13</li> <li>○ Stroke, TIA, or RIND</li> </ul>
<b>Cardiorespiratory</b>	<ul style="list-style-type: none"> <li>○ Chest pain/dyspnoea</li> <li>○ Oxygen saturation &lt; 97%</li> </ul>	<ul style="list-style-type: none"> <li>○ Uncontrolled severe hypertension (over a period of 12hr despite use of three antihypertensive agents),</li> <li>○ Oxygen saturation &lt; 90%, need for ≥ 50% oxygen for &gt; 1hr, intubation (other than for Caesarean section), pulmonary oedema</li> <li>○ Positive inotropic support</li> <li>○ Myocardial ischaemia or infarction</li> </ul>
<b>Haematological</b>	<ul style="list-style-type: none"> <li>○ Elevated WBC count</li> <li>○ Elevated INR or aPTT</li> <li>○ Low platelet count</li> </ul>	<ul style="list-style-type: none"> <li>○ Platelet count &lt; 50x10<sup>9</sup>/L</li> <li>○ Transfusion of any blood product</li> </ul>
<b>Renal</b>	<ul style="list-style-type: none"> <li>○ Elevated serum creatinine</li> <li>○ Elevated serum uric acid</li> </ul>	<ul style="list-style-type: none"> <li>○ Acute kidney injury (creatinine &gt; 150 μM with no prior renal disease)</li> <li>○ New indication for dialysis</li> </ul>
<b>Hepatic</b>	<ul style="list-style-type: none"> <li>○ Nausea or vomiting</li> <li>○ RUQ or epigastric pain</li> <li>○ Elevated serum AST, ALT, LDH, or bilirubin</li> <li>○ Low plasma albumin</li> </ul>	<ul style="list-style-type: none"> <li>○ Hepatic dysfunction (INR &gt; 2 in absence of DIC or warfarin)</li> <li>○ Hepatic haematoma or rupture</li> </ul>
<b>Feto-placental</b>	<ul style="list-style-type: none"> <li>○ Non-reassuring FHR</li> <li>○ IUGR</li> <li>○ Oligohydramnios</li> <li>○ Absent or reversed end-diastolic flow by Doppler velocimetry</li> </ul>	<ul style="list-style-type: none"> <li>○ Abruption with evidence of maternal or fetal compromise</li> <li>○ Reverse ductus venosus A wave</li> <li>○ Stillbirth</li> </ul>

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

A number of patients seen at Tygerberg Hospital only book after a gestational age of 20 weeks. The ISSHP classification systems does not have a class for these patients. We will classify these patients according to the classification of Davey and MacGillivray.<sup>37</sup>

**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 after 6 weeks after the expected due date 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 believes 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:**

- The mother must be able to understand the information provided, with the use of an interpreter if needed, and 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.

- 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 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.<sup>38</sup>
  - 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  $50 \times 10^9$  (platelet aggregation excluded)
  - Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than  $100 \times 10^9/L$ , aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase > 600 µ/L or haemolysis on a peripheral blood smear.
  - 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 metformin or a clinical indication for the use of metformin
- Contraindications to the use of metformin
  - Renal disease or dysfunction, suggested by a creatinine level greater than 124µmol/L
  - Known hypersensitivity to metformin
  - Acute or chronic metabolic acidosis, including diabetic ketoacidosis
- Current use of a drug that may be affected by metformin
  - Glyburide

- Furosemide
- Cationic drugs (amiloride, digoxin, morphine, procainamide, quinidine, quinine, rantidine, triamterene, trimethoprim and vancomycin)

# 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

### 6.2.1. Composite maternal outcome:

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

### 6.2.2. Composite neonatal 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
  - Significant neonatal sepsis as defined by the attending paediatrician.

## 6.3 Exploratory outcomes

### 6.3.1 Exploratory maternal outcomes

- Maternal death
- Eclampsia
- Pulmonary oedema (oxygen saturation  $\leq 90\%$ , with clinical signs and symptoms requiring treatment)
- Severe renal impairment or the need for dialysis
- Cerebral vascular event
- Liver haematoma or rupture
- Placental abruption
- 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  $125 \mu\text{mol/l}$
- Proteinuria greater than or equal to  $3\text{g}/24\text{h}$
- Hypertension with a systolic blood pressure greater than  $160\text{mmHg}$  or a diastolic blood pressure greater than  $110\text{mmHg}$  despite anti-hypertensive treatment
- Disseminated intravascular coagulation
- Platelet count less than  $50 \times 10^9$
- Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than  $100 \times 10^9/\text{L}$ , aspartate aminotransferase greater than  $60 \mu/\text{L}$ , and haemolysis as demonstrated by lactate dehydrogenase greater than or equal to  $600 \mu/\text{L}$  or haemolysis on a peripheral blood smear or a raised haptoglobin level)
- Liver transaminases  $> 500\text{IU}/\text{L}$
- 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 500mls at vaginal delivery and 1000 mls at caesarean section in the first 24 hours postpartum)
- Thromboembolic disease (defined as a deep-vein thrombosis, pulmonary embolism or both)
- Moderate or severe ascites noted on ultrasound or at delivery

### 6.3.2 Exploratory 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. In addition, longitudinal fetal heart rate changes are assessed. 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/efmguidelinerkog.pdf](http://www.nice.org.uk/nicemedia/pdf/efmguidelinerkog.pdf))

### 6.3.3 Exploratory neonatal 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 hospital stay
- Incidence of retinopathy of prematurity

## 6.4 Biomarkers:

- Maternal plasma samples: sFlt1, sEng, endothelin 1 and metformin levels.
- Placental samples: mRNA and protein expression of HO-1, sFlt1 and endothelin 1.



(These will be addressed in a separate trial protocol)

## **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 treatment team.

Normal clinical care will be maintained throughout the study. The use of steroids, antihypertensive agents 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 photocopied so that a signed copy can be given to the participant, a copy will be put in the clinical trial notes and a copy kept by the study investigators. The consent forms will be translated into English and Afrikaans. Only the study investigators and research midwives will be able to take consent. Patients will be given as much time as they need to decide whether they would like to be involved. Once the participant has signed consent she will be enrolled into 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**

### **7.2.1 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 metformin 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. 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 by the trial pharmacist. Once completed the trial drugs will be collected from the pharmacy and will be stored in a locked temperature monitored cabinet. Once a participant has been randomised a unique treatment pack number will be allocated to them.

### **7.2.3 Route of administration, dosage regimen and treatment period**

Each participant will be given a treatment pack by the study nurse. A copy of the randomisation number (from the randomisation website) will be placed in the participants trial folder. The treatment pack will be labelled with the participants name and trial number. The treatment pack will contain treatment for 21 days. Treatment will then be taken twice daily until the pregnancy has ended. Trial drugs will be kept in the packaging they are provided in and will under no circumstances 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 to 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.

#### **7.2.5 Dosage**

Metformin use is associated with side effects that include nausea and diarrhoea. We will start all participants on six tablets a day in divided doses. If a participant does experience side effects, we will decrease until the side effects improve. We may then increase the medication back up to 6 tablets a day if the side effects improve and the patient is willing to increase the medication again.

### **7.3 Expectant management for pre-eclampsia**

#### **7.3.1 Routine management**

Expectant management for early onset pre-eclampsia involves admission to hospital until delivery with 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 as per hospital protocol.

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. Expectant management will usually end at a gestation of 34 weeks with planned delivery.

## **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. 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<sub>1</sub>, sEng, and endothelin 1.

Tubes will be labelled with the participant's trial number and the date of collection. The samples will be spun and split into two aliquots. The aliquots will be stored in a minus 80-degree freezer and will be analysed at the end of the study.

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
- ***Metformin levels in the blood in a subcohort cohort of patients:***

There is little data available on the pharmacokinetics of metformin in pre-eclampsia. We propose to perform pharmacokinetic testing on a subgroup to determine if there are differences in the pharmacokinetics of metformin in the pregnant pre-eclamptic population and to confirm that the levels found in preeclampsia are similar to the levels found in healthy controls.

Fifteen patients will undergo pharmacokinetic testing before the PI2 trial is started. They will all be given metformin and will not be included in the 150 participants for PI2. If consent is given, blood will be drawn from an indwelling catheter in a forearm vein at 5 minutes at the following dosing interval: ,2,4,6,7,8 and 24 hours after the initial dose is given. A further single sample will be drawn on day 5 with a butterfly needle. Each sample will be four milliliters. The total amount of blood taken will be 28 mls. Cord blood samples will also be obtained from the umbilical cord to determine the fetal exposure.

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 metformin. The pharmacokinetic sampling will enable us to determine the pharmacokinetics of metformin in the pre-eclamptic population. This has not been described yet in the literature.

## **Cord Blood Samples**

Cord Blood Samples will be collected at delivery and will be labelled with the participant's trial number and date of collection. These samples will also be stored in a -80-degree freezer. We plan to measure metformin levels in the cord blood obtained at the time of the delivery.

## **Placental samples**

Placental samples will be collected at delivery from women who have consented for this to be performed. We will take 1cm<sup>3</sup> full thickness biopsies of the placenta. Each sample will be initially

placed in RNAlater and will then be frozen and stored in -80-degree freezer within a P12 Trial storage box.

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.

## **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 further clinical data and include them in our analyses. Basic clinical data and samples already collected will be included in the analysis in accord with the consent obtained at trial entry.

# 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 REDCap data base. All data will be considered strictly confidential and only the investigators will have access to the data base.

## 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. The aim here is to obtain a complete dataset with minimal incorrect entries due to either trial-based recording or data entry errors. All data will be double checked for accuracy.

## 8.5 Analysis plan

The analyses will be on an intention-to-treat principle with comparisons made between metformin and placebo. Comparisons will be expressed as geometric mean ratios or median differences with associated 95% confidence intervals.

## 8.6 Statistical methods

The patient characteristics at randomization, by treatment group, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution. The distribution of baseline characteristics between treatment groups will not be subject to hypothesis testing.



The primary outcome will be measured from the time of randomisation to delivery. Secondary maternal and neonatal outcomes will be measured from the time of recruitment until six weeks after the due date. The significance level for the primary outcome is set at 0.05 and all hypothesis testing will be two-sided. If, and only if, there is a significant difference for the primary outcome, the two secondary outcomes will be tested at a significance level of 0.025. Standard 95% confidence interval (CI) will be used to present the analysis of the primary outcome. The width of the CI used will be adjusted for the secondary outcomes if tested. For raw results data, the primary outcome will be summarized using median [25th – 75th percentile] along with minimum and maximum values.

All secondary outcomes and exploratory outcomes of interest will be presented by number (%) by treatment arm. Primary outcome effect size will be presented as difference in medians and 95% CI, adjusted for gestational age strata based upon the quantile regression model. P-values will be reported with associated with 95% CI for the secondary outcomes if tested. Exploratory outcomes will be presented with 95% CI.

Modelling will use quantile regression to assess difference in median prolongation between groups. This will be supplemented by survival analysis (i.e. time until delivery), using Cox proportional hazards regression (CPH) and Kaplan-Meier survivorship curves. Survival outcomes will not be subject to hypothesis testing. For both quantile regression and survival analysis effect estimates will be presented as group difference with 95% CI.

The number of events for each of the composite binary outcomes is likely to be small. An exact logistic regression model containing treatment group and gestational age strata will be used. In the event that it is not possible to obtain estimates, due to small counts each hypothesis will be tested, unadjusted for gestational strata, using Fisher's exact test. Point estimates of percentage and exact 95%CI will also be provided.

For the primary outcome a covariate adjusted analysis with both treatment group and gestational age design-based strata modelled as fixed will constitute the primary analysis outcome. Unadjusted and adjusted CPH analysis are only supplemental to this primary analysis. For the secondary outcomes composite binary outcomes will be modelled using a logistic regression model containing both treatment group and gestation aged based strata.

The sensitivity analyses that will be conducted on the primary outcome are firstly the treatment received, secondly gestational age as an effect modifier, thirdly covariate adjusted for an estimated fetal weight less than the 10th and 3rd centile on ultrasound at the time of randomisation and absent umbilical artery end diastolic flow as these are more likely to be associated with shorter randomisation to delivery times and lastly a survival analysis will be

performed using Cox proportional hazards regression (CPH) adjusted for gestational age strata and Kaplan-Meier survivorship curves.

No sensitivity analysis for primary outcome outcomes is planned for missing data as we expect these mothers not to have missing outcome data or baseline measured pre-treatment covariates. If the neonate is lost to follow-up the outcome will be set to missing, a sensitivity analysis will be performed to assess strength of inference given pattern of missingness by setting missing values to no event, mean event rate or event to assess dependence on inference on the unknown outcomes.

Statistical software used is Stata v 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). More details on the statistical analysis can be found in the PI2 statistical analysis plan (SAP).

# 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 complications of existing disease or illness.

These events will be recorded on the 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.<sup>39</sup> These procedures have been used in previous multicentre trials and proven to be efficient and compliant with the GCP principles and data management.

## **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 always be accessible to the principal 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.

## **Duration of the project**

It is anticipated that the study can be completed in approximately 2-3 years (2018 – 2021). The duration of the study has been conservatively approximated using data from the similar PIE study.

Recruitment will begin as soon as we have approval from Human Research Ethics, the South African Medicines Control Council, Tygerberg Hospital and once the PIE trial has completed randomisation. 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.

## **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 (<http://www.consort-statement.org/>).

## **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 every 4 to 6 months to discuss the progress of the trial. They will be supplied with reports of progress prior to each meeting. 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 and drug storage
- 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



## Ethical aspects

Approval will be obtained from the Human Research Ethics Committee at Stellenbosch University, 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

### 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). Data collection sheets will carry the Participant Identification number. A separate log linking the patient identification characteristics and the Participant Identification number will be kept in a secure location to which only the PI has access. Patient confidentiality will further 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 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.

## 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 of the neonatal services considerably.

## Resources and strengths of the study

### 14.1 Strengths of our study

1) **The 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 that 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).<sup>40</sup> Data on the number of cases of early onset pre-eclampsia managed at Tygerberg Hospital that have been included in the PIE trial suggest that this trial can be feasibly performed in just one hospital. This is in stark contrast with the incidence of early onset pre-eclampsia in the developed world where a number of recruiting sites would be needed. As an example, the STAMP trial (assessing the treatment of early onset pre-eclampsia with pravastatin) aimed to recruit 120 women in the UK. They had over 15 recruiting sites and were only able to recruit just over 60 patients.

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 and a proven track record. It has 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 metformin 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.

## **Publication of results**

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

## **Role of study funders**

The funders of the trial have had no role in the study design.

The funders will have no role in the collection, management, analysis and interpretation of the data. They will also have no role in writing the report and the decision to submit the report for publication.

## References

- 1 Dolea C, Abouzahr C. Global burden of hypertensive disorders of pregnancy in the year 2000. *Glob Burd Dis* 2003; : 1–11.
- 2 Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. *Annu Rev Pathol Mech Dis* 2010; 5: 173–92.
- 3 Bakker R, Pluimgraaff LE, Steegers EAP, et al. Associations of light and moderate maternal alcohol consumption with fetal growth characteristics in different periods of pregnancy: The Generation R Study. *Int J Epidemiol* 2010; 39: 777.
- 4 Steegers EAP, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet (London, England)* 2010; 376: 631–44.
- 5 Redman CW. Latest Advances in Understanding Preeclampsia. *Science (80- )* 2005; 308: 1592–4.
- 6 NCCEMD NC on CE into MD. Saving mothers 2008–2010: fifth report on the confidential enquiries into maternal deaths in South Africa. Pretoria, South Africa: National Department of Health, South Africa (in press)., 2012 DOI:10.2337/dc14-S014.
- 7 American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in Pregnancy. *Obstet Gynecol* 2013; 122: 1122–31.
- 8 B.M. S. Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol* 2003; 102: 181–92.
- 9 Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet (London, England)* 2005; 365: 785–99.
- 10 Zhang J, Meikle S, Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. *Hypertens pregnancy* 2003; 22: 203–12.
- 11 Cluver CA, Walker SP, Mol BW, et al. Double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): a study protocol. *BMJ Open* 2015; 5: e008211-2015–008211.
- 12 Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med Exp Biol Med (New York, NY)* 1999; 222: 222–35.

- 13 Hung T-H, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. *Taiwan J Obstet Gynecol* 2006; 45: 189–200.
- 14 Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006; 355: 992–1005.
- 15 Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; 350: 672–83.
- 16 Brownfoot FC, Hastie R, Hannan NJ, et al. Metformin as a prevention and treatment for preeclampsia: effects on soluble fms-like tyrosine kinase 1 and soluble endoglin secretion and endothelial dysfunction. *Am J Obstet Gynecol* 2016; 214: 356. e1-356. e15.
- 17 Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a Disease of the Maternal Endothelium The Role of Antiangiogenic Factors and Implications for Later Cardiovascular Disease. *Circulation* 2011; 123: 2856–69.
- 18 Maynard SE, Karumanchi SA. Angiogenic factors and preeclampsia. In: *Seminars in nephrology*. Elsevier, 2011: 33–46.
- 19 Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003; 111: 649–58.
- 20 Venkatesha S, Toporsian M, Lam C, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006; 12: 642–9.
- 21 Brodsky S V, Goligorsky MS. Endothelium under stress: local and systemic messages. In: *Seminars in nephrology*. Elsevier, 2012: 192–8.
- 22 Rowan JA, Hague WM, Gao W, Battin MR, Moore MP. Metformin versus insulin for the treatment of gestational diabetes. *N Engl J Med* 2008; 358: 2003–15.
- 23 Syngelaki A, Nicolaides KH, Balani J, et al. Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus. *N Engl J Med* 2016; 374: 434–43.
- 24 Chiswick C, Reynolds RM, Denison F, et al. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. *lancet Diabetes Endocrinol* 2015; 3: 778–86.
- 25 Committee TSPA. FDA classification of drugs for teratogenic risk: Teratology society public affairs committee. *Teratology* 1994; 49: 446–7.
- 26 Choi YH, Kim SG, Lee MG. Dose-independent pharmacokinetics of metformin in rats: Hepatic and gastrointestinal first-pass effects. *J Pharm Sci* 2006; 95: 2543–52.



- 27 Package insert: Metformin hydrochloride. New York, Bristol-Myers Squibb. .
- 28 Cassina M, Dona M, Di Gianantonio E, Litta P, Clementi M. First-trimester exposure to metformin and risk of birth defects: a systematic review and meta-analysis. *Hum Reprod Update* 2014; 20: 656–69.
- 29 Coetzee EJ, Jackson WPU. Pregnancy in established non-insulin-dependent diabetics. A five-and-a-half year study at Groote Schuur Hospital. *South African Med J* 1980; 58: 795–802.
- 30 Gilbert C, Valois M, Koren G. Pregnancy outcome after first-trimester exposure to metformin: a meta-analysis. *Fertil Steril* 2006; 86: 658–63.
- 31 Hughes RCE, Rowan JA. Pregnancy in women with Type 2 diabetes: who takes metformin and what is the outcome? *Diabet Med* 2006; 23: 318–22.
- 32 Charles B, Norris R, Xiao X, Hague W. Population pharmacokinetics of metformin in late pregnancy. *Ther Drug Monit* 2006; 28: 67–72.
- 33 The American College of Obstetricians and Gynecologists. Practice Bulletin: Gestational Diabetes Mellitus. *Obstet Gynecol* 2013; 122: 406–16.
- 34 Newson R. Generalized power calculations for generalized linear models and more. *Stata J* 2004; 4: 379–401.
- 35 Hall DR, Odendaal HJ, Kirsten GF, Smith J, Grove D. Expectant management of early onset, severe pre-eclampsia: perinatal outcome. *Bjog* 2000; 107: 1258–64.
- 36 Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy. *Pregnancy Hypertens An Int J Women's Cardiovasc Heal* 2014; 4: 105–45.
- 37 Davey D, McGillivray I. The classification and definition of the hypertensive disorders of pregnancy. *Am J Obstet Gynecol* 1988; 158: 892–8.
- 38 Mattar F, Sibai BM. EclampsiaVIII. Risk factors for maternal morbidity. *Am J Obstet Gynecol* 2000; 182: 307–12.
- 39 Idänpään-heikkilä JE. WHO Guidelines for Good Clinical Practice (GCP) for Trials on Pharmaceutical Products: Responsibilities of the Investigator. *Ann Med* 1994; 26: 89–94.
- 40 Duley LM. The Global Impact of Pre-eclampsia and Eclampsia. In: *Seminars in Perinatology*. Elsevier, 2009: 130–7.