OBSTETRICS

Esomeprazole to treat women with preterm preeclampsia: a randomized placebo controlled trial

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BACKGROUND: Preterm preeclampsia has a high rate of fetal death or disability. There is no treatment to slow the disease, except delivery. Preclinical studies have identified proton pump inhibitors as a possible treatment.

OBJECTIVE: The purpose of this study was to examine whether esomeprazole could prolong pregnancy in women who have received a diagnosis of preterm preeclampsia.

STUDY DESIGN: We performed a double-blind, randomized controlled trial at Tygerberg Hospital in South Africa. Women with preterm preeclampsia (gestational age 26 weeks+0 days to 31 weeks+6 days) were assigned randomly to 40-mg daily esomeprazole or placebo. The primary outcome was a prolongation of gestation of 5 days. Secondary outcomes were maternal and neonatal outcomes. We compared circulating markers of endothelial dysfunction that was associated with preeclampsia and performed pharmacokinetic studies.

RESULTS: Between January 2016 and April 2017, we recruited 120 participants. One participant was excluded because of incorrect randomization, which left 59 participants in the esomeprazole and 60 participants in the placebo group. Median gestational age at enrolment was 29+4 weeks gestation. There were no between-group differences in median time from randomization to delivery: 11.4 days (interquartile range, 3.6−19.7 days) in the esomeprazole group and 8.3 days (interquartile range, 3.8−19.6 days) in the placebo group (3 days longer in the esomeprazole arm; 95% confidence interval, −2.9−8.8; P=.31). There were no placental abruptions in the esomeprazole group and 6 (10%) in the placebo group (P=.01, P=.14 adjusted). There were no differences in other maternal or neonatal outcomes or markers of endothelial dysfunction. Esomeprazole and its metabolites were detected in maternal blood among those treated with esomeprazole, but only trace amounts in the umbilical cord blood.

CONCLUSION: Daily esomeprazole (40 mg) did not prolong gestation in pregnancies with preterm preeclampsia or decrease circulating soluble fms-like tyrosine kinase 1 concentrations. Higher levels in the maternal circulation may be needed for clinical effect.

Key words: esomeprazole, trial, preterm preeclampsia, sFlt1, pharmacokinetics

Preeclampsia is one of the most serious complications of pregnancy. It affects 3−8 % of pregnancies and is a leading cause of maternal, fetal, and neonatal morbidity.[1,2] There is no treatment that can slow disease progression, and the only treatment option is to deliver the pregnancy. For preeclampsia that occurs at preterm gestations, clinicians are often required to deliver the fetus early, which results in iatrogenic prematurity with a risk of major disability that includes cerebral palsy, intracerebral bleeding, retinopathy of prematurity, chronic lung disease, and death. The risks of these complications are higher if pregnancies are delivered at earlier gestations.[3] If a treatment were available that temporizes disease progression, it could be used to safely delay delivery to gain gestation, thereby decreasing the degree of prematurity and improving perinatal outcomes.

The preeclamptic placenta releases elevated levels of soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin into the maternal circulation.[4] These antiangiogenic factors cause maternal endothelial dysfunction, hypertension, and multiorgan injury.[5] Esomeprazole is a proton pump inhibitor (PPI) that is prescribed widely in pregnancy to relieve symptomatic gastric reflux. Members of our team have performed preclinical laboratory studies that have shown that PPIs such as esomeprazole are a candidate therapeutic for preeclampsia.[6] Esomeprazole, in particular, has been shown to have diverse biologic actions. Firstly esomeprazole decreases sFlt1 and soluble endoglin production and release from primary trophoblast cells and placental tissue explants and primary endothelial cells/tissues in both normal and preeclamptic pregnancies. Secondly esomeprazole was able to dilate whole human vessels from both normal pregnancies treated with a constrictor and vessels that were obtained from women with preeclampsia. Thirdly, preclinical studies also showed that esomeprazole decreased endothelial dysfunction by mitigating tumor necrosis α−induced endothelial injury, as demonstrated by reducing expression of endothelial vascular cell adhesion molecule-1 and reduced leucocyte adhesion to the endothelium. Lastly important animal studies clearly show that esomeprazole reduces blood pressure in a transgenic mouse model of preeclampsia in which human sFlt1 is overexpressed in the placenta and released in excess into the maternal blood, as seen in women with preeclampsia.[6] Others have subsequently found decreased circulating sFlt1 and soluble endoglin levels in an existing cohort of bloods of women with suspected or confirmed preeclampsia that were coincidentally taking PPIs.[7]

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AJOG at a Glance

Why was this study conducted?

Preeclampsia has high rates of fetal death or disability. There is no treatment to slow the disease, except delivery. Preclinical studies have identified proton pump inhibitors as a possible treatment.

Key findings

Daily oral esomeprazole (40 mg) did not prolong gestation in pregnancies with preterm preeclampsia or decrease circulating soluble fms-like tyrosine kinase 1 concentrations.

What does this add to what is known?

This is the first trial for preterm preeclampsia that has integrated clinical outcomes, mechanistic studies, and pharmacokinetics. Oral esomeprazole (40 mg) may be too low a dose to treat preterm preeclampsia; higher doses may still be effective. This may be the fastest completed randomized clinical trial of a treatment for preterm preeclampsia. It is possible to complete clinical trials for preterm preeclampsia in a reasonable timeframe by running the trials in settings in which the incidence of disease is high.

These promising preclinical data suggest that esomeprazole is a potential candidate treatment; we therefore set out to examine whether oral esomeprazole may be an effective treatment for preterm preeclampsia.

Methods

Trial design

In this single-site phase II double-blind, randomized, placebo-controlled clinical trial, we compared oral esomeprazole with placebo. A 40 mg daily dose was selected based on pharmacokinetic data that showed effective suppression of gastrointestinal symptoms in nonpregnant patients and on reassuring data that showed no adverse effects if taken during pregnancy.[8-11] The trial site was Tygerberg Hospital, Cape Town, South Africa, which is a large academic referral center that is situated in a region with high rates of preeclampsia. We have published the protocol,[12] and the trial was registered with the Pan African Clinical Trials Registry (PACTR201 504000771349).

Pregnant women with singleton pregnancies were invited to participate if they had been diagnosed with preterm preeclampsia between 26+0 and 31+6 weeks gestation. The gestation at enrolment was determined by either menstrual dates (if the women was certain of her last menstrual period) or by an early or mid-trimester pregnancy ultrasound examination. Both the managing perinatologist and neonatologist had to agree that expectant management could benefit the fetus.

Women were not eligible if they had an indication for immediate delivery because they could not be treated expectantly to gain further fetal maturity. Exclusion criteria therefore included established maternal or fetal compromise that necessitated delivery, the current use or contraindications to the use of PPIs, and the use of medications that could interact with PPIs (which included warfarin, ketoconazole, voriconazole, atazanavir, nelfinavir, saquinavir, digoxin, St John's Wort, rifampin, cilostazol, diazepam, tacrolimus, erlotinib, methotrexate, and clopidogrel). Specific clinical exclusion criteria included eclampsia, severe hypertension not be controlled within 48 hours of admission, a cerebrovascular event, posterior reversible encephalopathy syndrome, severe renal impairment with a creatinine >125 μmol/L, pulmonary edema, disseminated intravascular coagulation, hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, liver hematoma or rupture, severe ascites on ultrasound examination. We excluded pregnancies with a suspicion of a major fetal anomaly or malformation. Expectant management involved hospital admission with close maternal and fetal surveillance. Maternal surveillance involved 4 hourly blood pressure measurement, twice daily clinical assessments, daily urinalysis, and twice weekly biochemical testing. Fetal surveillance involved 6 hourly cardiotocography and ultrasound assessments every 2 weeks or more frequently, if indicated. To enhance fetal lung maturity, all participants received 2 doses of betamethasone that were given 24 hours apart, followed by a single repeat dose 1 week later if not delivered, as per local protocol.[13] Expectant management ended at 34 weeks gestation; women who reached this gestation were delivered. Delivery at <34 weeks gestation was a clinical decision made by the patient's treating team.

The study participants provided written informed consent. The study had Health Research Ethics Committee (HREC) approval, was approved by the South African Medicines Control Council. Study data were collected and managed with the use of REDCap electronic data capture tools.[14]

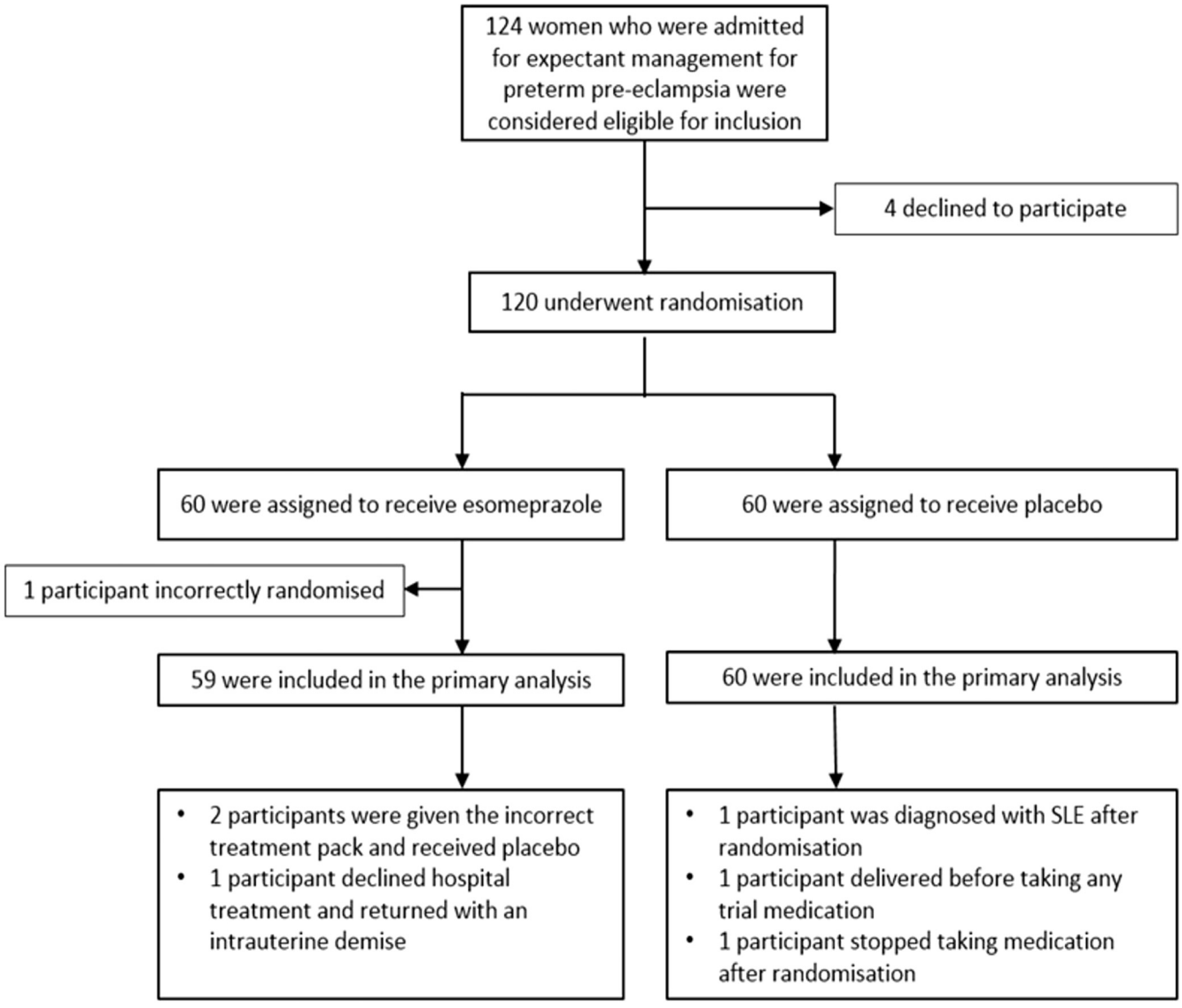
Randomization and masking

Randomization was performed in a 1:1 ratio with the use of an online, web-based sequence generator. Because gestation at randomization could possibly impact the length of pregnancy prolongation, randomization was stratified (strata 1 was ≤28+6 weeks; strata 2 was 29+0 until 31+6 weeks gestation). Randomization was done within blocks of random size within 4−6. The tablets and treatment packs were manufactured, packed, and labelled by the Institute of Drug Technology Limited (en.idtaus.com.au) in Victoria, Australia, and were identical with respect to variables such as size, thickness, physical properties, and appearance. The investigators had no access to the randomization list, and allocation concealment was maintained throughout the trial.

Placental and blood collection to measure angiogenic markers of preeclampsia and endothelial dysfunction and to perform pharmacokinetics

Plasma samples to measure circulating preeclampsia and angiogenic biomarkers were collected at randomization and twice weekly until delivery. Placental tissue

FIGURE 1
Flowchart of screening, randomization, and follow up



The flowchart summarizes the screening, randomization, allocation to esomeprazole or placebo, exclusion after randomization, complications and follow up of the study.

SLE, systemic lupus erythematosus.

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samples and umbilical artery cord blood were collected at delivery when possible. After recruitment was completed, circulating concentrations and placental expression of molecules that are markers of preeclampsia and endothelial dysfunction were measured.

Pharmacokinetics was performed in a subgroup of patients who had been administered esomeprazole. Plasma samples were drawn from a catheter in a forearm vein at the following dosing interval: predose, at 15, 30, and 45 minutes; postdose, at 1, 1.5, 2, 4, 8, and 24 hours. Levels were measured in batch after the trial was completed (the Supplemental Material provides further details on how the esomeprazole was measured).

Outcome measures

The primary outcome was prolongation of pregnancy, and the study was powered to show a prolongation of 5 days. Secondary outcomes included composite and individual maternal, fetal, and neonatal outcomes, maternal biomarkers, pharmacokinetics, and placental samples.

After completion of the trial, we measured the plasma circulating concentrations of the following markers of preeclampsia: sFlt1, soluble endoglin, placental growth factor (PlGF) with the

TABLE 1
Characteristics of trial participants at enrolment

Characteristics	Esomeprazole (n=59)	Placebo (n=60)
Gestation at randomization, wk+d		
Median [interquartile range]	29+4 [27+6–30+6]	29+5 [28+1–30+5]
Mean (standard deviation)	29.4 (1.65)	29.4 (1.66)
Gestation <29 weeks at randomization, n (%)[a]	20 (33.9)	20 (33.3)
Maternal age (y), median [interquartile range]	24 [21–31]	30 [25–34]
Body mass index (kg/m^2), median [interquartile range]	29.4 [24.8–33.3]	29.0 [24.0–35.2]
Race or ethnicity, n (%)		
Black	34 (57.6)	33 (55)
Colored (multiracial ethnic group native to Southern Africa)	25 (42.4)	27 (45.0)
Smoking, n (%)	8 (13.6)	4 (6.7)
Aspirin use, n (%)	1 (1.7)	0
Calcium use, n (%)	1 (1.7)	0
HIV positive, n (%)	8 (13.6)	12 (20.0)
Chronic hypertension, n (%)	13 (22.0)	21 (35.0)
Nulliparous, n (%)	26 (44.1)	12 (20)
Multiparous, n (%)		
Without hypertension in a previous pregnancy	25 (42.4)	27 (45)
With hypertension in a previous pregnancy	8 (13.6)	21 (34.9)
New paternity in current pregnancy, n (%)	11/37 (29.7)	17/48 (35.4)
Highest systolic blood pressure before randomization (mm Hg), mean (standard deviation)	166 (17.5)	168 (16.4)
Highest diastolic blood pressure before randomization (mm Hg), mean (standard deviation)	103 (13.4)	103 (11.4)
24-Hour protein creatinine ratio at enrolment (g/24 hr), median [interquartile range]	1.46 [0.62-3.16]	1.06 [0.57-16.86]
Hemoglobin (g/dL), mean (standard deviation)	12.3 (1.5)	11.6 (1.4)
Platelet count (10^9/L), mean (standard deviation)	207 (59.9)	222 (67.2)
Urea (mmol/L), mean (standard deviation)	4.0 (1.64)	3.7 (1.4)
Creatinine (mg/dL), mean (standard deviation)	0.05 (0.015)	0.05 (0.013)
Estimated fetal weight (g), mean (standard deviation)	1153 (300.4)	1153 (217.7)
Fetal weight percentile, median [interquartile range]	6.0 [2.1-24.8]	9.5 [1.7-22.5]
Absent blood flow on umbilical artery Doppler, n (%)	2 (3.4)	4 (6.7)

[a] Percentage of each group.

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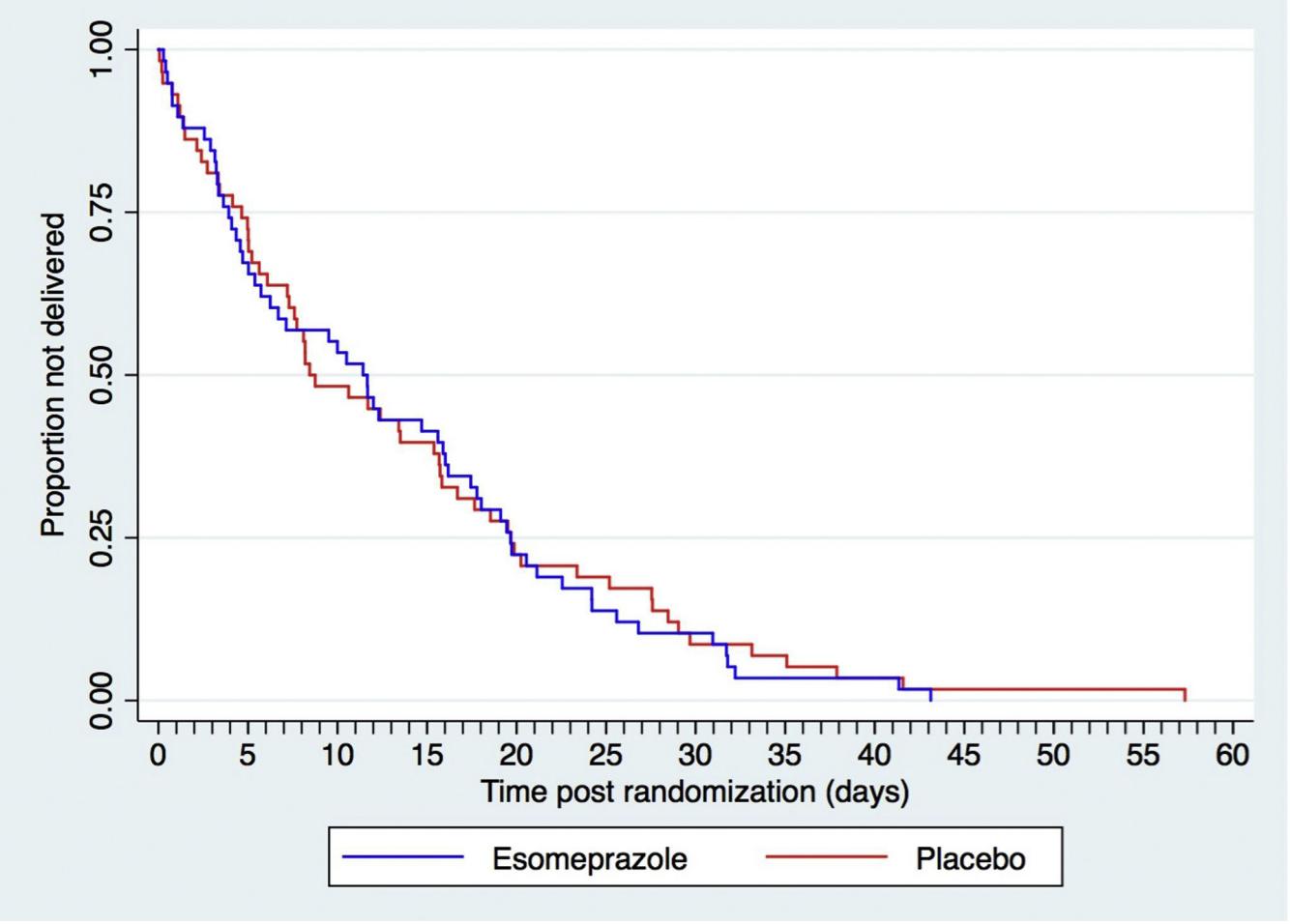
use of commercially available enzyme-linked immunosorbent assays. We also measured markers of endothelial dysfunction: endothelin-1, vascular endothelial cell adhesion molecule-1 (VCAM-1).

Total RNA was extracted from the placental biopsy specimens that were collected at delivery; the expression of sFlt1, PlGF, vascular endothelial growth factor-1, and the anti-oxidant molecule heme oxygenase-1 was measured by polymerase chain reaction (Supplemental Material).

Adherence and adverse events

Medication adherence was checked daily. After delivery, the treatment packs were collected, and the remaining tablets were counted. The trial midwife reviewed participants daily for adverse events. Serious adverse events were reported to the Data Monitoring and Safety Committee and Health Research Ethics Committee and were handled in accordance with Good Clinical Practice guidelines.

FIGURE 2
Survival curve



Survival curve shows the proportion of trial participants who remained undelivered, graphed against the number of days of gestation after randomization. *Blue* indicates the women who were treated with esomeprazole; *red* indicates the women who were treated with placebo.

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Statistical analysis

The sample size was based on data on the duration of expectant management at Tygerberg Hospital.[15] To identify a gain in gestation of 5 days, we needed to recruit 86 women (90% power, 2-sided alpha 0.05). This sample size was multiplied by 1.15 to statistically correct for non-normality. An additional 10 per arm were added to account for anticipated drop-outs. Thus, a total of 120 participants (60 per arm) had to be recruited.

Statistical analyses were performed on an intention-to-treat principle. A 2-sided *P*-value $<.05$ was considered to indicate statistical significance. The primary outcome was tested with the use of quantile regression analysis with the treatment group and gestational strata as covariates. Results are presented as median group difference with 95% confidence interval (95% CI). Survival analyses were done with Cox proportional hazards regression and graphed with Kaplan-Meier survivorship curves. Continuous variables were compared with either *t*-test (normally distributed variables) or Mann-Whitney *U* (nonnormally distributed data). Categoric values were compared with the use of the Fisher's exact test.

For circulating biomarker studies, between-group comparisons of circulating analyte concentrations were performed by a marginal mean model that was estimated with the use of generalized estimating equations to allow for both within patient correlation and missing samples. Graphic presentation used median, 25th, and 75th percentiles that were calculated from samples that were available at each day after random assignment. A smoothed scatterplot of these quantiles was constructed with the use of kernel-weighted local polynomial regression over a prespecified number of time units each side of the time of interest. The analysis used an Epanechnikov kernel function, automatic optimization of the degree of polynomials, and a bandwidth of 4 days.

Results

Trial participants

Participants were recruited from January 2016 until April 2017

TABLE 2
Outcomes according to trial group

Outcome	Esomeprazole (n=59)	Placebo (n=60)	*P* value
Primary			
Prolongation of gestation, d			
Median [interquartile range]	11.4 [3.6–19.7]	8.3 [3.8–19.6]	.31
Mean (standard deviation)	12.9 (10.8)	13.1(12.2)	
Gestation at delivery (wk+d), median [interquartile range]	31+2 [29+3–33+3]	31+3 [29+3–33+4]	.93
Secondary			
Composite maternal outcome, n (%)[a]	1 (1.7)	4 (6.7)	.36
Individual maternal outcomes			
Eclampsia, n (%)	0	3 (5.0)	.24
Pulmonary edema, n (%)	1 (1.7)	1 (1.7)	.99
Admission to high care unit or intensive care unit, n (%)	3 (5.1)	6 (10.0)	.49
Proteinuria ≥3g/24h, n (%)	22 (37.3)	24 (40)	.85
Systolic blood pressure >160 mm Hg, n (%)	29 (49.2)	24 (40.0)	.36
Diastolic blood pressure >110 mm Hg, n (%)	13 (22.0)	8 (13.3)	.24
Highest systolic blood pressure during trial (mm Hg), mean (standard deviation)	160 (11.9)	160 (12.3)	.91
Highest diastolic blood pressure during trial (mm Hg), mean (standard deviation)	102 (10.6)	101 (8.7)	.57
Platelet count <50×109, n (%)	0	1 (1.7)	.99
HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome, n (%)	5 (8.5)	3 (5.0)	.49
Aspartate aminotransferase (level) >60 μ/L, n (%)	3 (5.1)	1 (1.7)	.30
Hemolysis (lactate dehydrogenase >600 μ/L) or hemolysis on peripheral blood smear or decreased haptoglobin, n (%)	2 (3.4)	3 (5.0)	.99
Placental abruption, n (%)	0	6 (10.0)	.03
Major postpartum hemorrhage, n (%)	0	3 (5.0)	.24
Thromboembolic disease, n (%)	1 (1.7)	0	.99
Moderate-to-severe ascites, n (%)	7 (11.9)	4 (6.7)	.36
Composite fetal outcome, n (%)[b]	49 (83.1)	45 (75)	.37
Individual fetal outcomes			
Persistent absent flow in umbilical artery Doppler, n (%)	4 (6.8)	7 (11.7)	.53
Redistribution in the middle cerebral artery, n (%)	28 (47.5)	27 (45)	.85
Growth restriction (estimated fetal weight <10th percentile), n (%)	38 (64.4)	30 (50)	.14
Significant changes in fetal heart rate pattern necessitating delivery, n (%)	28 (47.5)	26 (43.3)	.74
Intrauterine death, n (%)	1 (1.7)	1 (1.7)	.99
Neonatal composite outcome, n (%)[c]	10 (16.9)	11 (18.3)	.88

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(Figure 1). Of 124 women who were admitted with preterm preeclampsia who were considered eligible, 4 women declined to participate (96.7% recruitment rate). One participant in the esomeprazole group was excluded after randomization because it was later discovered that she did not meet the trial criteria for a diagnosis of preeclampsia because she did not have significant hypertension and proteinuria. This left 59 women in the esomeprazole group. Two participants in this group were given the incorrect treatment pack and received placebo.

TABLE 2
Outcomes according to trial group *(continued)*

Outcome	Esomeprazole (n=59)	Placebo (n=60)	*P* value
Individual neonatal outcomes			
Neonatal death within 6 weeks after the due date, n (%)	7 (11.9)	9 (15.0)	.67
Grade III or IV intraventricular hemorrhage, n (%)	2 (3.4)	0	.24
Necrotizing enterocolitis, n (%)	4 (6.8)	3 (5.0)	.72
Bronchopulmonary dysplasia, n (%)	1 (1.7)	0	.50
Apgar score <7 at 5 minutes, n (%)	1 (1.7)	7 (11.7)	.06
Umbilical artery pH <7.05, n (%)	1/35 (2.9)	2/34 (5.9)	.61
Surfactant use, n (%)	14 (23.7)	9 (15.0)	.25
Neonatal intensive care unit admission, n (%)	8 (13.6)	4 (6.7)	.24
High care unit admission, n (%)	53 (89.8)	45 (75.0)	.05
Intubation and mechanical ventilation, n (%)	6 (10.2)	6 (10.0)	.99
Continuous positive airway pressure support, n (%)	46 (78.0)	39 (65.0)	.16
Grade III or IV hyaline membrane disease, n (%)	7 (11.9)	9 (15.0)	.79
Retinopathy of prematurity, n (%)	2 (3.4)	0	.24
Neonatal sepsis, n (%)	9 (15.3)	5 (8.3)	.27
Birthweight (g), mean (standard deviation)	1343 (466.5)	1379 (441.3)	.54
Discharge time (d), median [interquartile range]	3 (3–5)	3 (3–4)	.24

NOTE: No participant had any of the following outcomes: maternal death, severe renal impairment, cerebral vascular event, liver hematoma or rupture, posterior reversible encephalopathy syndrome, left ventricular failure, serum creatinine >125 μmol, disseminated intravascular coagulation, home oxygen support, persistent reversed flow in the umbilical artery Doppler.

[a] Included the occurrence of any of the following serious maternal outcomes: maternal death, eclampsia, pulmonary edema (oxygen saturation ≤90%, with clinical signs and symptoms that required treatment), severe renal impairment or the need for dialysis, a cerebral vascular event, and liver hematoma or rupture; [b] Reversed a-wave in the ductus venosus, significant changes in fetal heart rate pattern that necessitated delivery, intrauterine fetal death, fetal growth restriction, persistent reversed flow in the umbilical artery, redistribution in the middle cerebral artery Doppler, reversed a-wave in the ductus venosus Doppler; [c] Neonatal death within 6 weeks after the expected due date, grade III or IV intraventricular hemorrhage, necrotizing enterocolitis; and bronchopulmonary dysplasia.

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One participant in this group declined hospital treatment 1 week after randomization, left the hospital, and returned with a stillbirth. Sixty women were allocated to placebo, and all were included in the analysis. One participant delivered before taking her trial medication, and 1 participant was diagnosed with systemic lupus erythematosus after randomization. One participant in this group stopped taking her medication a few days before delivery. The maternal characteristics and obstetrics history of the cohort are shown in Table 1.

The median gestational age at randomization was 29 weeks 4 days in the esomeprazole group and 29 weeks 5 days in the placebo group. The placebo group had a higher median maternal age at enrolment. There were also more multiparous women, women with underlying hypertension, and women who had a previous pregnancy complicated by hypertension in the placebo group.

Primary outcome

The median time from randomization to delivery was 11.4 days (mean, 12.9 days) in the esomeprazole group vs 8.3 days (mean, 13.1 days) in the placebo group. There was no significant difference in median prolongation between treatment groups either unadjusted (median difference, 3.0; 95% CI, −2.9 to 8.8; *P*=.31) or adjusted for gestational age strata (median difference, 0.81; 95% CI, −5.1 to 6.7; *P*=.79). There was also no difference in the median prolongation between strata when adjusted for treatment group (median difference, 3.0; 95% CI, −3.2 to 9.2; *P*=.34) days.

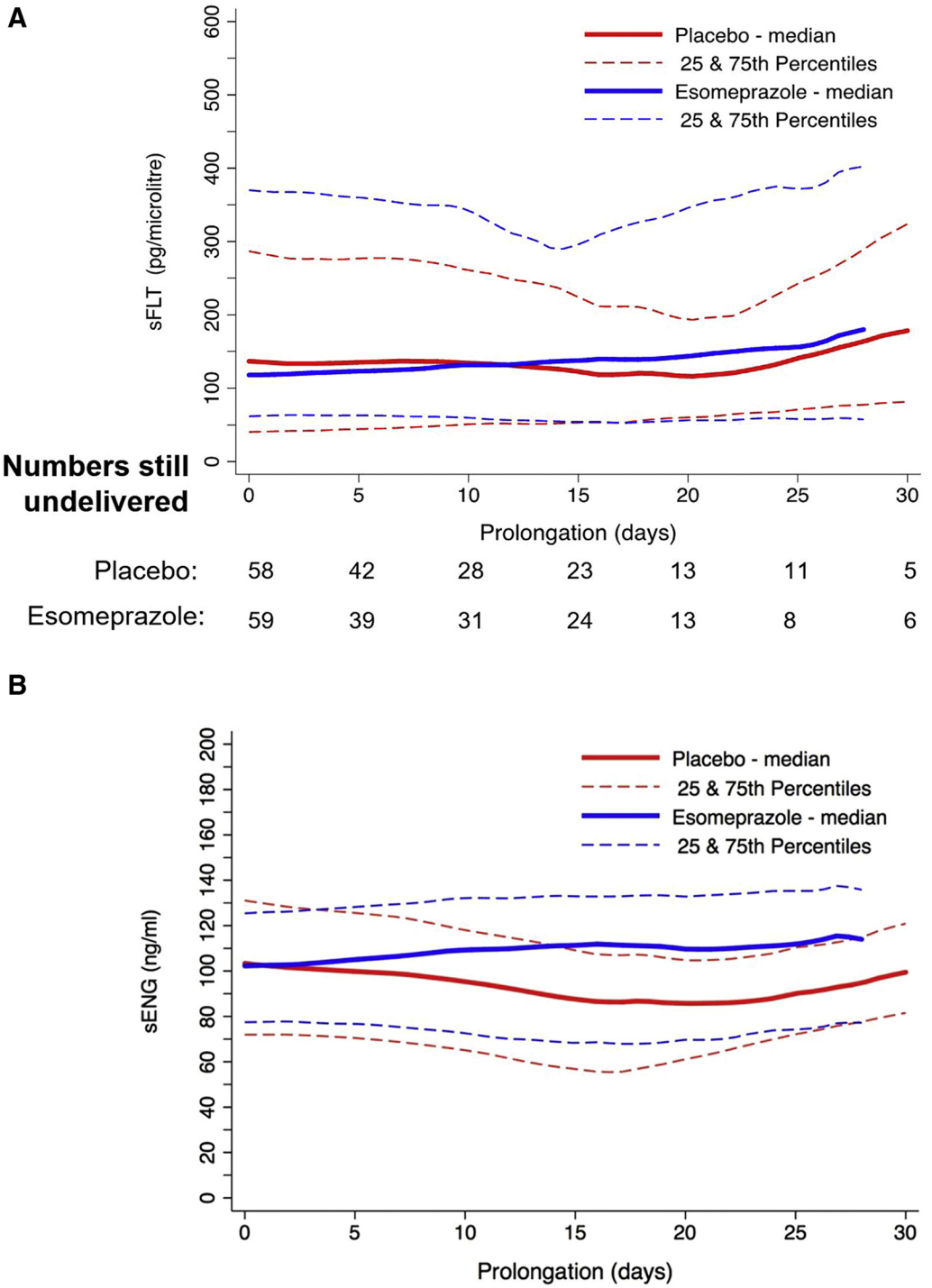
There was no difference in the instantaneous hazard of delivery, at any time, between the 2 treatment arms for either stratum (Figure 2). The estimated hazard ratio was 1.13 (95% CI, 0.70–2.17; *P*=.70) for <29 weeks and 1.07 (95% CI, 0.68–1.68; *P*=.78) for ≥29 weeks.

Secondary outcomes

There were no significant differences between treatment groups for any of the maternal, fetal, and neonatal composite or individual outcomes (Table 2), except for placental abruption. There were no placental abruptions (0/59) in the esomeprazole group and 10% (6/60) in the placebo group (*P*=.01), which was not significant when we adjusted for the fact that we performed multiple comparisons for other secondary outcomes (*P*=.14).

SFlt1 and soluble endoglin are antiangiogenic factors that are increased

FIGURE 3
Circulating plasma levels of antiangiogenic factors in women who were treated with either placebo or esomeprazole



A, Median circulating plasma soluble fms-like tyrosine kinase 1 concentrations (*solid lines*) and 25th and 75th percentiles (*dotted lines*) among participants administered placebo (*red*) or esomeprazole (*blue*). There were no differences in circulating soluble fms-like tyrosine kinase 1 levels between groups. **B,** Median circulating plasma soluble endoglin concentrations (*solid line*), and 25th and 75th percentiles (*dotted line*) among participants administered placebo (*red*) or esomeprazole (*blue*). There were no differences in circulating soluble endoglin levels between groups. Numbers that were still undelivered at each 5-day time point and that could have contributed to the data for soluble fms-like tyrosine kinase 1 or soluble endoglin are shown in **A.**

sENG, soluble endoglin; *sFlt1*, soluble fms-like tyrosine kinase 1.

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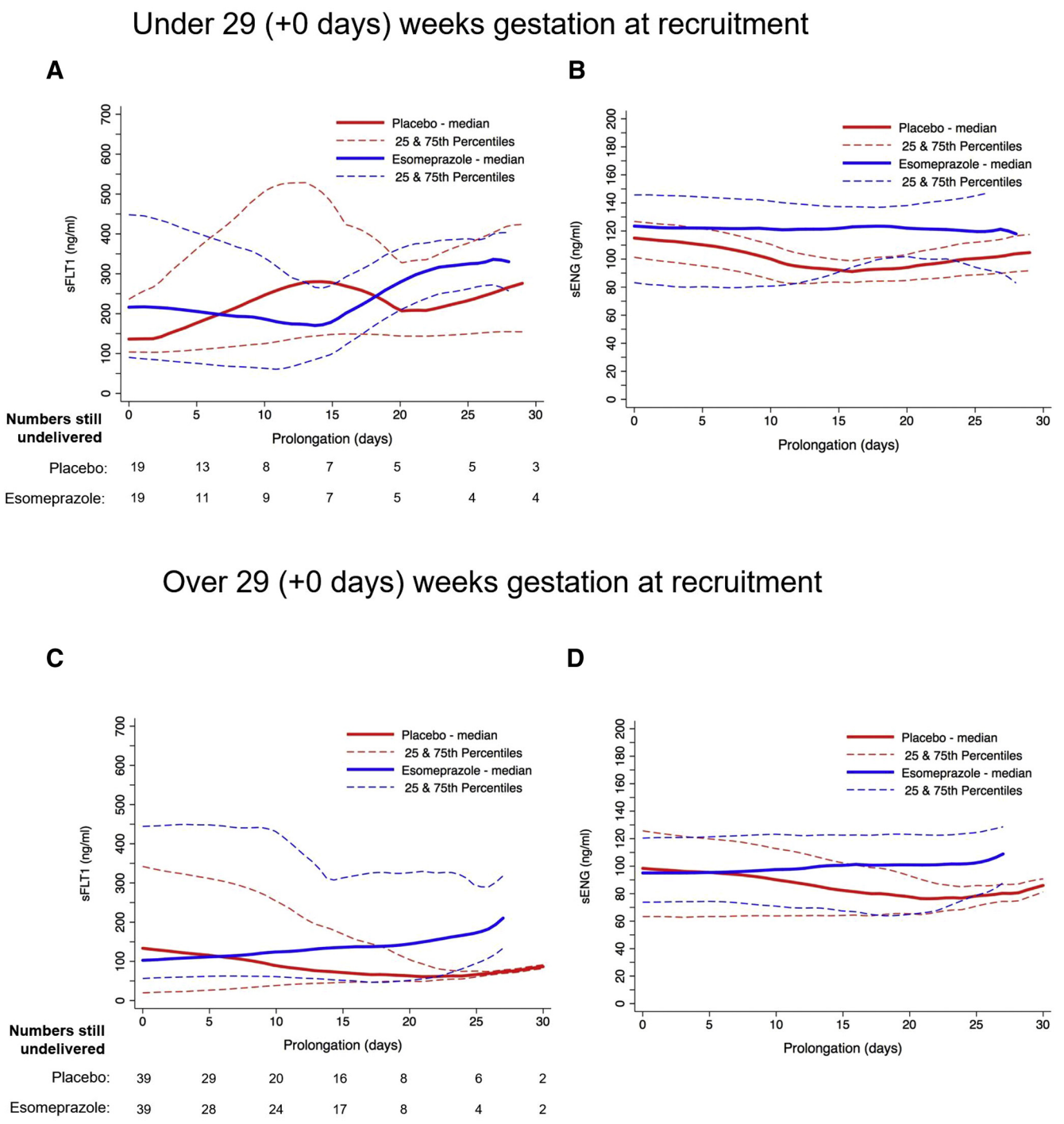
FIGURE 4
Circulating plasma levels of antiangiogenic factors in women who were treated with either placebo or esomeprazole



The graphs show analyte concentrations among the subcohorts of women who were either under (**A** and **B**) or over (**C** and **D**) 29 weeks gestation at recruitment. All graphs depict median circulating plasma concentrations (*solid lines*) of analytes and the 25th and 75th percentiles (*dotted lines*). None of the comparisons between esomeprazole (*blue*) and placebo (*red*) were significant. Numbers that were still undelivered at each 5-day time point and that could have contributed to the data are shown in **A** and **C** for soluble fms-like tyrosine kinase 1. The numbers that were left undelivered for soluble endoglin for **B** are the same as that shown in **A** for soluble fms-like tyrosine kinase 1; and the numbers that were left undelivered for **D** are the same as that shown in **C** for soluble fms-like tyrosine kinase 1.

sENG, soluble endoglin; *sFlt1*, soluble fms-like tyrosine kinase 1.

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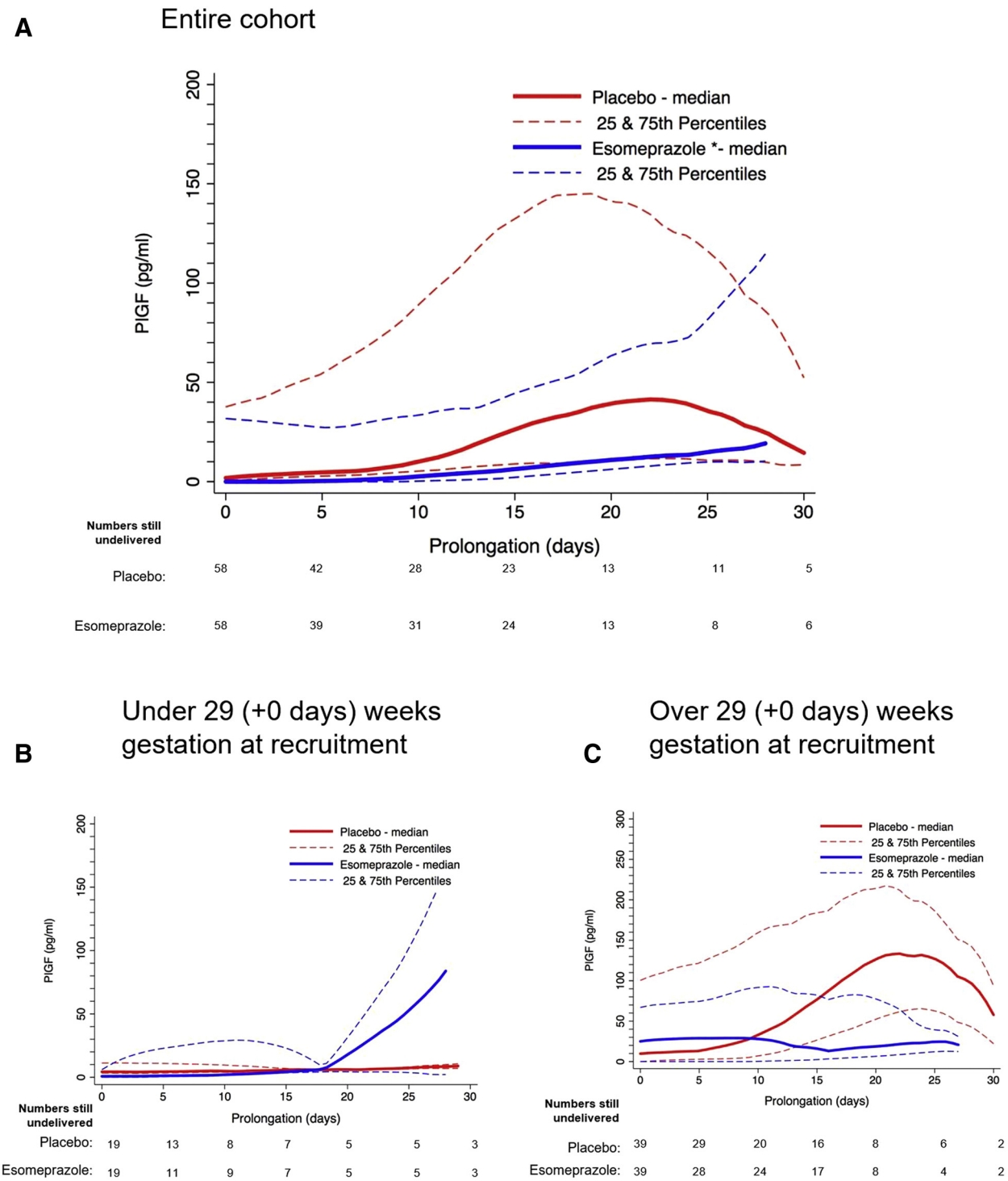
FIGURE 5
Circulating plasma levels of placental growth factor in women who were treated with either placebo or esomeprazole



The graphs show analyte for **A,** the entire cohort or the entire cohort split according to whether women were **B,** under or **C,** over 29 weeks gestation at recruitment. All graphs depict median circulating plasma concentrations (*solid lines*) of analytes and the 25th and 75th percentiles (*dotted lines*). None of the comparisons between esomeprazole (*blue*) and placebo (*red*) were significant. Numbers that were still undelivered at each 5-day time point and could have contributed to the data are shown below each graph.

PlGF, placental growth factor.

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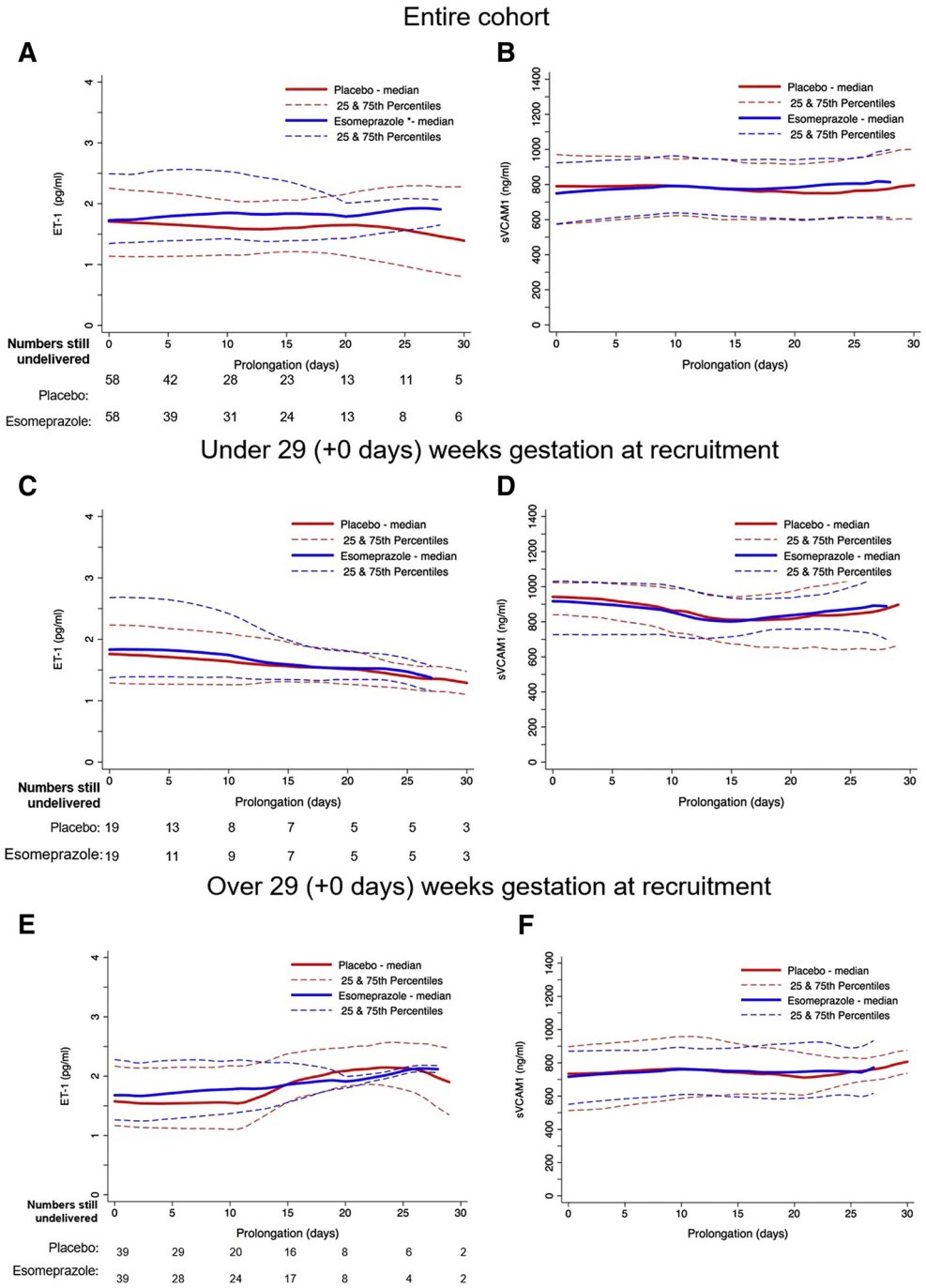
FIGURE 6
Circulating plasma levels of endothelin1 and soluble vascular cell adhesion molecule-1 in women who were treated with either placebo or esomeprazole



The graphs show analyte concentrations among **A** and **B,** the entire cohort or the entire cohort split according to whether women were **C** and **D,** under or **E** and **F,** over 29 weeks gestation at recruitment. All graphs depict median circulating plasma concentrations (*solid lines*) of analytes, and the 25th and 75th centiles (dotted lines). None of the comparisons between esomeprazole (*blue*) and placebo (*red*) were significant. Numbers that were still undelivered at each 5-day time point and that could have contributed to the data for **A** and **B** are shown below graph **A**; numbers that were still undelivered at each 5-day time point and that could have contributed to the data for **C** and **D** are shown below graph **C**; numbers that were still undelivered at each 5-day time point and that could have contributed to the data for **E** and **F** are shown below graph **E**.

ET-1, endothelin -1; *sVCAM1*, soluble vascular cell adhesion molecule-1.

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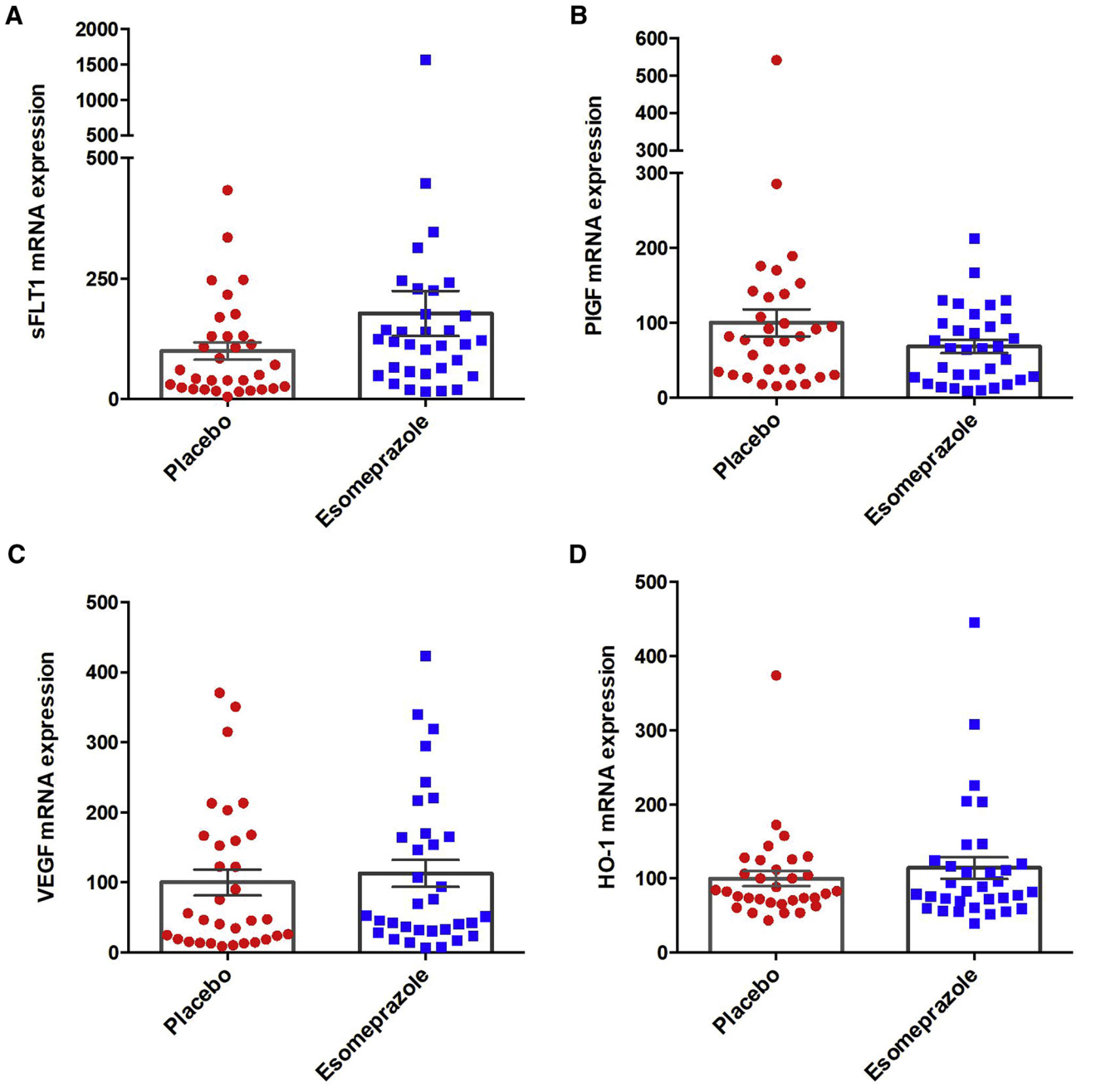
FIGURE 7
Placental messenger RNA expression



A, Soluble fms-like tyrosine kinase 1, **B,** placental growth factor, **C,** vascular endothelial growth factor, and **D,** heme oxygenase -1 in placental tissues that were collected from women who received placebo (n=32) or esomeprazole (n=33). None of the comparisons were significant. Data are mean fold change±standard error of the mean.

HO-1, heme oxygenase-1; *mRNA*, messenger RNA; *PlGF*, placental growth factor; *sFlt1*, soluble fms-like tyrosine kinase 1; *VEGF*, vascular endothelial growth factor.

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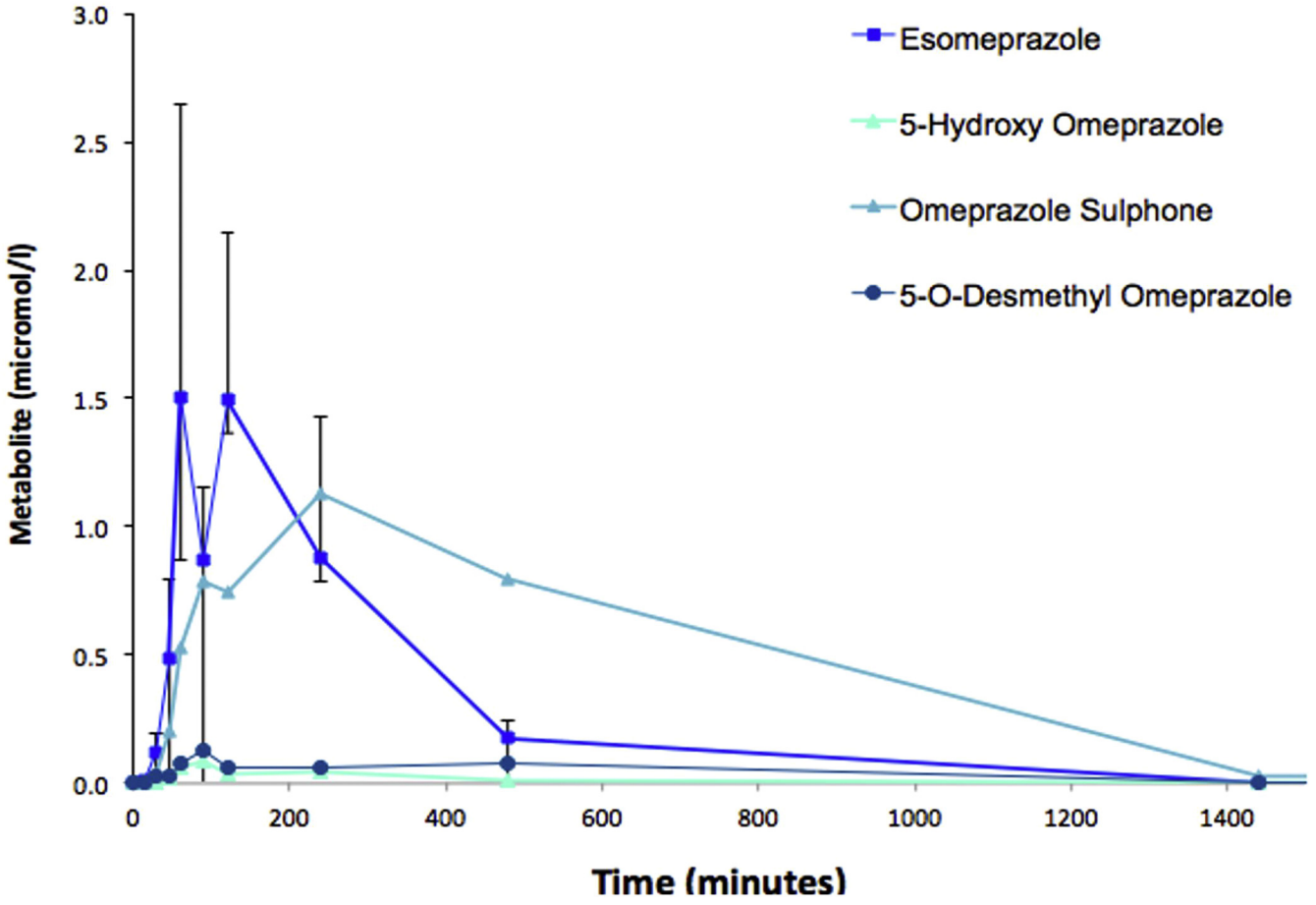
significantly in the circulation of pregnant women with preeclampsia and may have a role in the pathophysiology of the disease. Circulating sFlt1 and soluble endoglin concentrations were extremely high among trial participants, and there were no significant differences in concentrations on serial samples (which were obtained from those who were still undelivered at each time point) between the groups (Figure 3; Figure 4 shows analyte concentrations split into the 2

FIGURE 8
Pharmacokinetic analysis



Pharmacokinetic analysis showed that esomeprazole was detectable in the maternal circulation, with levels peaking soon after administration and a decline in concentration by 500 minutes after administration. Metabolites of esomeprazole (5-hydroxy, 5-O-desmethyl and omeprazole sulphone) were also detectable at lower levels soon after administration with overall higher levels of the metabolite omeprazole sulphone and a steady decrease across the first 1400 minutes.

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gestational age strata). Concentrations of both rapidly declined after delivery, as expected. There were also no differences in circulating levels of PlGF (a proangiogenic factor that is decreased in preeclampsia; Figure 5), endothelin 1 (endogenous vasoconstriction factor that is increased in preeclampsia), or vascular cell adhesion molecule-1 (associated with endothelial dysfunction; Figure 6). Analysis of placental messenger RNA expression of sFlt1, PlGF, vascular endothelial growth factor (proangiogenic factor) and heme oxygenaes-1 (endogenous antioxidant molecule) showed no differences between the esomeprazole and placebo arms (Figure 7).

Esomeprazole pharmacokinetics

Esomeprazole and its metabolites were measured in 10 participants who were assigned randomly to esomeprazole; exposure was similar to that of healthy nonpregnant volunteers with area under the curve geometric means of 5.88 μmol•h/L (95% CI, 2.96−11.68 μmol•h/L; Figure 8).[16] In contrast, esomeprazole and these metabolites were all undetectable in 9 participants who were administered placebo. Concentrations of esomeprazole and the metabolites were extremely low in the umbilical cord blood taken at birth.

Adverse events and adherence

Adherence was excellent. Only 1 participant in the placebo group stopped taking the trial medication. There were no significant differences in the incidences of serious adverse events between the 2 groups (Table 3).

Comment

In our trial, a daily dose of 40 mg of oral esomeprazole did not prolong gestation statistically further than expectant management alone. Additionally, there

TABLE 3
Severe adverse events

Adverse event	Esomeprazole (n=59), n (%)	Placebo (n=60), n (%)	*P* value
Maternal			
Eclampsia	0	3 (5)	.24
Pulmonary edema	1 (1.7)	1 (1.7)	.99
Blood loss of >1000 mL	0	3 (5)	.24
Fetal/neonatal			
Intrauterine death	1 (1.7)	1 (1.7)	.99
Neonatal death	7 (11.9)	9 (15.0)	.67
Necrotizing enterocolitis	4 (6.8)	3 (5)	.68
Neonatal sepsis	9 (15.3)	5 (8.3)	.24
Intracranial hemorrhage	2 (3.4)	0	.15

NOTE: No participant had any of the following serious adverse events: maternal death, severe renal impairment, cerebral vascular event, liver or rupture, posterior reversible encephalopathy syndrome, left ventricular failure, disseminated intravascular coagulation, fetal or neonatal congenital anomaly.

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was no difference in any of the biomarker outcomes or secondary maternal, fetal, and neonatal outcomes, except for placental abruption. However, this is a secondary outcome and did not remain significant on an adjusted analysis.

Ours is only one of very few completed randomized trials to explore treatments for preterm preeclampsia. We have completed perhaps the fastest recruitment for a randomized trial of a drug treatment for preterm preeclampsia, and we achieved this at 1 site by undertaking our study in an area with a very high incidence of disease. It is also the first completed randomized treatment trial of preterm preeclampsia in which blood biomarkers of preeclampsia or endothelial dysfunction were measured, as well as placental messenger RNA expression of genes that are relevant to the pathophysiology of preeclampsia.

There was a nonsignificant trend in median prolongation in the esomeprazole group of 3 days; however, to show that such a difference is significant, we would have needed 402 participants in each arm (alpha error, 5% for 90% power; a post hoc analysis that was calculated from the actual length of gestation observed in the current trial). Despite this, there were no trends in the mean prolongation or the instantaneous hazard of delivery to support this. There was a decrease in the incidence of placental abruption, but this difference was no longer significant after we adjusted for the fact that we performed multiple comparisons for all the different secondary outcomes. Therefore, the significance of this finding, if any, is uncertain.

Esomeprazole is 97% bound to protein and 80% renally excreted. We were concerned that the significant proteinuria that often is associated with preterm preeclampsia may alter esomeprazole pharmacokinetics. Those who received esomeprazole had exposure levels similar to healthy nonpregnant volunteers that had been reported previously.[17] The esomeprazole concentrations that were observed in our participants were around the lower range of concentrations that were used in our preclinical in vitro studies.[6] Thus, although 40 mg may be an optimal dose that is effective in decreasing gastric pH,[18] it is possible that a higher dose or an intravenous dose, which has a higher exposure over time and peak concentration,[16] may be effective in treating preeclampsia.

There is now strong (though circumstantial) evidence that placental secretion of sFlt1 (which causes endothelial dysfunction) may be a significant driver of the disease.[5,19] We and others have pegged decreasing sFlt1 secretion as a strategy to treat preeclampsia.[6,20-24] We did not find changes in any of these markers, which provides biologic evidence to support our clinical findings that 40 mg of oral esomeprazole does not seem to arrest the disease course of preeclampsia once it is diagnosed.

We note that rescuing a pregnancy with advanced preterm disease with severe placental involvement may be a difficult proposition. It has been reported recently that proton inhibitor use, to combat reflux, was associated with decreased sFlt1, soluble endoglin, and endothelin-1 levels.[7] We believe it remains possible that a 40-mg dose may still have merit as a preventative treatment for preeclampsia and may be more realistic. Whether this is the case will also require clinical trials.

Esomeprazole is prescribed widely during pregnancy, and levels in the umbilical cord have not been reported previously. It was reassuring therefore that there was very little, or no, esomeprazole detected in umbilical cord blood that was sampled at birth among those who received the drug. It provides further reassurance that there is likely to be minimal fetal exposure and is consistent with epidemiologic data that show no adverse effects of PPIs on fetal development.[9-11]

There have not been many completed phase II clinical trials that have tested candidate treatments for preterm preeclampsia. Previous trials have met problems with recruitment. One of the main difficulties is that the incidence of disease is low in the developing world. Sildenafil was assessed in a single-site, double-blind randomized controlled trial in Brazil.[25] Over a 28-month period, 100 women were recruited. There was a significant prolongation of gestation in the sildenafil group of 4 days; however, 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 temporize disease progression. Antithrombin was assessed to treat preterm preeclampsia in the PRESERVE-1 trial

that enrolled 120 women from 23 tertiary hospitals in the United States over 28 months (ISRCTN23410175).[26] There was no difference in prolongation of pregnancy or composite neonatal outcomes.[27] Trials that have assessed serelaxin (NCT01566630), pravastatin, high doses of antithrombin,[28] and celecoxib (NCT00442676) have been attempted, but all were terminated, perhaps because of poor recruitment.

A potential limitation of our trial is that we were powered to detect a 5-day prolongation of pregnancy and therefore cannot exclude the possibility that 40 mg of esomeprazole may be effective in prolonging pregnancy by 3 days (there was a nonsignificant median difference of 3 days). However, given the findings of pharmacokinetic and biomarker studies, we are inclined to pursue further trials with higher doses rather than to repeat this same trial with a larger number of participants.

Our trial has several strengths. As noted, we performed an integrated trial in which we not only obtained data on clinical outcomes but also derived important insights by undertaking biomarker studies and pharmacokinetics that will inform our next trial. Furthermore, it was run at 1 center, which allowed us to obtain a high recruitment rate, to closely monitor compliance, and to collect uniform high-quality data. Importantly, by basing this trial at an academic center that is embedded within a population with a high incidence of preterm preeclampsia, we overcame the problem faced by previous trials of low recruitment.

In conclusion, in women with a diagnosis of preterm preeclampsia at 26−32 weeks gestation, a daily oral dose of 40 mg of esomeprazole did not prolong pregnancies. Circulating levels of sFlt1 and other antiangiogenic markers were extremely high among the cohort and were not lowered by esomeprazole. The drug appears safe and is well tolerated. In pharmacokinetic studies, we found that esomeprazole was present in the maternal circulation, but concentrations were relatively low compared with those required to elicit tissue/cell responses in our previous laboratory studies. This raises the possibility that higher doses may be effective. Reassuringly, levels of esomeprazole in the umbilical cord blood were very low, or not detectable, which provides further reassurance that very little reaches the fetal compartment.

Furthermore, we have developed and successfully completed a new protocol to evaluate drugs to treat preterm preeclampsia that embeds mechanistic insights and pharmacokinetics with clinical endpoints. We also completed recruitment in a reasonable timeframe by performing this trial in an area where the incidence of preterm preeclampsia is very high. We propose this may be an optimal approach when designing clinical trials for preterm preeclampsia. ■

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References

1. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol 2013;170:1–7.
2. Duley LM. The global impact of pre-eclampsia and eclampsia. Semin Perinatol 2009;33:130–7.
3. Baschat AA, Cosmi E, Bilardo CM, et al. Predictors of neonatal outcome in early- onset placental dysfunction. Obstet Gynecol 2007;109:253–61.
4. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.
5. Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation 2011;123:2856–69.
6. Onda K, Tong S, Beard S, et al. Proton pump inhibitors decrease soluble fms-like tyrosine kinase-1 and soluble endoglin secretion, decrease hypertension, and rescue endothelial dysfunction. Hypertension 2017;69:457–68.
7. Saleh L, Samantar R, Garrelds IM, van den Meiracker AH, Visser W, Danser AHJ. Low soluble fms-like tyrosine kinase-1, endoglin, and endothelin-1 levels in women with confirmed or suspected preeclampsia using proton pump inhibitors. Hypertension 2017;70:594–600.
8. Andersson T, Röhss K, Bredberg E, Hassan-Alin M. Pharmacokinetics and pharmacodynamics of esomeprazole, the S-isomer of omeprazole. Aliment Pharmacol Ther 2001;15:1563–9.
9. Matok I, Levy A, Wiznitzer A, Uziel E, Koren G, Gorodischer R. The safety of fetal exposure to proton-pump inhibitors during pregnancy. Dig Dis Sci 2012;57:699–705.
10. Gill SK, O'Brien L, Einarson TR, Koren G. The safety of proton pump inhibitors (PPIs) in pregnancy: a meta-analysis. Am J Gastroenterol 2009;104:1541–5.
11. Pasternak B, Hviid A. Use of proton-pump inhibitors in early pregnancy and the risk of birth defects. N Engl J Med 2010;363:2114–23.
12. Cluver CA, Walker SP, Mol BW, et al. Double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): a study protocol. BMJ Open 2015;5:e008211.
13. Hall DR. Understanding expectant management of pre-eclampsia. Obstet Gynaecol Forum 2016;26:22–7.
14. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap): a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377–81.
15. Hall DR, Odendaal HJ, Steyn DW, Grove D. Expectant management of early onset, severe pre-eclampsia: maternal outcome. BJOG 2000;107:1252–7.
16. Hassan-Alin M, Andersson T, Bredberg E, Röhss K. Pharmacokinetics of esomeprazole after oral and intravenous administration of single and repeated doses to healthy subjects. Eur J Clin Pharmacol 2000;56:665–70.
17. Chunduri RHB, Dannana GS. Development and validation of a high throughput UPLC−MS/MS method for simultaneous quantification of esomeprazole, rabeprazole and levosulpiride in human plasma. J Pharm Anal 2016;6:190–8.
18. Röhss K, Hasselgren G, Hedenström H. Effect of esomeprazole 40 mg vs omeprazole 40 mg on 24-hour intragastric ph in patients with symptoms of gastroesophageal reflux disease. Dig Dis Sci 2002;47:954–8.
19. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649–58.
20. Brownfoot FC, Tong S, Hannan NJ, Hastie R, Cannon P, Kaitu'u-Lino TJ. Effects of simvastatin, rosuvastatin and pravastatin on soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sENG) secretion from human umbilical vein endothelial cells, primary trophoblast cells and placenta. BMC Pregnancy Childbirth 2016;16:117.

21. Brownfoot FC, Hastie R, Hannan NJ, et al. Metformin as a prevention and treatment for preeclampsia: effects on soluble fms-like tyrosine kinase 1 and soluble endoglin secretion and endothelial dysfunction. Am J Obstet Gynecol 2016;214:356.e1–15.

22. Brownfoot FC, Tong S, Hannan NJ, et al. Effects of pravastatin on human placenta, endothelium, and women with severe preeclampsia. Hypertension 2015;66:687–97;discussion 445.

23. Hannan NJ, Brownfoot FC, Cannon P, et al. Resveratrol inhibits release of soluble fms-like tyrosine kinase (sFlt-1) and soluble endoglin and improves vascular dysfunction - implications as a preeclampsia treatment. Sci Rep 2017;7:1819.

24. Thadhani R, Kisner T, Hagmann H, et al. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. Circulation 2011;124:940–50.

25. Vigil-De Gracia P, Ludmir J. Perinatal and hemodynamic evaluation of sildenafil citrate for preeclampsia treatment. Obstet Gynecol 2016;128:1181–2.

26. Paidas MJ, Sibai BM, Triche EW, Frieling J, Lowry S; PRESERVE-1 Study Group. Exploring the role of antithrombin replacement for the treatment of preeclampsia: a prospective randomized evaluation of the safety and efficacy of recombinant antithrombin in very preterm preeclampsia (PRESERVE-1). Am J Reprod Immunol 2013;69:539–44.

27. Sibai B, Paidas MJ. LB02: randomized double-blind placebo controlled evaluation of the safety and efficacy of recombinant antithrombin vs placebo in preterm preeclampsia. Am J Obstet Gynecol 2017;216(suppl):S559–60.

28. D'Angelo A, Valsecchi L. High dose antithrombin supplementation in early preeclampsia: a randomized, double blind, placebo-controlled study. Thromb Res 2016;140:7–13.

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Supplemental Material

Measuring plasma concentrations of esomeprazole and its metabolites

Plasma concentrations of esomeprazole and its metabolites (5-hydroxy omeprazole, omeprazole sulphone, and 5-O-desmethyl omeprazole) were determined with the use of a validated ultra-performance liquid chromatography–tandem mass spectrometry method. A Waters Acquity ultra-performance liquid chromatograph (Waters Corporation, Milford, MA) with a Waters HSS T3 column was linked to a Xevo TQ-S mass spectrometer (Waters Corporation). A gradient of 0.1% formic acid to acetonitrile was used, with d3-esomeprazole as the internal standard. In brief, the drugs were extracted with a buffered (2 mmol/L ammonium formate; pH 5.5) acetonitrile 60% solution, and the precipitated plasma proteins were separated by centrifugation (12 000*g*). The intra- and interday accuracy of the quality control samples was >90% and 85%, respectively; the intra- and interday precision was <11% and <15%, except for 5-O-desmethyl omeprazole that was 20% at the lower limit of quantification. The limit of quantitation was 1 ng/mL for all analytes. Phoenix WinNonlin software (version 9.0; Certara, Princeton, NJ) was used to characterize the pharmacokinetic parameters of esomeprazole with the use of non-compartmental analyses. The area under the plasma concentration-time curve was calculated for the 24-hr dosing interval with the log-linear trapezoidal method. Pharmacokinetic data were summarized as geometric mean values with 95% confidence intervals.

Preparation of placental tissue for analysis

Placental tissue was dissected from the whole placenta. Four pieces were dissected from distant sites; the tissue pieces were then washed in sterile phosphate-buffered saline solution, and smaller pieces were then dissected (to allow appropriate penetration of RNA preservation buffer [RNAlater]). Each piece was immersed in RNAlater according to manufacturer's instruction. Tissue samples were then blotted dry, snap frozen, and stored at −80°C until subsequent analysis.

Measuring analytes in the plasma by enzyme-linked immunosorbent assay

Patient plasma was assessed with the use of enzyme-linked immunosorbent assay for the presence of the following soluble factors: soluble Flt-1 (DuoSet VEGF R1/Flt-1 kit; R&D Systems by Bioscience, Waterloo, Australia), soluble endoglin (DuoSet Human Endoglin CD/105; R&D Systems), placental growth factor (P DuoSet PlGF; R&D Systems), endothelin-1 (Quantikine endothelin-1; R&D Systems), and soluble vascular cell adhesion molecule-1 (human VCAM-1/CD106 DuoSet; R&D Systems). Optical density for enzyme-linked immunosorbent assays was determined with a BioRad X-Mark microplate spectrophotometer (BioRad Laboratories, Inc, Hercules, CA). Protein levels were determined with BioRad Microplate Manager software (version 6; BioRad Laboratories, Inc).

Measuring expression of genes in placental tissue

Total RNA was extracted from placental tissue (from placebo [n=32] and esomeprazole [n=33] treated women) with the use of the RNeasy mini kit (Qiagen, Valencia, CA) and was quantified with a Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE). RNA (0.2 μg) was converted to complementary DNA with the use of a high-capacity complementary DNA reverse transcriptase kit (Applied Biosystems Life Technologies Corporation, Carlsbad, CA), according to manufacturer guidelines.

Quantitative polymerase chain reaction was performed with the use of Taqman gene expression assays for the following genes: *sFlt1*, *HO-1*, *PlGF* and *VEGFA*. Polymerase chain reaction was performed on the CFX 384 (BioRad Laboratories, Inc) using FAM-labeled Taqman universal polymerase chain reaction mastermix (Applied Biosystems) with the following run conditions: 50°C for 2 minutes, 95°C for 10 minutes, 95°C for 15 seconds, 60°C for 1 minute (40 cycles). All data were normalized to the housekeeping genes *TOP1* and *CYC1* as an internal control and calibrated against the average cycle threshold of the control samples. The results were expressed as fold-change relative to control subjects. All samples were run in triplicate.