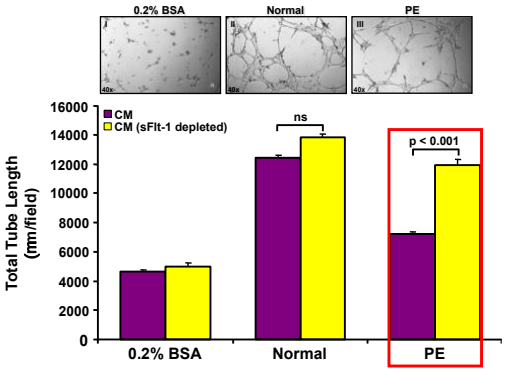
Scientific Evidence

Our studies show that preeclampsia to be a 'double hit' vascular disease with defects in the protective pathways of heme oxygenase-1 (Hmox1/HO-1)/carbon monoxide (CO) and cystathionine γ-lyase (CSE) / hydrogen sulfide (H_2S) as well as Vascular Endothelial Growth Factor (VEGF) signalling. This allows for the development of a novel therapy to target these proteins and ameliorate the disease in different disease models, in addition to identifying a subset of patients who may potentially benefit from this treatment.

In 1997, Ahmed speculated that loss of VEGF activity may cause preeclampsia due to a possible rise in soluble Flt-1 (sFlt-1). Work in the 1990s from the laboratories led by Prof Stephen K. Smith in Cambridge UK and Prof Asif Ahmed in Birmingham UK were the first to identify importance of VEGF receptor and sFlt-1 in placenta. Ahmed showed that removal of sFlt-1 per se from the conditioned media of preeclampsia placenta restored angiogenic balance (Fig. 1). Today, it is generally accepted that the primary culprit for preeclampsia is sFlt-1, the natural antagonist of VEGF. Soluble Flt-1 is increased several weeks before the onset of the disease. This leads to loss of VEGF activity causing maternal vascular damage.



Ahmad & Ahmed, Circ Res. 95:884-91, 2004.

Figure 1. The removal of soluble Flt-1 (sFlt-1) restore angiogenic balance by readdressing the angiogenic imbalance in preeclampsia placenta. Endothelial cells were exposed to conditioned media (CM) from normal (Normal) and preeclampsia (PE). Removal of sFlt-1 (Yellow column) from the placental CM was performed by immunoprecipitation with a specific antibody. Angiogenesis was measured by endothelial cell in vitro tube formation on growth-factor reduced Matrigel in 24-well plates.

In 2000, Ahmed and team had shown that human placenta is protected from damaging effects of hypoxia and cytokines by the enzyme, Heme Oxygenase. Later, Ahmed's laboratory demonstrated in two seminal papers in the journal Circulation that the two protective enzymes, HO-1 and CSE act as negative regulators of sFlt-1. This was shown by adenoviral overexpression of HO-1 and CSE or knockdown of these enzymes using siRNA in endothelial cells resulting in changes in sFlt-1 release (**see Fig 2**).

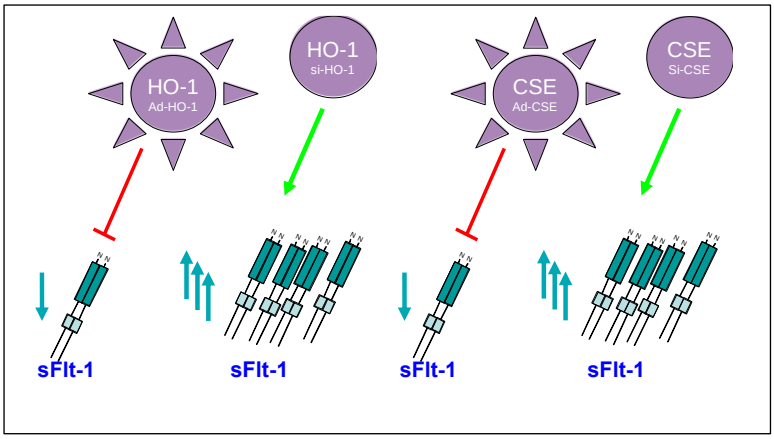


Figure 2. Heme oxygenase-1 (HO-1) and cystathionine γ-lyase (CSE) negatively regulate soluble Flt-1 (sFlt-1). Endothelial cells were either infected with adenovirus (Ad) AdHO-1 and AdCSE or siRNA was used to knockdown HO-1 or CSE. After 24 hours, sFlt-1 release from endothelial cells was assayed by ELISA.

The enzymatic activities of these enzymes are reduced in preeclamptic women along with their by-products such as H_2S. The first direct evidence that a dysfunctional CSE / H_2S pathway contributes to the pathogenesis of preeclampsia was first demonstrated using CSE inhibition, which induced preeclampsia-like features in pregnant mice owing to the inhibition of H_2S production. Intraperitoneal injection of a slow-releasing H_2S-generating compound GYY4137 restored fetal growth and reduced sFlt-1 levels in pregnant animals. These studies prompted us to proposed that preeclampsia was a double hit vascular disease (**Fig. 3**) and that a loss of a single HO-1 allele will result in both poor maternal and fetal outcomes under a high sFlt-1 environment.

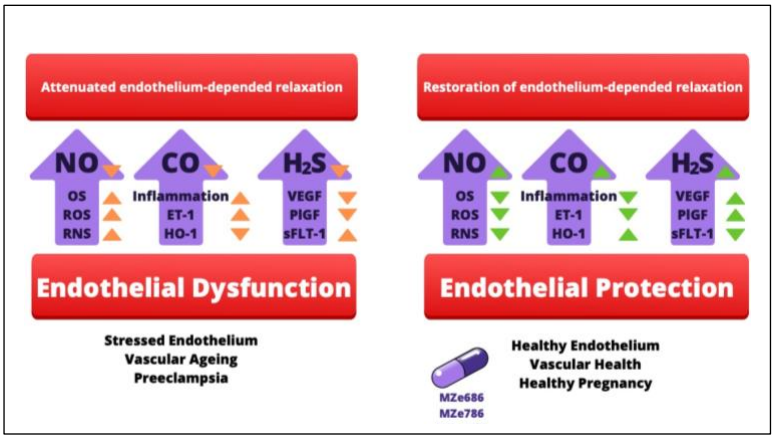


Figure 3. Preeclampsia is a 'double hit' vascular disease. The decrease in nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S) systems lead to increase in inflammation, oxidative stress (OS), soluble Flt-1 (sFlt-1) and a decrease in placenta growth factor (PlGF). There is loss of vascular endothelial growth factor (VEGF) activity and an increase generation of reactive oxygen species (ROS) leading to endothelial dysfunction that contributes to the pathogenesis of preeclampsia.

MZe786 is an orally active hydrogen sulfide-releasing molecule with a backbone of an aspirin structure sometimes dubbed 'Supercharged Aspirin®'. This aspirin analogue contains a diothiolethione moiety, which releases hydrogen sulfide (H_2S). 2-acetyloxybenzoic acid 4-(3-thioxo-3H-1,2-dithiol-5-ylphenyl ester (MZe786) was synthesised using aspirin linked by ester linkage to 5-(4-hydroxyphenyl)-1,2-dithiol-3-thione, which was demethylated from anethole trithione.

To determine the therapeutic potential of MZe786 in preeclampsia, two separate mouse models of preeclampsia were used: (1) pregnant mice lacking a single allele of heme oxygenease-1 ($Hmox1^{+/-}$) under high levels of soluble Flt-1 (sFlt-1) and (2) a surgically-induced approach that leads to Reduced Uterine Perfusion Pressure

(RUPP). The mice were treated with either a drug carrier (Control) or 50mg/kg MZe786 or 23mg/kg aspirin from day E11.5 - E17.5 (mid – end of pregnancy) through oral administration.

The first line of evidence of this innovation lies in the ability of globally patented therapy of MZe786 preventing preeclampsia in two separate animal models of preeclampsia (**Fig. 4**) and inhibits the culprit protein soluble Flt-1 (**Fig. 5**), which causes preeclampsia symptoms.

Preclinical studies demonstrated that MZe786 is effective in improving both the maternal and fetal outcomes in these experimental models of preeclampsia. The blood pressure, which is one of the main symptoms of preeclampsia was reduced. The glumeruli damage and Kidney Injury Marker-1 (KIM-1) were also significantly reduced in mice treated with MZe786. Soluble endoglin (sEng) and E-selectin are markers of endothelial activation and are increased in preeclampsia and both were significatly reduced by MZe786.

Fetal outcome were also assessed in these models of preeclampsia. Overexpression of sFlt-1 in Hmox1$^{+/-}$ and mRUPP led to fetal loss and fetal growth restriction. MZe786 rescued fetal loss and improved fetal weight. The Fetal:Placental ratio is a health indicator reflecting the balance between fetal and placental growth. The ratio was significantly reduced in the high sFlt-1 environment, and this was rescued by administration of MZe786. The effects on fetal health are a critical differentiator to other approaches that target only the maternal symptoms.

The protective role of MZe786 was further confirmed by its ability to reduce sFlt-1, a validated disease biomarker. The results from the studies support the further development of this novel molecule in the prevention and treatment of the condition. MZe786 appears to be a better therapeutic agent at preventing preeclampsia than aspirin alone.

Figure 4. Partial loss of heme oxygenase-1 under high soluble Flt-1 causes severe preeclampsia compared to high sFlt-1 alone. MZe786, hydrogen sulfide releasing aspirin, prevents preeclampsia by suppressing maternal hypertension and kidney injury and improves fetal outcome (Redox Biology 2021).

Second line of concreate evidence is that the Medicines and Healthcare products Regulatory Agency (MHRA) has awarded its first Innovation Passport for pregnancy use for the molecule MZe786 for the treatment of preeclampsia. MirZyme has proven using mouse models backed by human studies to show that preeclampsia is a 'double hit' vascular disease due to a defect in HO-1 and VEGF signalling (**Fig. 2**). This led to the development of a novel therapy, MZe786, to target the culprit protien to ameliorate the disease in different disease models and identify a subset of patients who may potentially benefit from such a treatment (**Fig. 6**).

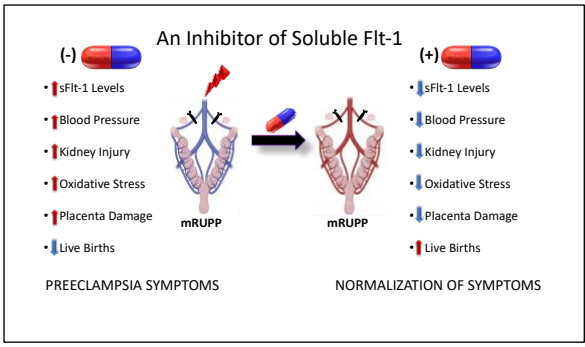


Figure 5. Refined mouse reduced uterine perfusion pressure (mRUPP) model exhibits preeclampsia symptoms. Mouse RUPP induces maternal hypertension, kidney injury, elevates circulating sFlt-1 levels. H_2S releasing aspirin, MZe786, acts as an inhibitor of sFlt-1 to successfully prevent preeclampsia and improve fetal outcome (Redox Biology 2021).

MirZyme has been granted global patents in 30 countries, the know-how and the MHRA 'Innovation Passport' award for the first pregnancy molecule to be granted a fast-track development award for the treatment of preeclampsia provides further sound evidence for this being unique and highly innovative approach to move research to reality. MirZyme to use MZe786 as a novel drug for oral use in the treatment for preeclampsia. We aim to improve global pregnancy outcomes, reduce the cost burden to healthcare systems and create new high skill employment.

MZe786 is the first in the world to be granted a fast track approval for the development in pregnancy by a regulatory body. It may also be the first orally active drug to be approved for such development in the treatment of preeclampsia. With over 18 million women suffering from hypertensive disorder of pregnancy, MZe786 has the potential to mitigate the suffurings of women and famalies in pregnancy. This combined with MirZyme's digital diagnostic tool will be able to stratify and identify patients specificaly for the optimal treatmen of preeclampsia.

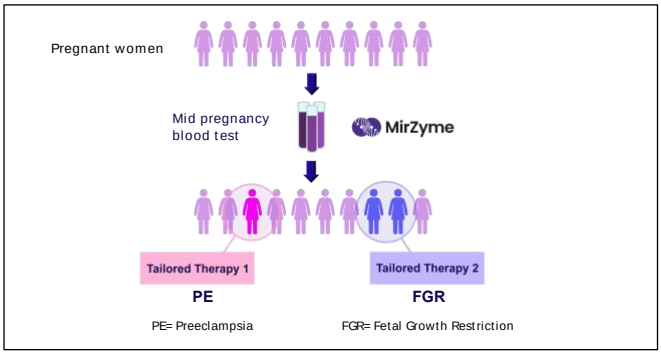


Figure 6. A stratified medicine approach for the treatment of preeclampsia. One test, one pill, save two lives.