Pravastatin, proton-pump inhibitors, metformin, micronutrients, and biologics: new horizons for the prevention or treatment of preeclampsia

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There has been increasing research momentum to identify new therapeutic agents for the prevention or treatment of preeclampsia, drugs that can affect the underlying disease pathophysiology. Molecular targets of candidate treatments include oxidative stress, antiangiogenic factors, and the angiotensin, nitric oxide, and proinflammatory pathways. The proposed treatments undergoing preclinical and clinical trial evaluation are thought to act on placental or endothelial disease or both. Most have adopted the pragmatic strategy of repurposing drugs. Of all the therapeutic agents proposed, pravastatin has received the most interest. There are preclinical studies showing that it has pleiotropic actions that favorably impact on multiple molecular targets and can resolve a preeclampsia phenotype in many animal models. An early phase clinical trial suggests that it may have therapeutic activity. Several large prevention trials are planned or ongoing and, when completed, could definitively address whether pravastatin can prevent preeclampsia. Proton-pump inhibitors, metformin, and sulfasalazine are other drugs with preclinical evidence of multiple molecular actions that could resolve the pathophysiology of preeclampsia. These agents are also currently being evaluated in clinical trials. There have been many recent preclinical studies identifying the potential of numerous natural compounds to treat preeclampsia, such as plant extracts and micronutrients that have potent anti-inflammatory or antioxidant activity. Recent preclinical studies have also proposed novel molecular-targeted strategies, such as monoclonal antibodies targeting tumor necrosis factor alpha, placental growth factor, and short interfering RNA technology, to silence the gene expression of soluble fms-like tyrosine kinase-1 or angiotensinogen. Other treatment approaches that have transitioned to human trials (ranging from single-arm to phase III trials that have been completed or are ongoing) include folic acid, nitric oxide donors (such as L-arginine), recombinant antithrombin III, digoxin immune antigen-binding fragment, and melatonin. There have been case series showing the removal of circulating soluble fms-like tyrosine kinase-1 may help stabilize the disease and prolong pregnancy. Interestingly, there are case reports suggesting that monoclonal antibody eculizumab (complement inhibitor) may have therapeutic potential. If new agents are discovered that are proven to be effective in preventing or treating preeclampsia, the potential to improve global maternal and perinatal health will be significant.

Key words: antioxidants, antithrombin III, apheresis, digoxin immune antigen-binding fragment, esomeprazole, L-arginine, melatonin, metformin, nitric oxide, pravastatin, preeclampsia, short interfering RNAs, soluble fms-like tyrosine kinase-1, treatment

Introduction

Preeclampsia complicates around 3% to 5% of pregnancies.[1,2] Unlike most major pregnancy complications, it can place women and fetuses at risk,[3] and its aftereffects can linger for decades in the mother (increases the risk of major cardiovascular diseases[4,5]) and child (increases the risk of chronic disabilities and developmental delays arising from fetal growth restriction and prematurity).

There are a dearth of drugs to treat the pathophysiological progression of preeclampsia. Only 1 drug, aspirin, clearly prevents the condition, and none has conclusively shown to improve the disease. A high-quality Cochrane systematic review that synthesized data from 60 trials (36,716 participants) concluded that aspirin modestly reduces proteinuric preeclampsia by around 18% (relative risk [RR] 0.82; 95% confidence interval [CI], 0.77–0.88).[6] Although the RR reduction for preterm preeclampsia is greater,[7,8] this subtype only represents a small fraction of cases. Furthermore, although the Cochrane review shows encouraging trends, it remains unclear whether calcium prevents preeclampsia.[9,10] Therefore, there is a pressing need to identify new therapeutic agents to decrease the global burden of the disease.

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Here, we will review research efforts to develop new prevention strategies and treatments for preeclampsia. An overview of the pathogenesis of preeclampsia will be presented first. We will then discuss various candidate therapeutic agents, summarizing which steps in the pathophysiological pathway they are thought to act on and present the preclinical and clinical evidence. Figure 1 provides a broad overview of the classes of candidate therapeutic agents that will be discussed. As the scope of this review will be to examine emerging therapeutic agents, we will not cover aspirin, calcium, or low-molecular-weight heparin.

Search strategy

To prepare this review, we performed a systematic search using the National Library of Medicine, National Center for Biotechnology Information. The following search terms were used: "preeclampsia or preeclampsia" and "treatment or drug or prevention or therapeutic." There were 17,557 hits with years spanning from 1914 to 2020. Examining the breakdown of articles published per year, there was a peak of manuscripts published that spanned from the years 2015 to 2019 (with 2020 tracking similar to 2019). Given the number of total articles and the intent to provide a contemporaneous update, we focused our systematic search on studies published from 2015. We identified 5075 articles published between 2015 and 2020. We screened these articles by reviewing the title and abstract. Relevant articles were then examined in depth and, if still relevant, discussed in this review. We also manually searched references before 2015. In addition, we also searched the US, African, European, and Australian clinical trial registries. For this review, we prioritized drugs with in vivo preclinical data, and we only included trials that had been preregistered.

Steps in the pathogenesis of preeclampsia being pursued as therapeutic targets

During embryo implantation in early pregnancy, the extravillous cytotrophoblast from the placenta invade and remodel the uterine spiral arteries in the myometrium. This creates a high-flow, low resistance blood supply to the maternal compartment that can adequately perfuse the placenta and sustain the pregnancy. Poor cytotrophoblast invasion that leads to poor placental implantation is the initial pathogenic event that gives rise to preeclampsia.[11]

Poor placentation in early pregnancy results in persistent placental hypoxia, ischemia-reperfusion injury, and placental oxidative stress (Figure 2).[12] The preeclamptic placenta reacts by releasing excessive amounts of soluble factors that enter the maternal circulation and cause endothelial dysfunction, maternal vascular injury, and hypertension.

Placentally derived soluble factors include antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1)[13,14] and soluble endoglin (sEng).[15] These factors bind and neutralize the actions of proangiogenic factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor beta. There are other placentally derived soluble factors, such as proinflammatory cytokines and placental debris.[16]

The soluble factors from the placenta can reduce the production of important vasoactive molecules (including the vasodilator, nitric oxide) and cause the local release of endothelially derived factors that further exacerbate the endothelial dysfunction. These include thromboxane and endothelin-1 (ET-1) (both induce vasoconstriction) and proinflammatory cytokines.

Although it is clearly not the primary event triggering the disease, sFlt-1 is likely to be a central disease driver of preeclampsia as it fulfills most criteria for establishing disease causation proposed by Sir Bradford Hill.[17] Circulating sFlt-1 is reliably increased with most cases of preeclampsia (consistency)[18,19]; levels are increased preceding clinical disease (temporality)[20,21]; levels correlate with disease severity (strength and biologic gradient)[14]; the link is biologically plausible (plausibility); and overexpressing sFlt-1 in animal models can recapitulate clinical features of the disease (experiment).[22,23] Consequently, sFlt-1 has been an important target for many striving to identify new therapeutic agents.

The presence of an increased proinflammatory response (both in the placenta and in the vasculature [systemic]) and oxidative stress in preeclampsia is well established.[24] Normal pregnancy evokes a proinflammatory state, which is exaggerated in preeclampsia.[25] Monocytes and granulocytes are activated, and an imbalance of circulating pro- and anti-inflammatory cytokines ensues, including an increase in tumor necrosis factor alpha (TNF-α) and interleukin-6.[26] This proinflammatory state in the blood vessels results in endothelial activation, local endothelial secretion of inflammatory cytokines and adhesion molecules, and a reduction in the vasodilators nitric oxide and prostaglandins.

The initial events of poor placental implantation and perfusion are thought to give rise to oxidative stress and free radical tissue damage, which cumulate in placental insufficiency.[27] This is evidenced in the placenta by an up-regulation of xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase (important sources of superoxide), increased lipid peroxidation, and activation of apoptotic pathways.[28–30] Because of placental oxidative stress and endothelial activation, oxidative stress is also present in the maternal vasculature, with elevated lipid peroxidation markers and decreased antioxidant capacity.[31]

Further to inflammation and oxidative stress, exacerbated hypercoagulation also results from endothelial activation.[32] Injury to the endothelium results in increased platelet adhesion, reduced fibrinolysis, and activation of the clotting cascade.[32] This is evidenced among women with preeclampsia by reduced antithrombin III[33] and platelet counts and an elevated fibronectin[34] and is especially apparent among women who develop HELLP syndrome (hemolysis, elevated liver enzymes, and

low platelet count) and disseminated intravascular coagulation.

Consequently, identifying drugs that dampen the inflammatory response or resolve oxidative stress has been another common therapeutic strategy.

Drug candidates to treat or prevent preeclampsia

Pravastatin

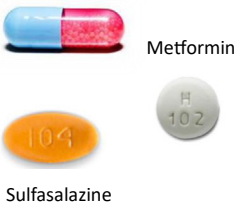
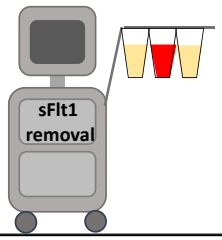
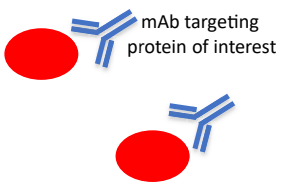
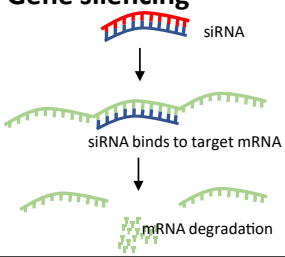
Pravastatin is a lipid-lowering drug widely taken to reduce the risk of cardiovascular events.[35] Over the past decade, it has received the most attention of all candidate drugs (besides aspirin and calcium) for its potential to treat or prevent preeclampsia.

Preclinical studies suggest that pravastatin may have beneficial actions on both placental and maternal vascular diseases. In vitro, the administration of pravastatin to placental tissues or cells up-regulates antioxidant pathways and can promote a favorable angiogenic profile by decreasing the secretion of sFlt-1 and sEng.[36] There is also experimental in vitro evidence that it resolves endothelial dysfunction in several experimental assays (reduces expression of vascular cell adhesion molecule 1 [VCAM-1] and ET-1 and leukocyte adhesion on endothelial cells and increases endothelial cell migration and adhesion).[36,37] This means that it could beneficially act on both the placental and maternal vascular diseases (Figure 1).

Several groups have reported that pravastatin can resolve the preeclampsia phenotype in various animal models (such as hypertension and proteinuria).[22,23,38–40] In these animal models, there is also evidence that pravastatin can reduce circulating sFlt-1, decrease inflammation, and up-regulate nitric oxide synthase (the enzyme in the endothelium that makes nitric oxide).[22,23,38–40]

Treatment using pravastatin during pregnancy may even improve long-term outcomes after pregnancy. Pravastatin given during pregnancy in a mouse model of preeclampsia (complement knockout model) resolved hypertension, left ventricular remodeling, and maternal renal lesions.[41] Pravastatin administered during pregnancy and 28 days after delivery in a rat model of preeclampsia (human angiotensinogen gene knocked in) resolved cardiovascular

FIGURE 1
Classes of therapeutic interventions being evaluated to treat preeclampsia

Type of therapeutic intervention	Description	Examples
Repurposed drugs Proton pump inhibitors  	The most commonly pursued approach **Advantage:** some drug safety information will be known, allowing efficacy trials to be fast tracked **Disadvantage:** Limits preclinical discovery to drugs that are already formulated (see comment section)	Pravastatin Esomeprazole Metformin Antithrombin III Sulfasalazine Melatonin
Plasma apheresis  	Filtration of the blood to remove lipids or excessive anti-angiogenic factors. **Advantage:** Small trials have shown it can remove circulating sFlt-1 (though transiently) **Disadvantage:** Invasive	Apheresis using columns that are designed to remove sFlt-1
Monoclonal antibodies  	Monoclonal antibodies are highly specific and can be engineered to limit placental transfer **Advantage:** Exquisitely specific **Disadvantage:** Expensive, and still needs more safety data in pregnancy	Etenercept (anti-TNFα) Eculizumab (complement inhibitor)
Gene silencing  	siRNA are short strands of RNA that exploit intracellular machinery to reduce the expression of target genes and proteins **Advantage:** Exquisitely specific **Disadvantage:** Still requires 'first in human' phase I trials	siRNAs targeting sFlt-1 *or* angiotensinogen

Of the examples listed, only siRNA technologies have not been tested in pregnant women.

maB, monoclonal antibody; *mRNA*, messenger RNA; *SFlt-1*, soluble fms-like tyrosine kinase-1; *siRNA*, short interfering RNA; *TNF-α*, tumor necrosis factor alpha.

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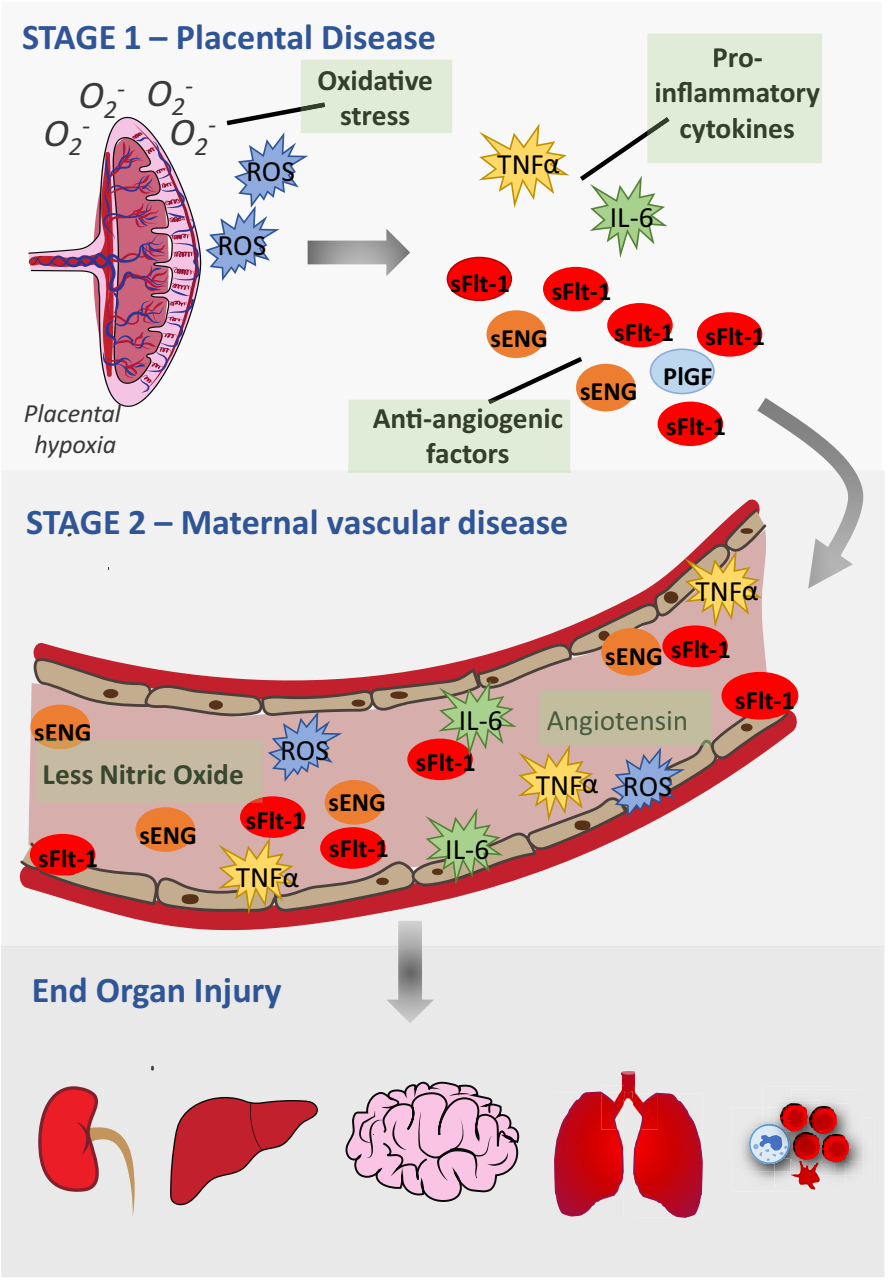
FIGURE 2
Pathogenesis of preeclampsia: a 2-stage process



The first stage is placental disease. During embryo implantation, there is poor cytotrophoblast invasion and remodeling of the maternal spiral arteries, resulting in placental hypoxia, oxidative stress, and the production of ROS. The preeclamptic placenta then releases elevated levels of proinflammatory cytokines (such as TNF-α and IL-6) and antiangiogenic factors (such as sFlt-1 and sEng). There is also reduced placental release of PlGF. The second stage of preeclampsia is maternal vessel disease and endothelial dysfunction. There is a significant injury to the maternal blood vessels caused by the circulating soluble factors from the preeclamptic placenta. Among many pathologic changes in the vessels, endothelial release of nitric oxide is decreased, and activity of the renin-angiotensin system is increased, promoting vasoconstriction. This vascular injury causes hypertension and end-organ injury that are seen in clinical diseases. Many potential therapeutic strategies are being tested to either treat or prevent preeclampsia. Most therapeutic strategies target the 5 aspects of the pathogenesis of preeclampsia that are highlighted in the *green boxes* and are discussed throughout the review.

IL-6, interleukin-6; *PlGF*, placental growth factor; *ROS*, reactive oxygen species; *sEng*, soluble endoglin; *SFlt-1*, soluble fms-like tyrosine kinase-1; *TNF-α*, tumor necrosis factor alpha.

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dysfunction detected by echocardiography and histologically (less cardiac hypertrophy and interstitial fibrosis).[42]

There have also been early phase trials of pravastatin. Costantine et al[43] reported a small randomized trial where 10 mg of daily pravastatin or placebo (commenced at 12−16 weeks' gestation) was administered to 20 participants with a history of preterm preeclampsia. The half-life was 2 to 3 hours, and pravastatin appeared safe. Furthermore, 4 of 10 participants who received placebo developed preeclampsia but none for those who received pravastatin. Circulating sFlt-1 levels trended toward a nonsignificant reduction.

Lefkou et al[44] reported an unblinded, uncontrolled study of 11 participants with antiphospholipid syndrome who received 20 mg of daily pravastatin and compared them with 10 participants who did not receive the drug. The differences in clinical outcomes were striking—only 6 of 11 neonates on placebo survived, whereas all 11 participants on pravastatin had healthy live births at term. There was also ultrasound evidence demonstrating that pravastatin improved uterine blood flow (where serial ultrasounds performed at least a week apart demonstrated an improvement in the mean uterine artery pulsatility index for all 11 participants who received pravastatin). Although encouraging, the findings require validation.

There has been 1 randomized treatment trial. The statins to ameliorate early-onset preeclampsia (StAmP) trial was a multicenter trial in the United Kingdom, where 40 mg of daily pravastatin or placebo was administered to 62 participants with preeclampsia diagnosed between 24 and 31 weeks' gestation.[45] There was no change in the primary outcome which was a reduction in plasma sFlt-1 3 days after randomization. Pravastatin also did not affect perinatal outcomes. However, this small trial was almost certainly underpowered to definitely rule out the potential of pravastatin to treat preeclampsia. In a case series of 4 women with preterm preeclampsia, pravastatin (40 mg daily) may have stabilized levels of circulating levels of sFlt-1.[36]

Pravastatin was originally assigned a category X rating, suggesting that it should be avoided during pregnancy. However, a systematic review concluded that there is no association between fetal abnormalities and prenatal pravastatin administration (although the number of pregnancies where pravastatin was administered was just 500−600, meaning that studies so far may be underpowered to detect any increased risk of structural anomalies).[46] Reassuringly, animal toxicology studies have suggested that the drug is safe,[46] pravastatin is not detectable[43] or at very low concentrations[45] in the umbilical cord at birth, and placental perfusion studies suggest that there may be active efflux of the drug back into the maternal compartment.[47] However, given it is possible that the developing fetal brain may be sensitive to drugs that target lipid metabolism (and there are in vitro studies showing pravastatin may increase death in mouse fetal neural stem cells[48]), large trials of pravastatin should ideally monitor neonatal and childhood health.

Trials underway provide hope that there will be a clear answer as to whether pravastatin prevents preeclampsia (the Table summarizes all the randomized trials in progress discussed in this review). A large multicenter trial in Europe (EudraCT 2016-005206-19) aims to randomize 1120 participants identified as high risk of developing preeclampsia at 35 to 36 weeks' gestation (based on a screening algorithm) to 20 mg of pravastatin or placebo. A phase I pharmacokinetic and safety trial in the United States will randomize 48 participants with a history of preterm preeclampsia at 12 to 16 weeks' gestation to either placebo, 10, 20, or 40 mg of daily pravastatin (NCT01717586). Excitingly, a large trial also in the United States will randomize 1550 participants with a previous history of preeclampsia (requiring delivery before 34 weeks' gestation) to 20 mg pravastatin or placebo (NCT03944512). An unblinded Indonesian trial will randomize 280 women at 10 to 20 weeks' gestation to daily 40 mg of pravastatin or no drug (INOVASIA trial; NCT03648970). There is no treatment trial registered for pravastatin.

Proton-pump inhibitors

Proton-pump inhibitors are widely taken to relieve symptomatic gastric acid reflux by decreasing acid secretion, including during pregnancy. We have published preclinical evidence[49] suggesting, similar to pravastatin, proton-pump inhibitors (lansoprazole, rabeprazole, and esomeprazole) can decrease placental release of sFlt-1 and sEng in vitro (at lower concentrations than pravastatin[36]). Using primary tissues, the report also demonstrated that proton-pump inhibitors reduce placental and vascular proinflammatory cytokine production and mitigate endothelial dysfunction (reducing VCAM-1 and ET-1 expression, reducing leucocyte adhesion, and enhancing endothelial cell proliferation and endothelial tube formation). The report also provided evidence that esomeprazole may facilitate favorable vascular dynamics as it up-regulates phosphorylated nitric oxide synthase in endothelial cells (an enzyme producing potent nitric oxide) and vasodilates human omental blood vessels ex vivo (obtained during cesarean delivery).[49] Furthermore, esomeprazole has been shown to resolve the hypertensive phenotype in 2 animal models of preeclampsia.[49,50] Hence, proton-pump inhibitors may have the potential to treat or prevent preeclampsia.

Proton-pump inhibitors are well tolerated. Furthermore, there are safety data from very large cohorts concluding that there is no teratogenic risk, even after first-trimester exposure.[51,52] A metaanalysis has reported an association between prenatal exposure and childhood asthma,[53] but it is unclear whether the link is causative or whether confounding variables may explain the finding.

Saleh et al[54] examined a cohort of women with confirmed or suspected preeclampsia and found that those coincidently taking proton-pump inhibitors had a favorable angiogenic profile (decreased sFlt-1, sEng, and ET-1) compared with those who did

not. Epidemiologic data from a Swedish cohort of 157,720 pregnancies provided a mixed picture[55]: women coincidently on proton-pump inhibitors had, in fact, an increased risk of preeclampsia (adjusted odds ratio [aOR], 1.17; 95% CI, 1.04−1.32) but a reduced risk of preterm preeclampsia diagnosed at <37 weeks' gestation (aOR, 0.63; 95% CI, 0.41−0.96) or at <34 weeks' gestation (aOR, 0.41; 95% CI, 0.20−0.82).

We have reported a clinical trial performed in Cape Town, South Africa, where we randomized 120 participants diagnosed with preterm preeclampsia at 26 to 32 weeks' gestation to 40 mg of daily esomeprazole or placebo.[56] The median prolongation in gestation (the primary outcome) was 11.4 days (interquartile range [IQR] 3.6−19.7) in the esomeprazole arm and 8.3 days (IQR, 2.8−19.6) among those who received the placebo, a nonsignificant increase of 3 days (95% CI, 2.9−8.8; P=.31). Although a likely explanation for our findings is that 40 mg of esomeprazole cannot resolve preterm preeclampsia, it is possible that the study was underpowered. We followed up the randomized trial with pharmacokinetic studies and found that circulating concentrations of esomeprazole were minimal 7 hours after administration.[56] Hence, a higher dose, twice daily dosing, or intravenous administration may have efficacy, although this would require evaluation. Reassuringly, levels of esomeprazole were undetectable in the umbilical cord at birth, suggesting that there is no significant transplacental passage at this oral dose.[56]

There are further large trials evaluating 40 mg of daily esomeprazole registered (Table). A trial in Australia aims to randomize 480 participants identified as high risk from a first-trimester screening algorithm (the primary outcome is a difference in blood pressure at 36 weeks' gestation; ANZCTR12618001755224). A placebo-controlled prevention trial in Egypt aims to randomize 1000 participants (NCT03717740), and a treatment trial aims to randomize 390 women with preterm preeclampsia (NCT03213639).

Metformin and sulfasalazine

Metformin is an oral hypoglycemic agent used to treat type II diabetes and gestational diabetes. Similar to pravastatin and proton-pump inhibitors, it also reduces the secretion of antiangiogenic factors from the placenta in a dose-dependent manner. It can also mitigate endothelial dysfunction (reduces VCAM-1 expression) and can promote vasodilation in whole maternal omental blood vessels.[57] It may also have angiogenic properties as it can promote vessel outgrowths from aortic rings obtained from mice.[57] Furthermore, there is evidence that it resolves the preeclampsia phenotype in animal models.[58,59]

A randomized trial by Syngelaki et al[60] examining the potential of metformin to decrease maternal and fetal weight gain in women who are obese reported a remarkable 76% reduction in rates of preeclampsia (OR, 0.24; 95% CI, 0.10−0.61; P<.001). A metaanalysis of trials assessing metformin vs insulin concluded that rates of hypertensive disorders are decreased among those administered metformin (n=836; RR, 0.68; 95% CI, 0.48−0.95),[61] but a metaanalysis of trials where women received metformin or placebo did not find a difference in hypertensive disorders[61] (n=609; RR, 0.86; 95% CI, 0.33−2.26; P=.76). This is despite the inclusion of the trial by Syngelaki et al[60] where the treatment effect was significant. Although there are some promising leads, it must be noted that no metformin prevention trial has evaluated the risk of developing preeclampsia as the primary outcome.

A large cohort that evaluated childhood outcomes (growth, emotional and developmental) concluded that prenatal administration of metformin is safe.[62] However, preclinical and clinical data also exist that raise safety concerns.[63] A drawback to metformin is that it has a high prevalence of gastrointestinal side effects, which may decrease compliance.

We have just completed recruitment of 180 participants in a randomized placebo trial of 3000 mg of extended-release metformin or placebo administered in divided doses, in South Africa (PACTR20168001752102; results of the trial are yet to be reported) (Table).[64] In light of our experience from the esomeprazole trial,[56] we started with pharmacokinetic studies (before embarking on the randomized trial) in a cohort of 15 women with preterm preeclampsia and confirmed that there were good circulating drug levels.[65] There is another treatment for preterm preeclampsia trial registered that will compare the combination of esomeprazole (40 mg) and metformin (1000 mg) with placebo (NCT03717701) (Table). A North American open-label trial of metformin will recruit 60 pregnant women with type 1 diabetes (primary outcome is the rate of hypertensive disorders; NCT03570632).

Sulfasalazine, an anti-inflammatory drug used to treat inflammatory bowel disease, has also been demonstrated to reduce placental secretion of sFlt-1, increase placental growth factor (PlGF), and decrease endothelial dysfunction.[66] Its potential advantage is that its potent anti-inflammatory properties may help resolve the placental and systemic inflammation present in preeclampsia. However, it is unclear how much drug is absorbed into the circulation. Given this information, we have commenced a pharmacokinetic trial of sulfasalazine among women with preterm preeclampsia (ACTRN12617000226303).

There are other small molecules also reported to decrease sFlt-1 secretion (such as sofalcone, YC1, and ouabain),[67−69] but they may be distant from the translation given that their safety remains uncertain.

Mechanisms of action by which pravastatin, proton-pump inhibitors, metformin, and sulfasalazine decrease soluble fms-like tyrosine kinase-1

The question arises as to how drugs with such disparate actions—from lipid-lowering, proton-pump inhibition to glycemic control—converge to reduce sFlt-1 secretion. Identifying common intracellular targets is not just an issue of scientific curiosity: if found, it strengthens biologic plausibility and may uncover new drug targets. We have

TABLE
Ongoing randomized, placebo-controlled prevention and treatment trials for preeclampsia that are registered

Drug	Registration number	Location	Description Primary outcome for prevention trials: rates of preeclampsia; Primary outcome for treatment trials: prolongation of pregnancy (unless otherwise stated)	Planned end date[a]
Prevention				
Pravastatin (20 mg daily)	EudraCT 2016-005206-19	Europe	1120 participants, randomized at 35–36 wk of gestation, identified at higher risk based on a screening algorithm[b]	Recruiting; end date not stated
	NCT03944512	United States	1550 participants randomized at 12–16 wk of gestation. Identified at higher risk based on a history of preterm preeclampsia requiring birth at <34 wk of gestation	Planned period of recruitment June 2026–June 2031
Esomeprazole (40 mg daily)	ANZCTR12618001755224	Australia	480 participants randomized at <16 wk of gestation, identified at higher risk based on a first-trimester screening algorithm[c] The primary outcome is blood pressure at 36 wk of gestation	Recruiting; end date not stated
	NCT03717740	Egypt	1000 participants, identified at higher risk based on clinical factors (NICE guidelines)	January 2022
Treatment				
Metformin (3000 mg daily, in divided doses)	PACTR20168001752102	South Africa	180 participants with preterm preeclampsia (PI2 trial), randomized at 26–32 wk of gestation	August 2020 (recruitment is now complete)
Esomeprazole (40 mg daily)	NCT03213639	Egypt	390 participants with preterm preeclampsia (ESOPE trial), randomized at 28–32 wk of gestation	October 2020
Metformin (1000 mg) and esomeprazole (40 mg)	NCT03717701	Egypt	120 participants with preterm preeclampsia, randomized to esomeprazole and metformin or placebo at 28–32 wk of gestation	November 2020
Broccoli sprout extract tablet (8 mg twice daily)	ACTRN12618000216213	Australia	180 participants with preterm preeclampsia, randomized at 24–34 wk of gestation	Not yet started; no end date set
Digoxin immune Fab (AMAG-423) (intravenous infusion)	NCT03008616	United States	200 participants with preterm preeclampsia, randomized at 23-32 wk of gestation The primary outcome is the proportion of infants who have an intraventricular hemorrhage and necrotizing enterocolitis or have died at 36 wk of gestation (corrected gestational age)	March 2020
Recombinant antithrombin III (KW-3357) (intravenous infusion)	NCT04182373	Japan	200 participants with preterm preeclampsia, randomized at 24–32 wk of gestation	May 2022

ESOPE, Esomeprazole in Treatment of Early Onset Preeclampsia; *NICE*, National Institute for Health and Care Excellence; *PIGF*, placental growth factor.

[a] Planned end date, stated as part of clinical trials registration; [b] The third-trimester screening algorithm combines mean arterial pressure, maternal history, clinical characteristics, and circulating levels of PIGF and soluble fms-like tyrosine kinase-1; [c] The first-trimester screening algorithm combines mean arterial pressure, maternal history, clinical characteristics, uterine artery pulsatility index measured by ultrasound, and circulating levels of PIGF and pregnancy-associated plasma protein A.

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identified 2 molecular targets where some of these drugs are acting.

We have shown that inhibiting the electron transport chain of the mitochondria (which produces energy) decreases placental sFlt-1 secretion, suggesting that the mitochondria positively regulate the secretion of sFlt-1.[57] We have also demonstrated that metformin[57] and esomeprazole[70] decrease sFlt-1 production by inhibiting mitochondrial function. Although this identifies the mitochondria as a theoretical drug target, deliberately pursuing other drugs that perturb the function of this subcellular organ will need to be done with care given its critical role in generating intracellular energy.

The epidermal growth factor receptor pathway (EGFR) is a major cell signaling pathway that promotes cell survival and coordinates many intracellular processes.[70] We have shown that the EGFR pathway also positively regulates the secretion of sFlt-1[70] and that statins, esomeprazole,[70] and sulfasalazine[71] may inhibit EGFR signaling to reduce sFlt-1 secretion.

Gefitinib is a molecularly targeted drug designed to inhibit EGFR to treat lung cancers. We have shown that gefitinib is potentially the most potent inhibitor of sFlt-1 of any small-molecule drug where very low drug concentrations are required to decrease sFlt-1 secretion in vitro.[70] However, because EGFR signaling plays an important role in placental biology, any proposed human trial of gefitinib would need to be carefully designed with fetal safety as a major outcome.

There are likely to be other molecular circuits regulating sFlt-1 secretion besides the mitochondria and EGFR. For instance, we[36] and others[72] have demonstrated that direct inhibition of the 3-hydroxy-methylglutaryl coenzyme A reductase pathway (the direct target of statins) can decrease sFlt-1 secretion. Furthermore, inhibiting the hypoxia-inducible factor-1 alpha (HIF-1α) pathway also reduces sFlt-1 secretion.[68] Given that proton-pump inhibitors[49] and metformin[73–75] inhibit HIF-1α expression, this may be another mechanism by which these drugs inhibit sFlt-1 release.

Antioxidants, plant extracts, and micronutrients

Given the role of oxidative stress in the pathogenesis of preeclampsia, many drugs with antioxidant actions have been evaluated for their potential to prevent or treat preeclampsia. A very large clinical trial[76] (n=10,154; RR of preeclampsia in the vitamin arm, 1.07; 95% CI, 0.93−1.24; P=.33) dashed hope raised from an earlier trial[77] that antioxidants, vitamin C and E, prevents preeclampsia.

Melatonin is a naturally occurring compound released by the pineal gland that regulates circadian rhythm. It is well known for its antioxidant properties. In preclinical studies, melatonin reduced placental oxidative stress and up-regulated molecules involved in the antioxidant response.[78] In a phase I single-arm trial, the team reported that 10 mg of oral melatonin (administered 3 times a day) to 20 women with preterm preeclampsia prolonged gestation by a median of 6±2.3 days and reduced the need for antihypertensive agents compared with that of 48 historic controls.[78] No further trial of melatonin is registered.

MitoQ is a small molecule available as a nutritional supplement that targets mitochondrial oxidative stress (a major source of reactive oxygen species that cause intracellular oxidative stress). Yang et al[79] recently showed that MitoQ reduced placental oxidative stress and resolved preeclampsia in a mouse model only if given later in pregnancy. Interestingly, it exacerbated the preeclampsia phenotype if commenced in early pregnancy, and they reasonably hypothesize that it was because oxidative stress plays a positive role in early placental development.

Polyphenols are micronutrients with antioxidant properties found in foods, such as berries, beans, vegetables, tea, nuts, and red wine. Flavonoids are antioxidants that confer fruit and vegetables their vivid colors. We have shown that adding the polyphenol resveratrol to placental cytotrophoblast up-regulates intracellular molecules involved in the antioxidant response and reduces sFlt-1 secretion,[80] whereas others have shown that resveratrol resolves the hypertensive phenotype in a pregnant rat model.[81] There have now been many studies reporting that a number of micronutrients or plant-based extracts (including polyphenols and flavonoids) can resolve preeclampsia in animal models.[82–86] A randomized treatment trial of broccoli sprout (enriched in the antioxidant sulforaphane[87]) has been registered (ANZCTR12618000216213) (Table).[88]

Regarding other micronutrients, high-dose folic acid supplementation had been proposed as a preventative treatment, but disappointingly, a large phase III trial did not find it to be beneficial (n=2464; RR of preeclampsia among women given 4 mg of folic acid was 1.10; 95% CI, 0.90−1.34; P=.37).[89] Nicotinamide (vitamin B3) had been shown to antagonize vasoconstriction and resolve the preeclampsia phenotype in 2 animal models.[90] A small single-arm trial of nicotinamide administered to 25 women with preeclampsia is registered (NCT3419364).

Hence, micronutrients and plant-based extracts may merit testing in clinical trials given that they are widely consumed and well tolerated. However, given that preclinical studies have identified so many candidates with therapeutic potential, the challenge is to triage which ones should be tested in clinical trials. Perhaps a systematic preclinical screen comparing the candidates in similar models may be a pragmatic approach to choose which ones are prioritized for human trials.

Drugs targeting the nitric oxide synthase pathway

Enhancing elements of the nitric oxide synthase pathway has been a focus of therapeutic strategies given that increasing circulating levels of nitric oxide (made by nitric oxide synthase) may resolve maternal vascular disease. Considerable optimism was generated from a randomized trial comparing 50 mg of oral sildenafil given 3 times a day (enhances the action of nitric oxide) with placebo administered to 100 participants with preterm preeclampsia. Pregnancy duration was significantly

lengthened by 4 days among those administered sildenafil (14.4 days [95% CI, 12.5−16.6] vs 10.4 days [95% CI, 8.4−12.3]; P=.008), and there was evidence of reduced blood flow resistance of the uterine and umbilical arteries (measured by ultrasound).[91] Unfortunately, since that trial, concerns have been raised regarding a possible association between prenatal exposure of sildenafil and neonatal death from pulmonary hypertension.[92] Given this information, it is difficult to envisage that there could be enthusiasm to pursue sildenafil as a treatment for preeclampsia.

Vadillo-Ortega et al[93] published a randomized blinded prevention trial (n=222 per arm), which found that edible bars with L-arginine (a substrate of nitric oxide) administered to women with a history of preeclampsia resulted in a remarkable reduction in rates of preeclampsia (RR, 0.17; 95% CI, 0.12−0.21). A small prevention trial reached a similar conclusion,[94] and there is preclinical support that L-arginine could prevent preeclampsia.[95,96] These leads make L-arginine a promising concept. It is therefore disappointing that no large clinical trial appears to be registered.

A small trial of intravenous S-nitrosoglutathione (a nitric oxide donor) in 6 participants with preterm preeclampsia demonstrated a decreased blood pressure and showed trends toward improving several indices that reflect disease severity.[97] In contrast, a blinded, randomized prevention trial of 100 participants comparing oral isosorbide mononitrate (nitric oxide donor) with placebo did find a difference in the incidence of preeclampsia, but it was likely to be underpowered.[98]

Relaxin is an endogenous peptide hormone that increases nitric oxide production and may reduce endothelial dysfunction.[99] A phase I trial of recombinant human relaxin administered to women with preterm preeclampsia was suspended without the results being reported (NCT00333307).

Apheresis

Apheresis was initially proposed as a treatment for preeclampsia in 2003[100] and 2006[101] based on the premise that it removed lipids that cause cardiovascular disease. Since those periods, the focus of treating preeclampsia with apheresis has shifted to its potential to remove sFlt-1 from the circulation. Thadhani et al[102] modified dextran sulfate columns used for apheresis to preferentially remove sFlt-1 and conducted a pilot study in women with preterm preeclampsia. There was a 15% to 28% reduction in circulating sFlt-1 levels following a single apheresis treatment. Furthermore, 3 more women underwent serial apheresis treatments: circulating sFlt-1 transiently reduced after each treatment and the pregnancies lasted 15 to 23 days.[102] They subsequently reported the outcomes of 11 more women with preterm preeclampsia who had apheresis treatment (once or multiple times),[103] and the group had pregnancies that potentially lasted longer than a contemporaneous preeclamptic cohort that did not have the treatment.[103] There is a single-arm trial of apheresis treatment in 23 participants registered (healthy volunteers and women with preeclampsia) where safety is the primary outcome (NCT02923296).

Apheresis is invasive, and it will be challenging to undertake large clinical trials. Even without such trials, it may arguably find a role in treating disease occurring at very preterm gestations, where options are limited and the prognosis is grim (the analogy may be abdominal cerclage, offered to women who have had recurrent preterm birth at very early gestations even though evidence supporting this technique is only from case series[104]).

Monoclonal antibodies

Monoclonal antibodies may be an attractive drug class to treat preeclampsia as they are highly specific and can, in theory, be engineered so that they do not cross the placenta. TNF-α is a cytokine that is increased in preeclampsia[26] and postulated to play an important role in the proinflammatory response associated with the disease. Etanercept is a fusion protein (used clinically to treat rheumatoid arthritis) where a portion of the TNF-α receptor is fused to the constant (Fc) portion of the immunoglobulin G antibody. The TNF receptor on etanercept binds to circulating TNF-α and neutralizes its activity. Two teams have shown that etanercept can resolve the preeclampsia phenotype in rat models.[105,106]

Preeclampsia is associated with complement activation, and perturbing this system in a mouse model can produce clinical features of preeclampsia.[107] There has been a case report where serial doses of the complement inhibitor eculizumab (a recombinant humanized monoclonal antibody (maB) used for hemolytic uremic syndrome) was used to treat a patient at 26 3/7 weeks' gestation with HELLP syndrome. Following treatment, her biochemistry (including significantly deranged liver function tests and low platelet counts) normalized, and she gained 17 days in gestation before being delivered at 29 2/7 weeks' gestation.[108] There have also been 2 case reports where eculizumab was administered soon after birth to treat acute kidney injury and poor renal function arising from preeclampsia after they had failed to respond to dialysis.[109,110] Both cases recovered fully. Although eculizumab may merit further investigation for its potential to treat preeclampsia, a downside is that it is exceptionally expensive.

Digoxin immune antigen-binding fragment (Fab), immunoglobulin fragments derived from sheep and used to treat digoxin toxicity, has been proposed as a treatment. The rationale is that endogenous digitalis-like factors block intracellular sodium pumps in the endothelium, leading to vasoconstriction. Digoxin immune Fab blocks these factors. There is a randomized trial that aims to administer digoxin immune Fab (NCT03008616) (Table) or placebo to 200 women with preterm preeclampsia. It follows a pilot trial that suggests that it is safe and possibly improved renal blood flow (improved creatinine clearance), but it did not affect the use of antihypertensive medications.[111]

Antithrombin III

Recombinant antithrombin III has been proposed as a treatment for

preeclampsia given that it is postulated that its anti-inflammatory and anticoagulant properties may be beneficial. Unfortunately, a multicenter placebo-controlled treatment trial in the United States of women with preterm preeclampsia did not find a difference in the median increase in gestational age (5.0 days [range, 0−75] in the treatment group and 6.0 days [range, 0−85] in the placebo group; P=.95).[112] A randomized treatment trial in Japan of another recombinant antithrombin III drug, KW-3357, is registered (NCT04182373) (Table).

Placental growth factor

Administering PlGF may help overcome the loss of VEGF that is neutralized by circulating sFlt-1. This may restore blood vessel homeostasis and be a way to treat the disease. Makris et al[113] showed administering PlGF to a baboon model of preeclampsia (produced by ligating a uterine artery to induce placental hypoxia) resolved clinical parameters, including hypertension. Although encouraging, it is enormously costly and challenging to upscale the manufacture of whole proteins, such as PlGF, for human administration.

Short interfering RNAs

Recently, Turanov et al[114] published the use of short interfering RNA (siRNA) technology targeting sFlt-1. These are short RNA strands that exploit existing intracellular machinery to reduce the expression of the target proteins. The team generated siRNAs targeting sFlt-1, chemically modified so it can resist degradation and survive longer in the circulation, and demonstrated that it reduced sFlt-1 expression in mice. The siRNA also reduced circulating sFlt-1 and decreased blood pressure and proteinuria in a baboon model of preeclampsia.

Haase et al[115] also used siRNA technology and showed the silencing angiotensinogen resolved the preeclamptic phenotype in animal models, including proteinuria, hypertension, and fetal growth restriction (perturbations in the renin-angiotensin system causing endothelial dysfunction are a likely feature of preeclampsia[116]). It is a clever strategy given that most siRNAs administered into the venous circulation are taken up by the liver, which just happens to be where circulating angiotensinogen is synthesized. This means that the siRNAs can be simply administered intravenously and most will find its way to the liver. siRNA treatments can be produced at a scale and may prove an exciting development if translated into clinical trials.

Other novel approaches

Other scientifically sophisticated preclinical approaches shown to resolve a preeclampsia phenotype in animal models (but may still be remote from translation) include stem cells,[117] nanoparticle delivery of siRNAs,[118] and a drug-carrying biopolymer that can retain an active drug (a peptide fragment that inhibits NF-κB, a master regulator of the proinflammatory response) in the maternal vascular compartment to inhibit maternal systemic inflammation (but prevent potentially deleterious placental transfer).[119]

Considerations in the design of future clinical trials

Almost all randomized treatment trials completed[56,112] or in progress (Table) have been for preterm preeclampsia, where the primary outcome has been mostly focused on prolonging gestation (the StAmP trial that evaluated oral pravastatin was an exception, where a difference in circulating sFlt-1 was the primary outcome[45]). Gaining gestation seems an appropriate outcome for current treatment trials trying to identify drugs that can mitigate the pathophysiology of the disease (given none has been identified to date). For any that are discovered, they may need to be further evaluated in subsequent large phase III trials to see whether their administration can improve clinically relevant endpoints, such as maternal and neonatal outcomes.

There have been no treatment trials of drugs that may mitigate disease progression in cohorts with preeclampsia diagnosed at term gestation. Although the course of the disease itself will be time limited (as women are usually delivered soon after diagnosis), preeclampsia with severe features can still occur, resulting in significant maternal morbidity. There is potentially an unmet demand for a drug that can temporize the disease at term gestation.

Interestingly, some prevention trials have taken the novel approach of a biomarker strategy to identify those at risk of preeclampsia. For instance, a phase II trial of esomeprazole is randomizing those identified at increased risk of preeclampsia on the basis of a first-trimester screening algorithm (combines maternal characteristics, ultrasound findings, and circulating levels of PlGF and pregnancy-associated plasma protein A; ANZCTR12618001755224) (Table), and a phase III trial of pravastatin is identifying women on the basis of a screening test at 35 to 36 weeks' gestation (combines maternal characteristics and circulating levels of sFlt-1 and PlGF; EudraCT 2016-005206-19) (Table). Given screening based on clinical history alone only has a modest positive predictive value (meaning most who are randomized are not destined to develop the disease) and poor sensitivity,[120] incorporating biomarkers to identify women at increased risk may be a valuable approach to design large prevention trials.

Comment

There has been an encouraging escalation in research activity focused on identifying new treatment strategies for preeclampsia. The rise in the number of preclinical reports over recent years has been quite steep. In fact, there are now quite a bewildering number of candidates that seem to resolve various complications in animal models. It is further encouraging that there has also been an increase in clinical trials published over the last decade[45,56,91,112] and others that are ongoing (Table).

Here, we wish to discuss a few points that may help sharpen the translational strategy for research teams pursuing preclinical concepts or who are contemplating clinical trials.

First, it is evident that most candidate therapeutic agents for preeclampsia being examined are repurposed drugs: that is, drugs licensed to treat another medical condition and now identified for their potential to treat preeclampsia. Drug repurposing is a pragmatic way forward as it can fast-track promising preclinical concepts to trials given that safety data for the drug may exist. Hence, repurposing drugs can save many years of toxicologic and phase I safety trials.[121] It is a strategy well worth pursuing.

However, it should also be noted that a disadvantage of the repurposing strategy is that it limits preclinical discovery to drugs already formulated. Major advances in therapeutic strategies for oncology have been made via molecularly targeted approaches[122,123]: generating new compounds tailored to target specific molecules that are known disease drivers. It is possible that repurposing may be effective in preventing preeclampsia (to modify the disease early in its pathogenesis), but more molecularly targeted approaches will be needed to treat preeclampsia because by the time the disease is clinically apparent, the placental and maternal vascular pathologies will be very advanced. This is may mean only molecularly targeted approaches will be potent enough to make an effective impact on clinical disease progression. The siRNA studies targeting sFlt-1[114] and angiotensinogen[115] are examples of molecularly targeted approaches tailored specifically to preeclampsia, but research activity in this area arguably trails the field of oncology by decades.[124] We hope that more molecular-targeted approaches will be discovered and evaluated in trials.

Second, as we ponder which drugs may be best suited to be taken to clinical trials, it may be worth highlighting the distinction among developing drugs to prevent preeclampsia vs treating it. The first is efficacy: as noted, it may be more realistic to find agents to prevent preeclampsia compared with finding effective treatments to resolve advanced placental and maternal vascular diseases that are present by the time the disease clinically manifests.

Conversely, for a new drug to be clinically acceptable to prevent preeclampsia, it would need to be exceptionally safe. The reason is that all the screening approaches to identify women at risk of preeclampsia have modest value in accurately predicting preeclampsia; that is, a low positive predictive value.[120] This means any preventative treatment will be administered for many months to far more pregnancies that were never destined to develop the condition than those that were. In contrast, the threshold at which we may tolerate uncertainties around safety may be lower when contemplating treatments for clinical preeclampsia. This is because for treatment trials, it would be anticipated that the drug will be given to a select few (all of whom have the disease) for a few weeks, if not days, and well clear of early pregnancy, when the teratogenic risk is greatest. When considering these points, siRNA, monoclonal antibodies, and apheresis approaches may be better suited for treatment trials, whereas repurposing drugs may be better suited for large prevention trials.

Our final comment is that although there has been a pleasing increase in clinical trials of new candidates to treat or prevent preeclampsia, the overall number remains modest when compared with other specialties. A review in the field of oncology lamented that of 5000 to 10,000 drugs in oncology approved by the Federal Drug Administration, only 5% survive trials to reach the market.[121] If our own therapeutics field was functioning even at 1% of this scale, it would mean, in theory, that we will have around 3 to 5 additional agents to use to treat or prevent preeclampsia, to add to the sole agent we have (aspirin).

With the raft of new candidate treatments, it may be time that we gather into large, international consortiums that remain together beyond the life of 1 trial. Our research field may benefit by forming expert committees (agnostic to any specific treatment option) to objectively prioritize which of the many preclinical offerings may be the lead candidates. Furthermore, the clinicians run trials to test them, 1 by 1. If these were done with success, the discovery of new agents that resolve the disease course of preeclampsia could make a lasting impact, saving the lives of babies and mothers and decreasing the lifelong sequelae brought by this condition. ■

GLOSSARY

aOR: adjusted odds ratio
CI: confidence interval
EGFR: epidermal growth factor receptor inhibitor
Fab: antigen-binding fragment
HELLP: hemolysis, elevated liver enzymes, low platelet count
IL-6: interleukin-6
IQR: interquartile range
NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells
PlGF: placental growth factor
RR: relative risk
ROS: reactive oxygen species
sEng: soluble endoglin
SFlt-1: soluble fms-like tyrosine kinase-1
siRNA: short interfering RNA
TNF-α: tumor necrosis factor alpha
VEGF: vascular endothelial growth factor

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