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Complete List of Authors:	Cluver, Catherine; Stellenbosch University Faculty of Science, Obstetrics and Gynaecology; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Translational Obstetrics Group Walker, Susan; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Translational Obstetrics Group; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Obstetrics and Gynaecology Mol, Ben; School of Medicine, Monash University, Melbourne, Australia, Obstetrics and Gynaecology Hall, David; Stellenbosch University Faculty of Science, Obstetrics and Gynaecology Hiscock, Richard; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Anaesthetics Brownfoot, Fiona; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia., Translational Obstetrics Group; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Obstetrics and Gynaecology Kaitu'u-Lino, Tu'uhevaha; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia., Translational Obstetrics Group and Department of Obstetrics and Gynaecology; University of Melbourne, Obstetrics and Gynaecology Tong, Stephen; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Translational Obstetrics Group; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Obstetrics and Gynaecology
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A double blind, randomised, placebo-controlled trial to evaluate the efficacy of metformin to treat early onset pre-eclampsia (PI2 Trial): study protocol

Authors:

Catherine A Cluver,[1,2] Susan P Walker,[3] Ben W Mol,[4] David R Hall,[1] Richard Hiscock,[5] Fiona C Brownfoot,[2,3] Tu'uhevaha J Kaitu'u-Lino,[2,3] Stephen Tong[2,3]

Author Affiliations:

[1] Department of Obstetrics and Gynaecology, Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa.

[2] Translational Obstetrics Group, University of Melbourne, Mercy Hospital for Women, 163 Studley Road, Heidelberg, Victoria 3084, Australia.

[3] Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne, 163 Studley Road, Heidelberg, Victoria 3084, Australia.

[4] Department of Obstetrics and Gynaecology, Monash School of Medicine, Monash University, Melbourne, Victoria, Australia.

[5] Department of Anaesthetics, Mercy Hospital for Women, University of Melbourne, Victoria 3084, Australia.

Corresponding author:

Dr Catherine Anne Cluver

Department of Obstetrics and Gynaecology, Stellenbosch University, Tygerberg Hospital

Francie van Zyl Drive, Tygerberg 7505, South Africa

Email: cathycluver@hotmail.com

Phone: +27823210298

ABSTRACT

Introduction

Pre-eclampsia is a major complication of pregnancy, globally responsible for 60,000 maternal deaths per year, and far more fetal losses. There is no definitive treatment other than delivery. A therapeutic that could quench the disease process would be useful to treat preterm pre-eclampsia, as it could allow these pregnancies to safely continue to a gestation where fetal outcomes are significantly improved. We have published preclinical data to show that metformin, a drug known to be safe in pregnancy and commonly used to treat gestational diabetes, has potent biological effects making it another promising candidate to treat pre-eclampsia. Here, we describe a phase II clinical trial to examine whether administering extended release metformin may be effective in treating women with preterm pre-eclampsia (PI2 Trial).

Methods

The PI2 Trial is a phase II, double blind, randomised controlled trial that aims to recruit 150 women with early onset pre-eclampsia (gestational age 26+0 to 31+6 weeks) who are being managed expectantly. Participants will be randomised to receive either 3 grams metformin or placebo daily. The primary outcome is time from randomisation till delivery. A delay in delivery of 5 days is assumed to be clinically relevant. Secondary outcomes include maternal, fetal and neonatal composite and individual outcomes. We will examine whether metformin can decrease serum sFlt-1 and s-endoglin levels and record adverse events.

Ethics and dissemination

This study has ethical approval (Protocol number M16/09/037 Federal Wide Assurance Number 00001372, Institutional Review Board Number IRB0005239), is registered with the Pan African

Clinical Trial Registry (PACTR201608001752102) and the South African Medicine Control Council (20170322). Data will be presented at international conferences and published in peer-reviewed journals.

ARTICLE SUMMARY

Article focus

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

Key Messages

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

Strengths and limitation of this study

- This is a protocol for a randomised, double blind, placebo controlled clinical trial.
- This is the first trial to assess whether metformin is a treatment option for pre-eclampsia.
- We plan to recruit 150 participants which provides sufficient power for our primary outcome (time from randomisation to delivery).
- It may be underpowered to show improvements in maternal and perinatal outcomes. Therefore, if the trial yields a positive result, larger multi-centre studies will be needed.

INTRODUCTION

Pre-eclampsia is one of the most serious complications of pregnancy affecting 5% of pregnancies worldwide.[1] It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit.[2] 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.[3] Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[4] and it is estimated that for every pre-eclampsia related death there are 50-100 other women who experience significant morbidity associated with pre-eclampsia.[5]

At present, the only treatment available for pre-eclampsia is termination/delivery of the pregnancy. This poses a difficult clinical dilemma in the setting of early onset pre-eclampsia. Clinicians are often forced to deliver early to prevent disease progression and major maternal morbidity (ie severe maternal organ injury), but in doing so, inflict severe prematurity on the fetus. In particular, fetuses delivered at less than 33 weeks' gestation are at significant risk of severe disability including cerebral palsy, stroke (intracerebral bleeding), retinopathy of prematurity, chronic lung disease and death.[6]

We have recently completed a phase II, double blind, randomised controlled trial, the Pre-eclampsia Intervention with Esomeprazole (PIE) trial, to evaluate whether 40 mg of esomeprazole was a possible treatment for early onset pre-eclampsia. This trial showed no between-group differences in median time from randomization to delivery (11·4 days in the esomeprazole group, and 8·3 days in the placebo group [95% confidence interval -2·9-8·8, P=0·31]).[7] Here, we propose metformin as the next therapeutic candidate to test in a clinical trial for early onset pre-eclampsia.

Metformin is a promising therapeutic candidate as it has been shown to reduce soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) secretion from endothelial cells and primary trophoblasts.[8] These key anti-angiogenic molecules released from the placenta are responsible for the widespread endothelial dysfunction and impaired vascular relaxation observed in pre-eclampsia. Reduced angiogenesis is also thought to contribute to placental hypoxia and to the development of pre-eclampsia. Metformin has also been shown to improve endothelial dysfunction, improve blood vessel relaxation and promote angiogenesis.[8] Given these multiple potential effects, metformin is a particularly promising therapeutic candidate.

OBJECTIVES

The primary objective is to examine whether 3 grams of metformin XR can safely prolong gestation for an additional 5 days among women with early onset pre-eclampsia diagnosed 26+0 – 31+6 weeks being managed expectantly, compared to expectant management alone.

The secondary objectives are to examine whether metformin can improve maternal and neonatal outcomes, and to determine whether metformin can significantly decrease levels of circulating sFlt-1 and/or sEng.

METHODS

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

Study design

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

Study population

Pregnant women diagnosed with early onset pre-eclampsia at a gestational age between 26+0 weeks to 31+6 weeks at Tygerberg hospital (Western Cape Provence of South Africa) will be invited to participate. To be eligible for this study the treating clinicians need to have made an initial assessment and deemed that the patient is suitable for expectant management and delivery is not immediately required or anticipated in the next 48 hours.

Inclusion criteria:

We will recruit women with a singleton pregnancy diagnosed with pre-eclampsia, defined according to the criteria published by The International Society for the Study of Hypertension In Pregnancy (ISSHP),[9] and require the presence of significant proteinuria (more than 300 milligrams in a 24 hour urine collection).

We will recruit women with pregnancies at a gestational age between 26 + 0 weeks and 31 + 6 weeks, determined by either period dates (if the woman is certain of her last menstrual period) or by an early, or mid trimester pregnancy ultrasound. If the gestational age is uncertain, we will recruit participants with an estimated fetal weight between 500 and 1800 grams, determined by ultrasound performed at presentation. A full list of inclusion criteria is provided in Table 1.

Exclusion criteria:

Exclusion criteria include women with established maternal or fetal compromise that necessitates delivery within 48 hours, the current use of metformin, contraindications to the use of metformin or the use of medications that interact with metformin. A full list of exclusion criteria is provided in Table 2.

Participant enrolment

Participants will be identified after they have been admitted to the tertiary referral centre with a diagnosis of early onset pre-eclampsia for expectant management. An information leaflet will be given to all potential participants and written informed consent will be obtained (Supplementary information 2 and 3).

Randomisation and allocation concealment

After informed consent, participants will be randomised to metformin or placebo in a 1:1 ratio. An online, web-based sequence generator system linked with codes for placebo and treatment tablets will be used. Both researchers and participants will be blinded.

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

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

Each participant will be given an individual treatment pack containing either 500mg metformin XR or identical placebo tablets which have been produced by Merck

(http://www.merck.com/index.html). Labelling, storage and preparation will be done according to the requirements of the Medicines for Human Use (Clinical Trials) regulations. Participants will be started on one tablet twice and day and the dose will be increased every 24 to 48 hours until side effects develop or a maximum dose of 3 tablets twice a day is reached.

Co-interventions

Participants will remain under the care of the hospital treating team and the study will not alter or interfere with the routine care provided for women with early onset pre-eclampsia, including the decision for delivery. Management for early onset pre-eclampsia involves admission to hospital, and close maternal and fetal surveillance. Maternal surveillance includes four hourly blood pressure measurement, twice daily clinical assessment, daily urinalysis, and twice weekly assessments with blood tests (full blood count, renal function tests and hepatocellular enzymes if HELLP syndrome is suspected) and 24-hour urinary protein measurement on admission. Fetal surveillance includes six-hourly cardiotocography and ultrasound assessments every two weeks (or more frequently if clinically indicated) for Doppler velocimetry of the umbilical artery, middle cerebral artery, ductus venosus, amniotic fluid volume assessment and fetal 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 for the neonate. A single repeat dose will be given one week later. Most participants will be on antihypertensive treatment, and the dose and number of antihypertensives will be recorded. All women should already be receiving calcium, iron and folic acid supplementation.

Clinical care will be left up to the discretion of the clinical team. The indication for delivery will be a clinical decision. Indications for delivery may include inability to achieve blood pressure

control, the development of major maternal or fetal complications, or intrauterine fetal death. Expectant management will usually end at a gestation of 34 weeks.

Sample collection

Blood samples will be routinely collected twice a week. Two sets of specimens will be drawn, the routine pre-eclamptic monitoring samples and the trial samples. The routine blood samples include measurements of the haemoglobin, the platelet count and the urea and creatinine levels. These will be used by the managing clinicians to determine disease severity and may trigger delivery. The second set of samples will be the trial blood samples. These include measurements of sFlt-1, sEng, and endothelin-1. Placental samples will be collected at delivery and placed in RNA later and then frozen for long-term storage at -80°C. These trial blood and placental samples will only be analysed once the trial is complete.

Outcomes

The primary outcome is time from randomisation till delivery. We believe that a further 5 days would have significant clinical benefits for the neonate, potentially improving perinatal outcome. The secondary outcomes include maternal, fetal and neonatal mortality and major morbidity, maternal serum biomarkers (including sFlt-1, sEng and endothelin-1) and placental samples (See Tables 3 to 6 for more information regarding secondary outcomes). We will present a composite outcome of the important maternal outcomes, which includes maternal death, eclampsia, pulmonary oedema, severe renal impairment or the need for dialysis, cerebral vascular event, and the development of a liver haematoma or rupture. We will also compare the individual components of the composite maternal outcome. Other individual maternal outcomes to be compared are listed in Table 3. The fetal composite outcome will include ultrasound features, intrauterine fetal demise and fetal growth restriction. These will also be assessed as individual outcomes (see Table 4). The composite neonatal outcome will include neonatal death

within 6 weeks after the due date, severe intraventricular haemorrhage, necrotizing enterocolitis or bronchopulmonary dysplasia. We will also compare the individual components of the composite neonatal outcome. Other individual neonatal outcomes to be compared are listed in Table 5. We will measure the biomarkers sFlt-1, sEng and endothelin-1 which are anti-angiogenic factors likely to play a role in the maternal endothelial dysfunction that is central to the pathophysiology of pre-eclampsia (i.e. it would be advantageous if treatments can decrease circulating levels of these circulating factors). The placenta will be examined for changes in mRNA and protein expression of total Flt-1 and splice variants sFlt-1 e15a and sFlt- i13 and Endoglin (see Table 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 withdrawal, we will seek the patient's permission to use their clinical information and laboratory samples collected thus far in subsequent analyses.

Duration of the trial

It is anticipated that the study will be completed in approximately 3 years (2018 – 2020). In our previous PIE study, we included 120 women in 15 months.

Ethical approval and dissemination

This study has ethical approval (Stellenbosch University HREC Protocol number M16/09/037 Federal Wide Assurance Number 00001372, Institutional Review Board Number IRB0005239) and is registered with the Pan African Clinical Trial Registry (Application ID PACTR201608001752102) and the South African Medicine Control Council (20170322). Data will be presented at international conferences and published in peer-reviewed journals.

Confidentiality

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

Data management and statistical analysis

Data will be collected prospectively using a REDCap database.[10] Data entry and checking will be continuous, and queries will be addressed contemporaneously to ensure clarification without delay. All data will be double checked for accuracy. Intention to treat analyses will be performed comparing metformin and placebo, for primary and secondary outcomes. Comparisons will be expressed as geometric mean ratios or median differences with associated 95% confidence intervals.

The patient characteristics, by treatment group at randomisation, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution.

For the primary outcome we plan to perform two analyses: 1) primary intention to treat analysis (ITT) and 2) a treatment received (TR) analysis to examine response among those who actually took all tablets. The primary outcome, time from randomization until delivery, will be assessed using linear regression modelling by (a) unadjusted analysis with treatment as the sole covariate; (b) a covariate adjusted analysis with both treatment group and gestational age design-based strata; and (c) a covariate adjusted analysis that includes treatment group, the gestational age design-based strata and absent flow on umbilical artery Doppler (UAD) examination measured at the time of randomization. The trial investigators consider that a pregnancy with absent UAD flow is more likely to be associated with shorter randomization to

delivery times. This pre-specified covariate adjustment will increase the precision of the primary outcome measure if there is a correlation between UA flow and delivery time. By design the stratification variable, gestational age, will be correlated with delivery time, this correlation is accounted for by including it as a covariate in the adjusted analyses (b) and (c). Given the likely skewed distribution in prolongation times, modelling will use quantile regression to assess differences in median prolongation between groups and transformation of primary outcome, to achieve adequate distributions of residuals, will be performed if indicated. Standard regression diagnostics will be performed to assess model fit.

Results will be presented as group difference with 95% confidence intervals (95%CI). Sensitivity analyses may be performed to assess (i) the effect of any chance covariate imbalance between treatment arms, using inverse probability weighted propensity scores, as the sole covariate apart from treatment and stratum assignment and (ii) a treatment received analysis should this differ substantially from treatment assignment, to examine response in those who actually took the active medication. Sensitivity analyses will use the same quantile regression model described above. We will also present a survival analysis (i.e. time until delivery), using adjusted Cox proportional hazards regression (CPH) and Kaplan-Meier survivorship curves. The adjusted CPH model will include both treatment group and gestational aged based strata as covariates. If a fetus does demise during expectant management, we will give it a length of pregnancy prolongation of zero and will include it in the primary analysis.

Secondary outcomes may be composite or single. Composite binary outcomes will be modelled using a logistic regression model containing both treatment group and gestational age based strata and presented as RR (95% CI). Continuous single outcome variables will be compared using either the unpaired t-test (for normally distributed variables) or Mann-Whitney U (non-normally distributed) whilst categorical values will be compared using likelihood-based chi-

squared test. For the longitudinal data of plasma sFlt-1, sEng and endothelin 1 levels, we will present the data graphically and assess differences in levels between groups adjusted for gestational age and strata by a marginal mean model using generalized estimating equations. Comparison of levels between groups at delivery will use quantile median regression as described for the primary outcome. Finally, the placental expression of sFlt-1, sEng and HO-1 will be compared using simple statistics.

The significance level is set at 0.05 and all hypothesis testing will be two-sided. Primary and composite outcomes will not be adjusted for multiple comparisons. Non-composite secondary outcomes will be adjusted for multiple comparisons using the Holm's step procedure. Statistical software used is Stata v 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Sample size and stratification according to gestation at recruitment

The primary research question is whether metformin will result in a gain in the median length of gestation of greater than or equal to 5 days compared to placebo therapy. 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) and the Powercal program running in Stata v15 (Stata statistical software. 2017 Release 15. Stata Corp. College Station, TX: StataCorp LLC).[11]

We have two sources for data on the prolongation of gestation in preeclamptic mothers receiving expectant management which were both performed at the same hospital where we plan to run this trial (Tygerberg Hospital). Hall et al, in the largest descriptive study on expectant

management of preterm pre-eclampsia, reported a mean gestation prolongation of 11 (SD 7) days equivalent to a CV = 0.64,[12] and the PIE trial, where patients randomized to the placebo arm had an overall mean prolongation of 13.1 (SD 12.2) days, a CV = 0.93.[13] In this study for mothers entering at gestations greater than 29 weeks, the CV = 0.8. We have therefore 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.

For 90% power, with a two-sided alpha set at 0.05, control median prolongation of 8.3 days and prolongation of gestation of 5 days with the measure of variability used in this analysis being the Coefficient of Variation (CV) = (data SD)/ data Mean) we require 65 participants per group. Allowing for 10 dropouts per group we require 75 participants per arm and therefore a total of 150 participants.

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. 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. 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. Stratum 1 will include pregnancies of a gestational age of 26 weeks 0 days up to and including 28 weeks and 6 days. Stratum 2 will include pregnancies from 29 weeks and 0 days up to and including 31 weeks and 6 days.

Adverse events

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

Unblinding

Given that the safety profile of metformin in pregnancy is well established, we anticipate the need for unblinding to be unlikely. However, we will have the following procedures in place should unblinding be required. Sheets with lists of 25 randomisation codes each will be sealed in individual, signed, numbered envelopes. If unblinding is requested, the relevant envelope will be opened by a person not involved in the trial in the presence of two witnesses, the group allocation read, and the list re-sealed in an envelope and signed. The randomisation envelopes will be accessible at all times to the principal investigator who will be contactable by mobile telephone. The principal investigator will co-ordinate this process.

Early termination of the trial

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

DISCUSSION

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

Recently a number of clinical trials have been proposed for assessing novel treatments for pre-eclampsia but only a few have been completed. Our group has recently completed the PIE trial assessing esomeprazole to treat preterm pre-eclampsia.[13] In this trial a daily dose of 40mg esomeprazole did not prolong gestation or have any effect on biomarkers associated with pre-eclampsia. Sildenafil was assessed in a single site, double blind, randomised controlled trial in Brazil.[14] Over a 28-month period, 100 women were recruited, and results were available for 93 women. There was a significant prolongation of gestation in the sildenafil group of 4 days but given that sildenafil is a vasodilator it is possible that this prolongation in gestation may have occurred because the drug decreased blood pressure and mitigated a clinical reason to deliver, rather than temporising disease progression. Antithrombin was assessed to treat preterm pre-eclampsia in the PRESERVE-1 trial which enrolled 120 women from 23 tertiary hospitals over 28 months (ISRCTN23410175).[15] There was no difference in prolongation of pregnancy or composite neonatal outcomes.[16] Trials assessing serelaxin (NCT01566630), pravastatin, high doses of antithrombin,[17] and celecoxib (NCT00442676) have been attempted but all were terminated due to poor recruitment.

It is known that the pre-eclamptic placenta releases anti-angiogenic sFlt-1 and sEng into the maternal circulation, causing widespread maternal endothelial dysfunction and organ injury.[18] A drug that can decrease sFlt-1 and sEng production and decrease endothelial dysfunction may be a potential treatment for pre-eclampsia. We have generated preclinical data suggesting metformin may have such actions.[8] Based on this preclinical data, we are now proposing to undertake this phase II randomized clinical trial.

We propose recruiting 150 women at Tygerberg Hospital, in the Western Cape Provence of South Africa. There are advantages to running the trial at this site. South Africa has a very high incidence of pre-eclampsia. Tygerberg Hospital is a tertiary referral centre with a neonatal intensive care unit, a maternal critical care unit and an adult intensive care unit. It is an academic centre that actively contributes to the global scientific literature and our team has recently completed the PIE trial at this research unit. We will make use of the sytems set up by this trial. Thus, we believe running this trial at Tygerberg Hospital represents a balance between obtaining sufficient number of cases of preterm pre-eclampsia, performing the trial in a cost-effective manner and offering modern obstetric and perinatal care, making the results potentially generalizable to both developed and developing countries.

Metformin is known to be safe in pregnancy and is considered a category B drug in pregnancy (by the Food and Drug Administration (FDA)).[19] There are numerous studies of metformin for the treatment of diabetes and polycystic ovarian syndrome in pregnancy which have shown no adverse fetal effects when used in the first, second and third trimesters.[20–25]

In strong support of our preclinical data are meta-analyses of clinical trial data that have shown a decrease in the incidence of hypertensive disorders when metformin has been used for other indications.[26] Excitingly, a randomised trial by the Fetal Medicine Foundation (for obese women

without diabetes) reported that metformin significantly reduced the incidence of pre-eclampsia by as much as 76%.[27] The EMPOWaR trial also evaluated maternal and fetal effects of metformin in obese women.[28] While reporting no decrease in the incidence of hypertensive disorders of pregnancy with metformin, the most likely reason for this difference was poor compliance.[29]

We have powered our study for the primary outcome, which is to show whether metformin can safely prolong gestation for a further five days in mothers being expectantly managed for preterm pre-eclampsia. A limitation in this trial is that we have not specifically powered to detect improvements in maternal or neonatal outcomes (although these are planned secondary outcomes). If this trial yields a positive result, a further phase III multi-centre randomised trial that is sufficiently powered, may be required to be sufficiently powered to demonstrate improvements in clinical outcomes.

As we did for the PIE trial, we will measure biomarkers that reflect disease severity. We will measure circulating levels of sFlt-1 and sEng, among others, in serial samples obtained from the mother. These anti-angiogenic factors are considered to play an important role in inciting maternal endothelial dysfunction and end-organ injury seen in pre-eclampsia. Furthermore, we will measure expression of key molecules in the placental samples obtained at delivery.

Pre-eclampsia kills mothers, fetuses and neonates and is responsible for severe maternal and neonatal morbidity. This is especially the case in the developing world where there is a lack of resources, including staff, equipment and finances. If a treatment were to be discovered, it would have dramatic effects on maternal and neonatal outcomes. Metformin has shown potential as a therapeutic agent in preclinical work on pre-eclampsia. Further advantages of metformin XR are that it is available in a long acting formulation, it is safe during pregnancy and

it is not expensive. This makes it an ideal candidate as a global therapeutic for pre-eclampsia. It is therefore imperative for this trial to be performed. If metformin were proven to be effective at prolonging gestation in early onset pre-eclampsia it could play an important role in decreasing the clinical burden of this dangerous condition.

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TABLES

Table 1: Inclusion criteria

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

Table 2: Exclusion criteria

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

Table 3: Maternal Secondary Outcomes

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

Thromboembolic disease
Moderate or severe ascites

Table 4: Fetal outcomes

Neonatal outcomes	
Fetal composite outcome:	Reversed a-wave in the ductus venosus on fetal ultrasound
	Significant changes in fetal heart rate patterns on the non-stress test that necessitate delivery
	Intrauterine fetal demise
	Fetal growth restriction at birth
	Persistent reversed flow in the umbilical artery
	Redistribution in the middle cerebral artery
Fetal individual outcomes:	Each of the components of the composite neonatal outcome

Table 5: Neonatal outcomes

Neonatal outcomes	
Neonatal composite outcome:	Neonatal death within 6 weeks after the due date
	Grade III or IV intraventricular haemorrhage
	Necrotizing enterocolitis
	Bronchopulmonary dysplasia
Neonatal individual outcomes:	Each of the components of the composite neonatal outcome
	APGAR score < 7 at 5 minutes
	Umbilical artery pH < 7.05
	Umbilical artery lactate
	Surfactant use

Neonatal intensive care or special care unit admission
Intubation and mechanical ventilation
Continuous positive airway pressure (CPAP) support
Grade III/IV hyaline membrane disease
Length of oxygen treatment
Hospital stay
Incidence of retinopathy of prematurity
Neonatal sepsis

Table 6: Biomarker outcomes

Maternal plasma: Maternal plasma sFlt1 sEng Placental Growth Factor (PGF) Endothelin 1 (these analytes will be measured in serial samples collected during expectant managements)
Placental samples: Heme Oxygenase-1 Flt-1 sFlt-1 e15a sFlt-1 i13 Endoglin Vascular Endothelial Growth Factor Placental Growth Factor Hypoxia inducible factor-1 **α** (mRNA and protein levels will be measured in these placental samples taken at delivery)

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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

Full title of trial	Pre-eclampsia Intervention 2 (PI2) Trial: a double blind randomised, placebo-controlled trial of Metformin to treat early onset pre-eclampsia
Short title	PI2 trial
Version and date	19 February 2018 Version 1.1
Ethics approval	Protocol number: (M16/09/037) Federal Wide Assurance Number 00001372 Institutional Review Board (IRB) Number: IRB0005239
Pan African Clinical Trial Registry ID	PACTR201608001752102
Medicine Control Council Reference number	20170322 N2/19/8/2 Approved 12 May 2017
Trial medication	Metformin
Phase of trial	Phase II
Principal investigator	Dr Catherine Anne Cluver
Supervisors/ Co-investigators	Professor Stephen Tong Professor Susan Walker Prof Ben Mol Prof DR Hall Dr T Kaitu'u-Lino Dr N Hannan Dr H Hiscock Dr F Brownfoot
Funders	Mercy Perinatal Foundation South African Medical Research Council

CONTACT DETAILS

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

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

Prof Susan Walker
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: spwalker@unimelb.edu.au
Prof Ben Mol
The Robinson Institute, School of Paediatrics and Reproductive Health
University of Adelaide
Phone: +61 434122170
Email: ben.mol@adelaide.edu.au

Prof David Hall
Tygerberg Hospital
University of Stellenbosch
Tel: +27 21 938 9059
Email: drh@sun.ac.za

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

Dr Tu'uhevaha Kaitu'u-Lino
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: t.klino@unimelb.edu.au

Dr Fiona Brownfoot
Mercy Hospital for Women
University of Melbourne
Tel: +614 1813 0139
Email: Fiona.brownfoot@gmail.com

Trial Design and Statistics

Dr Richard Hiscock
Mercy Hospital for Women
Email: richardjhiscock@gmail.com

Laboratory Coordination

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

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

TRIAL COMMITTEES

Trial Steering Committee

Chair:

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

Members:

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

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

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

Data Monitoring Committee

Chair:

Prof Gerhard Theron
Consultant Obstetrician and Gynaecologist
Stellenbosch University and Tygerberg Hospital
Email: gbth@sun.ac.za

Tel: +27 219389217

Members

Dr Adrie Bekker
Consultant Paediatrician
Stellenbosch Hospital and Tygerberg Hospital
Email: adrie@sun.ac.za
Tel: +27 722125453

Mr Chris Muller
Biostatistician
Stellenbosch University
Tel +27824303101
Email: cmuller@sun.ac.za

Protocol Versions

1.1

Signatures

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

Principal investigator:

Date: 19/02/2018

Abbreviations

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

CONTENTS

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 1α (HIF 1α)
- 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.[1] It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit.[2] 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.[3] Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[4,5] and in South Africa hypertensive disorders of pregnancy are responsible for 14% of maternal deaths.[6] In the United States it is estimated that for every pre-eclampsia related death there are probably 50-100 other women who experience significant morbidity associated with pre-eclampsia.[7]

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

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

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.[11]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.[12,13] 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.[14,15] 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.[2,9]

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 1α (HIF 1α), 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.[16] These characteristics make it a potential therapeutic to treat pre-eclampsia. Possible biological explanations include the following:

- Metformin inhibits hypoxic inducible factor 1α (HIF 1α)
- 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 1α (HIF 1α)

HIF 1α 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 1α the secretion of sFlt is decreased which may improve clinical outcomes in pre-eclampsia.[16] Metformin is an ideal HIF 1α inhibitor as it is safe in pregnancy. In contrast other HIF 1α 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.[17] Their effect is probably mediated via anti-angiogenic mechanisms.

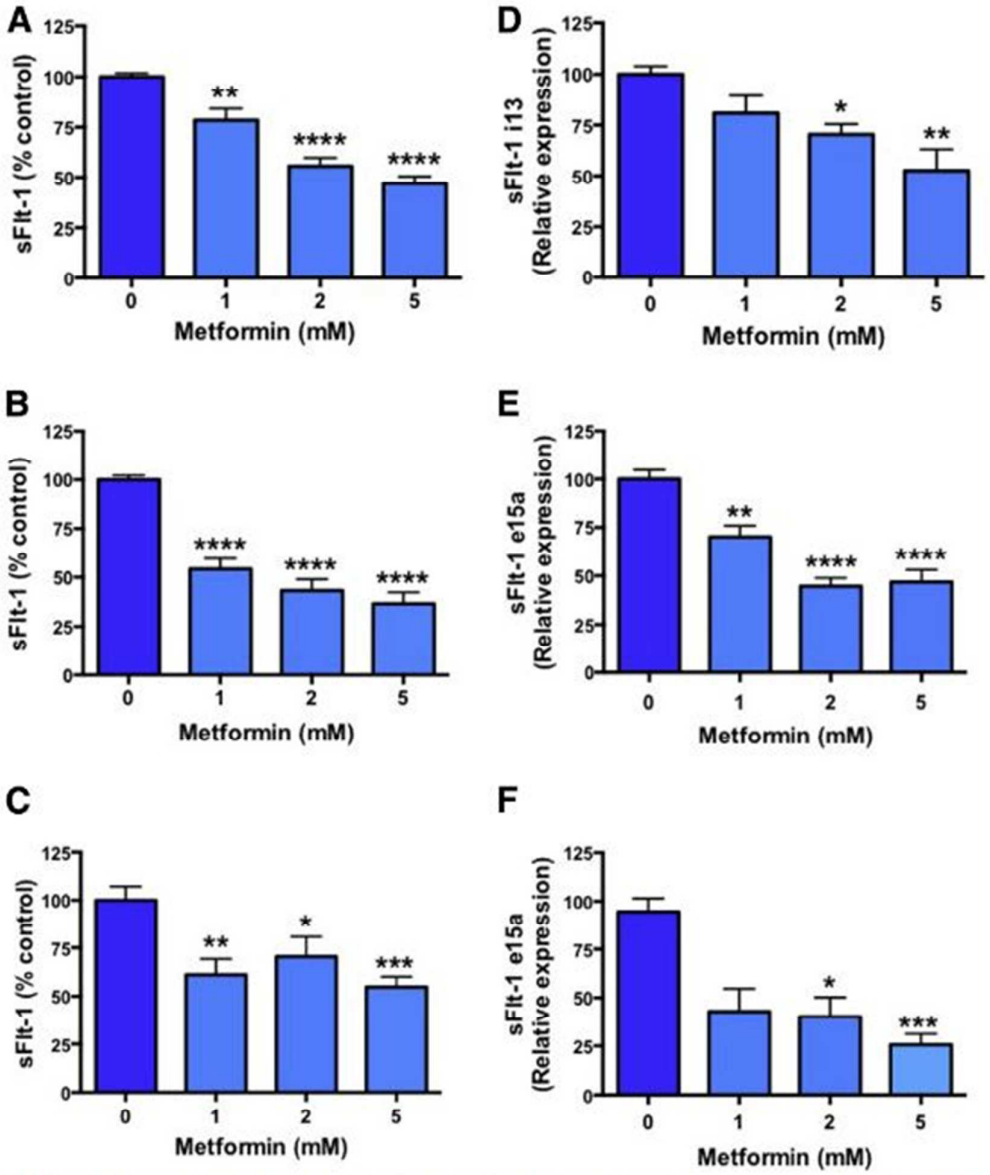
The evidence implicating sFlt-1 and sEng as central to the pathogenesis of pre-eclampsia is compelling.[18] Serum sFlt-1 and sEng are increased in women with pre-eclampsia many weeks preceding clinical disease and there is a dose dependent relationship between serum levels and disease severity.[14] sFlt-1 administered *in vivo* to pregnant rats induces hypertension and proteinuria.[19] 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.[20] sFlt-1 and sEng are, by far and away, the most studied molecules in the field of pre-eclampsia.

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

The acceptance of the strategy to reduce levels of these anti-angiogenic factors 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).[16]

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

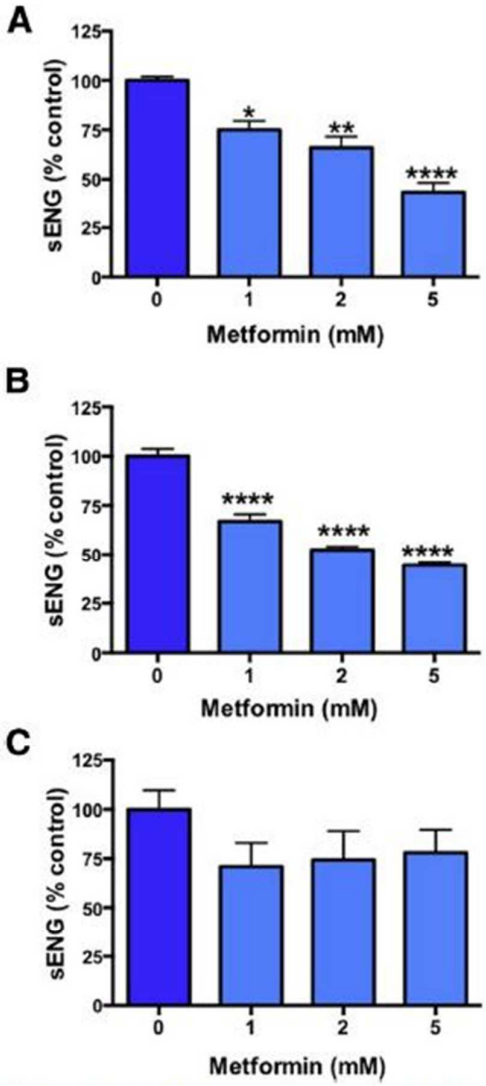


Metformin (0, 1, 2, 5 mmol/L) dose-dependently reduced soluble fms-like tyrosine kinase 1 secretion from **A,** endothelial cells, **B,** villous cytotrophoblast cells, and **C,** preterm preeclamptic placental villous explants. Metformin reduced endothelial cell expression of **D,** *sFlt-1 i13* isoform, **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.

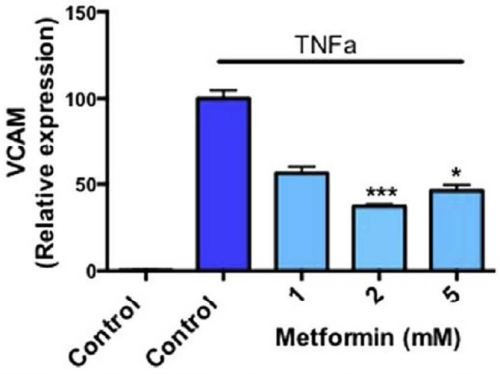
2.3.3 Metformin reduces VCAM-1 expression on endothelial cells

A hallmark of pre-eclampsia is endothelial dysfunction, a form of maternal blood vessel injury.[17] 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.[21]

It has recently been discovered that metformin potently blocks up-regulation of VCAM-1 induced by Tumour Necrosis Factor-α (TNF-α), 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 α 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 $P < .0001$.

TNFα, tumor necrosis factor α; *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.[16]

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.[22] 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.[23] 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.[24]

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.[25]

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.[26,27]

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.[28]

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.[22,29–32] 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.[33]

2.5.2 Metformin drug information

Product information on metformin can be found at the following link:
http://packageinserts.bms.com/pi/pi_glucophage_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.[32]

2.5.2.2 Contraindications

Metformin is contraindicated in

- Renal disease or renal dysfunction (suggested by serum creatinine levels ≥123umol/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 insuffiency 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 Cmax 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 Cmax and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax 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 1α (HIF 1α)
- 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 (Cmax), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (Tmax) following administration of a single 850mg tablet of metformin Cmax 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 µg/mL. During controlled clinical trials, maximum plasma levels did not exceed 5 µg/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) and the Powercal program running in Stata v15(Stata statistical software. 2017 *Release 15*. Stata Corp. College Station, TX: StataCorp LLC)[34].

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.[35] 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.[11] 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	**65**	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

	Gestational age (GA)	**EFW if GA is unknown**
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.[36] 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[36]

Classification of the HDP.

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

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

Table 3: ISSHP classification of adverse conditions and severe complications of preeclampsia[36]

Adverse conditions and severe complications of preeclampsia.

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

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

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

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.[38]
  - Severe hypertension defined as a systolic blood pressure greater than or equal to 160 mmHg or diastolic blood pressure greater than or equal to 110 mmHg that cannot be controlled with antihypertensive medication within 48 hours of admission.
  - Cerebrovascular event defined as an ischaemic or haemorrhagic stroke associated with clinical symptoms and definitive signs on imaging.
  - Posterior reversible encephalopathy syndrome (PRES) associated with pre-eclampsia defined on imaging as reversible vasogenic oedema, usually in the occipital or parietal lobes.
  - Severe renal impairment with a creatinine level of greater or equal to 125 µmol/l or a need for dialysis.
  - Signs of left ventricular failure which include pulmonary oedema requiring treatment or oxygen saturations of less than 90% caused by left sided heart failure.
  - Disseminated intravascular coagulation defined as an INR greater than 2
  - Platelet count at presentation less than $50x10^9$ (platelet aggregation excluded)
  - Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100×10^9/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase > 600 µ/L or haemolysis on a peripheral blood smear.
  - 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 124umol/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

Maternal:

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

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

- Use of antihypertensive agents (number of agents and daily dose at delivery)
- Major postpartum haemorrhage (defined as blood loss of more than 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

Fetal:

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

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

There is no standard classification system for the non-stress test. The attending clinician usually assess the heart rate patterns by looking at the baseline, assessing for baseline shifts, by assessing the variability, by looking at the presence or absence of accelerations and the presence of decelerations and by assessing for a sinusoidal pattern. 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/efmguidelinercog.pdf)

Neonatal:

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

- Neonatal death within 6 weeks after the due date
- Grade III or IV intraventricular haemorrhage defined on imaging as enlarged ventricles associated with haemorrhage or when the haemorrhage extends into the cerebral tissue around the ventricles
- Necrotizing enterocolitis diagnosed on radiographic studies

- Bronchopulmonary dysplasia defined as needing oxygen at day 28 of life, either on a ventilator, by CPAP or via a nasal catheter
- Significant neonatal sepsis as defined by the attending paediatrician.

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

- APGAR score of less than 7 at 5 minutes
- Umbilical artery pH below 7.05
- Umbilical artery lactate
- Surfactant use
- Neonatal intensive care admission or special care unit admission
- Intubation and mechanical ventilation or continuous positive airway pressure (CPAP) support
- Grade III/IV hyaline membrane disease
- Length of hospital stay
- Incidence of retinopathy of prematurity

Biomarkers:

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

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. To avoid these side effects, we will use a step-up approach in dosing. We will start all participants on one tablet twice a day. If the medication is tolerated after 24 to 48 hours the dose will be increased to two tablets twice a day. Then after 24 to 48 hours we will then increase the dose to three tablets twice a day if tolerated. If a participant does experience side effects, we will decrease the dose back to the dose that was previously well tolerated. We may then increase the medication back up to 3 tablets twice a day if the side effects are improved and the patient is willing to increase the medication again. The maximum dose of the active tablte (metformin) will be 1.5gm daily.

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, and close maternal and fetal surveillance.

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

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

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

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

Clinical care will be left up to the discretion of the clinical team. The indication for delivery will be a clinical decision. Indications for delivery may include failure to control blood pressure, the development of major maternal or fetal complications. 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, 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[2] 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.

7.5.2 Urine samples

Urine samples will be collected 2 times per week and sent for spot protein: creatinine ratios. 24-hour protein excretion is routinely measured only once on admission. This will be performed at the Tygerberg Laboratory.

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 $1cm^3$ 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 PI2 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. 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, for primary and secondary outcomes. 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.

Primary outcome

We plan to perform two analyses: 1) primary intention to treat analysis (ITT) and 2) a treatment received (TR) analysis to examine response among those who actually took the tablets. The primary outcome, the difference in time from randomization until delivery, will be assessed using linear regression modelling by (a) unadjusted analysis with treatment as the sole covariate; (b) a covariate adjusted analysis with both treatment group and gestational age design-based strata; and (c) a covariate adjusted analysis that includes treatment group, the gestational age design-based strata and absent flow on umbilical artery Doppler (UAD) examination measured at the time of randomization. The trial investigators consider that a mother with absent UAD flow likely was more likely to be associated with shorter randomization to delivery times. This pre-specified covariate adjustment will increase the precision of the primary outcome measure if there is a correlation between UA flow and delivery time. By design the stratification variable, gestational age, will be correlated with delivery time, this correlation is accounted for by including it as a covariate in the adjusted analyses (b) and (c). Given the likely skewed distribution in prolongation times, modelling will use quantile regression to assess difference in median prolongation between groups and transformation of primary outcome, to achieve adequate distributions of residuals, will be performed if indicated. Standard regression diagnostics will be performed to assess model fit.

Results will be presented as group difference with 95% confidence intervals (95%CI). Sensitivity analyses may be performed to asses (i) the effect of any chance covariate imbalance between treatment arms, using inverse probability weighted propensity scores, as the sole covariate apart from treatment and statum assignment and (ii) an intention received analysis should this differ substantially from treatment assignment, to examine response in those who actually took the active medication. Sensitivity analyses will use the same quantile regression model described above. We will also present survival a analysis (ie time until delivery), using adjusted Cox proportional hazards regression (CPH) and Kaplan-Meier survivorship curves. The adjusted CPH model will include both treatment group and gestational aged based strata as covariates.

If a fetus does demise during expectant management, we will give it a length of pregnancy prolongation of zero and will include it in the primary analysis.

Secondary Outcomes

Secondary outcomes may be composite or single, listed as per 6.2. Composite binary outcomes will be modelled using a logistic regression model containing both treatment group and gestation aged based strata and presented as RR (95% CI). Continuous single outcome variables will be compared using either unpaired t-test (for normally distributed variables) or Mann-Whitney U (non-normally distributed) whilst categorical values will be compared using likelihood-based chi-squared test. For the longitudinal data of plasma sFlt-1, sEng and endothelin 1 levels, we will present the data graphically and assess differences in levels between groups adjusted for gestational age and strata by a marginal mean model estimated using generalized estimating equations. Comparison of levels between groups at delivery will use quantile median regression as described for the primary outcome. Finally, the placental expression of sFlt-1, sEng and HO-1 will be compared using simple statistics.

The significance level is set at 0.05 and all hypothesis testing will be two-sided. Primary and composite outcomes will not be adjusted for multiple comparisons. Non-composite secondary outcomes will be adjusted for multiple comparisons using the Holm's step procedure. Statistical software used is Stata v 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC)

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.[39] 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 be accessible at all times 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 monthsto 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 while 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).[40] 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 facilitates 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 publically 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.

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PATIENT INFORMATION LEAFLET: PI2 TRIAL

SUMMARY

Name of principal investigator: Dr Cathy Cluver

Name of research midwife: ……..

Contact number: ……

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

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

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

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

BACKGROUND INFORMATION ON PRE-ECLAMPSIA

What is pre-eclampsia?

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

How do we treat pre-eclampsia?

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

When will I have my baby?

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

What treatment will I receive?

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

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

INVITATION TO BE INVOLVED IN THE PI2 TRIAL

You are being invited to participate in a research project to find out whether a drug called metformin can be used to treat pre-eclampsia. The name of the study is the Pre-eclampsia Intervention 2 (PI2) Trial. It is important that you read all the information provided about the trial before you decide to take part and that you understand why we are doing the research and what you would need to do if you were involved in the study. If you have any questions about the trial you can ask your doctor, the midwives or any of the staff from the research project. You can talk to any of the other women who have decided to be involved or not involved in the study.

Why are we doing this study?

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

What is the purpose of this study?

Recent research has shown that there are substances produced by the placenta that can cause pre-eclampsia. Work done in a laboratory in Melbourne, Australia has shown that these substances may be reduced by metformin. The PI2 trial is the first trial in the world that will look to see if metformin can be used to treat pre-eclampsia.

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

You have been invited to be in this study as you have been diagnosed with pre-eclampsia. We would like to include 150 women with pre-eclampsia in this study.

Do I have to be in the study?

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

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

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

You will then need to take extra tablets each day until the baby is delivered. One of the research team will visit you every day to see how you are feeling and to collect information about your pregnancy. When your routine blood tests are done we will take an extra sample of blood for the study. On the first day that you take the medication we will need to take an extra sample of blood. We may need to do extra ultrasound examinations of your baby. Once your baby is born and the cord has been cut we will take a small amount of blood from the placenta. We will take a small sample from the placenta which will be sent for testing.

We will follow you up after the delivery of the baby. The samples taken may be sent overseas for further testing to try to find a treatment for pre-eclampsia. Only tests related to finding a cure for pre-eclampsia will be performed on the samples taken.

Is metformin treatment safe in pregnancy?

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

Are there any side effects of metformin?

All drugs may have side effects. Side effects that have been associated with the use of metformin include headache, diarrhea, nausea, flatulence, abdominal pain, constipation and a dry mouth. There are certain drugs that cannot be used with metformin. If you are taking one of these drugs you will not be asked to participate in the trial. A very rare complication that occurs in 3 per 100 000 women taking metformin is lactic acidosis. This can present with malaise, muscle pains, breathing difficulty and abdominal pain. We will monitor you for any signs of this very rare complication. We will provide you with a copy of the product information leaflet if you would like more information.

Will I receive the placebo treatment or the metformin treatment?

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

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

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

Will my information be kept confidential?

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

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

Only half of the women in this trial will be given metformin. If you do receive this treatment you may or may not benefit from the effects of this drug and you may or may not have improvement in your pre-eclampsia. There are no other direct benefits for you being

involved in this study. By being involved you may help us find a treatment for pre-eclampsia which could help many pregnant mothers in your situation in the future.

What are the disadvantages of being in the study?

You will need to take extra tablets and we may need to take a few extra samples of your blood for testing.

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

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

What will happen with the results of this research project?

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

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

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

Who has paid for this study?

This study has been funded by grants from the University of Melbourne. Dr Cluver's salary has been paid for by grants from the Discovery Foundation and the South African Medical Association.

Who do I contact if I have a problem?

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

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

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

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

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

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

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

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

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

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

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

Thank you for taking the time to read this information leaflet about the PI2 trial.

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

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

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

INFORMED CONSENT FORM

PRINCIPAL INVESTIGATOR: Dr Catherine Anne Cluver

RESEARCH MIDWIFE: Name: ……
Contact number: ……
Email: …….
ADDRESS: Department of Obstetrics and Gynaecology
Tygerberg Hospital and University of Stellenbosch

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

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

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

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

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

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

Declaration by participant

By signing below, I …… agree to take part in a research study entitled: Pre-eclampsia Intervention 2 (PI2) trial: a double blind randomised, controlled trial of metformin to treat early onset preeclampsia.

I declare that:

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

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

....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* …… declare that:

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

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

....

Signature of investigator **Signature of witness**

Declaration by interpreter

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

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

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

....

Signature of interpreter **Signature of witness**

BMJ Open

A double blind, randomised, placebo-controlled trial to evaluate the efficacy of metformin to treat preterm pre-eclampsia (PI2 Trial): study protocol

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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	pre-eclampsia, metformin, preterm preeclampsia, treatment, Clinical trials < THERAPEUTICS, preeclampsia

A double blind, randomised, placebo-controlled trial to evaluate the efficacy of metformin to treat preterm pre-eclampsia (PI2 Trial): study protocol

Authors:

Catherine A Cluver,[1,2] Susan P Walker,[3] Ben W Mol,[4] David R Hall,[1] Richard Hiscock,[5] Fiona C Brownfoot,[2,3] Tu'uhevaha J Kaitu'u-Lino,[2,3] Stephen Tong[2,3]

Author Affiliations:

[1] Department of Obstetrics and Gynaecology, Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa.

[2] Translational Obstetrics Group, University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia.

[3] Department of Obstetrics and Gynaecology, University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia.

[4] Department of Obstetrics and Gynaecology, Monash School of Medicine, Monash University, Melbourne, Victoria, Australia.

[5] Department of Anaesthetics, University of Melbourne, Mercy Hospital for Women, Victoria 3084, Australia.

Corresponding author:

Dr Catherine Anne Cluver

Department of Obstetrics and Gynaecology, Stellenbosch University, Tygerberg Hospital

Francie van Zyl Drive, Tygerberg 7505, South Africa

Email: cathycluver@hotmail.com

Phone: +27823210298

ABSTRACT

Introduction

Pre-eclampsia is a major complication of pregnancy, globally responsible for 60,000 maternal deaths per year, and far more fetal losses. There is no definitive treatment other than delivery. A therapeutic that could quench the disease process would be useful to treat preterm pre-eclampsia, as it could allow these pregnancies to safely continue to a gestation where fetal outcomes are significantly improved. We have published preclinical data to show that metformin, a drug known to be safe in pregnancy and commonly used to treat gestational diabetes, has potent biological effects making it another promising candidate to treat pre-eclampsia. Here, we describe a phase II clinical trial to examine whether administering extended release metformin may be effective in treating women with preterm pre-eclampsia (PI2 Trial).

Methods

The PI2 Trial is a phase II, double blind, randomised controlled trial that aims to recruit 150 women with preterm pre-eclampsia (gestational age 26+0 to 31+6 weeks) who are being managed expectantly. Participants will be randomised to receive either 3 grams metformin or placebo daily. The primary outcome is time from randomisation till delivery. A delay in delivery of 5 days is assumed to be clinically relevant. The secondary outcomes will be a maternal composite and neonatal composite outcome. All other outcomes will be exploratory. We will record adverse events.

Ethics and dissemination

This study has ethical approval (Protocol number M16/09/037 Federal Wide Assurance Number 00001372, Institutional Review Board Number IRB0005239), is registered with the Pan African Clinical Trial Registry (PACTR201608001752102) and the South African Medicine Control

Council (20170322). Data will be presented at international conferences and published in peer-reviewed journals.

ARTICLE SUMMARY

Article focus

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

Key Messages

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

Strengths and limitation of this study

- This is a protocol for a randomised, double blind, placebo controlled clinical trial.
- This is the first trial to assess whether metformin is a treatment option for pre-eclampsia.
- We plan to recruit 150 participants which provides sufficient power for our primary outcome (time from randomisation to delivery).
- It may be underpowered to show improvements in maternal and perinatal outcomes. Therefore, if the trial yields a positive result, larger multi-centre studies will be needed.

INTRODUCTION

Pre-eclampsia is one of the most serious complications of pregnancy affecting 5% of pregnancies worldwide.[1] It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit.[2] 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.[3] Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[4] and it is estimated that for every pre-eclampsia related death there are 50-100 other women who experience significant morbidity associated with pre-eclampsia.[5]

At present, the only treatment available for pre-eclampsia is termination/delivery of the pregnancy. This poses a difficult clinical dilemma in the setting of preterm pre-eclampsia. Clinicians are often forced to deliver early to prevent disease progression and major maternal morbidity (ie severe maternal organ injury), but in doing so, inflict severe prematurity on the fetus. In particular, fetuses delivered at less than 33 weeks' gestation are at significant risk of severe disability including cerebral palsy, stroke (intracerebral bleeding), retinopathy of prematurity, chronic lung disease and death.[6]

We have recently completed a phase II, double blind, randomised controlled trial, the Pre-eclampsia Intervention with Esomeprazole (PIE) trial, to evaluate whether 40 mg of esomeprazole was a possible treatment for early onset pre-eclampsia. This trial showed no between-group differences in median time from randomization to delivery (11·4 days in the esomeprazole group, and 8·3 days in the placebo group [95% confidence interval -2·9-8·8, P=0·31]).[7] Here, we propose metformin as the next therapeutic candidate to test in a clinical trial for preterm pre-eclampsia.

Metformin is a promising therapeutic candidate as it has been shown to reduce soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) secretion from endothelial cells and primary trophoblasts.[8] These key anti-angiogenic molecules released from the placenta are responsible for the widespread endothelial dysfunction and impaired vascular relaxation observed in pre-eclampsia. Reduced angiogenesis is also thought to contribute to placental hypoxia and to the development of pre-eclampsia. Metformin has also been shown to improve endothelial dysfunction, improve blood vessel relaxation and promote angiogenesis.[8] Given these multiple potential effects, metformin is a particularly promising therapeutic candidate.

OBJECTIVES

The primary objective is to examine whether 3 grams of metformin XR can safely prolong gestation among women with preterm pre-eclampsia who are being managed expectantly, compared to expectant management alone. A prolongation of 5 or more days will be considered clinically significant.

The secondary objectives are to examine whether metformin can improve maternal and neonatal composite outcomes.

METHODS

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

Study design

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

Study population

Pregnant women diagnosed with pre-eclampsia at a gestational age between 26+0 weeks to 31+6 weeks at Tygerberg hospital (Western Cape Provence of South Africa) will be invited to participate. To be eligible for this study the treating clinicians need to have made an initial assessment and deemed that the patient is suitable for expectant management and delivery is not immediately required or anticipated in the next 48 hours.

Inclusion criteria:

We will recruit women with a singleton pregnancy diagnosed with pre-eclampsia and chronic hypertension with superimposed preeclampsia defined according to the criteria published by The International Society for the Study of Hypertension In Pregnancy (ISSHP),[9] and require the presence of significant proteinuria (more than 300 milligrams in a 24 hour urine collection). Some women only present for antenatal care after 20 weeks gestation. We will include these women and classify these women as unclassified proteinuric hypertension during the pregnancy according to the classification of Davey and MacGillvray.[10] Postpartum, these women will be reclassified as having had pre-eclampsia or chronic hypertension based on their blood pressure and need for medication more than 6 week after the due date.

Gestational age will be determined by either period dates (if the woman is certain of her last menstrual period) or by an early, or mid trimester pregnancy ultrasound. If the gestational age is uncertain, we will recruit participants with an estimated fetal weight between 500 and 1800 grams, determined by ultrasound performed at presentation. A full list of inclusion criteria is provided in Table 1.

Exclusion criteria:

Exclusion criteria include women with established maternal or fetal compromise that necessitates delivery within 48 hours, the current use of metformin, contraindications to the use

of metformin or the use of medications that interact with metformin. A full list of exclusion criteria is provided in Table 2.

Participant enrolment

Participants will be identified after they have been admitted to the tertiary referral centre with a diagnosis of preterm pre-eclampsia for expectant management. An information leaflet will be given to all potential participants and written informed consent will be obtained (Supplementary information 2 and 3).

Randomisation and allocation concealment

After informed consent, participants will be randomised to metformin or placebo in a 1:1 ratio. An online, web-based sequence generator system linked with codes for placebo and treatment tablets will be used. Both researchers and participants will be blinded.

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

Once the participants have been randomised the treatment pack with the same code will be allocated to the participant. All treatment packs will be identical and will contain either active

tablets or placebo. The researchers will have no access to the randomisation list. This process will ensure that there is allocation concealment throughout the conduct of the trial.

Each participant will be given an individual treatment pack containing either 500mg metformin XR or identical placebo tablets which have been produced by Merck (http://www.merck.com/index.html). Labelling, storage and preparation will be done according to the requirements of the Medicines for Human Use (Clinical Trials) regulations. Participants will be started on six tablets daily in divided doses. If side effects develop the dose will be decreased until the side effects improve and then increased again if tolerated.

Co-interventions

Participants will remain under the care of the hospital treating team and the study will not alter or interfere with the routine care provided for women with preterm pre-eclampsia, including the decision for delivery. Management for preterm pre-eclampsia involves admission to hospital 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 fetal 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 for the neonate. A single repeat dose will be given one week later as per hospital protocol.[11] Most participants will be on antihypertensive treatment, and the dose and number of

antihypertensives will be recorded. All women should already be receiving calcium, iron and folic acid supplementation.

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

Sample collection

Blood samples will be routinely collected twice a week. . The routine blood samples include measurements of the haemoglobin, the platelet count and the urea and creatinine levels. These will be used by the managing clinicians to determine disease severity and may trigger delivery.

Outcomes

The primary outcome is to examine whether up to 3 grams of metformin daily can safely prolong gestation in women with preterm pre-eclampsia diagnosed between 26+0 to 31+6 weeks gestation, compared to standard of care, expectant management alone. This is set in a superiority framework with two-sided hypothesis regions for all statistical testing. A prolongation of 5 days or longer will be considered clinically significant.

The secondary outcome is to determine whether metformin improves maternal composite and neonatal composite outcomes. The composite maternal outcome includes maternal death, eclampsia, pulmonary oedema, severe renal impairment or the need for dialysis, a cerebral vascular event, the development of a liver haematoma or rupture and placental abruption. The composite neonatal outcome will include neonatal death within 6 weeks after the due date, grade III or IV intraventricular haemorrhage defined enlarged ventricles associated with

haemorrhage or haemorrhage extending into the cerebral tissue around the ventricles on imaging, necrotizing enterocolitis defined on radiographic studies or bronchopulmonary dysplasia defined as needing oxygen at day 28 of life, either on a ventilator, by CPAP or via a nasal catheter. See Table 3.

All other outcomes, including outcomes nested within the composite secondary outcomes will be classified as exploratory outcomes. These are listed in Table 4.

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 withdrawal, we will seek the patient's permission to use their clinical information and laboratory samples collected thus far in subsequent analyses.

Duration of the trial

It is anticipated that the study will be completed in approximately 3 years (2018 – 2020). In our previous PIE study, we included 120 women in 15 months.[7]

Ethical approval and dissemination

This study has ethical approval (Stellenbosch University HREC Protocol number M16/09/037 Federal Wide Assurance Number 00001372, Institutional Review Board Number IRB0005239) and is registered with the Pan African Clinical Trial Registry (Application ID PACTR201608001752102) and the South African Medicine Control Council (20170322). Data will be presented at international conferences and published in peer-reviewed journals.

Confidentiality

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

Data management and statistical analysis

Data will be collected prospectively using a REDCap database.[12] Data entry and checking will be continuous, and queries will be addressed contemporaneously to ensure clarification without delay. All data will be double checked for accuracy.

The patient characteristics, by treatment group at randomisation, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution.

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 intertest 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). The trial presentation will meet the CONSORT statement requirements (http://www.consort-statement.org/).

Sample size and stratification according to gestation at recruitment

The primary research question is whether metformin will result in a gain in the median length of gestation compared to placebo therapy. A prolongation of more than 5 days will be considered clinically significant. 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) and the Powercal program running in Stata v15 (Stata statistical software. 2017 Release 15. Stata Corp. College Station, TX: StataCorp LLC).[13]

We have two sources for data on the prolongation of gestation in preeclamptic mothers receiving expectant management which were both performed at the same hospital where we plan to run

this trial (Tygerberg Hospital). Hall et al, in the largest descriptive study on expectant management of preterm pre-eclampsia, reported a mean gestation prolongation of 11 (SD 7) days equivalent to a Coefficient of Variation (CV) = 0.64,[14] and the PIE trial[7], where patients randomized to the placebo arm had an overall mean prolongation of 13.1 (SD 12.2) days, a CV = 0.93.[7] In this study for mothers entering at gestations greater than 29 weeks, the CV = 0.8. We have therefore 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.

For 90% power, with a two-sided alpha set at 0.05, control median prolongation of 8.3 days and prolongation of gestation of 5 days with the measure of variability used in this analysis being the CV = (data SD)/ data Mean) we require 65 participants per group. Allowing for 10 dropouts per group we require 75 participants per arm and therefore a total of 150 participants.

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. 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. 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. Stratum 1 will include pregnancies of a gestational age of 26 weeks 0 days up to and including 28 weeks and 6 days. Stratum 2 will include pregnancies from 29 weeks and 0 days up to and including 31 weeks and 6 days.

More detail is available in the trial statistical analysis plan (Supplementary Information 4).

Adverse events

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

Unblinding

Given that the safety profile of metformin in pregnancy is well established, we anticipate the need for unblinding to be unlikely. However, we will have the following procedures in place should unblinding be required. Sheets with lists of 25 randomisation codes each will be sealed in individual, signed, numbered envelopes. If unblinding is requested, the relevant envelope will be opened by a person not involved in the trial in the presence of two witnesses, the group allocation read, and the list re-sealed in an envelope and signed. The randomisation envelopes will be accessible at all times to the principal investigator who will be contactable by mobile telephone. The principal investigator will co-ordinate this process.

Early termination of the trial

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

DISCUSSION

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

Recently a number of clinical trials have been proposed for assessing novel treatments for pre-eclampsia but only a few have been completed. Our group has recently completed the PIE trial assessing esomeprazole to treat preterm pre-eclampsia.[7] In this trial a daily dose of 40mg esomeprazole did not prolong gestation or have any effect on biomarkers associated with pre-eclampsia. Sildenafil was assessed in a single site, double blind, randomised controlled trial in Brazil.[15] Over a 28-month period, 100 women were recruited, and results were available for 93 women. There was a significant prolongation of gestation in the sildenafil group of 4 days but given that sildenafil is a vasodilator it is possible that this prolongation in gestation may have occurred because the drug decreased blood pressure and mitigated a clinical reason to deliver, rather than temporising disease progression. Antithrombin was assessed to treat preterm pre-eclampsia in the PRESERVE-1 trial which enrolled 120 women from 23 tertiary hospitals over 28 months (ISRCTN23410175).[16] There was no difference in prolongation of pregnancy or composite neonatal outcomes.[17] Trials assessing serelaxin (NCT01566630), pravastatin, high doses of antithrombin,[18] and celecoxib (NCT00442676) have been attempted but all were terminated due to poor recruitment.

It is known that the pre-eclamptic placenta releases anti-angiogenic sFlt-1 and sEng into the maternal circulation, causing widespread maternal endothelial dysfunction and organ injury.[19] A drug that can decrease sFlt-1 and sEng production and decrease endothelial dysfunction may be a potential treatment for pre-eclampsia. We have generated preclinical data suggesting metformin may have such actions.[8] Based on this preclinical data, we are now proposing to undertake this phase II randomized clinical trial.

We propose recruiting 150 women at Tygerberg Hospital, in the Western Cape Provence of South Africa. There are advantages to running the trial at this site. South Africa has a very high incidence of pre-eclampsia. Tygerberg Hospital is a tertiary referral centre with a neonatal intensive care unit, a maternal critical care unit and an adult intensive care unit. It is an academic centre that actively contributes to the global scientific literature and our team has recently completed the PIE trial at this research unit. We will make use of the sytems set up by this trial. Thus, we believe running this trial at Tygerberg Hospital represents a balance between obtaining sufficient number of cases of preterm pre-eclampsia, performing the trial in a cost-effective manner and offering modern obstetric and perinatal care, making the results potentially generalizable to both developed and developing countries.

Metformin is known to be safe in pregnancy and is considered a category B drug in pregnancy (by the Food and Drug Administration (FDA)).[20] There are numerous studies of metformin for the treatment of diabetes and polycystic ovarian syndrome in pregnancy which have shown no adverse fetal effects when used in the first, second and third trimesters.[21–26] Metformin does cross the placental barrier and it's long term effect on fetal development is unknown but a recent ACOG practice bulletin has concluded that it can reasonably be used in pregnancy.[27]

In strong support of our preclinical data are meta-analyses of clinical trial data that have shown a decrease in the incidence of hypertensive disorders when metformin has been used for other

indications.[28] Excitingly, a randomised trial by the Fetal Medicine Foundation (for obese women without diabetes) reported that metformin significantly reduced the incidence of pre-eclampsia by as much as 76%.[29] The EMPOWaR trial also evaluated maternal and fetal effects of metformin in obese women.[30] While reporting no decrease in the incidence of hypertensive disorders of pregnancy with metformin, the most likely reason for this difference was poor compliance.[31]

We have powered our study for the primary outcome, which is to show whether metformin can safely prolong gestation for a further five days in mothers being expectantly managed for preterm pre-eclampsia. This surrogate primary outcome marker has been chosen as it provides a sample size that is feasible and attainable for a phase II study to assess efficacy. A limitation in this trial is that we have not specifically powered to detect improvements in maternal or neonatal outcomes (although these are planned secondary outcomes). If this trial yields a positive result, a further phase III multi-centre randomised trial that is sufficiently powered, may be required to be sufficiently powered to demonstrate improvements in clinical outcomes.

As we did for the PIE trial, we will measure biomarkers that reflect disease severity. We will measure circulating levels of sFlt-1 and sEng, among others, in serial samples obtained from the mother. These anti-angiogenic factors are considered to play an important role in inciting maternal endothelial dysfunction and end-organ injury seen in pre-eclampsia. Furthermore, we will measure expression of key molecules in the placental samples obtained at delivery.

Pre-eclampsia kills mothers, fetuses and neonates and is responsible for severe maternal and neonatal morbidity. This is especially the case in the developing world where there is a lack of resources, including staff, equipment and finances. If a treatment were to be discovered, it would have dramatic effects on maternal and neonatal outcomes. Metformin has shown

potential as a therapeutic agent in preclinical work on pre-eclampsia. Further advantages of metformin XR are that it is available in a long acting formulation, it is safe during pregnancy and it is not expensive. This makes it an ideal candidate as a global therapeutic for pre-eclampsia. It is therefore imperative for this trial to be performed. If metformin were proven to be effective at prolonging gestation in early onset pre-eclampsia it could play an important role in decreasing the clinical burden of this dangerous condition.

CONTRIBUTORSHIP STATEMENT

CC, SW and ST developed and designed the trial and obtained funding for the trial.

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

BM and DH assisted with the study design.

RH designed the statistical aspects of this protocol.

FB and TK were involved in the design of the laboratory arm of this trial.

All were involved in revision of the manuscript.

All authors approved the final version to be submitted.

CC and RH responded to the reviewer's comments.

COMPETING INTERESTS

The authors report no competing interests or conflict of interest.

FUNDING

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DATA SHARING STATEMENT

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

PATIENT AND PUBLIC INVOLVEMENT

Patients and the public were not involved in the development of the research question, the design of the study, the conduct or recruitment and the burden of the intervention was not assessed by the patients or the public. The results of the study will be emailed to the participants.

TABLES

Table 1: Inclusion criteria

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

Table 2: Exclusion criteria

Any of the following at the initial assessment:
Patient is unable or unwilling to give consent
Established fetal compromise that necessitates delivery
The presence of: Eclampsia
Severe hypertension, 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
Posterior reversible encephalopathy syndrome (PRES)
Severe renal impairment, defined as a creatinine level ≥ 125 µmol/l or a need for dialysis.
Pulmonary oedema
Left sided heart failure
Disseminated intravascular coagulation
Platelet count < $50x10^9$
Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome
Liver transaminases > 500IU/L
Liver haematoma or rupture
Severe ascites
Diabetes
Current use of metformin
Contraindications or a hypersensitivity reaction to the use of metformin
Current use of a drug that may be affected by metformin

Table 3: Secondary Outcomes

Secondary outcomes	
Composite maternal outcome:	Maternal death
	Eclampsia
	Pulmonary oedema
	Severe renal impairment or dialysis
	Cerebral vascular event
	Liver haematoma or rupture
	Placental abruption
Composite neonatal outcome:	Neonatal death within 6 weeks after the due date
	Grade III or IV intraventricular haemorrhage
	Necrotizing enterocolitis (diagnosed on radiological studies)
	Bronchopulmonary dysplasia (defined as needing oxygen at day 28 of life, either on a ventilator, by CPAP or via a nasal catheter)

Table 4: Exploratory outcomes

Exploratory outcomes

Maternal exploratory outcomes: Maternal death

Eclampsia

Pulmonary oedema

Severe renal impairment or dialysis

Cerebral vascular event

Liver haematoma or rupture

Abruptio placentae

Admission to a high care or intensive care unit

Posterior reversible encephalopathy syndrome

Left ventricular failure

Serum creatinine > 125 µmol/l

Proteinuria >3g/24h

Severe hypertension

Disseminated intravascular coagulation

Platelet count less than 50x109

HELLP syndrome

Liver transaminases >500IU/L

Placental abruption

Mode of delivery

Use of antihypertensive agents

Major postpartum haemorrhage

Thromboembolic disease

Moderate or severe ascites

Fetal exploratory outcomes: Reversed a-wave in the ductus venosus on fetal ultrasound

	Significant changes in fetal heart rate patterns on the non-stress test that necessitate delivery
	Intrauterine fetal demise
	Fetal growth restriction at birth
	Persistent reversed flow in the umbilical artery
	Redistribution in the middle cerebral artery
Neonatal exploratory outcomes:	Neonatal death within 6 weeks after the due date
	Grade III or IV intraventricular haemorrhage
	Necrotizing enterocolitis
	Bronchopulmonary dysplasia
	APGAR score < 7 at 5 minutes
	Umbilical artery pH < 7.05
	Umbilical artery lactate
	Surfactant use
	Neonatal intensive care or special care unit admission
	Intubation and mechanical ventilation
	Continuous positive airway pressure (CPAP) support
	Grade III/IV hyaline membrane diseaseHospital stay
	Incidence of retinopathy of prematurityNeonatal sepsis

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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

Full title of trial	Pre-eclampsia Intervention 2 (PI2) Trial: a double blind randomised, placebo-controlled trial of Metformin to treat early onset pre-eclampsia
Short title	PI2 trial
Version and date	30 January 2019 Version 1.2
Ethics approval	Protocol number: (M16/09/037) Federal Wide Assurance Number 00001372 Institutional Review Board (IRB) Number: IRB0005239
Pan African Clinical Trial Registry ID	PACTR201608001752102
Medicine Control Council Reference number	N2/19/8/2 Approved 12 May 2017
Trial medication	Metformin
Phase of trial	Phase II
Principal investigator	Dr Catherine Anne Cluver
Supervisors/ Co-investigators	Professor Stephen Tong Professor Susan Walker Prof Ben Mol Prof DR Hall Dr T Kaitu'u-Lino Dr N Hannan Dr H Hiscock Dr F Brownfoot
Funders	Mercy Perinatal Foundation mercyperinatal@mercy.com.au South African Medical Research Council http://www.mrc.ac.za/

CONTACT DETAILS

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

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

Prof Susan Walker
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: spwalker@unimelb.edu.au
Prof Ben Mol
The Robinson Institute, School of Paediatrics and Reproductive Health
University of Adelaide
Phone: +61434122170
Email: ben.mol@adelaide.edu.au

Prof David Hall
Tygerberg Hospital
University of Stellenbosch
Tel: +27 21 938 9059
Email: drh@sun.ac.za

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

Dr Tu'uhevaha Kaitu'u-Lino
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: t.klino@unimelb.edu.au

Dr Fiona Brownfoot
Mercy Hospital for Women
University of Melbourne
Tel: +614 1813 0139
Email: Fiona.brownfoot@gmail.com

Trial Design and Statistics

Dr Richard Hiscock
Mercy Hospital for Women
Email: richardjhiscock@gmail.com

Laboratory Coordination

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

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

TRIAL COMMITTEES

Trial Steering Committee

Chair:

Prof Ben Mol
Professor of Obstetrics and Gynaecology
Evidenced based Women's Health Care Research Group
Monash University
Phone: +613 857 22836
Email: Ben.Mol@monash.edu

Members:

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

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

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

Data Monitoring Committee

Chair:

Prof Gerhard Theron
Consultant Obstetrician and Gynaecologist
Stellenbosch University and Tygerberg Hospital
Email: gbth@sun.ac.za

Tel: +27 219389217

Members

Dr Adrie Bekker
Consultant Paediatrician
Stellenbosch Hospital and Tygerberg Hospital
Email: adrie@sun.ac.za
Tel: +27 722125453

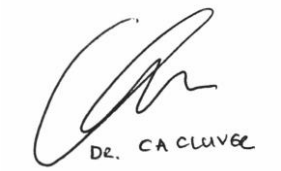
Mr Chris Muller
Biostatistican
Stellenbosch University
Tel +27824303101
Email: cmuller@sun.ac.za

Protocol Versions

1.2

Signatures

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



Principal investigator:

Date: 30/01/2019

Abbreviations

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

CONTENTS

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 1α (HIF 1α)
- 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.[1] It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit.[2] 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.[3] Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[4,5] and in South Africa hypertensive disorders of pregnancy are responsible for 14% of maternal deaths.[6] In the United States it is estimated that for every pre-eclampsia related death there are probably 50-100 other women who experience significant morbidity associated with pre-eclampsia.[7]

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

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

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.[11]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.[12,13] 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.[14,15] 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.[2,9]

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 1α (HIF 1α), 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.[16] These characteristics make it a potential therapeutic to treat pre-eclampsia. Possible biological explanations include the following:

- Metformin inhibits hypoxic inducible factor 1α (HIF 1α)
- 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 1α (HIF 1α)

HIF 1α 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 1α the secretion of sFlt is decreased which may improve clinical outcomes in pre-eclampsia.[16] Metformin is an ideal HIF 1α inhibitor as it is safe in pregnancy. In contrast other HIF 1α 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.[17] Their effect is probably mediated via anti-angiogenic mechanisms.

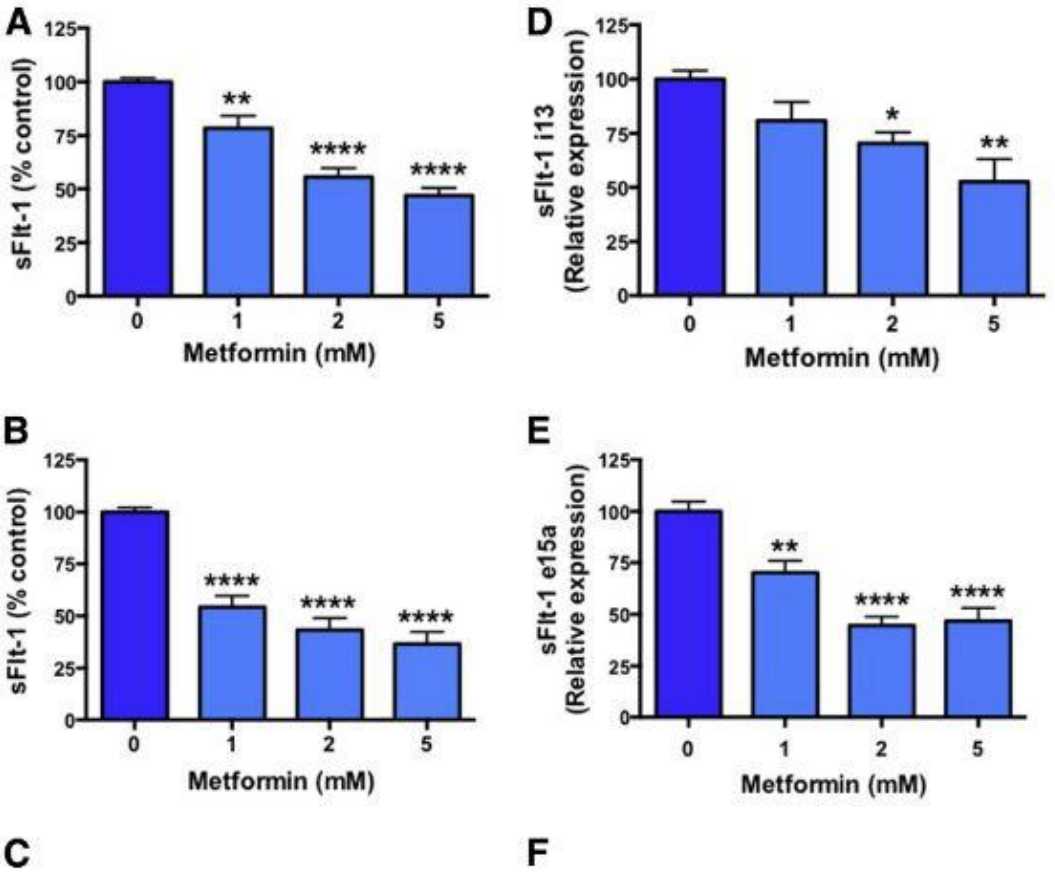
The evidence implicating sFlt-1 and sEng as central to the pathogenesis of pre-eclampsia is compelling.[18] Serum sFlt-1 and sEng are increased in women with pre-eclampsia many weeks preceding clinical disease and there is a dose dependent relationship between serum levels and disease severity.[14] sFlt-1 administered *in vivo* to pregnant rats induces hypertension and proteinuria.[19] 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.[20] sFlt-1 and sEng are, by far and away, the most studied molecules in the field of pre-eclampsia.

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

The acceptance of the strategy to reduce levels of these anti-angiogenic factors 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).[16]

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

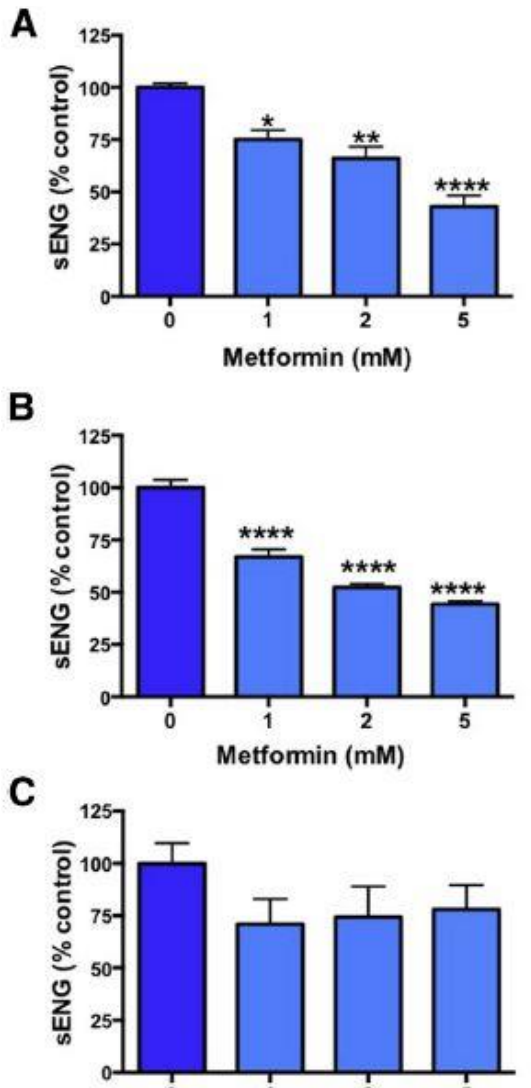


Metformin (0, 1, 2, 5 mmol/L) dose-dependently reduced soluble fms-like tyrosine kinase 1 secretion from **A,** endothelial cells, **B,** villous cytotrophoblast cells, and **C,** preterm preeclamptic placental villous explants. Metformin reduced endothelial cell expression of **D,** *sFlt-1 i13* isoform, **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.

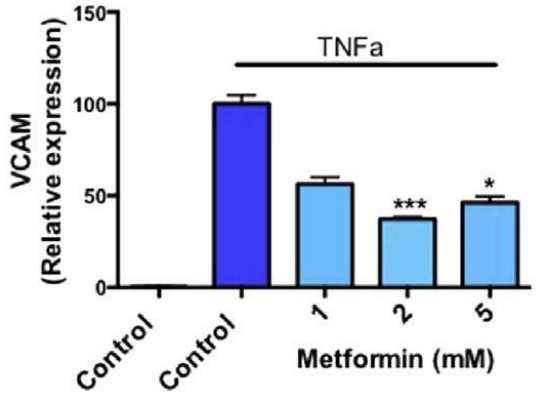
2.3.3 Metformin reduces VCAM-1 expression on endothelial cells

A hallmark of pre-eclampsia is endothelial dysfunction, a form of maternal blood vessel injury.[17] 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.[21]

It has recently been discovered that metformin potently blocks up-regulation of VCAM-1 induced by Tumour Necrosis Factor-α (TNF-α), 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 α 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 $P < .0001$.

TNFα, tumor necrosis factor α; *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.[16]

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.[22] 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.[23] 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.[24]

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.[25]

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.[26,27]

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.[28]

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.[22,29–32] 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.[33]

2.5.2 Metformin drug information

Product information on metformin can be found at the following link: http://packageinserts.bms.com/pi/pi_glucophage_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.[32]

2.5.2.2 Contraindications

Metformin is contraindicated in

- Renal disease or renal dysfunction (suggested by serum creatinine levels ≥123umol/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 insuffiency 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 Cmax 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 Cmax and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax 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 1α (HIF 1α)
- 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 (Cmax), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (Tmax) following administration of a single 850mg tablet of metformin Cmax 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 µg/mL. During controlled clinical trials, maximum plasma levels did not exceed 5 µg/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) and the Powercal program running in Stata v15(Stata statistical software. 2017 *Release 15*. Stata Corp. College Station, TX: StataCorp LLC)[34].

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.[35] 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.[11] 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	**65**	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

	Gestational age (GA)	**EFW if GA is unknown**
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.[36] 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[36]

Classification of the HDP.

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

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

Table 3: ISSHP classification of adverse conditions and severe complications of preeclampsia[36]

Adverse conditions and severe complications of preeclampsia.

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

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

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

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.[38]
  - Severe hypertension defined as a systolic blood pressure greater than or equal to 160 mmHg or diastolic blood pressure greater than or equal to 110 mmHg that cannot be controlled with antihypertensive medication within 48 hours of admission.
  - Cerebrovascular event defined as an ischaemic or haemorrhagic stroke associated with clinical symptoms and definitive signs on imaging.
  - Posterior reversible encephalopathy syndrome (PRES) associated with pre-eclampsia defined on imaging as reversible vasogenic oedema, usually in the occipital or parietal lobes.
  - Severe renal impairment with a creatinine level of greater or equal to 125 µmol/l or a need for dialysis.
  - Signs of left ventricular failure which include pulmonary oedema requiring treatment or oxygen saturations of less than 90% caused by left sided heart failure.
  - Disseminated intravascular coagulation defined as an INR greater than 2
  - Platelet count at presentation less than $50x10^9$ (platelet aggregation excluded)
  - Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100×10^9/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase > 600 µ/L or haemolysis on a peripheral blood smear.
  - 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 124umol/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 ≤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 ≤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 µmol/l
- Proteinuria greater than or equal to 3g/24h
- Hypertension with a systolic blood pressure greater than 160mmHg or a diastolic blood pressure greater than 110mmHg despite anti-hypertensive treatment
- Disseminated intravascular coagulation
- Platelet count less than 50x109
- Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100 × 109/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase greater than or equal to 600 µ/L or haemolysis on a peripheral blood smear or a raised haptoglobin level)
- Liver transaminases > 500IU/L
- Placental abruption defined as a retroplacental clot covering more than 15% of the maternal surface or a diagnosis on histology
- Mode of delivery
- Use of antihypertensive agents (number of agents and daily dose at delivery)
- Major postpartum haemorrhage (defined as blood loss of more than 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/efmguidelinercog.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, 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^3 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 PI2 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 intertest 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.[39] 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 while 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).[40] 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 facilitates 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.

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PATIENT INFORMATION LEAFLET: PI2 TRIAL

SUMMARY

Name of principal investigator: Dr Cathy Cluver

Name of research midwife: ……..

Contact number: ……

What is pre-eclampsia?

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

The trial:

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

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

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

BACKGROUND INFORMATION ON PRE-ECLAMPSIA

What is pre-eclampsia?

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

How do we treat pre-eclampsia?

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

When will I have my baby?

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

What treatment will I receive?

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

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

INVITATION TO BE INVOLVED IN THE PI2 TRIAL

You are being invited to participate in a research project to find out whether a drug called metformin can be used to treat pre-eclampsia. The name of the study is the Pre-eclampsia Intervention 2 (PI2) Trial. It is important that you read all the information provided about the trial before you decide to take part and that you understand why we are doing the research and what you would need to do if you were involved in the study. If you have any questions about the trial you can ask your doctor, the midwives or any of the staff from the research project. You can talk to any of the other women who have decided to be involved or not involved in the study.

Why are we doing this study?

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

What is the purpose of this study?

Recent research has shown that there are substances produced by the placenta that can cause pre-eclampsia. Work done in a laboratory in Melbourne, Australia has shown that these substances may be reduced by metformin. The PI2 trial is the first trial in the world that will look to see if metformin can be used to treat pre-eclampsia.

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

You have been invited to be in this study as you have been diagnosed with pre-eclampsia. We would like to include 150 women with pre-eclampsia in this study.

Do I have to be in the study?

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

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

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

You will then need to take extra tablets each day until the baby is delivered. One of the research team will visit you every day to see how you are feeling and to collect information about your pregnancy. When your routine blood tests are done we will take an extra sample of blood for the study. On the first day that you take the medication we will need to take an extra sample of blood. We may need to do extra ultrasound examinations of your baby. Once your baby is born and the cord has been cut we will take a small amount of blood from the placenta. We will take a small sample from the placenta which will be sent for testing.

We will follow you up after the delivery of the baby. The samples taken may be sent overseas for further testing to try to find a treatment for pre-eclampsia. Only tests related to finding a cure for pre-eclampsia will be performed on the samples taken.

Is metformin treatment safe in pregnancy?

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

Are there any side effects of metformin?

All drugs may have side effects. Side effects that have been associated with the use of metformin include headache, diarrhea, nausea, flatulence, abdominal pain, constipation and a dry mouth. There are certain drugs that cannot be used with metformin. If you are taking one of these drugs you will not be asked to participate in the trial. A very rare complication that occurs in 3 per 100 000 women taking metformin is lactic acidosis. This can present with malaise, muscle pains, breathing difficulty and abdominal pain. We will monitor you for any signs of this very rare complication. We will provide you with a copy of the product information leaflet if you would like more information.

Will I receive the placebo treatment or the metformin treatment?

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

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

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

Will my information be kept confidential?

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

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

Only half of the women in this trial will be given metformin. If you do receive this treatment you may or may not benefit from the effects of this drug and you may or may not have improvement in your pre-eclampsia. There are no other direct benefits for you being

involved in this study. By being involved you may help us find a treatment for pre-eclampsia which could help many pregnant mothers in your situation in the future.

What are the disadvantages of being in the study?

You will need to take extra tablets and we may need to take a few extra samples of your blood for testing.

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

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

What will happen with the results of this research project?

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

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

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

Who has paid for this study?

This study has been funded by grants from the University of Melbourne. Dr Cluver's salary has been paid for by grants from the Discovery Foundation and the South African Medical Association.

Who do I contact if I have a problem?

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

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

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

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

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

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

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

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

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

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

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

Thank you for taking the time to read this information leaflet about the PI2 trial.

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

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

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

INFORMED CONSENT FORM

PRINCIPAL INVESTIGATOR: Dr Catherine Anne Cluver

RESEARCH MIDWIFE: Name: ……
Contact number: ……
Email: …….
ADDRESS: Department of Obstetrics and Gynaecology
Tygerberg Hospital and University of Stellenbosch

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

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

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

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

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

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

Declaration by participant

By signing below, I …….. agree to take part in a research study entitled: Pre-eclampsia Intervention 2 (PI2) trial: a double blind randomised, controlled trial of metformin to treat early onset preeclampsia.

I declare that:

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

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

…… ……

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* …… declare that:

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

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

…… ……

Signature of investigator **Signature of witness**

Declaration by interpreter

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

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

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

....

Signature of interpreter **Signature of witness**

Statistical Analysis Plan (SAP)
Pre-eclampsia Intervention 2 trial (PI2)

Contents

Section A. Administrative

1. Title:

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

2. Trial registration:

Pan African Clinical Trials Registry ID: PACTR201608001752102

3. SAP version:

Version: 1.0 Date: 4th January 2019

4. Protocol Version:

This document has been written based on information contained in the study protocol version 1.1 dated 19th February 2018.

5. SAP Revisions

Revision history, with justification and timing: Not applicable

6. Roles and Responsibility:

Document prepared by Dr Richard Hiscock, Mercy Hospital for Women, University of Melbourne and Dr Cathy Cluver, Stellenbosch University.
Email: richardjhiscock@gmail.com, cathycluver@hotmail.com

Signatures:

Signature of senior statistician responsible *Date 10 January 2018*

Signature of chief investigator/clinical lead *Date: 20 December 2018*

Section B: Introduction and Objectives

7. Synopsis of trial background and rationale:

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: (i) inhibits hypoxic inducible factor 1α (HIF 1α); (ii) reduces sFlt-1 and sEng secretion from primary endothelial cells and placental tissue; (iii) reduces VCAM-1 expression on endothelial cells; and (iv) 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 fewer 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 (excerpted from trial protocol p 11).

8. Research hypothesis and objectives:

Research hypothesis:
In women with preterm pre-eclampsia undergoing expectant management, a daily dose of 3 grams of metformin prolongs gestation by at least 5 days.

Study objectives:
Primary objective:

- Examine whether up to 3 grams of metformin daily can safely prolong gestation for 5 days in women with early onset pre-eclampsia diagnosed between 26+0 to 31+6 weeks gestation, compared to standard of care, expectant management alone. This is set in a superiority framework with two-sided hypothesis regions for all statistical testing.

Secondary objectives:

- Determine whether metformin improves maternal and neonatal composite outcomes in early onset pre-eclampsia compared to placebo.

Section C: Trial Methods

9. Trial design: description of trial design

This is a single center (hospital based) phase II parallel group placebo-controlled, double-blind randomized control trial. The allocation ratio is 1:1. Mothers are randomized to receive either metformin or placebo tablets.

10. Randomization and allocation concealment:

Randomisation will be performed in an equal ratio of metformin to placebo, stratified into two strata by gestational age using blocks of 4 to 6 with the size and order randomly assigned within each stratum. Stratification details are:

	Gestational age (GA)	EFW if GA is unknown
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

An online, web-based sequence generator system will be used. This system will be managed by the South Australian Health and Medical Research Institute (SAHMRI) (https://www.sahmri.org) and is a REDCap based system. Codes will be generated for each packet of placebo and treatment tablets. The treatment tablets and placebo will be manufactured by Merck Pharmaceuticals. The trial medication will be packed and labelled by the trial pharmacist. Once randomized, the treatment pack with the same code will be allocated to the participant. The randomization list and matching treatment code will be stored by the pharmacy and by SAHMRI and only released to the researchers once the data has been finalized and locked for editing (including neonatal follow-up) or to the DMSC (closed sessions) upon request. The researchers, clinicians and participants will remain blinded until completion of the trial.

11. Sample size

Sample size calculation is determined by the primary research question: does treatment with metformin result in a gain in the length of gestation of greater than or equal to 5 days compared to the mother receiving placebo therapy. For each mother her gestational age at diagnosis (trial enrolment) sets the maximum allowable length of pregnancy prolongation. For those with PE 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 setting a ceiling for maximal prolongation of 15 days. Therefore, the maximum potential gestation prolongation is 63 days for stratum 1 and 42 days for stratum 2 mothers. The primary outcome will be the median prolongation of gestation, reflecting the skewed distribution of the gestation prolongation seen in published clinical trials.[1] The researchers believe that median prolongation a more clinically meaningful measure of change than either raw mean or ratio change. Sample size calculations were based upon the Geometric Mean Ratio (GMR), an appropriate measure for differences in median. Analysis was performed both using PASS 13 (Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA, 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).

Power calculations settings were: 90% power, with two-sided alpha set at 0.05, median prolongation of 8.3 days in the control arm and a clinically important gestation prolongation of 5 days. The measure of variability used in this analysis is the coefficient of variation (CV) in mothers treated with standard expectant

[1] Hall DR, Odendaal HJ, Kirsten GF, et al. Expectant management of early onset, severe pre-eclampsia:

management (control group). Two studies were used provided estimates for the expected gestational prolongation and associated CV in pre-eclamptic mothers receiving expectant management after PE diagnosis (control group) on two studies [2]. The first by Hall et al reported a mean gestation prolongation of 11 (SD 7) days equivalent to a CV = 0.64, whilst the second by Cluver et al (PIE 1 study) reported an overall mean prolongation of 13.1 (SD 12.2) days in the placebo arm, a CV = 0.93. In the PIE 1 study mothers entering at a gestation greater than 29 weeks had a CV = 0.8. We have chosen to use a CV = 1.0, slightly larger than the 0.93 found in the overall PIE study, to maintain adequate power if slightly greater variability in prolongation times occurs. The results of sample size estimates for a limited range of GMR and two CVs are presented in the following table:

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	**65**	61	49

*placebo group median = 8.32 days

Therefore, at analysis we require 65 patients per trial arm. Allowing for 10 dropouts within each arm we plan to recruit 150 patients (75 patients in the placebo & 75 in the treatment arms). The dropouts, including patient requested trial withdrawal and delivery before any trial medication was given occurred in 6/119 (5.0%) of mothers.

Whilst presentation of results will include Kaplan-Meier survivorship curves and adjusted hazard ratios for a Cox proportional hazards model the following power analysis based upon expected events indicates that this study is not adequately powered to use survivorship as the primary endpoint. In the setting of this trial the Hazard Ratio (HR) defined as the time for control/time for Rx at the same proportion of survival. For example, taking the median survival time of 9 days in the control group from the Hall study we can assess the HR detected for the predetermined group size:

Time above median (days)	Median time in Rx group (days)	HR	Group size (equal group size)
3	12	0.75	360
4	13	0.69	225
5	14	0.64	158
6	15	0.60	120
7	16	0.56	96
8	17	0.53	80
9	**18**	**0.50**	**68**
13.5	22.5	0.40	42

[2] Cluver CA, Hannan NJ, van Papendorp E, et al. Esomeprazole to treat women with preterm preeclampsia: a randomized placebo-controlled trial. Am J Obstet Gynecol 2018;219:388.e1-17

Given the sample size of 65 per arm this study has a power of 0.9 to detect a difference of 9 days which translates to an ability to detect only a Hazard Ratio ~ 0.5 or smaller.

All secondary outcomes are binary with expected incidence less than 0.05. At the projected sample size of 65 the power to detect a 50% change in incidence is low at 0.12.

12. Framework

This trial uses a superiority hypothesis testing framework between treatment groups for all outcomes.

13. Statistical Interim analyses and stopping guidance

This is a phase II trial with mothers under direct in hospital observation which facilitates immediate detection and management of adverse events. For the planned sample size only a very large treatment effect size would lead to an indication to cease the trial for efficacy at information fraction of 60 – 80%. Given the phase II nature of the study, the complete observation of mothers whilst under trial treatments, there is no planned interim analysis for efficacy. There is no planned interim assessment for futility. The DMC will monitor for adverse events and will provide stopping guidance should this be deemed necessary. See separate DMC charter (PI2 DMSC Charter version 16 June 2018).

14. Timing of final analysis

Unblinding and final analysis will be performed only after all participants have being delivered and all neonatal follow-up has been completed. Neonatal death is one of the outcomes within the prespecified composite neonatal outcome (see protocol p 38 – 39) and is assessed at six weeks post-delivery. Therefore, analysis is will not commence prior to six weeks after the delivery of the last recruited trial patient. Publication of biomarker results may be published separately from the clinical trial outcomes.

15. Timing of outcome assessments.

The primary outcome is measured from time of randomization to delivery. Secondary maternal and neonatal composite outcomes are measured from time of recruitment until six weeks after the due date, with timing dependent upon outcome measures.

Section D: Statistical Principles

16. Confidence intervals and p-value

The significance level is set at 0.05 and all hypothesis testing will be two-sided. A single primary outcome is tested at a significance level of 0.05. If and only if a significant difference is found will the secondary outcomes be tested. The two secondary outcomes will each be tested at a significance level of 0.025.

17. Description of any planned adjustment for multiplicity, and if so, including how the type 1 error is to be controlled

No adjustment for multiplicity will be performed**.** The strategy for outcome testing is pre-specified and maintains the overall trial error rate at 0.05.

18. Confidence intervals (CI) to be reported

Standard 95% confidence intervals will be used to present analysis of the primary adjusted outcome. For secondary outcomes it is planned to the adjusted width of CI used.[3]

19. Adherence and Protocol Deviations

Adherence:
Trial medication will be administered to mothers on an inpatient basis. Measures to monitor adherence are: 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. The dosing schedule details a programmed escalation of dose in the of side effects. We will use two measures of dosing in the metformin arm: (i) average daily dose of tablets = number of ingested medications/days of therapy and (ii) percentage of maximum dose = ingested medications/ total maximum medications.

Description of how adherence to the intervention will be presented:
Adherence will be presented on the two measures detailed above, using descriptive statistics (N, mean, SD, median, minimum, maximum) converted to metformin dose for the intervention arm.

Definition of protocol deviation for the trial and description in trial results:
Protocol deviations due to errors in applying inclusion/exclusion criteria, the wrong intervention being administered will be documented in trial results either in patient flow diagram or text. The primary outcome will not be subject to ascertainment error due to its unambiguous nature. Prior to unblinding of treatment all protocol deviations will be assessed, and determination made about inclusion of these participants within the trial analysis populations. Determinations will be presented in the research results in the patient flow diagram and possibly in the text.

[3] Ludbrook J. Multiple inferences using confidence intervals. Clin Exp Pharmacol Physiol 2000;27:212 - 215.

19. Analysis populations.

The intention-to-treat population will include all randomized patients, regardless of their eligibility, according to the treatment they were randomized to receive. Primary outcome will also be tested on trial arms based upon treatment received as a sensitivity analysis.

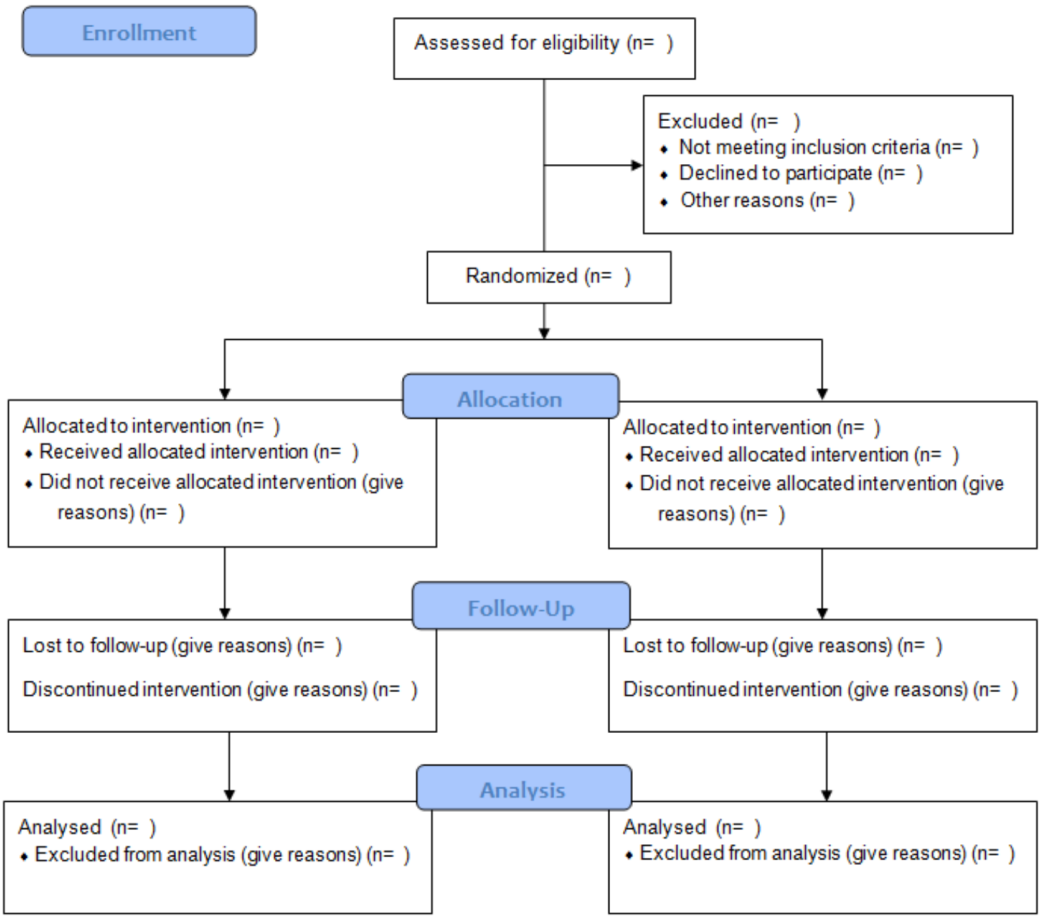
Section E: Trial Population

20. Screening Data

Screening data will be collected and reported. The name of patients assessed for eligibility and the reason for not enrolling will be recorded and reported. The trial inclusion and exclusion criteria are specified in the protocol. A CONSORT diagram will be used to summarize and present this data.

21. CONSORT flow diagram template for the PI2 trial

The following CONSORT flow diagram will be used to detail enrollment, randomization, treatment allocation, reasons for not receiving treatment, follow up and analysis



22. Baseline characteristics

List of Baseline characteristic to be summarized by treatment groups and presented in tables:

- Gestational age at randomization
- Maternal age in years
- BMI
- Ethnicity (black, colored, other)

- Smoking
- Aspirin usage
- Calcium usage
- HIV status
- Chronic hypertension
- Parity (Nulliparity; Multiparity +/- hypertension in previous pregnancy)
- New paternal parity in current pregnancy
- Pre-randomization highest systolic and diastolic blood pressure
- 24 hour Creatinine ratio
- Haemoglobin, platelet count, urea and creatinine before randomization
- Estimated fetal weight on ultrasound
- Presence of absent flow on umbilical artery doppler.

Details of how baseline characteristics will be descriptively summarized:

Categorical data will be summarized by numbers and percentages. Continuous data will be summarized by mean, SD and range if data are normal and median, IQR and range if data are skewed. Minimum and maximum values will also be presented for continuous data. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

Section F: Analysis

23. Outcome definitions

Primary outcome definition:

The primary outcome is time from randomization until delivery, measured in hours and presented in days. The summary of interest is median prolongation and data will not be transformed prior to analysis. If a fetus does demise during expectant management, we will give it a length of pregnancy prolongation of zero and will include it in the primary analysis.

Composite secondary outcome definitions:

(i) Maternal composite outcome: the occurrence of any of the following serious maternal outcomes: maternal death, eclampsia, pulmonary oedema (oxygen saturation ≤90%, with clinical symptoms requiring treatment), severe renal impairment or the need for dialysis, a cerebral vascular event, placental abruption and liver haematoma or rupture.

(ii) Neonatal composite outcome: the occurrence of any of the following serious neonatal outcomes: neonatal death within 6 weeks after the expected due date, grade III or IV intraventricular haemorrhage, necrotizing enterocolitis and bronchopulmonary dysplasia

Exploratory outcomes:

All other outcomes, including outcomes nested within individual secondary outcomes or outcomes with less clinical interest than secondary outcomes are classified as exploratory outcomes. These will not be subject to hypothesis testing, but precision of point estimates and may be presented using unadjusted 95%CI.

24. Statistical Analysis methods

Raw data primary outcome:
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 intertest 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 associated with 95%CI for all testable outcomes.

Primary outcome:
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% confidence intervals (95%CI).

Secondary outcomes:
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**.**

Adjustment for covariates for each primary and secondary outcomes:
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.

Secondary outcomes: Composite binary outcomes will be modelled using a logistic regression model containing both treatment group and gestation aged based strata.

Methods used for assumptions to be checked for statistical methods:
Quantile regression makes no distributional assumptions (assessed using residuals) apart from assuming the outcome is continuous. Secondary outcomes tested using exact logistic regression or Fisher's exact test make no distributional assumptions.

Sensitivity analyses:
Sensitivity analysis will be conducted on the primary outcome are:
(i) Treatment received Analysis will be adjusted by median quantile regression as already specified for the primary outcome intention to treat analysis. Treatment received, given varying dosage schedule is defined as an average daily dose.
(ii) Gestational age as an effect modifier. An interaction between treatment and gestational age strata will be using the same model as the primary outcome. Given study size it is acknowledged that this has low power to detect an interaction effect.
(iii) Covariate adjusted. The trial investigators consider that a mother with absent UAD flow, an estimated fetal weight less than the 10th centile and an estimated fetal weight less than the 3rd centile are more likely to

be associated with shorter randomization to delivery times. Covariate adjustment for these factors may increase the precision of the primary outcome measure if there is a correlation between them and delivery time.
(iv) 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 pretreatment 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.

Subgroup analyses
Gestational age at randomization has been incorporated in the prespecified primary outcome analysis. No other subgroup analyses are planned.

25. Missing data reporting and assumptions/statistical methods to handle missing data

In this study primary outcome will not have missing data, both gestational age at randomization and treatment are predetermined and all mothers will deliver in the acute in hospital setting. Based upon our experience in the PIE 1 trial (at the same institution) we expect no of missing covariate values for the three components required for analysis of the POM – group assignment, gestational age-based strata and time to delivery (delivery time – admission time). Imputation will not be performed. As described above, if an FDIU occurs it will be allocated a time of zero prolongation.

Secondary outcomes that are pre-specified for hypothesis testing use the same pre-randomization covariates and all outcomes are identifiable. If 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.

26. Additional Analyses

For the primary outcome a prespecified analysis assessing effect of absent uterine artery flow on outcome will be performed by a covariate adjusted analysis that includes treatment group, the gestational age design-based strata, fetal growth restriction (defined as an estimated fetal weight less than the 10th centile and less than the 3rd centile and absent flow on umbilical artery Doppler (UAD) examination measured at the time of randomization. The trial investigators consider that a mother with fetal growth restriction or absent UAD flow was more likely to be associated with shorter randomization to delivery times. This pre-specified covariate adjustment will increase the precision of the primary outcome measure if there is a correlation.

27. Harms

The number (and percentage) of patients experiencing serious adverse events (SAE) will be presented for each treatment arm. These are: maternal death, fetal death, event resulting threat to life of mother or baby, event that causes prolonged hospital stay, event resulting in significant disability to mother and congenital birth defect not previously detected on ultrasound. All will be listed by trial arm in

supplementary trial results and no formal statistical testing will be undertaken. Note that for maternal and neonatal composite secondary outcomes and non-composite 'pre-specified testable' secondary outcomes results will be reported with the primary outcome.

28. Statistical Software

Statistical software used will be Stata v 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) or R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Packages used will be detailed.

29. References

All methods used are standard.

30. Reference to Data Management Plan

Details on data handling and cleaning is specified in the PI2 Protocol.

31. Reference to the Trial Master File and Statistical Master File

The Trial Master File is kept and updated by the Principle Investigator (Dr C Cluver).

32. Reference to other Standard Operating Procedures or documents

Not applicable

Draft Tables for PI2 Trial

Table 1: Characteristics of trial participants at enrolment
Table 2: Primary and secondary outcomes
Table 3: Severe adverse events
Table 4 : Side effects from the medication

Table 1: Characteristics of Trial Participants at enrolment			
Characteristics		**Metformin (n=)**	**Placebo (n=)**
Gestation at randomisation	Median [IQR]		
	Mean (SD)		
Gestation < 29 weeks at randomisation	n (% of each group)		
Maternal age in years	Median [IQR]		
Body mass index	Median [IQR]		
Race or ethnicity: Black	n (%)		
Coloured	n (%)		
Smoking	n (%)		
Aspirin use n (%)	n (%)		
Calcium use n (%)	n (%)		
HIV positive n (%)	n (%)		
Chronic hypertension n (%)	n (%)		
Nulliparous	n (%)		
Multiparous without hypertension in a previous pregnancy	n (%)		
Multiparous with hypertension in a previous pregnancy	n (%)		
New paternity in current pregnancy	n (%)		
Highest systolic blood pressure before randomisation (mm Hg)	Mean (SD)		
Highest diastolic blood pressure before randomisation (mm Hg)	Mean (SD)		
24-hour protein creatinine ratio at enrolment (g/24 hours)	Median [IQR]		

Table 2: Primary and Secondary outcomes				
		Metformin (n=)	Placebo (n=60)	P value
PRIMARY OUTCOME				
Prolongation of gestation (days)	Median [IQR]			
	Mean (SD)			
SECONDARY OUTCOMES				
Composite maternal outcome	n (%)			
Composite neonatal outcome	n (%)			
Haemoglobin (g/dl)	Mean (SD)			
Platelet count ($(10^9$/L)	Mean (SD)			
Urea (mmol/L)	Mean (SD)			
Creatinine (mg/dL)	Mean (SD)			
Estimated Fetal weight (g)	Mean (SD)			
Fetal weight centile	Median [IQR]			
Absent flow on umbilical artery Doppler	n (%)			

Table 3: Severe adverse events (presented as number with percentages)		
	Metformin (n=)	Placebo (n=)
MATERNAL		
Maternal death		
Eclampsia		
Cerebral vascular event		
Posterior reversible encephalopathy syndrome		
Left ventricular failure		
Pulmonary oedema		
Severe renal impairment		
Blood loss of more than 1000mls		
Disseminated intravascular coagulation		
Liver haematoma or rupture		
FETAL/NEONATAL		

Intrauterine demise		
Fetal or neonatal congenital anomaly		
Neonatal death		
Necrotising enterocolitis		
Neonatal sepsis		
Intracranial haemorrhage		

Table 4: Side effects from medication (presented as number with percentages)		
	Metformin (n=)	Placebo (n=)
Nausea		
Vomiting		
Diarrhoea		
Headache		
Decreased tablets		
Stopped medication		

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number of the PI2 Clinical Trial Protocol
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2-4
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	62
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	4-5
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	15-16
	6b	Explanation for choice of comparators	16-26
Objectives	7	Specific objectives or hypotheses	27-28

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	29
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	30
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	33-37
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	42-47
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	44
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	43-44
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	44-45
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	38-40
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	42-45
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	29-31
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	29
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	31
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	31
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	31-32
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	31-32
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	52
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	45, 47
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	48
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	48-50
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	PI2 Statistical Analysis Plan Version 1.0

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	PI2 Statistical Analysis Plan Version 1.0
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	4-5, 55
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	52
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	51-52
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	55
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	55-57
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	42
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	57
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	N/A

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	57 No contractual agreements that limit access
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	61
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See Informed consent form and patient information leaflet
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	45-47

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

BMJ Open

A double blind, randomised, placebo-controlled trial to evaluate the efficacy of metformin to treat preterm pre-eclampsia (PI2 Trial): study protocol

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Complete List of Authors:	Cluver, Catherine; Stellenbosch University Faculty of Science, Obstetrics and Gynaecology; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Translational Obstetrics Group Walker, Susan; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Translational Obstetrics Group; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Obstetrics and Gynaecology Mol, Ben; School of Medicine, Monash University, Melbourne, Australia, Obstetrics and Gynaecology Hall, David; Stellenbosch University Faculty of Science, Obstetrics and Gynaecology Hiscock, Richard; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Anaesthetics Brownfoot, Fiona; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia., Translational Obstetrics Group; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Obstetrics and Gynaecology Kaitu'u-Lino, Tu'uhevaha; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia., Translational Obstetrics Group and Department of Obstetrics and Gynaecology; University of Melbourne, Obstetrics and Gynaecology Tong, Stephen; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Translational Obstetrics Group; University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia, Obstetrics and Gynaecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	pre-eclampsia, metformin, preterm preeclampsia, treatment, Clinical trials < THERAPEUTICS, preeclampsia

A double blind, randomised, placebo-controlled trial to evaluate the efficacy of metformin to treat preterm pre-eclampsia (PI2 Trial): study protocol

Authors:

Catherine A Cluver,[1,2] Susan P Walker,[3] Ben W Mol,[4] David R Hall,[1] Richard Hiscock,[5] Fiona C Brownfoot,[2,3] Tu'uhevaha J Kaitu'u-Lino,[2,3] Stephen Tong[2,3]

Author Affiliations:

[1] Department of Obstetrics and Gynaecology, Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa.

[2] Translational Obstetrics Group, University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia.

[3] Department of Obstetrics and Gynaecology, University of Melbourne, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia.

[4] Department of Obstetrics and Gynaecology, Monash School of Medicine, Monash University, Melbourne, Victoria, Australia.

[5] Department of Anaesthetics, University of Melbourne, Mercy Hospital for Women, Victoria 3084, Australia.

Corresponding author:

Dr Catherine Anne Cluver

Department of Obstetrics and Gynaecology, Stellenbosch University, Tygerberg Hospital

Francie van Zyl Drive, Tygerberg 7505, South Africa

Email: cathycluver@hotmail.com

Phone: +27823210298

ABSTRACT

Introduction

Pre-eclampsia is a major complication of pregnancy, globally responsible for 60,000 maternal deaths per year, and far more fetal losses. There is no definitive treatment other than delivery. A therapeutic that could quench the disease process would be useful to treat preterm pre-eclampsia, as it could allow these pregnancies to safely continue to a gestation where fetal outcomes are significantly improved. We have published preclinical data to show that metformin, a drug known to be safe in pregnancy and commonly used to treat gestational diabetes, has potent biological effects making it another promising candidate to treat pre-eclampsia. Here, we describe a phase II clinical trial to examine whether administering extended release metformin may be effective in treating women with preterm pre-eclampsia (PI2 Trial).

Methods

The PI2 Trial is a phase II, double blind, randomised controlled trial that aims to recruit 150 women with preterm pre-eclampsia (gestational age 26+0 to 31+6 weeks) who are being managed expectantly. Participants will be randomised to receive either 3 grams metformin or placebo daily. The primary outcome is time from randomisation till delivery. A delay in delivery of 5 days is assumed to be clinically relevant. The secondary outcomes will be a maternal composite and neonatal composite outcome. All other outcomes will be exploratory. We will record adverse events.

Ethics and dissemination

This study has ethical approval (Protocol number M16/09/037 Federal Wide Assurance Number 00001372, Institutional Review Board Number IRB0005239), is registered with the Pan African Clinical Trial Registry (PACTR201608001752102) and the South African Medicine Control

Council (20170322). Data will be presented at international conferences and published in peer-reviewed journals.

ARTICLE SUMMARY

Article focus

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

Key Messages

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

Strengths and limitation of this study

- This is a protocol for a randomised, double blind, placebo controlled clinical trial.
- This is the first trial to assess whether metformin is a treatment option for pre-eclampsia.
- We plan to recruit 150 participants which provides sufficient power for our primary outcome (time from randomisation to delivery).
- Our trial may be underpowered to show improvements in maternal and perinatal outcomes.
- If the trial yields a positive result, larger multi-centre studies will be needed.

INTRODUCTION

Pre-eclampsia is one of the most serious complications of pregnancy affecting 5% of pregnancies worldwide.[1] It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit.[2] 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.[3] Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[4] and it is estimated that for every pre-eclampsia related death there are 50-100 other women who experience significant morbidity associated with pre-eclampsia.[5]

At present, the only treatment available for pre-eclampsia is termination/delivery of the pregnancy. This poses a difficult clinical dilemma in the setting of preterm pre-eclampsia. Clinicians are often forced to deliver early to prevent disease progression and major maternal morbidity (ie severe maternal organ injury), but in doing so, inflict severe prematurity on the fetus. In particular, fetuses delivered at less than 33 weeks' gestation are at significant risk of severe disability including cerebral palsy, stroke (intracerebral bleeding), retinopathy of prematurity, chronic lung disease and death.[6]

We have recently completed a phase II, double blind, randomised controlled trial, the Pre-eclampsia Intervention with Esomeprazole (PIE) trial, to evaluate whether 40 mg of esomeprazole was a possible treatment for early onset pre-eclampsia. This trial showed no between-group differences in median time from randomization to delivery (11·4 days in the esomeprazole group, and 8·3 days in the placebo group [95% confidence interval -2·9-8·8, P=0·31]).[7,8] Here, we propose metformin as the next therapeutic candidate to test in a clinical trial for preterm pre-eclampsia.

Metformin is a promising therapeutic candidate as it has been shown to reduce soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) secretion from endothelial cells and primary trophoblasts.[9] These key anti-angiogenic molecules released from the placenta are responsible for the widespread endothelial dysfunction and impaired vascular relaxation observed in pre-eclampsia. Reduced angiogenesis is also thought to contribute to placental hypoxia and to the development of pre-eclampsia. Metformin has also been shown to improve endothelial dysfunction, improve blood vessel relaxation and promote angiogenesis.[9] Given these multiple potential effects, metformin is a particularly promising therapeutic candidate.

OBJECTIVES

The primary objective is to examine whether 3 grams of metformin XR can safely prolong gestation among women with preterm pre-eclampsia who are being managed expectantly, compared to expectant management alone. A prolongation of 5 or more days will be considered clinically significant.

The secondary objectives are to examine whether metformin can improve maternal and neonatal composite outcomes.

METHODS

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

Study design

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

Study population

Pregnant women diagnosed with pre-eclampsia at a gestational age between 26+0 weeks to 31+6 weeks at Tygerberg hospital (Western Cape Provence of South Africa) will be invited to participate. To be eligible for this study the treating clinicians need to have made an initial assessment and deemed that the patient is suitable for expectant management and delivery is not immediately required or anticipated in the next 48 hours.

Inclusion criteria:

We will recruit women with a singleton pregnancy diagnosed with pre-eclampsia and chronic hypertension with superimposed preeclampsia defined according to the criteria published by The International Society for the Study of Hypertension In Pregnancy (ISSHP),[10] and require the presence of significant proteinuria (more than 300 milligrams in a 24 hour urine collection). Some women only present for antenatal care after 20 weeks gestation. We will include these women and classify these women as unclassified proteinuric hypertension during the pregnancy according to the classification of Davey and MacGillvray.[11] Postpartum, these women will be reclassified as having had pre-eclampsia or chronic hypertension based on their blood pressure and need for medication more than 6 week after the due date.

Gestational age will be determined by either period dates (if the woman is certain of her last menstrual period) or by an early, or mid trimester pregnancy ultrasound. If the gestational age is uncertain, we will recruit participants with an estimated fetal weight between 500 and 1800 grams, determined by ultrasound performed at presentation. A full list of inclusion criteria is provided in Table 1.

Exclusion criteria:

Exclusion criteria include women with established maternal or fetal compromise that necessitates delivery within 48 hours, the current use of metformin, contraindications to the use

of metformin or the use of medications that interact with metformin. A full list of exclusion criteria is provided in Table 2.

Participant enrolment

Participants will be identified after they have been admitted to the tertiary referral centre with a diagnosis of preterm pre-eclampsia for expectant management. An information leaflet will be given to all potential participants and written informed consent will be obtained (Supplementary information 2 and 3).

Randomisation and allocation concealment

After informed consent, participants will be randomised to metformin or placebo in a 1:1 ratio. An online, web-based sequence generator system linked with codes for placebo and treatment tablets will be used. Both researchers and participants will be blinded.

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

Once the participants have been randomised the treatment pack with the same code will be allocated to the participant. All treatment packs will be identical and will contain either active

tablets or placebo. The researchers will have no access to the randomisation list. This process will ensure that there is allocation concealment throughout the conduct of the trial.

Each participant will be given an individual treatment pack containing either 500mg metformin XR or identical placebo tablets which have been produced by Merck (http://www.merck.com/index.html). Labelling, storage and preparation will be done according to the requirements of the Medicines for Human Use (Clinical Trials) regulations. Participants will be started on six tablets daily in divided doses. If side effects develop the dose will be decreased until the side effects improve and then increased again if tolerated.

Co-interventions

Participants will remain under the care of the hospital treating team and the study will not alter or interfere with the routine care provided for women with preterm pre-eclampsia, including the decision for delivery. Management for preterm pre-eclampsia involves admission to hospital 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 fetal 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 for the neonate. A single repeat dose will be given one week later as per hospital protocol.[12] Most participants will be on antihypertensive treatment, and the dose and number of

antihypertensives will be recorded. All women should already be receiving calcium, iron and folic acid supplementation.

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

Sample collection

Blood samples will be routinely collected twice a week. The routine blood samples include measurements of the haemoglobin, the platelet count and the urea and creatinine levels. These will be used by the managing clinicians to determine disease severity and may trigger delivery.

Outcomes

The primary outcome is to examine whether up to 3 grams of metformin daily can safely prolong gestation in women with preterm pre-eclampsia diagnosed between 26+0 to 31+6 weeks gestation, compared to standard of care, expectant management alone. This is set in a superiority framework with two-sided hypothesis regions for all statistical testing. A prolongation of 5 days or longer will be considered clinically significant.

The secondary outcome is to determine whether metformin improves maternal composite and neonatal composite outcomes. The composite maternal outcome includes maternal death, eclampsia, pulmonary oedema, severe renal impairment or the need for dialysis, a cerebral vascular event, the development of a liver haematoma or rupture and placental abruption. The composite neonatal outcome will include neonatal death within 6 weeks after the due date, grade III or IV intraventricular haemorrhage defined enlarged ventricles associated with

haemorrhage or haemorrhage extending into the cerebral tissue around the ventricles on imaging, necrotizing enterocolitis defined on radiographic studies or bronchopulmonary dysplasia defined as needing oxygen at day 28 of life, either on a ventilator, by CPAP or via a nasal catheter. See Table 3.

All other outcomes, including outcomes nested within the composite secondary outcomes will be classified as exploratory outcomes. These are listed in Table 4.

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 withdrawal, we will seek the patient's permission to use their clinical information and laboratory samples collected thus far in subsequent analyses.

Duration of the trial

It is anticipated that the study will be completed in approximately 3 years (2018 – 2020). In our previous PIE study, we included 120 women in 15 months.[8]

Confidentiality

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

Data management and statistical analysis

Data will be collected prospectively using a REDCap database.[13] Data entry and checking will be continuous, and queries will be addressed contemporaneously to ensure clarification without delay. All data will be double checked for accuracy.

The patient characteristics, by treatment group at randomisation, will be presented as mean (SD), median [25th – 75th percentile], minimum, maximum and count (%) depending upon type and distribution.

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 intertest 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). The trial presentation will meet the CONSORT statement requirements (http://www.consort-statement.org/).

Sample size and stratification according to gestation at recruitment

The primary research question is whether metformin will result in a gain in the median length of gestation compared to placebo therapy. A prolongation of more than 5 days will be considered clinically significant. 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) and the Powercal program running in Stata v15 (Stata statistical software. 2017 Release 15. Stata Corp. College Station, TX: StataCorp LLC).[14]

We have two sources for data on the prolongation of gestation in preeclamptic mothers receiving expectant management which were both performed at the same hospital where we plan to run this trial (Tygerberg Hospital). Hall et al, in the largest descriptive study on expectant management of preterm pre-eclampsia, reported a mean gestation prolongation of 11 (SD 7) days equivalent to a Coefficient of Variation (CV) = 0.64,[15] and the PIE trial[8], where patients randomized to the placebo arm had an overall mean prolongation of 13.1 (SD 12.2) days, a CV = 0.93.[8] In this study for mothers entering at gestations greater than 29 weeks, the CV = 0.8. We have therefore 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.

For 90% power, with a two-sided alpha set at 0.05, control median prolongation of 8.3 days and prolongation of gestation of 5 days with the measure of variability used in this analysis being the CV = (data SD)/ data Mean) we require 65 participants per group. Allowing for 10 dropouts per group we require 75 participants per arm and therefore a total of 150 participants.

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. 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. 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. Stratum 1 will include pregnancies of a gestational age of 26 weeks 0 days up to and including 28 weeks and 6 days. Stratum 2 will include pregnancies from 29 weeks and 0 days up to and including 31 weeks and 6 days.

More detail is available in the trial statistical analysis plan (Supplementary Information 4).

Adverse events

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

Unblinding

Given that the safety profile of metformin in pregnancy is well established, we anticipate the need for unblinding to be unlikely. However, we will have the following procedures in place should unblinding be required. Sheets with lists of 25 randomisation codes each will be sealed in individual, signed, numbered envelopes. If unblinding is requested, the relevant envelope will

be opened by a person not involved in the trial in the presence of two witnesses, the group allocation read, and the list re-sealed in an envelope and signed. The randomisation envelopes will be accessible at all times to the principal investigator who will be contactable by mobile telephone. The principal investigator will co-ordinate this process.

Early termination of the trial

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

Patient and public involvement

Patients and the public were not involved in the development of the research question, the design of the study, the conduct or recruitment and the burden of the intervention was not assessed by the patients or the public. The results of the study will be emailed to the participants

ETHICS AND DISSEMINATION

This study has ethical approval (Stellenbosch University HREC Protocol number M16/09/037 Federal Wide Assurance Number 00001372, Institutional Review Board Number IRB0005239) and is registered with the Pan African Clinical Trial Registry (Application ID PACTR201608001752102) and the South African Medicine Control Council (20170322). Data will be presented at international conferences and published in peer-reviewed journals.

DISCUSSION

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

Recently a number of clinical trials have been proposed for assessing novel treatments for pre-eclampsia but only a few have been completed. Our group has recently completed the PIE trial assessing esomeprazole to treat preterm pre-eclampsia.[8] In this trial a daily dose of 40mg esomeprazole did not prolong gestation or have any effect on biomarkers associated with pre-eclampsia. Sildenafil was assessed in a single site, double blind, randomised controlled trial in Brazil.[16] Over a 28-month period, 100 women were recruited, and results were available for 93 women. There was a significant prolongation of gestation in the sildenafil group of 4 days but given that sildenafil is a vasodilator it is possible that this prolongation in gestation may have occurred because the drug decreased blood pressure and mitigated a clinical reason to deliver, rather than temporising disease progression. Antithrombin was assessed to treat preterm pre-eclampsia in the PRESERVE-1 trial which enrolled 120 women from 23 tertiary hospitals over 28 months (ISRCTN23410175).[17] There was no difference in prolongation of pregnancy or composite neonatal outcomes.[18] Trials assessing serelaxin (NCT01566630), pravastatin, high doses of antithrombin,[19] and celecoxib (NCT00442676) have been attempted but all were terminated due to poor recruitment.

It is known that the pre-eclamptic placenta releases anti-angiogenic sFlt-1 and sEng into the maternal circulation, causing widespread maternal endothelial dysfunction and organ injury.[20] A drug that can decrease sFlt-1 and sEng production and decrease endothelial dysfunction may be a potential treatment for pre-eclampsia. We have generated preclinical data suggesting metformin may have such actions.[9] Based on this preclinical data, we are now proposing to undertake this phase II randomized clinical trial.

We propose recruiting 150 women at Tygerberg Hospital, in the Western Cape Provence of South Africa. There are advantages to running the trial at this site. South Africa has a very high incidence of pre-eclampsia. Tygerberg Hospital is a tertiary referral centre with a neonatal intensive care unit, a maternal critical care unit and an adult intensive care unit. It is an academic centre that actively contributes to the global scientific literature and our team has recently completed the PIE trial at this research unit. We will make use of the sytems set up by this trial. Thus, we believe running this trial at Tygerberg Hospital represents a balance between obtaining sufficient number of cases of preterm pre-eclampsia, performing the trial in a cost-effective manner and offering modern obstetric and perinatal care, making the results potentially generalizable to both developed and developing countries.

Metformin is known to be safe in pregnancy and is considered a category B drug in pregnancy (by the Food and Drug Administration (FDA)).[21] There are numerous studies of metformin for the treatment of diabetes and polycystic ovarian syndrome in pregnancy which have shown no adverse fetal effects when used in the first, second and third trimesters.[22–27] Metformin does cross the placental barrier and it's long term effect on fetal development is unknown but a recent ACOG practice bulletin has concluded that it can reasonably be used in pregnancy.[28]

In strong support of our preclinical data are meta-analyses of clinical trial data that have shown a decrease in the incidence of hypertensive disorders when metformin has been used for other

indications.[29] Excitingly, a randomised trial by the Fetal Medicine Foundation (for obese women without diabetes) reported that metformin significantly reduced the incidence of pre-eclampsia by as much as 76%.[30] The EMPOWaR trial also evaluated maternal and fetal effects of metformin in obese women.[31] While reporting no decrease in the incidence of hypertensive disorders of pregnancy with metformin, the most likely reason for this difference was poor compliance.[32]

We have powered our study for the primary outcome, which is to show whether metformin can safely prolong gestation for a further five days in mothers being expectantly managed for preterm pre-eclampsia. This surrogate primary outcome marker has been chosen as it provides a sample size that is feasible and attainable for a phase II study to assess efficacy. A limitation in this trial is that we have not specifically powered to detect improvements in maternal or neonatal outcomes (although these are planned secondary outcomes). If this trial yields a positive result, a further phase III multi-centre randomised trial that is sufficiently powered, may be required to be sufficiently powered to demonstrate improvements in clinical outcomes.

As we did for the PIE trial, we will measure biomarkers that reflect disease severity. We will measure circulating levels of sFlt-1 and sEng, among others, in serial samples obtained from the mother. These anti-angiogenic factors are considered to play an important role in inciting maternal endothelial dysfunction and end-organ injury seen in pre-eclampsia. Furthermore, we will measure expression of key molecules in the placental samples obtained at delivery.

Pre-eclampsia kills mothers, fetuses and neonates and is responsible for severe maternal and neonatal morbidity. This is especially the case in the developing world where there is a lack of resources, including staff, equipment and finances. If a treatment were to be discovered, it would have dramatic effects on maternal and neonatal outcomes. Metformin has shown

potential as a therapeutic agent in preclinical work on pre-eclampsia. Further advantages of metformin XR are that it is available in a long acting formulation, it is safe during pregnancy and it is not expensive. This makes it an ideal candidate as a global therapeutic for pre-eclampsia. It is therefore imperative for this trial to be performed. If metformin were proven to be effective at prolonging gestation in early onset pre-eclampsia it could play an important role in decreasing the clinical burden of this dangerous condition.

CONTRIBUTORSHIP STATEMENT

CC, SW and ST developed and designed the trial and obtained funding for the trial.

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

BM and DH assisted with the study design.

RH designed the statistical aspects of this protocol.

FB and TK were involved in the design of the laboratory arm of this trial.

All were involved in revision of the manuscript.

All authors approved the final version to be submitted.

CC and RH responded to the reviewer's comments.

COMPETING INTERESTS

The authors report no competing interests or conflict of interest.

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DATA SHARING STATEMENT

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

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TABLES

Table 1: Inclusion criteria

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

Table 2: Exclusion criteria

Any of the following at the initial assessment:
Patient is unable or unwilling to give consent
Established fetal compromise that necessitates delivery
The presence of: Eclampsia
Severe hypertension, 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
Posterior reversible encephalopathy syndrome (PRES)
Severe renal impairment, defined as a creatinine level ≥ 125 µmol/l or a need for dialysis.
Pulmonary oedema
Left sided heart failure
Disseminated intravascular coagulation
Platelet count < $50x10^9$
Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome
Liver transaminases > 500IU/L
Liver haematoma or rupture
Severe ascites
Diabetes
Current use of metformin
Contraindications or a hypersensitivity reaction to the use of metformin
Current use of a drug that may be affected by metformin

Table 3: Secondary Outcomes

Secondary outcomes	
Composite maternal outcome:	Maternal death
	Eclampsia
	Pulmonary oedema
	Severe renal impairment or dialysis
	Cerebral vascular event
	Liver haematoma or rupture
	Placental abruption
Composite neonatal outcome:	Neonatal death within 6 weeks after the due date
	Grade III or IV intraventricular haemorrhage
	Necrotizing enterocolitis (diagnosed on radiological studies)
	Bronchopulmonary dysplasia (defined as needing oxygen at day 28 of life, either on a ventilator, by CPAP or via a nasal catheter)

Table 4: Exploratory outcomes

Exploratory outcomes

Maternal exploratory outcomes: Maternal death

Eclampsia

Pulmonary oedema

Severe renal impairment or dialysis

Cerebral vascular event

Liver haematoma or rupture

Abruptio placentae

Admission to a high care or intensive care unit

Posterior reversible encephalopathy syndrome

Left ventricular failure

Serum creatinine > 125 µmol/l

Proteinuria >3g/24h

Severe hypertension

Disseminated intravascular coagulation

Platelet count less than 50x109

HELLP syndrome

Liver transaminases >500IU/L

Placental abruption

Mode of delivery

Use of antihypertensive agents

Major postpartum haemorrhage

Thromboembolic disease

Moderate or severe ascites

Fetal exploratory outcomes: Reversed a-wave in the ductus venosus on fetal ultrasound

Significant changes in fetal heart rate patterns on the non-stress test that necessitate delivery

Intrauterine fetal demise

Fetal growth restriction at birth

Persistent reversed flow in the umbilical artery

Redistribution in the middle cerebral artery

Neonatal exploratory outcomes: Neonatal death within 6 weeks after the due date

Grade III or IV intraventricular haemorrhage

Necrotizing enterocolitis

Bronchopulmonary dysplasia

APGAR score < 7 at 5 minutes

Umbilical artery pH < 7.05

Umbilical artery lactate

Surfactant use

Neonatal intensive care or special care unit admission

Intubation and mechanical ventilation

Continuous positive airway pressure (CPAP) support

Grade III/IV hyaline membrane diseaseHospital stay

Incidence of retinopathy of prematurityNeonatal sepsis

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1

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

Full title of trial	Pre-eclampsia Intervention 2 (PI2) Trial: a double blind randomised, placebo-controlled trial of Metformin to treat early onset pre-eclampsia
Short title	PI2 trial
Version and date	30 January 2019 Version 1.2
Ethics approval	Protocol number: (M16/09/037) Federal Wide Assurance Number 00001372 Institutional Review Board (IRB) Number: IRB0005239
Pan African Clinical Trial Registry ID	PACTR201608001752102
Medicine Control Council Reference number	N2/19/8/2 Approved 12 May 2017
Trial medication	Metformin
Phase of trial	Phase II
Principal investigator	Dr Catherine Anne Cluver
Supervisors/ Co-investigators	Professor Stephen Tong Professor Susan Walker Prof Ben Mol Prof DR Hall Dr T Kaitu'u-Lino Dr N Hannan Dr H Hiscock Dr F Brownfoot
Funders	Mercy Perinatal Foundation mercyperinatal@mercy.com.au South African Medical Research Council http://www.mrc.ac.za/

CONTACT DETAILS

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

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

Prof Susan Walker
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: spwalker@unimelb.edu.au
Prof Ben Mol
The Robinson Institute, School of Paediatrics and Reproductive Health
University of Adelaide
Phone: +61434122170
Email: ben.mol@adelaide.edu.au

Prof David Hall
Tygerberg Hospital
University of Stellenbosch
Tel: +27 21 938 9059
Email: drh@sun.ac.za

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

Dr Tu'uhevaha Kaitu'u-Lino
Mercy Hospital for Women
University of Melbourne
Tel: +613 8458 4380
Email: t.klino@unimelb.edu.au

Dr Fiona Brownfoot
Mercy Hospital for Women
University of Melbourne
Tel: +614 1813 0139
Email: Fiona.brownfoot@gmail.com

Trial Design and Statistics

Dr Richard Hiscock
Mercy Hospital for Women
Email: richardjhiscock@gmail.com

Laboratory Coordination

South Africa:

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

Australia:

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

TRIAL COMMITTEES

Trial Steering Committee

Chair:

Prof Ben Mol
Professor of Obstetrics and Gynaecology
Evidenced based Women's Health Care Research Group
Monash University
Phone: +613 857 22836
Email: Ben.Mol@monash.edu

Members:

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

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

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

Data Monitoring Committee

Chair:

Prof Gerhard Theron
Consultant Obstetrician and Gynaecologist
Stellenbosch University and Tygerberg Hospital
Email: gbth@sun.ac.za

Tel: +27 219389217

Members

Dr Adrie Bekker
Consultant Paediatrician
Stellenbosch Hospital and Tygerberg Hospital
Email: adrie@sun.ac.za
Tel: +27 722125453

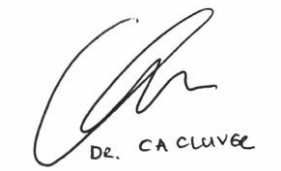
Mr Chris Muller
Biostatistican
Stellenbosch University
Tel +27824303101
Email: cmuller@sun.ac.za

Protocol Versions

1.2

Signatures

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



Principal investigator:

Date: 30/01/2019

Abbreviations

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

CONTENTS

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 1α (HIF 1α)
- 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.[1] It is a multi-system disorder involving maternal vessels (causing hypertension and endothelial dysfunction), the kidneys, the liver, the lungs, the haematological system, the cardiovascular system and the fetoplacental unit.[2] 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.[3] Globally, pre-eclampsia is responsible for >60,000 maternal deaths annually[4,5] and in South Africa hypertensive disorders of pregnancy are responsible for 14% of maternal deaths.[6] In the United States it is estimated that for every pre-eclampsia related death there are probably 50-100 other women who experience significant morbidity associated with pre-eclampsia.[7]

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

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

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.[11]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.[12,13] 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.[14,15] 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.[2,9]

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 1α (HIF 1α), 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.[16] These characteristics make it a potential therapeutic to treat pre-eclampsia. Possible biological explanations include the following:

- Metformin inhibits hypoxic inducible factor 1α (HIF 1α)
- 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 1α (HIF 1α)

HIF 1α 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 1α the secretion of sFlt is decreased which may improve clinical outcomes in pre-eclampsia.[16] Metformin is an ideal HIF 1α inhibitor as it is safe in pregnancy. In contrast other HIF 1α 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.[17] Their effect is probably mediated via anti-angiogenic mechanisms.

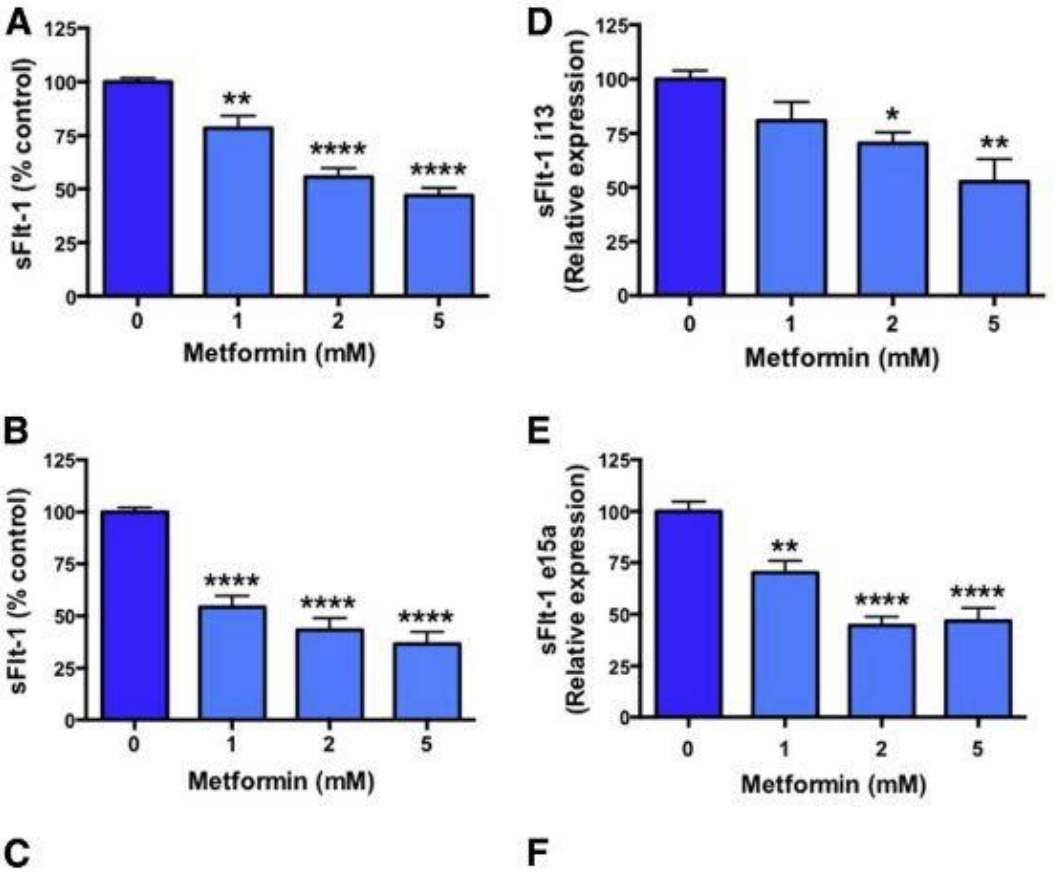
The evidence implicating sFlt-1 and sEng as central to the pathogenesis of pre-eclampsia is compelling.[18] Serum sFlt-1 and sEng are increased in women with pre-eclampsia many weeks preceding clinical disease and there is a dose dependent relationship between serum levels and disease severity.[14] sFlt-1 administered *in vivo* to pregnant rats induces hypertension and proteinuria.[19] 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.[20] sFlt-1 and sEng are, by far and away, the most studied molecules in the field of pre-eclampsia.

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

The acceptance of the strategy to reduce levels of these anti-angiogenic factors 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).[16]

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

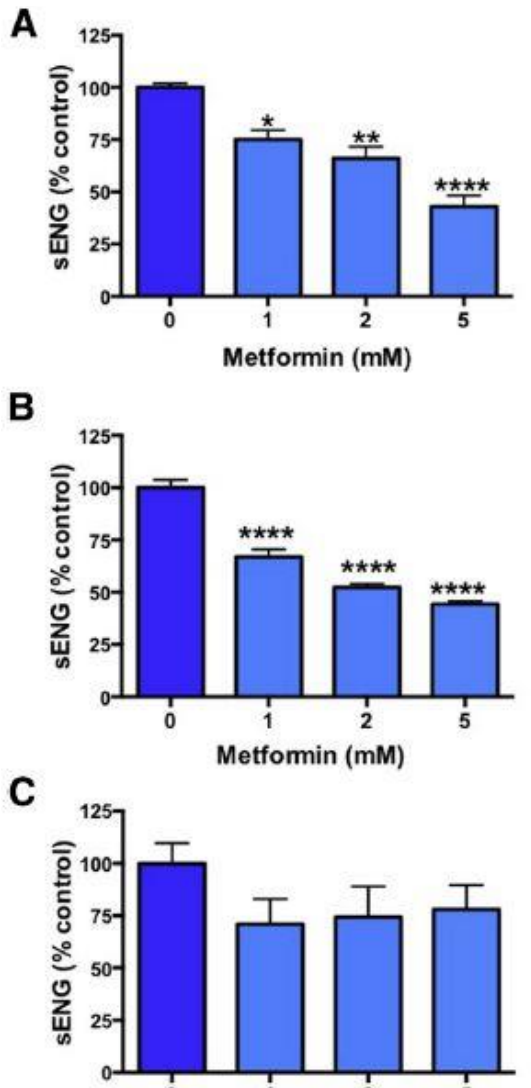


Metformin (0, 1, 2, 5 mmol/L) dose-dependently reduced soluble fms-like tyrosine kinase 1 secretion from **A,** endothelial cells, **B,** villous cytotrophoblast cells, and **C,** preterm preeclamptic placental villous explants. Metformin reduced endothelial cell expression of **D,** *sFlt-1 i13* isoform, **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.

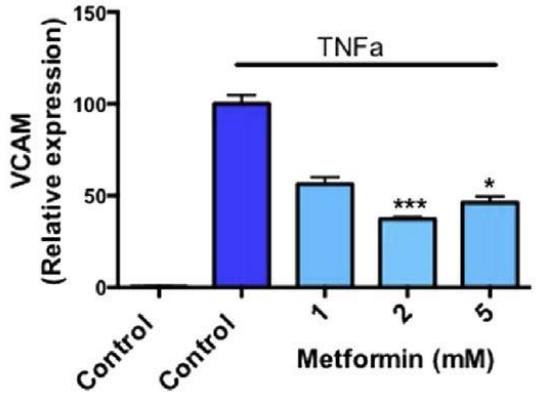
2.3.3 Metformin reduces VCAM-1 expression on endothelial cells

A hallmark of pre-eclampsia is endothelial dysfunction, a form of maternal blood vessel injury.[17] 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.[21]

It has recently been discovered that metformin potently blocks up-regulation of VCAM-1 induced by Tumour Necrosis Factor-α (TNF-α), 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 α 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 $P < .0001$.

TNFα, tumor necrosis factor α; *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.[16]

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.[22] 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.[23] 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.[24]

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.[25]

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.[26,27]

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.[28]

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.[22,29–32] 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.[33]

2.5.2 Metformin drug information

Product information on metformin can be found at the following link: http://packageinserts.bms.com/pi/pi_glucophage_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.[32]

2.5.2.2 Contraindications

Metformin is contraindicated in

- Renal disease or renal dysfunction (suggested by serum creatinine levels ≥123umol/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 insuffiency 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 Cmax 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 Cmax and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax 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 1α (HIF 1α)
- 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 (Cmax), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (Tmax) following administration of a single 850mg tablet of metformin Cmax 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 µg/mL. During controlled clinical trials, maximum plasma levels did not exceed 5 µg/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) and the Powercal program running in Stata v15(Stata statistical software. 2017 *Release 15*. Stata Corp. College Station, TX: StataCorp LLC)[34].

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.[35] 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.[11] 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	**65**	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

	Gestational age (GA)	**EFW if GA is unknown**
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.[36] 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[36]

Classification of the HDP.

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

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

Table 3: ISSHP classification of adverse conditions and severe complications of preeclampsia[36]

Adverse conditions and severe complications of preeclampsia.

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

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

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

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.[38]
  - Severe hypertension defined as a systolic blood pressure greater than or equal to 160 mmHg or diastolic blood pressure greater than or equal to 110 mmHg that cannot be controlled with antihypertensive medication within 48 hours of admission.
  - Cerebrovascular event defined as an ischaemic or haemorrhagic stroke associated with clinical symptoms and definitive signs on imaging.
  - Posterior reversible encephalopathy syndrome (PRES) associated with pre-eclampsia defined on imaging as reversible vasogenic oedema, usually in the occipital or parietal lobes.
  - Severe renal impairment with a creatinine level of greater or equal to 125 µmol/l or a need for dialysis.
  - Signs of left ventricular failure which include pulmonary oedema requiring treatment or oxygen saturations of less than 90% caused by left sided heart failure.
  - Disseminated intravascular coagulation defined as an INR greater than 2
  - Platelet count at presentation less than $50x10^9$ (platelet aggregation excluded)
  - Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100×10^9/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase > 600 µ/L or haemolysis on a peripheral blood smear.
  - 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 124umol/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 ≤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 ≤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 µmol/l
- Proteinuria greater than or equal to 3g/24h
- Hypertension with a systolic blood pressure greater than 160mmHg or a diastolic blood pressure greater than 110mmHg despite anti-hypertensive treatment
- Disseminated intravascular coagulation
- Platelet count less than 50x109
- Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome defined as a platelet count less than 100 × 109/L, aspartate aminotransferase greater than 60 µ/L, and haemolysis as demonstrated by lactate dehydrogenase greater than or equal to 600 µ/L or haemolysis on a peripheral blood smear or a raised haptoglobin level)
- Liver transaminases > 500IU/L
- Placental abruption defined as a retroplacental clot covering more than 15% of the maternal surface or a diagnosis on histology
- Mode of delivery
- Use of antihypertensive agents (number of agents and daily dose at delivery)
- Major postpartum haemorrhage (defined as blood loss of more than 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/efmguidelinercog.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, 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^3 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 PI2 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 intertest 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.[39] 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 while 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).[40] 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 facilitates 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.

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PATIENT INFORMATION LEAFLET: PI2 TRIAL

SUMMARY

Name of principal investigator: Dr Cathy Cluver

Name of research midwife: ……..

Contact number: ……

What is pre-eclampsia?

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

The trial:

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

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

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

BACKGROUND INFORMATION ON PRE-ECLAMPSIA

What is pre-eclampsia?

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

How do we treat pre-eclampsia?

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

When will I have my baby?

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

What treatment will I receive?

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

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

INVITATION TO BE INVOLVED IN THE PI2 TRIAL

You are being invited to participate in a research project to find out whether a drug called metformin can be used to treat pre-eclampsia. The name of the study is the Pre-eclampsia Intervention 2 (PI2) Trial. It is important that you read all the information provided about the trial before you decide to take part and that you understand why we are doing the research and what you would need to do if you were involved in the study. If you have any questions about the trial you can ask your doctor, the midwives or any of the staff from the research project. You can talk to any of the other women who have decided to be involved or not involved in the study.

Why are we doing this study?

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

What is the purpose of this study?

Recent research has shown that there are substances produced by the placenta that can cause pre-eclampsia. Work done in a laboratory in Melbourne, Australia has shown that these substances may be reduced by metformin. The PI2 trial is the first trial in the world that will look to see if metformin can be used to treat pre-eclampsia.

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

You have been invited to be in this study as you have been diagnosed with pre-eclampsia. We would like to include 150 women with pre-eclampsia in this study.

Do I have to be in the study?

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

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

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

You will then need to take extra tablets each day until the baby is delivered. One of the research team will visit you every day to see how you are feeling and to collect information about your pregnancy. When your routine blood tests are done we will take an extra sample of blood for the study. On the first day that you take the medication we will need to take an extra sample of blood. We may need to do extra ultrasound examinations of your baby. Once your baby is born and the cord has been cut we will take a small amount of blood from the placenta. We will take a small sample from the placenta which will be sent for testing.

We will follow you up after the delivery of the baby. The samples taken may be sent overseas for further testing to try to find a treatment for pre-eclampsia. Only tests related to finding a cure for pre-eclampsia will be performed on the samples taken.

Is metformin treatment safe in pregnancy?

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

Are there any side effects of metformin?

All drugs may have side effects. Side effects that have been associated with the use of metformin include headache, diarrhea, nausea, flatulence, abdominal pain, constipation and a dry mouth. There are certain drugs that cannot be used with metformin. If you are taking one of these drugs you will not be asked to participate in the trial. A very rare complication that occurs in 3 per 100 000 women taking metformin is lactic acidosis. This can present with malaise, muscle pains, breathing difficulty and abdominal pain. We will monitor you for any signs of this very rare complication. We will provide you with a copy of the product information leaflet if you would like more information.

Will I receive the placebo treatment or the metformin treatment?

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

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

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

Will my information be kept confidential?

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

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

Only half of the women in this trial will be given metformin. If you do receive this treatment you may or may not benefit from the effects of this drug and you may or may not have improvement in your pre-eclampsia. There are no other direct benefits for you being

involved in this study. By being involved you may help us find a treatment for pre-eclampsia which could help many pregnant mothers in your situation in the future.

What are the disadvantages of being in the study?

You will need to take extra tablets and we may need to take a few extra samples of your blood for testing.

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

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

What will happen with the results of this research project?

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

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

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

Who has paid for this study?

This study has been funded by grants from the University of Melbourne. Dr Cluver's salary has been paid for by grants from the Discovery Foundation and the South African Medical Association.

Who do I contact if I have a problem?

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

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

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

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

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

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

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

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

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

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

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

Thank you for taking the time to read this information leaflet about the PI2 trial.

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

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

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

INFORMED CONSENT FORM

PRINCIPAL INVESTIGATOR: Dr Catherine Anne Cluver

RESEARCH MIDWIFE: Name: ……
Contact number: ……
Email: …….
ADDRESS: Department of Obstetrics and Gynaecology
Tygerberg Hospital and University of Stellenbosch

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

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

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

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

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

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

Declaration by participant

By signing below, I …….. agree to take part in a research study entitled: Pre-eclampsia Intervention 2 (PI2) trial: a double blind randomised, controlled trial of metformin to treat early onset preeclampsia.

I declare that:

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

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

…… ……

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* …… declare that:

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

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

…… ……

Signature of investigator **Signature of witness**

Declaration by interpreter

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

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

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

....

Signature of interpreter **Signature of witness**

Statistical Analysis Plan (SAP)
Pre-eclampsia Intervention 2 trial (PI2)

Contents

Section A. Administrative

1. Title:

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

2. Trial registration:

Pan African Clinical Trials Registry ID: PACTR201608001752102

3. SAP version:

Version: 1.0 Date: 4th January 2019

4. Protocol Version:

This document has been written based on information contained in the study protocol version 1.1 dated 19th February 2018.

5. SAP Revisions

Revision history, with justification and timing: Not applicable

6. Roles and Responsibility:

Document prepared by Dr Richard Hiscock, Mercy Hospital for Women, University of Melbourne and Dr Cathy Cluver, Stellenbosch University.
Email: richardjhiscock@gmail.com, cathycluver@hotmail.com

Signatures:

Signature of senior statistician responsible *Date 10 January 2018*

Signature of chief investigator/clinical lead *Date: 20 December 2018*

Section B: Introduction and Objectives

7. Synopsis of trial background and rationale:

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: (i) inhibits hypoxic inducible factor 1α (HIF 1α); (ii) reduces sFlt-1 and sEng secretion from primary endothelial cells and placental tissue; (iii) reduces VCAM-1 expression on endothelial cells; and (iv) 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 fewer 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 (excerpted from trial protocol p 11).

8. Research hypothesis and objectives:

Research hypothesis:
In women with preterm pre-eclampsia undergoing expectant management, a daily dose of 3 grams of metformin prolongs gestation by at least 5 days.

Study objectives:
Primary objective:

- Examine whether up to 3 grams of metformin daily can safely prolong gestation for 5 days in women with early onset pre-eclampsia diagnosed between 26+0 to 31+6 weeks gestation, compared to standard of care, expectant management alone. This is set in a superiority framework with two-sided hypothesis regions for all statistical testing.

Secondary objectives:

- Determine whether metformin improves maternal and neonatal composite outcomes in early onset pre-eclampsia compared to placebo.

Section C: Trial Methods

9. Trial design: description of trial design

This is a single center (hospital based) phase II parallel group placebo-controlled, double-blind randomized control trial. The allocation ratio is 1:1. Mothers are randomized to receive either metformin or placebo tablets.

10. Randomization and allocation concealment:

Randomisation will be performed in an equal ratio of metformin to placebo, stratified into two strata by gestational age using blocks of 4 to 6 with the size and order randomly assigned within each stratum. Stratification details are:

	Gestational age (GA)	EFW if GA is unknown
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

An online, web-based sequence generator system will be used. This system will be managed by the South Australian Health and Medical Research Institute (SAHMRI) (https://www.sahmri.org) and is a REDCap based system. Codes will be generated for each packet of placebo and treatment tablets. The treatment tablets and placebo will be manufactured by Merck Pharmaceuticals. The trial medication will be packed and labelled by the trial pharmacist. Once randomized, the treatment pack with the same code will be allocated to the participant. The randomization list and matching treatment code will be stored by the pharmacy and by SAHMRI and only released to the researchers once the data has been finalized and locked for editing (including neonatal follow-up) or to the DMSC (closed sessions) upon request. The researchers, clinicians and participants will remain blinded until completion of the trial.

11. Sample size

Sample size calculation is determined by the primary research question: does treatment with metformin result in a gain in the length of gestation of greater than or equal to 5 days compared to the mother receiving placebo therapy. For each mother her gestational age at diagnosis (trial enrolment) sets the maximum allowable length of pregnancy prolongation. For those with PE 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 setting a ceiling for maximal prolongation of 15 days. Therefore, the maximum potential gestation prolongation is 63 days for stratum 1 and 42 days for stratum 2 mothers. The primary outcome will be the median prolongation of gestation, reflecting the skewed distribution of the gestation prolongation seen in published clinical trials.[1] The researchers believe that median prolongation a more clinically meaningful measure of change than either raw mean or ratio change. Sample size calculations were based upon the Geometric Mean Ratio (GMR), an appropriate measure for differences in median. Analysis was performed both using PASS 13 (Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA, 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).

Power calculations settings were: 90% power, with two-sided alpha set at 0.05, median prolongation of 8.3 days in the control arm and a clinically important gestation prolongation of 5 days. The measure of variability used in this analysis is the coefficient of variation (CV) in mothers treated with standard expectant

[1] Hall DR, Odendaal HJ, Kirsten GF, et al. Expectant management of early onset, severe pre-eclampsia:

management (control group). Two studies were used provided estimates for the expected gestational prolongation and associated CV in pre-eclamptic mothers receiving expectant management after PE diagnosis (control group) on two studies [2]. The first by Hall et al reported a mean gestation prolongation of 11 (SD 7) days equivalent to a CV = 0.64, whilst the second by Cluver et al (PIE 1 study) reported an overall mean prolongation of 13.1 (SD 12.2) days in the placebo arm, a CV = 0.93. In the PIE 1 study mothers entering at a gestation greater than 29 weeks had a CV = 0.8. We have chosen to use a CV = 1.0, slightly larger than the 0.93 found in the overall PIE study, to maintain adequate power if slightly greater variability in prolongation times occurs. The results of sample size estimates for a limited range of GMR and two CVs are presented in the following table:

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	**65**	61	49

*placebo group median = 8.32 days

Therefore, at analysis we require 65 patients per trial arm. Allowing for 10 dropouts within each arm we plan to recruit 150 patients (75 patients in the placebo & 75 in the treatment arms). The dropouts, including patient requested trial withdrawal and delivery before any trial medication was given occurred in 6/119 (5.0%) of mothers.

Whilst presentation of results will include Kaplan-Meier survivorship curves and adjusted hazard ratios for a Cox proportional hazards model the following power analysis based upon expected events indicates that this study is not adequately powered to use survivorship as the primary endpoint. In the setting of this trial the Hazard Ratio (HR) defined as the time for control/time for Rx at the same proportion of survival. For example, taking the median survival time of 9 days in the control group from the Hall study we can assess the HR detected for the predetermined group size:

Time above median (days)	Median time in Rx group (days)	HR	Group size (equal group size)
3	12	0.75	360
4	13	0.69	225
5	14	0.64	158
6	15	0.60	120
7	16	0.56	96
8	17	0.53	80
9	**18**	**0.50**	**68**
13.5	22.5	0.40	42

[2] Cluver CA, Hannan NJ, van Papendorp E, et al. Esomeprazole to treat women with preterm preeclampsia: a randomized placebo-controlled trial. Am J Obstet Gynecol 2018;219:388.e1-17

Given the sample size of 65 per arm this study has a power of 0.9 to detect a difference of 9 days which translates to an ability to detect only a Hazard Ratio ~ 0.5 or smaller.

All secondary outcomes are binary with expected incidence less than 0.05. At the projected sample size of 65 the power to detect a 50% change in incidence is low at 0.12.

12. Framework

This trial uses a superiority hypothesis testing framework between treatment groups for all outcomes.

13. Statistical Interim analyses and stopping guidance

This is a phase II trial with mothers under direct in hospital observation which facilitates immediate detection and management of adverse events. For the planned sample size only a very large treatment effect size would lead to an indication to cease the trial for efficacy at information fraction of 60 – 80%. Given the phase II nature of the study, the complete observation of mothers whilst under trial treatments, there is no planned interim analysis for efficacy. There is no planned interim assessment for futility. The DMC will monitor for adverse events and will provide stopping guidance should this be deemed necessary. See separate DMC charter (PI2 DMSC Charter version 16 June 2018).

14. Timing of final analysis

Unblinding and final analysis will be performed only after all participants have being delivered and all neonatal follow-up has been completed. Neonatal death is one of the outcomes within the prespecified composite neonatal outcome (see protocol p 38 – 39) and is assessed at six weeks post-delivery. Therefore, analysis is will not commence prior to six weeks after the delivery of the last recruited trial patient. Publication of biomarker results may be published separately from the clinical trial outcomes.

15. Timing of outcome assessments.

The primary outcome is measured from time of randomization to delivery. Secondary maternal and neonatal composite outcomes are measured from time of recruitment until six weeks after the due date, with timing dependent upon outcome measures.

Section D: Statistical Principles

16. Confidence intervals and p-value

The significance level is set at 0.05 and all hypothesis testing will be two-sided. A single primary outcome is tested at a significance level of 0.05. If and only if a significant difference is found will the secondary outcomes be tested. The two secondary outcomes will each be tested at a significance level of 0.025.

17. Description of any planned adjustment for multiplicity, and if so, including how the type 1 error is to be controlled

No adjustment for multiplicity will be performed**.** The strategy for outcome testing is pre-specified and maintains the overall trial error rate at 0.05.

18. Confidence intervals (CI) to be reported

Standard 95% confidence intervals will be used to present analysis of the primary adjusted outcome. For secondary outcomes it is planned to the adjusted width of CI used.[3]

19. Adherence and Protocol Deviations

Adherence:
Trial medication will be administered to mothers on an inpatient basis. Measures to monitor adherence are: 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. The dosing schedule details a programmed escalation of dose in the of side effects. We will use two measures of dosing in the metformin arm: (i) average daily dose of tablets = number of ingested medications/days of therapy and (ii) percentage of maximum dose = ingested medications/ total maximum medications.

Description of how adherence to the intervention will be presented:
Adherence will be presented on the two measures detailed above, using descriptive statistics (N, mean, SD, median, minimum, maximum) converted to metformin dose for the intervention arm.

Definition of protocol deviation for the trial and description in trial results:
Protocol deviations due to errors in applying inclusion/exclusion criteria, the wrong intervention being administered will be documented in trial results either in patient flow diagram or text. The primary outcome will not be subject to ascertainment error due to its unambiguous nature. Prior to unblinding of treatment all protocol deviations will be assessed, and determination made about inclusion of these participants within the trial analysis populations. Determinations will be presented in the research results in the patient flow diagram and possibly in the text.

[3] Ludbrook J. Multiple inferences using confidence intervals. Clin Exp Pharmacol Physiol 2000;27:212 - 215.

19. Analysis populations.

The intention-to-treat population will include all randomized patients, regardless of their eligibility, according to the treatment they were randomized to receive. Primary outcome will also be tested on trial arms based upon treatment received as a sensitivity analysis.

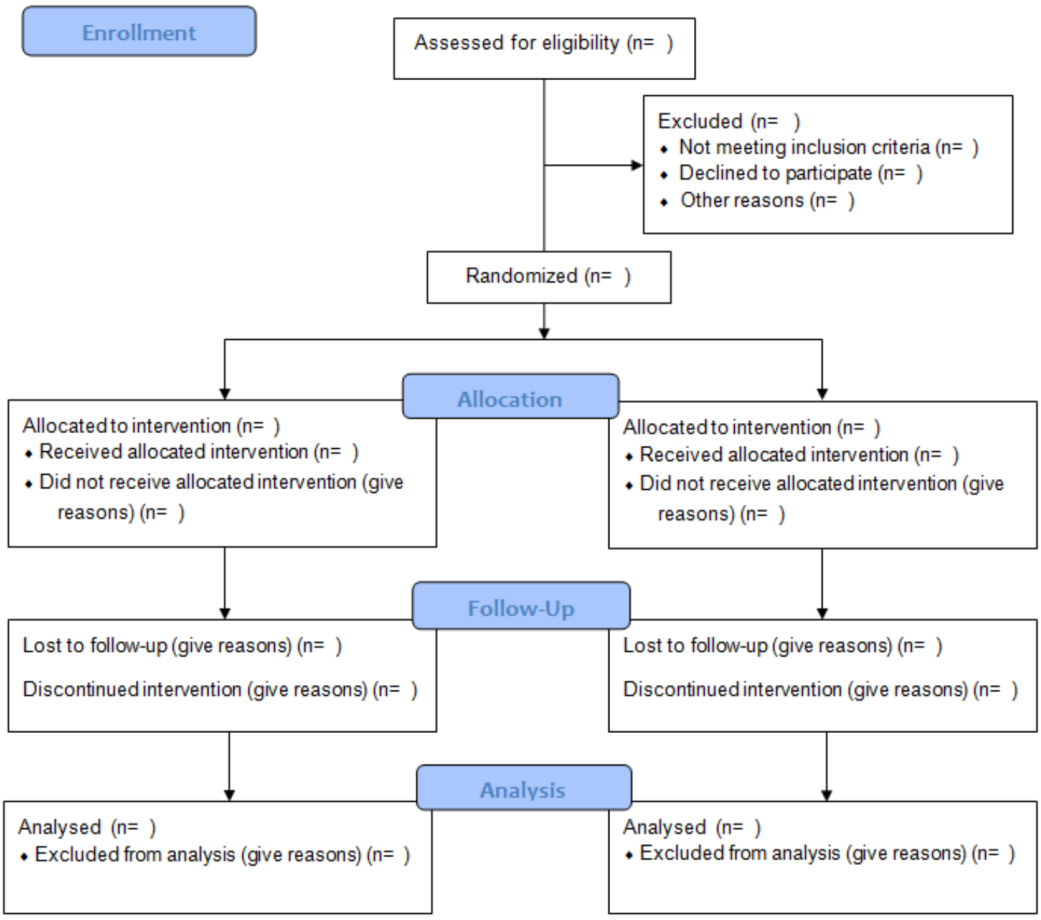
Section E: Trial Population

20. Screening Data

Screening data will be collected and reported. The name of patients assessed for eligibility and the reason for not enrolling will be recorded and reported. The trial inclusion and exclusion criteria are specified in the protocol. A CONSORT diagram will be used to summarize and present this data.

21. CONSORT flow diagram template for the PI2 trial

The following CONSORT flow diagram will be used to detail enrollment, randomization, treatment allocation, reasons for not receiving treatment, follow up and analysis



22. Baseline characteristics

List of Baseline characteristic to be summarized by treatment groups and presented in tables:

- Gestational age at randomization
- Maternal age in years
- BMI
- Ethnicity (black, colored, other)

- Smoking
- Aspirin usage
- Calcium usage
- HIV status
- Chronic hypertension
- Parity (Nulliparity; Multiparity +/- hypertension in previous pregnancy)
- New paternal parity in current pregnancy
- Pre-randomization highest systolic and diastolic blood pressure
- 24 hour Creatinine ratio
- Haemoglobin, platelet count, urea and creatinine before randomization
- Estimated fetal weight on ultrasound
- Presence of absent flow on umbilical artery doppler.

Details of how baseline characteristics will be descriptively summarized:

Categorical data will be summarized by numbers and percentages. Continuous data will be summarized by mean, SD and range if data are normal and median, IQR and range if data are skewed. Minimum and maximum values will also be presented for continuous data. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

Section F: Analysis

23. Outcome definitions

Primary outcome definition:

The primary outcome is time from randomization until delivery, measured in hours and presented in days. The summary of interest is median prolongation and data will not be transformed prior to analysis. If a fetus does demise during expectant management, we will give it a length of pregnancy prolongation of zero and will include it in the primary analysis.

Composite secondary outcome definitions:

(i) Maternal composite outcome: the occurrence of any of the following serious maternal outcomes: maternal death, eclampsia, pulmonary oedema (oxygen saturation ≤90%, with clinical symptoms requiring treatment), severe renal impairment or the need for dialysis, a cerebral vascular event, placental abruption and liver haematoma or rupture.

(ii) Neonatal composite outcome: the occurrence of any of the following serious neonatal outcomes: neonatal death within 6 weeks after the expected due date, grade III or IV intraventricular haemorrhage, necrotizing enterocolitis and bronchopulmonary dysplasia

Exploratory outcomes:

All other outcomes, including outcomes nested within individual secondary outcomes or outcomes with less clinical interest than secondary outcomes are classified as exploratory outcomes. These will not be subject to hypothesis testing, but precision of point estimates and may be presented using unadjusted 95%CI.

24. Statistical Analysis methods

Raw data primary outcome:
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 intertest 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 associated with 95%CI for all testable outcomes.

Primary outcome:
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% confidence intervals (95%CI).

Secondary outcomes:
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**.**

Adjustment for covariates for each primary and secondary outcomes:
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.

Secondary outcomes: Composite binary outcomes will be modelled using a logistic regression model containing both treatment group and gestation aged based strata.

Methods used for assumptions to be checked for statistical methods:
Quantile regression makes no distributional assumptions (assessed using residuals) apart from assuming the outcome is continuous. Secondary outcomes tested using exact logistic regression or Fisher's exact test make no distributional assumptions.

Sensitivity analyses:
Sensitivity analysis will be conducted on the primary outcome are:
(i) Treatment received Analysis will be adjusted by median quantile regression as already specified for the primary outcome intention to treat analysis. Treatment received, given varying dosage schedule is defined as an average daily dose.
(ii) Gestational age as an effect modifier. An interaction between treatment and gestational age strata will be using the same model as the primary outcome. Given study size it is acknowledged that this has low power to detect an interaction effect.
(iii) Covariate adjusted. The trial investigators consider that a mother with absent UAD flow, an estimated fetal weight less than the 10th centile and an estimated fetal weight less than the 3rd centile are more likely to

be associated with shorter randomization to delivery times. Covariate adjustment for these factors may increase the precision of the primary outcome measure if there is a correlation between them and delivery time.
(iv) 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 pretreatment 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.

Subgroup analyses
Gestational age at randomization has been incorporated in the prespecified primary outcome analysis. No other subgroup analyses are planned.

25. Missing data reporting and assumptions/statistical methods to handle missing data

In this study primary outcome will not have missing data, both gestational age at randomization and treatment are predetermined and all mothers will deliver in the acute in hospital setting. Based upon our experience in the PIE 1 trial (at the same institution) we expect no of missing covariate values for the three components required for analysis of the POM – group assignment, gestational age-based strata and time to delivery (delivery time – admission time). Imputation will not be performed. As described above, if an FDIU occurs it will be allocated a time of zero prolongation.

Secondary outcomes that are pre-specified for hypothesis testing use the same pre-randomization covariates and all outcomes are identifiable. If 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.

26. Additional Analyses

For the primary outcome a prespecified analysis assessing effect of absent uterine artery flow on outcome will be performed by a covariate adjusted analysis that includes treatment group, the gestational age design-based strata, fetal growth restriction (defined as an estimated fetal weight less than the 10th centile and less than the 3rd centile and absent flow on umbilical artery Doppler (UAD) examination measured at the time of randomization. The trial investigators consider that a mother with fetal growth restriction or absent UAD flow was more likely to be associated with shorter randomization to delivery times. This pre-specified covariate adjustment will increase the precision of the primary outcome measure if there is a correlation.

27. Harms

The number (and percentage) of patients experiencing serious adverse events (SAE) will be presented for each treatment arm. These are: maternal death, fetal death, event resulting threat to life of mother or baby, event that causes prolonged hospital stay, event resulting in significant disability to mother and congenital birth defect not previously detected on ultrasound. All will be listed by trial arm in

supplementary trial results and no formal statistical testing will be undertaken. Note that for maternal and neonatal composite secondary outcomes and non-composite 'pre-specified testable' secondary outcomes results will be reported with the primary outcome.

28. Statistical Software

Statistical software used will be Stata v 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) or R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Packages used will be detailed.

29. References

All methods used are standard.

30. Reference to Data Management Plan

Details on data handling and cleaning is specified in the PI2 Protocol.

31. Reference to the Trial Master File and Statistical Master File

The Trial Master File is kept and updated by the Principle Investigator (Dr C Cluver).

32. Reference to other Standard Operating Procedures or documents

Not applicable

Draft Tables for PI2 Trial

Table 1: Characteristics of trial participants at enrolment
Table 2: Primary and secondary outcomes
Table 3: Severe adverse events
Table 4 : Side effects from the medication

Table 1: Characteristics of Trial Participants at enrolment			
Characteristics		**Metformin (n=)**	**Placebo (n=)**
Gestation at randomisation	Median [IQR]		
	Mean (SD)		
Gestation < 29 weeks at randomisation	n (% of each group)		
Maternal age in years	Median [IQR]		
Body mass index	Median [IQR]		
Race or ethnicity: Black	n (%)		
Coloured	n (%)		
Smoking	n (%)		
Aspirin use n (%)	n (%)		
Calcium use n (%)	n (%)		
HIV positive n (%)	n (%)		
Chronic hypertension n (%)	n (%)		
Nulliparous	n (%)		
Multiparous without hypertension in a previous pregnancy	n (%)		
Multiparous with hypertension in a previous pregnancy	n (%)		
New paternity in current pregnancy	n (%)		
Highest systolic blood pressure before randomisation (mm Hg)	Mean (SD)		
Highest diastolic blood pressure before randomisation (mm Hg)	Mean (SD)		
24-hour protein creatinine ratio at enrolment (g/24 hours)	Median [IQR]		

Table 2: Primary and Secondary outcomes				
		Metformin (n=)	Placebo (n=60)	P value
PRIMARY OUTCOME				
Prolongation of gestation (days)	Median [IQR]			
	Mean (SD)			
SECONDARY OUTCOMES				
Composite maternal outcome	n (%)			
Composite neonatal outcome	n (%)			
Haemoglobin (g/dl)	Mean (SD)			
Platelet count ($(10^9$/L)	Mean (SD)			
Urea (mmol/L)	Mean (SD)			
Creatinine (mg/dL)	Mean (SD)			
Estimated Fetal weight (g)	Mean (SD)			
Fetal weight centile	Median [IQR]			
Absent flow on umbilical artery Doppler	n (%)			

Table 3: Severe adverse events (presented as number with percentages)		
	Metformin (n=)	Placebo (n=)
MATERNAL		
Maternal death		
Eclampsia		
Cerebral vascular event		
Posterior reversible encephalopathy syndrome		
Left ventricular failure		
Pulmonary oedema		
Severe renal impairment		
Blood loss of more than 1000mls		
Disseminated intravascular coagulation		
Liver haematoma or rupture		
FETAL/NEONATAL		

Intrauterine demise		
Fetal or neonatal congenital anomaly		
Neonatal death		
Necrotising enterocolitis		
Neonatal sepsis		
Intracranial haemorrhage		

Table 4: Side effects from medication (presented as number with percentages)		
	Metformin (n=)	Placebo (n=)
Nausea		
Vomiting		
Diarrhoea		
Headache		
Decreased tablets		
Stopped medication		

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number of the PI2 Clinical Trial Protocol
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2-4
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	62
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	4-5
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	15-16
	6b	Explanation for choice of comparators	16-26
Objectives	7	Specific objectives or hypotheses	27-28

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	29
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	30
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	33-37
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	42-47
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	44
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	43-44
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	44-45
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	38-40
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	42-45
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	29-31
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	29
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	31
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	31
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	31-32
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	31-32
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	52
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	45, 47
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	48
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	48-50
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	PI2 Statistical Analysis Plan Version 1.0

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	PI2 Statistical Analysis Plan Version 1.0
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	4-5, 55
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	52
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	51-52
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	55
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	55-57
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	42
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	57
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	N/A

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	57 No contractual agreements that limit access
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	61
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See Informed consent form and patient information leaflet
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	45-47

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