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:

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Signatures:

Signature of senior statistician responsible   *Date 10 January 2018*

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

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SAP version: Version: 1.0 Date: 4th January 2019
Pre-eclampsia Intervention 2 (PI2) Trial: a double blind randomized, placebo-controlled trial of Metformin to treat early onset pre-eclampsia.

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

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[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

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[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.

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[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
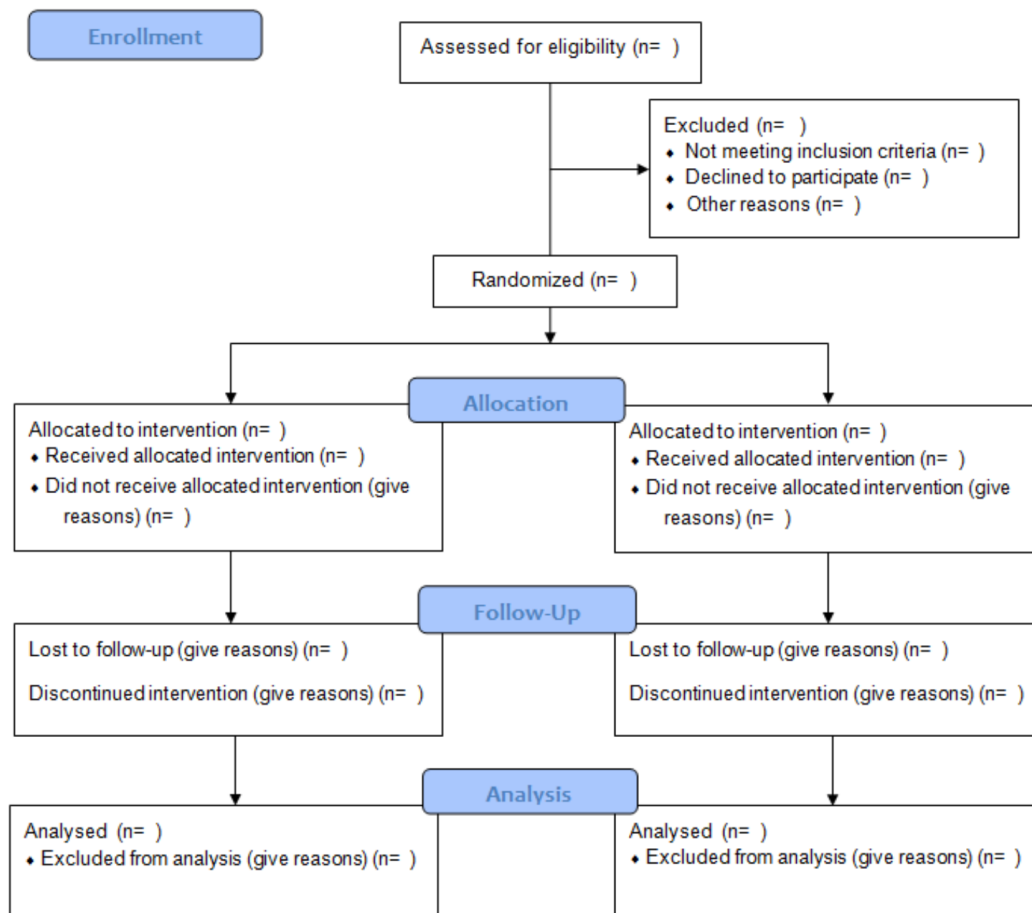
22. Baseline characteristics

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) <u>Treatment received</u> 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) <u>Gestational age as an effect modifier.</u> 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) <u>Covariate adjusted.</u> 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		