White paper for

DiaMedica THERAPEUTICS

# THE POTENTIAL OF DM199 TO TREAT PREECLAMPSIA

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# SYNOPSIS

Preeclampsia is a severe and life-threatening disease that occurs in pregnancy and affects the mother and unborn child. Classic signs of preeclampsia are hypertension and severe injury to the mother's vascular system, which damages multiple vital organs. Today, no treatment slows disease progression. DM199 is a recombinant tissue kallikrein-1 (rhKLK1) protein made by DiaMedica Therapeutics. It switches on molecular pathways in blood vessels to reduce blood pressure and improve blood vessel health. DM199 has exciting potential to be a novel drug treatment for preeclampsia.

Unlike small molecule antihypertensives (nearly all existing drugs on the market) that cross the placental barrier and reach the fetus, DM199 is a large protein molecule that cannot penetrate the placental surface. It cannot breach either the placenta or the fetus, meaning it offers a unique safety advantage in pregnancy. This white paper discusses the potential of DM199 to treat preeclampsia.

# SECTION 1: PREECLAMPSIA

## Preeclampsia is a condition that typically presents after 20 weeks of gestation in pregnancy and can be life-threatening to the mother and unborn child[1]

The most common presentations of preeclampsia are **maternal vascular disease and endothelial dysfunction**. Damage to the mother's blood vessels leads to **hypertension** and **injuries to many vital organs.**[2,3] The symptoms of preeclampsia may include liver failure, kidney failure, seizures, cerebral hemorrhaging, pulmonary edema, or blood clotting difficulties. Subsequently, the mother's compromised blood supply to her uterus and placenta can **affect the unborn baby**, causing fetal growth restriction and placental abruption as the placenta may dangerously sheer off the wall of the uterus before the baby is born. Both fetal growth restriction and placental abruption can result in stillbirth.[2] Severe maternal vascular dysfunction from preeclampsia leads to dangerous pregnancy complications. The difficulty in treating preeclampsia stems from it being both a placental disease and maternal vascular disease. Accordingly, both must be equally considered in a treatment plan.

## Preeclampsia is the only major pregnancy-related disease that is life-threatening to both mother and baby[2,3]

Preeclampsia develops in two stages: **stage 1 is placental disease**, and **stage 2 is maternal vascular disease**. Placental disease begins before the clinical diagnosis of preeclampsia. In early pregnancy, the placenta fails to implant properly on the inner lining of the uterus. The **preeclamptic placenta then releases damaging anti-angiogenic factors** (sFlt-1 and sEng), oxidative stress factors (ROS), and proinflammatory cytokines throughout the rest of the pregnancy.[4] These harmful factors then travel through the maternal circulatory system and cause widespread maternal vascular injury. However, preeclampsia becomes clinically apparent in **stage two** of the disease, when the mother's blood pressure rapidly

increases, and clinical laboratory tests uncover evidence of injury to her organs. At this point in disease progression, the standard of care is to administer medications immediately. These medications include hypertensive agents and magnesium sulfate to reduce the risk of stroke or seizures. However, these options treat the symptoms arising from end-organ damage but do not limit further damage.

Critically, no pharmaceutical agents on the market slow disease progression. Today, delivery through the induction of labor or a cesarean section are the only options to halt preeclampsia symptoms. This is due to removing the placenta from the mother, which is the source of anti-angiogenic factors responsible for vascular and organ damage.

## Preeclampsia is common

Preeclampsia is the second most common cause of maternal mortality throughout the world.[5] The disease occurs in 3-7% of all pregnancies and is estimated to cause at least 42,000 maternal deaths every year.[6-8] For every one maternal death related to preeclampsia, another 50 to 100 women are thought to suffer substantial morbidity. Globally, nearly 1.6 million cases of severe preeclampsia occur every year.[9] In the United States, preeclampsia is a cause of significant maternal-fetal morbidity and mortality.[10] Preeclampsia, along with related hypertensive disorders of pregnancy, affects approximately 180,000 to 300,000 births.[10] In 2017, the annual short-term costs of preeclampsia in the United States were estimated to be 2.2 billion dollars.[9] Given that the prevalence of preeclampsia has only increased, these healthcare costs will likely be far higher in 2024.

## Disparities in patient outcomes based on race

Globally, pregnant women experience health disparities based on their race. It is estimated that over 90% of deaths caused by preeclampsia occur in low and middle-income countries, which leads to the conclusion that preeclampsia disproportionately affects minority populations.[7] In the United States, **African American women and their unborn babies bear the brunt of preeclampsia.** They are twice as likely to develop preeclampsia compared to white women. African American mothers and their unborn babies are around three times more likely to die from preeclampsia compared to white women.[11]

## Preterm preeclampsia is a particularly dangerous subtype

Preeclampsia can occur at preterm gestation, before 37 weeks of pregnancy, or at term gestation once the pregnancy has reached at least 37 weeks. In the United States, there are up to 30,000 cases of preterm preeclampsia annually. Preterm preeclampsia can be especially dangerous as the mother and fetus are at higher risk of complications. To save the mother's life, clinicians are often forced to deliver the fetus at preterm gestation, well before the fetus has fully developed. **There are no treatment options that allow severe preterm preeclamptic patients to continue pregnancy to full-term gestation**.

Although preterm delivery is a life-saving intervention, it has poor health outcomes for the fetus depending on how early the baby is born.[12] Very preterm babies, such as those born between 24 and 32 weeks gestation, are at risk of cerebral palsy, intellectual deficits, blindness, deafness, death soon after birth, and severe developmental delays.[13] **Preeclampsia is one of the major causes of prematurity.** When preeclampsia occurs at a very preterm gestation, the standard of care is to leave the baby in utero for as long as possible. However, clinicians intensely monitor the mother and fetus in hopes that the preeclampsia does not progress. Once there is evidence of disease progression as severe organ injury, delivery of the baby must take place regardless of gestational age to save the mother's life.[14] Because very preterm babies will need intensive care for many months, the healthcare costs of preeclampsia in the United States disproportionately come from preterm preeclampsia. A study from California, based on data from 2009-2011, found that the average all-in maternal hospital delivery cost for infants born at less than 28 weeks gestation was $317,982.[9,15] These cases represented only 0.4% of all deliveries but accounted for 20% of the total delivery costs. Another retrospective cohort study from 2008-2016 involved 763,566 infants with insurance coverage through Aetna, Inc., and found that the average cost for the first six months of postnatal life was $484,640 for infants born between 24-28 weeks gestation.[16] The highest cost was for infants born at 24 weeks, at $603,788, which had a mortality rate of 24.4%.

## Preeclampsia's impact can last a lifetime

Once a pregnant woman is diagnosed with preeclampsia, she will have a **lifelong increased risk of chronic illnesses,** especially related to her cardiovascular health. Additionally, she will have a high risk of developing chronic hypertension, heart and renal failure, death from cardiovascular disease, and a two to four times increased

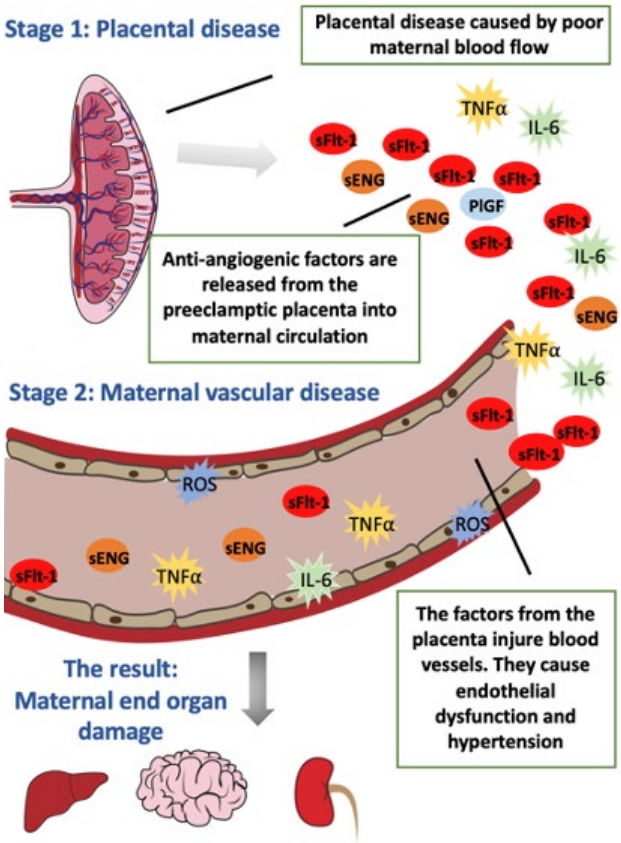
risk of stroke.[2,17] as mentioned, if the baby is premature and growth-restricted at birth, the baby will struggle with **lifelong adverse health effects as well.**

(Additional reading: comprehensive reviews in The Lancet, New England Journal of Medicine and British Medical Journal.[2,3,18])

# STAGES OF PREECLAMPSIA

## Preeclampsia advances in two stages: the first stage is a placental disease, and the second stage is a maternal vascular disease and subsequent endothelial dysfunction and organ injury

First proposed in 1993, a two-stage paradigm was developed as a useful way to conceptualize the pathogenesis of preeclampsia. The first stage is **poor early placental development**, followed by the second stage of systemic **maternal vascular and endothelial dysfunction** that leads to **critical maternal organ injury (Figure 1).**[19]




### Figure 1: Pathogenesis of preeclampsia

**Stage 1: placental disease.** Poor remodelling of the local maternal vasculature injures the placenta and causes 'syncytiotrophoblast stress' (see text). The unhealthy syncytiotrophoblast releases a raft of anti-blood vessel (anti- angiogenic) factors into the maternal circulation. These include **anti-angiogenic proteins** sFltl and sEng, **oxidative stress factors** (ROS) and **proinflammatory cytokines** (such as TNF-a and IL-6)

**Stage 2: maternal vessel disease and endothelial dysfunction.** There is a maternal vascular injury caused by the anti-angiogenic factors released from the preeclamptic placenta. This vascular injury causes **endothelial dysfunction, hypertension and injury to many of the mother's organs.**

Brown structures in the blood vessels depict endothelial cells
sFltl — Soluble fms like tyrosine kinase
sEng — soluble endoglin
ROS — reactive oxygen species
TNF a — Tumour necrosis factor alpha
IL 6 — Interleukin 6

### Stage 1: Placental disease

In normal early pregnancy, the developing placenta remodels the maternal vasculature inside the uterus to gain nutrients from the maternal bloodstream for growth. After the early placenta embeds in the uterus, columns of placental cells grow inward through the inner third of the uterus, then enter the lattice of local uterine blood vessels (**spiral arterioles**) and strip these vessels of their muscular walls, rendering them no longer able to contract (i.e., spiral arteriole remodeling).[20-22] The net result is significant remodeling of the maternal vascular architecture that directly borders the placenta. The remodeling leads to large sources of maternal blood that flow gently along the placental surface. This configuration sets up ideal conditions for transferring nutrients and oxygen from the maternal bloodstream to the placenta. Uterine vascular remodeling happens during the first trimester of pregnancy (up to 13 weeks gestation).

Without proper placental implantation, uterine vascular remodeling does not lead to a healthy exchange of nutrients from mother to fetus.[18,23] In preeclampsia, the spiral arteriole remodeling of the muscular layer is incomplete, so the maternal **spiral arterioles** retain the ability to contract. Contraction of the maternal blood vessels decreases how much blood can pass through them and increases blood flow pressures and speeds. Instead of large volumes of maternal blood gently flowing along the surface of the placenta, local blood flow is turbulent and has a high velocity.[22,24,25] The turbulent blood flow injures the delicate cellular surface of the placenta and the villous tree. It causes injury to the cellular layer that lines the placental surface (the **syncytiotrophoblast** layer). The syncytiotrophoblast cells then suffer a multitude of pathological cellular stresses (ischemia, senescence, endoplasmic reticulum stress, oxidative damage, and a pro-inflammatory state).[24-28] **Syncytiotrophoblast stress** is an overarching term used to describe this mechanism in preeclampsia **(Figure 1).**[3,29,30] Overall, stage 1 of preeclampsia, also known as placental disease, is illustrated by disrupted uterine vascular remodeling. This leads to poor maternal blood flow and nutrient exchange to the placenta and subsequent pathological cellular stresses.

Left in a situation of persistent low oxygenation and low nutrient levels, the **preeclamptic placenta releases damaging anti-angiogenic factors** (sFlt-1 and sEng), oxidative stress factors (ROS), pro-inflammatory cytokines, and exosomes or cellular debris throughout the rest of pregnancy.[4,31-34] These factors cause widespread vascular injury and hypertension, which develop into stage 2 of the disease.

### Stage 2: Maternal vascular disease and endothelial dysfunction

In stage 2 of preeclampsia, excessive secretion of factors in the preeclamptic placenta contributes to maternal vascular disease and subsequent endothelial dysfunction and hypertension.[29] a likely central driver of stage 2 is an overproduction of an **anti-angiogenic protein** called **soluble fms-like tyrosine kinase-1**, or **sFlt-1.**[35-37] sFlt-1 binds to the pro-angiogenic factor vascular endothelial growth factor-1 (**VEGF-1**) and renders it inactive **(Figure 3).** Vascular endothelial growth factor (**VEGF**) signaling on blood vessels is necessary to maintain good vascular health in a pro-angiogenic state. Neutralized by the binding of sFlt-1, VEGF-1 can no longer bind to its specific receptors studded along blood vessels to maintain healthy blood vessel homeostasis.[37]

In addition to sFlt-1, soluble endoglin (sEng) is another anti-angiogenic protein that may play an important role in the pathogenesis of preeclampsia.[38] Like sFlt-1, sEng is produced in excess from the preeclamptic placenta and may plausibly cause endothelial dysfunction.[37] Animal studies where sEng is over-expressed in pregnant mice reproduce all the features of preeclampsia in humans, including high blood pressure, protein the urine and even evidence of liver injury that occurs with severe preeclampsia.[39]

In addition to sFlt-1 and sEng, many other factors are overproduced by the diseased placenta and travel into the mother's circulation. The additional factors include oxidative stress factors (ROS), pro-inflammatory cytokines, and exosomes or cellular debris.[4,31,32,34] Subsequently, these factors incite widespread injury to the mother's blood vessels.[37] As mentioned, her heart, kidneys, lungs, and brain can become severely injured.

As the preeclamptic pregnancy continues into the second trimester, the placenta continues to produce harmful factors that travel through the maternal circulation, leading to more severe vascular disease and endothelial dysfunction in arterial blood vessels.

An arterial blood vessel has three main layers: the endothelial cell layer, the vascular smooth muscle cell layer, and the connective tissue layer **(Figure 2).**

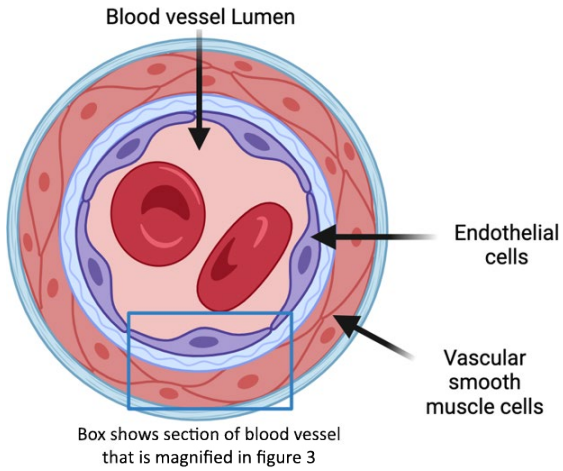
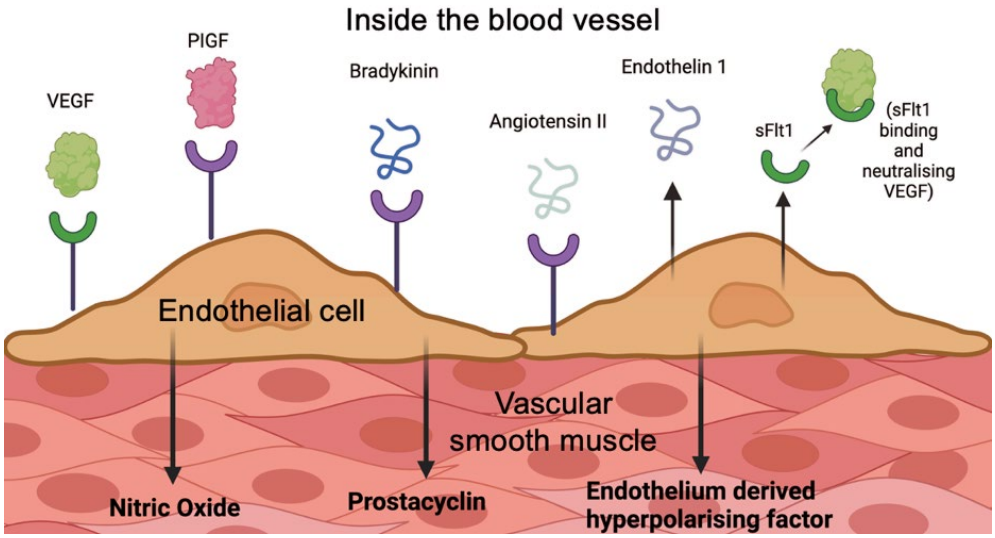



**Figure 2: Cross section of a blood vessel**

In preeclampsia, the two major layers of interest are the endothelial cell layer and the smooth muscle cell layer. In contact with the blood, the most proximal layer inside the vessel is comprised of **endothelial cells**. These cells are bound together to form a sealed sheath. The more distal (middle) second layer is comprised of **vascular smooth muscle cells**. Unlike endothelial cells, which are a single cellular layer, vascular smooth muscle cells create muscles through multiple layers of cells. Hence, one surface of the endothelial cell layer directly interacts with the blood moving through the vessel, and the other surface of the endothelial cell layer binds to the vascular smooth muscle cell layer of the blood vessel. The primary roles of the vascular smooth muscle cell layer are to contract, which narrows the diameter of the blood vessel and increases blood pressure, and to relax, which widens the diameter of the blood vessel and decreases blood pressure. Signals received from the endothelial cells inside the arterial wall dictate whether vascular smooth muscle cells contract or relax. The third, outermost layer comprises connective tissue, which connects the vessel to surrounding tissues.




## Figure 3: Endothelial cell signaling

**Endothelial cells are master controllers of blood vessels. Endothelial cells:**

**1) Receive signals from molecules in the blood:** Such as VEGF, PIGF, Bradykinin and Angiotensin II. These molecules latch onto specific receptors on the surface of endothelial cells

**2) Send signals locally to the underlying smooth muscle:** Such as nitric oxide, prostacyclin and endothelium derived hyperpolarising factor.

**3) Send signals into the bloodstream:** Such as endothelin 1 and sFItl and others.

VEGF — vascular endothelial Growth Factor
PIGF — Placental growth factor
sFlt-1 — soluble fms like tyrosine kinase

**Endothelial cells** are master regulators of blood vessels and take the lead in coordinating blood pressure and general vascular health. They receive messages from proteins and molecules in the bloodstream and react accordingly. They can send local messages to the underlying smooth muscle cells and relay distant information through the bloodstream **(Figure 3):**

1. **Receive signals from molecules in the blood:** Molecules travel through the bloodstream and bind to specific receptors on the surface of endothelial cells. The receptors will relay signals inside the cell to then issue further commands. These signals are relayed through sophisticated molecular circuitry inside the endothelial cells. Each molecule travels through the bloodstream and binds to corresponding receptors on the surface of endothelial cells. Examples of molecules that endothelial cells may receive from the bloodstream include **VEGF**, placental growth factor (**PIGF**), or **bradykinin,** which promote vasodilation. In opposition, angiotensin II (**ANG2**) binds to the angiotensin II type 1 receptor (**AT1R**) to promote vasoconstriction.

2. **Send signals locally to the underlying vascular smooth muscle:** Endothelial cells create and release molecules that instruct vascular smooth muscle cells to contract or relax. Three important molecules released by endothelial cells that stimulate the vascular smooth muscle cells are **nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF)**. These molecules promote local vascular relaxation, thereby reducing blood pressure. In opposition, **Endothelin-1** is a peptide that is one of the most potent vasoconstrictors released by the endothelial cells in blood vessels and may cause hypertension.[40] In addition, the renin-angiotensin system (RAS) is thought to be dysregulated in preeclampsia, which contributes to the pathogenesis of the disease. Several studies have revealed that women with preeclampsia generate a unique agonistic autoantibody, which activates **AT1R**, a key component in the RAS cascade leading to vasoconstriction.[41]

3. **Send distant signals into the bloodstream:** Endothelial cells can release specific molecules into the bloodstream that travel and send signals to far away endothelial cells. Examples of these molecules include **sFlt-1, sEng, endothelin-1**, and **cytokines**. These cause a pro-inflammatory state and lead to hypertension and endothelial dysfunction.

In summary, the three main functions of healthy endothelial cells are disrupted in preeclampsia. The preeclamptic mother's endothelial cells

receive pro-inflammatory molecules, which affect local tissues and travel through the bloodstream to negatively affect other tissues. Upon progression of the disease, in stage 2 of preeclampsia, maternal vascular disease continues endothelial dysfunction and causes hypertension and organ injury.

# SEARCHING FOR A BETTER TREATMENT OPTION

## An agent that slows the progression of preeclampsia would be a major breakthrough. It could save many lives and reduce healthcare costs

The current treatments for preeclamptic symptoms are hypertensive agents and possibly magnesium sulfate to reduce the risk of stroke or seizures. However, no agent on the market will **slow disease progression and ameliorate preeclampsia**. Without a standardized treatment, when preeclampsia is diagnosed, the clinical decision is to closely monitor the mother and hope the disease doesn't progress. If preeclampsia progresses, induction of labor or cesarean section is the standard of care to alleviate active symptoms. As mentioned on page 3 of this white paper, clinicians closely monitor the degree of maternal organ damage. If severe injury is apparent, the baby is prematurely delivered regardless of gestational age.

Two of the most popular hypertensive agents, ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are not used for preeclampsia due to their significant teratogenic effects and the potential for severe adverse outcomes for the fetus. These small molecule agents can cross the placenta and disrupt normal fetal development, particularly affecting the renal system, which can lead to conditions such as oligohydramnios (reduced amniotic fluid) and fetal renal failure. Additionally, ACEIs and ARBs are associated with increased risks of congenital malformations, fetal hypotension, intrauterine growth restriction (IUGR), preterm birth, and even fetal demise. These risks make them unsuitable for managing hypertension in pregnant women, especially those with preeclampsia. Treatment options today to lower blood pressure, often with limited effect, include methyldopa, labetalol, and nifedipine, which are preferred to control blood pressure without compromising fetal health.

An agent that maintains healthy blood pressure and reduces maternal blood vessel injury would save lives in preterm preeclampsia, term gestation preeclampsia, and postpartum.

**In preterm preeclampsia cases**, the fetus may have **increased gestation days in utero.** Allowing the pregnancy to safely continue to full-term gestation would reduce premature birth complications. A pharmaceutical agent that maintains healthy blood pressure and improves maternal blood vessel health could also reduce immediate healthcare costs from neonatal intensive care admission and give the baby better prospects for lifelong health. For mothers with **preeclampsia at term gestation**, diagnosed after 37 weeks of pregnancy, there is often evidence of severe organ injury.[14,42] Hence, the treatment with DM199 could quell the disease long enough for clinicians to have time (around 24-72 hours) to safely birth the baby. It could offer safer outcomes for the mother and unborn baby.

## Clinical trials investigating new agents

Unfortunately, phase 3 clinical trials testing preeclampsia agents have not been successful. Generally, the patient population treated in these trials are women with **preterm preeclampsia**. The primary outcome for most trials has been **to increase the gestation time** so the baby can fully develop in utero. At very premature gestations, such as 24-32 weeks gestation, the addition of 5-7 days in utero is likely to translate into significantly improved health outcomes for the newborn and reduce the risk of death or permanent chronic illnesses at the time of birth.

Current or completed clinical trials for agents to treat **preeclampsia are sildenafil, metformin, siRNAs, antithrombin, pravastatin, and esomeprazole**.

**1. Sildenafil:** Trapani et al. reported a randomized trial of 100 participants that found oral sildenafil (50mg, three times a day) given to women with preterm preeclampsia significantly lengthened gestation by four days.[43] This is notable as it was the first randomized trial to identify an agent that could increase gestation time. It was a hopeful discovery. The results indicate that sildenafil enhances the effect of **nitric oxide**, allowing for vasodilation and reducing the preeclamptic mother's blood pressure.[43] There has also been a worldwide effort to see whether oral sildenafil could be used to treat severe **fetal growth restriction** (where the baby undergrows and becomes sick because the placenta is poorly perfused with maternal blood). Unfortunately, these trials were abruptly stopped because a trial in the Netherlands raised concerns that sildenafil may cross into the placenta and cause serious harm to the fetal lungs through neonatal pulmonary hypertension and neonatal loss.[44] Thus, **sildenafil cannot be further considered an agent** to be administered during pregnancy.

The experience of the sildenafil clinical trials in obstetrics offers two lessons. The first is that finding an agent that can increase **nitric oxide release to treat preeclampsia** and rescue placental function may be promising. The second reminds researchers to be cautious if the investigational agent crosses over to the placenta and into the fetal circulation, as it may have untoward effects on the unborn baby.

**2. Metformin:** Cluver et al. published a randomized trial showing that oral metformin may lengthen gestation by seven days.[45] Traditionally, metformin is used to treat diabetes and gestational diabetes. This clinical trial was motivated by prior laboratory experiments that demonstrated the possibility that metformin may improve endothelial function and lead to vasodilation.[46] Since the initial trial, a large validation randomized trial has been initiated in Cape Town with the aim of recruiting 500 participants (**Trial registration: PACTR202104532026017**). Metformin seems to be the lead candidate as the first agent that may slow the disease progression of preeclampsia.

However, there are two points to note from the Cluver et al. trial.[45] Primarily, while there was an encouraging seven-day gain in gestation, many of the participants still went on to develop severe preeclampsia and needed urgent induced birth. This means that metformin alone is not enough to slow the disease progression of preeclampsia. Hence, even if the validation trial shows metformin is useful, **other more effective agents are urgently required**. Secondly, as metformin is a small molecule agent, it readily crosses the placenta, and the mother and fetus had similar circulating levels of metformin in the blood. While large epidemiological studies have been reassuring and not uncovered undue harm, there is an ongoing debate that metformin given during pregnancy may be risky for the fetus.[47,48]

**3. Short interfering RNAs (siRNA) targeting sFlt-1:** Comanche BioPharma is developing CBP-4888 for the treatment of preeclampsia. They recently completed a Phase 1 study (NCT05881993) involving healthy female volunteers of childbearing age, and have announced plans to dose their first pregnant preeclamptic patients in 2024.[49] CBP-4888 is a fixed-dose combination of two chemically synthesized, lipid-conjugated small interfering ribonucleic acid (siRNA) duplex oligonucleotides (siRNA-2283 and siRNA-2519) targeting two soluble fms-like tyrosine kinase-1 (sFlt-1) mRNA isoforms.[50] It is administered as a single subcutaneous injection and is intended to silence the gene responsible for the production of sFlt-1 in the placenta to reduce circulating levels of the sFlt-1 protein. As described above,

the preeclamptic placenta releases many harmful factors. sFLT-1 is one of two key antiangiogenic proteins (sENG being the other), in addition to inflammatory cytokines (TNF-α and IL-6) and harmful reactive oxygen species also released by the placenta.

**4. Antithrombin, Pravastatin, and Esomeprazole:** Randomized clinical trials of investigational agents include intravenous **antithrombin,** which is meant to dampen the immune system, but a USA-based, multi-center trial failed to find benefit, and an unpublished Japanese trial also found no benefit of antithrombin in preeclampsia treatment [agent name KW-3357].[51,52]

**Pravastatin,** a cholesterol-lowering agent, and oral esomeprazole, an agent used to treat gastric reflux, are postulated to inhibit the development of preeclampsia.[53-57] However, all trials using **antithrombin, pravastatin, and esomeprazole** to treat preeclampsia have **reported negative outcomes.**

(A review in the American Journal of Obstetrics and Gynecology details many other agents in preclinical development.[58])

# SECTION 2: THE POTENTIAL OF DM199 TO TREAT PREECLAMPSIA

**The ideal drug to treat preeclampsia is one that can**
**1) powerfully reduce blood pressure**
**2) increase blood flow to the placenta to reduce placental disease,**
**3) reduce endothelial injury**
**4) does not cross through the placenta at all AND**
**5) already shown to be safe when administered to humans in a non-pregnant population. No such drug has yet been proposed**

## DM199: a promising candidate treatment for preeclampsia

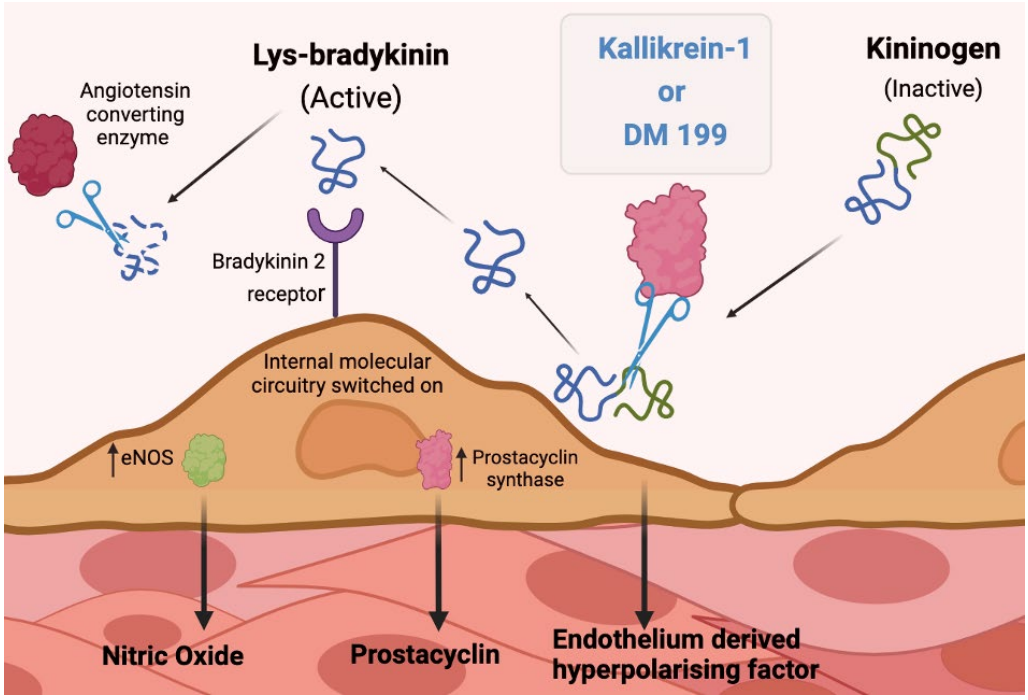
DiaMedica Therapeutics is developing DM199, a manufactured, synthetic version of tissue Kallikrein-1 (rhKLK1) with small modifications that increase its stability. KLK1 is an endogenous protein created and secreted from healthy endothelial cells and other tissues, such as the kidneys, pancreas, and lungs. **DM199 has potent blood pressure-lowering effects** by simply switching on existing natural molecular machinery within cells.

## The main role of KLK1 is to produce active bradykinins which dilate blood vessels and reduce blood pressure

As mentioned above, bradykinin is a circulating peptide that powerfully reduces blood pressure through vasodilation. Kininogens are inactive proteins released from the liver into the circulation. KLK1 binds to kininogens and cleaves the molecules to make kinins that include **bradykinin (Figure 4).**

Bradykinin is a short nine amino acid peptide that binds to and activates bradykinin 2 receptors that are studded on the surface of endothelial cells. Due to rapid degradation by inactivating enzymes, bradykinin exerts biological action locally at the site where they

are produced. Once activated, bradykinin 2 receptors trigger a molecular cascade within the endothelial cells that upregulates the production and release of three potent vasodilating molecules, **nitric oxide,** and **prostacyclin** and **endothelium-derived hyperpolarizing factor.**




## Figure 4: Kallikrein 1 and DM 199 signalling

**Kallikrein-1 and DM 199 makes lys-bradykinin, a potent blood pressure lowering molecule**

**Kallikrein-1** or **DM 199** (drug version of Kallikrein-1) cleave inactive kininogens to make active **bradykinin (or Lys-bradykinin).** This happens in the bloodstream.

**Bradykinin/Lys-bradykinin** then activates the **bradykinin 2 receptor** on the surface of endothelial cells. This switches on internal molecular circuitry which results in the production and secretion of the three major molecules that relaxes vascular smooth muscle cells to **reduce blood pressure.**

Activation of the bradykinin 2 receptor increases **endothelial nitric oxide synthase (eNOS)** which makes **nitric oxide,** and it increases the enzyme **prostacyclin synthase** which makes **prostacyclin.**

**Bradykinin** has a very short life life. It is broken down within a minute it is made by angiotensin converting enzyme.

**Other notes:**

Activation of the bradykinin 2 receptor may usefully reduce inflammation (not shown in this diagram).

A bradykinin 1 receptor exists but the bradykinin 2 receptor is thought to account for most of the vascular actions of the kininogen-kinin-receptor system. Bradykinin 1 receptor is specifically upregulated in pathological, pro- inflammatorv situations such as tissue trauma.

Nitric oxide is a short-lived gas made by **nitric oxide synthase (eNOS)**, a protein enzyme inside endothelial cells. The binding of bradykinin to the bradykinin 2 receptor activates **eNOS** by adding a phosphate molecule at one site (on the amino acid serine at position 1179) and removing a phosphate molecule at another site (from the amino acid threonine from position 497).[59] Adding or removing a phosphate molecule acts as an on-off switch for many proteins. In addition, binding the bradykinin 2 receptor sends a signal into the cell's nucleus to produce more **eNOS**. **Nitric oxide** and **prostacyclin** diffuse from endothelial cells into vascular smooth muscle cells and cause the muscle to relax. This dilation of the blood vessel increases blood flow[60]. Nitric oxide and prostacyclin are believed to work in synergy. In addition, there is evidence that activation of the bradykinin 2 receptor promotes the release of a third vasodilating molecule, **endothelium-derived hyperpolarizing factor (EDHF)** that works alongside NO and PGI2 to relax smooth muscle cells, leading to increased vasodilation and blood flow.[61]

DM199 switches on existing molecular circuits to promote **significant relaxation of blood vessels** and thus, **a reduction in blood pressure.** Potent blood pressure reductions have already been shown in multiple clinical trials of DM199 used to treat non-pregnancy conditions. Hence, **DM199 is an excellent candidate to reduce hypertension, which is a consistent feature in preeclampsia.**

Preeclampsia is a complex pregnancy complication primarily caused by poor maternal blood flow to the placenta, leading to placental ischemia and the subsequent release of anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng). These factors contribute to endothelial dysfunction and the clinical manifestations of preeclampsia. DM199 has potential to increase blood flow by promoting vasodilation and improving endothelial function. By enhancing maternal blood flow to the placenta, DM199 could reduce placental ischemia and prevent the release of harmful anti-angiogenic factors. This disease-potential modifying approach might prevent the development and progression of preeclampsia and release of sFlt-1, sEng, ROS and pro-inflammatory cytokines offering a novel therapeutic strategy that addresses the root cause of the condition rather than targeting the multifactorial downstream symptoms.

## Fetal growth restriction

NO, PGI2 and EDHF represent a trio of powerful vasodilators that could offer a comprehensive approach to treating fetal growth restriction (FGR) by enhancing

uteroplacental blood flow. NO induces vasodilation by relaxing vascular smooth muscles, thus improving blood flow to the placenta and increasing oxygen and nutrient delivery to the fetus. Prostacyclin not only facilitates vasodilation but also inhibits platelet aggregation and smooth muscle cell proliferation, further supporting placental blood flow and reducing the risk of thrombosis and vascular occlusion. EDHF, meanwhile, contributes to vascular relaxation through hyperpolarization of smooth muscle cells, working alongside NO and PGI2 to maintain low vascular resistance and optimal blood flow. By targeting multiple pathways that regulate vascular tone and endothelial function, the combination of NO, PGI2, and EDHF can synergistically enhance placental perfusion and mitigate the effects of endothelial dysfunction, offering a promising therapeutic strategy to improve fetal growth and outcomes in pregnancies complicated by FGR.

DM199 may also limit anti-angiogenic factors from causing widespread maternal vascular damage. As described above in stage 1 of preeclampsia, inadequate uterine vascular or spiral arteriole remodeling occurs during early pregnancy, increasing blood flow resistance. Subsequently, the **uterine arteries narrow,** and the lack of oxygen supply to the placenta causes the release of free radicals (oxidative stress)[25] that damage placental cells and activate many abnormal cellular pathways. Ultimately, this leads to syncytiotrophoblast stress[26,27] and the release of many anti-angiogenic factors[58]

There is circumstantial evidence to suggest that this placental dysfunction could be mitigated with increasing nitric oxide. In the abovementioned trial examining oral sildenafil to treat preterm preeclampsia[43], those given the oral sildenafil had improvements in uterine artery blood flow with a 22% reduction in the pulsatility index compared to a 2.1% reduction in those given a placebo (measured using Doppler ultrasound, $p<0.001$)[43]. Conversely, in the 135 patient STRIDER preeclampsia clinical trial of oral sildenafil to treat women with severe fetal growth restriction did not show significant improvements in uterine blood flow[62]. Given these conflicting trials, whether DM199 can increase blood flow in the uterine arteries is a hypothesis that needs to be evaluated in clinical trials.

Additionally, if the hypothesis is proven, DM199 may even have merit as a **treatment for fetal growth restriction** in the absence of preeclampsia. This would be a considerable breakthrough as fetal growth restriction is the leading cause of stillbirth worldwide as approximately 3 million babies are lost to stillbirth annually. The implication is that **DM199 could be a treatment for preeclampsia and intrauterine growth restriction**, which are two of the main obstetrical complications that plague pregnancies worldwide.

## DM199 could reduce endothelial cell dysfunction in maternal vascular disease

In stage 2 of preeclampsia, there is significant endothelial damage and subsequent injury to the mother's vital organs. DM199 administration activates bradykinin 2 receptors and may reduce endothelial dysfunction and vascular injury. **Activation of the bradykinin 2 receptors** promotes a pro-angiogenic state, reduces oxidative stress and inflammation, and increases insulin sensitivity and glucose uptake.

1. A large body of literature shows that tissue kallikrein-1 (or DM199) and VEGF likely play important roles in angiogenesis.[63,64] Activation of the **bradykinin 2 receptor** facilitates signaling of the **VEGF**, which is necessary for maintaining healthy vascular functioning in a pro-angiogenic state. VEGF is blocked by **sFlt-1** and is the target of the siRNA agent studied by Comanche Biopharma. The activation of the bradykinin 2 receptor facilitates VEGF signaling in two ways. First, it relays a signal to increase activation of the VEGF 2 receptor itself that sits on the cell membrane. The VEGF 2 receptor is switched on (or phosphorylated) in a process called transactivation.[65] The second way is that signals are relayed into the nucleus to produce more VEGF protein as well as its main receptor, the VEGF 2 receptor.[63]

2. The downstream intracellular molecules switched on by the bradykinin 2 receptor may **reduce oxidative stress**, commonly found in preeclampsia.[66,67] For example, bradykinin has been shown to reduce the presence of damaging free radicals in cells.[68] DM199 also stimulates nitric oxide and prostacyclin production, which is thought to reduce oxidative stress.[67]

3. The downstream intracellular molecules switched on by the bradykinin 2 receptor may also **reduce inflammation.** For example, nitric oxide acts as a vasodilator and dampens inflammation.[60]

4. Activation of the bradykinin 2 receptor also **increases insulin sensitivity, glucose uptake** and glycogen synthesis, which reduces glucose levels in the bloodstream.[69] High blood glucose can injure endothelial cells, and diabetic women are at a higher risk of developing preeclampsia when pregnant. Reducing blood glucose levels may further reduce endothelial dysfunction.

## DM199 is unlikely to cross the placenta due to its size and the absence of known active transport mechanisms

The overwhelming majority of current drugs cross the placenta. Most drugs are **small molecule drugs** (such as the many drugs sitting on the pharmacy shelves). Being tiny chemicals, small molecule drugs readily diffuse across the cell membrane of the placenta to enter the fetal circulation. While most won't cause harm to the fetus (just like adults, the fetus also has a liver and kidneys capable of metabolizing drugs to protect itself), some might. While small molecules passively cross the placental barrier, monoclonal antibodies, another broad class of medicines, pass through into the fetal circulation an active transport mechanism. Therefore, when new small molecule drugs or monoclonal antibodies are used during pregnancy, follow-up studies are essential to ensure there are no short- or long-term ill effects on the child.

## Hence, nearly all drugs cross into the placenta

Drugs that are either **peptides** (a short sequence of amino acids, the building blocks of proteins) or **proteins** (longer sequences of amino acids) **do not cross the placenta**. They are too big to diffuse through the cell surface and there are no known active transport mechanisms. Currently, there are very few protein and peptide drugs on the market. However, they are an exciting and rapidly growing portion of the drug market (their market growth is far outstripping new small molecule drugs). An example of a blockbuster peptide drug is Ozempic. Another example is oxytocin which has been used for decades in obstetrics to induce labor. **DM199 is a protein drug** and is particularly attractive because, as a protein consisting of around 238 amino acids, it is too large to passively diffuse across the placenta into the fetal circulation. This suggests that, unlike nearly all other drugs, we may need to be less concerned about untoward effects on the fetus.

## Most drugs currently used to treat pregnancy conditions are also drug versions of natural molecules

It is notable most drugs used to treat obstetric conditions are drug versions of natural molecules: these drugs commandeer the body's natural molecular machinery to exert beneficial effects. Examples include natural progesterone given in a vaginal pessary to reduce the risk of preterm birth, various types of prostaglandins given to prime the cervix in preparation for labor, and oxytocin given to stimulate labor.[70,71]

Hence, the concept of administering DM199, an analogue of a naturally occurring protein, is not radical and will enhance its acceptability among both clinicians and patients.

# DM199 HUMAN CLINICAL TRIALS AND REPRODUCTIVE TOXICOLOGY STUDIES

## Human clinical studies

To date, DM199 has been tested in over 275 humans across multiple dose ranges and for up to 95 days of administration. In general, DM199 has been found to be safe and well-tolerated. The most notable side effect and dose limiting tolerability was hypotension (low blood pressure), which only occurred when patients were given exceedingly high doses of DM199.

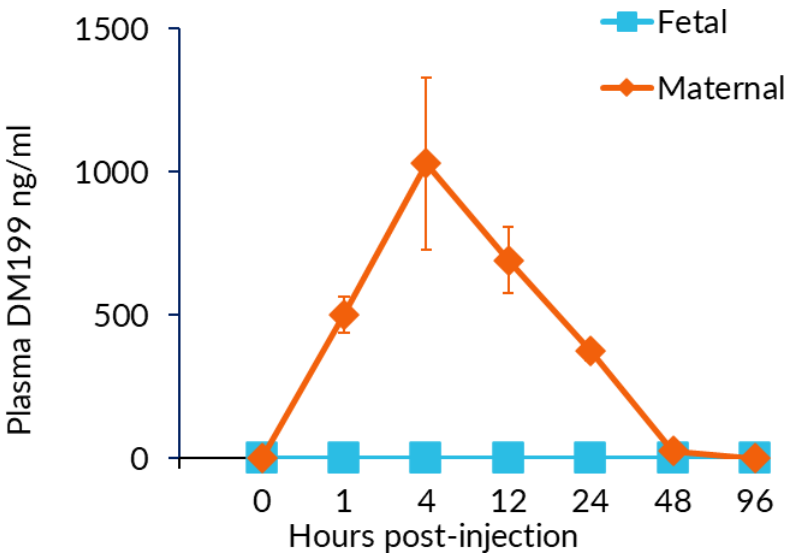
DiaMedica conducted Phase 2 studies in chronic kidney disease (CKD) (REDUX study, n=82) and acute ischemic stroke (AIS) (ReMEDy1 study, n=90). These studies met their primary endpoints of safety and tolerability, and preliminary signals of efficacy were observed in subgroup analyses. In the REDUX CKD study, a statistically significant reduction in blood pressure compared to baseline was observed over 95 days in the subgroup of patients whose baseline systolic blood pressure was greater than or equal to 130 mmHg (n=47). An even greater decrease in systolic blood pressure occurred in subjects with baseline systolic blood pressure above 140 mmHg (p=0.004) and above 150 mmHg (p=0.003). In the ReMEDy1 Phase 2 AIS study, the subgroup of patients who did not receive mechanical thrombectomy (n=46) showed better physical recovery endpoint of excellent outcomes based on modified rankin score of 0-1, a reduced number of deaths, and prevented stroke-in-evolution or recurrent ischemic events in the DM199-treated group compared to the placebo-treated group.

## Reproductive toxicology studies

DiaMedica has completed the pre-requisite developmental and reproductive toxicology (DART) studies which are necessary prior to dosing pregnant humans. These studies evaluated the potential of the drug to have an effect the 3 phases of reproduction, Segment I - effects on fertility, Segment II - effects on development of the embryo and fetus, and Segment III - effects on the fetus through sexual maturation of the offspring of treated mothers. The findings from

these studies support the potential safety in pregnant humans.

In addition, DiaMedica completed a placental transfer study in rodents to assess the potential of DM199 crossing the placental barrier. Pregnant rats were administered large doses of DM199 and sacrificed at different time points after administration. DM199 (KLK1) was subsequently measured in the serum of both the dams (maternal, n=30) and the pups (fetal, n=24). As shown in the exhibit below, a clear pharmacokinetic curve was observed in the dams but reassuringly, no detectable amount of DM199 was found in the pups at any time point. Below the level of quantification values were reported as zero in the graph below.




# POSSIBLE ADVERSE EVENTS OF DM199

## Possible release of prostaglandin E2

In a Phase 1b single dose study of DM199 in patients with CKD, there was a trend toward a rise in prostaglandin E2 levels in the blood. At 24 hours post-administration, median levels of prostaglandin E2 in the blood rose 8% from baseline levels. This rise from baseline was not statistically significant, but this study had a small number of patients, and the trend was noted. Relative to the vast scientific literature on bradykinin receptor signaling and nitric oxide production, research linking bradykinin 2 receptor with prostaglandin release is sparse. However, a laboratory

research study concluded that bradykinin 2 receptor signaling increased the release of prostaglandin E2 in human skeletal cell biopsies.[72] If **prostaglandin E2 were secreted** into the blood in biologically relevant amounts, it could travel to the uterus and **cause cervical ripening** (softening and widening of the cervix). Sometimes, this can lead to uterine contractions and labor.[71] However, the risk is low based on the modest rise of blood prostaglandin E2 seen in the DM199-treated CKD patients. In addition, in the reproductive toxicology studies described above no animals were reported to have had uterine contractions. Our proposed clinical trials (described below) have been designed to safely evaluate (and likely exclude) this potential adverse outcome.

## A worsening of inflammation

As previously mentioned, nitric oxide production may reduce the inflammatory response in endothelial cells. However, it is **unclear whether giving DM199 will reduce inflammation**. For example, bradykinin and bradykinin receptor activation are implicated in the progression of COVID-19.[73] In severe COVID-19 infections, these receptors indicate a pathological upregulation and an exaggerated response, leading to an increased immune response and hypotension.[73] However, much of the pathological immune response is thought to be mediated by the bradykinin 1 receptor, which is only upregulated in severe inflammatory disease states or severe infection. Reassuringly, in the early phase trials of DM199 in CKD patients, the circulating levels of c-reactive protein (a general biomarker of inflammation) did not increase. Hence, we consider an exacerbation of inflammation is unlikely.

## An excessive fall in the mother's blood pressure causing fetal distress

In an obstetric clinic, when hypertensive agents are given to preeclamptic women, the target blood pressure is around 130/80 mmHg. A pronounced fall in blood pressure may reduce the amount of blood pumping to the placenta and reduce oxygen delivery to the fetus. The fetus then becomes distressed and at risk for complications. This hypotension occurs if blood vessels across the body dilate significantly relative to the amount of blood in circulation.

DM199 can cause significant hypotension if overdosed, so identifying the appropriate dose level in pregnancy is essential. In addition, DM199 can cause hypotension if it is given alongside an angiotensin-converting enzyme (ACE) inhibitor. This is because ACE degrades bradykinin and lys-bradykinin and

renders it inactive. The inactivation of ACE means increased/prolonged bradykinin 2 receptor activation. **Fortunately, ACE inhibitors are contraindicated during pregnancy,** meaning there is no risk of both agents being given at the same time. The need to avoid ACE inhibitors in pregnancy is well-known to obstetricians.

# PLANNED CLINICAL TRIALS OF DM199 AS A TREATMENT FOR PREECLAMPSIA

## Human clinical studies

DiaMedica Therapeutics is collaborating with the authors of this white paper (CC, ST, SW) to undertake a Phase 2 clinical trial of DM199 in women with preeclampsia and potentially fetal growth restriction. A short description of these studies is included below, and more details will be shared in the future.

| | Participants | Summary |
|---|---|---|
| Part 1 | Up to 60 | Pregnant woman with preeclampsia between 27 to 42 weeks of gestation and are scheduled to deliver within 72 hours, SBP ≥150 mmHg. Part 1A involves an ascending dose-finding study recruiting up to 30 participants. Part 1B is an expansion cohort of an additional 30 participants at the dose established in Part 1A. Key data from Part 1 will be used to assess safety and tolerability, and to assess whether DM199 acutely lowers blood pressure, acutely dilates intrauterine arteries (measured with Doppler ultrasound), and whether it crosses the placental barrier (measured in cord blood), as well as other disease specific measurements and biomarkers. |
| Part 2 | Up to 30 | Pregnant woman with preeclampsia between 27 to 33 weeks gestation in the expectant management setting, aimed at safely prolonging the pregnancy. Key data from Part 2 is expected to include assessments of safety and tolerability, the number of days pregnancy is prolonged, changes in the urinary albumin-to-creatinine ratio over seven days compared to baseline, need to increase or decrease other antihypertensive agents, as well as other disease-specific measurements and biomarkers. |
| Part 3 | Up to 30 | Pregnant woman between 26 to 32 weeks of gestation with fetal growth restriction (FGR) but without preeclampsia, contingent upon observing if DM199 can enhance intrauterine blood flow as assessed by Doppler ultrasound evaluation in Part 1. Key data from Part 3 is expected to include changes in uterine artery and ophthalmic arterial blood flow (measured by Doppler ultrasound), birthweight centile and fetal growth trajectory. |

# DM199 IS A PROMISING NEW TREATMENT FOR PREECLAMPSIA

This white paper highlights DM199's potential to significantly reduce blood pressure and endothelial dysfunction, enhancing perfusion to both the fetus and maternal organs. These improvements could extend gestational days and potentially mitigate long-term cardiovascular side effects in mothers. Additionally, DM199, being a sizable protein composed of approximately 238 amino acids, is likely too large to passively diffuse across the placenta into fetal circulation, yielding a significant fetal safety profile.

# SUMMARY:

## DM199 is an attractive therapeutic candidate:

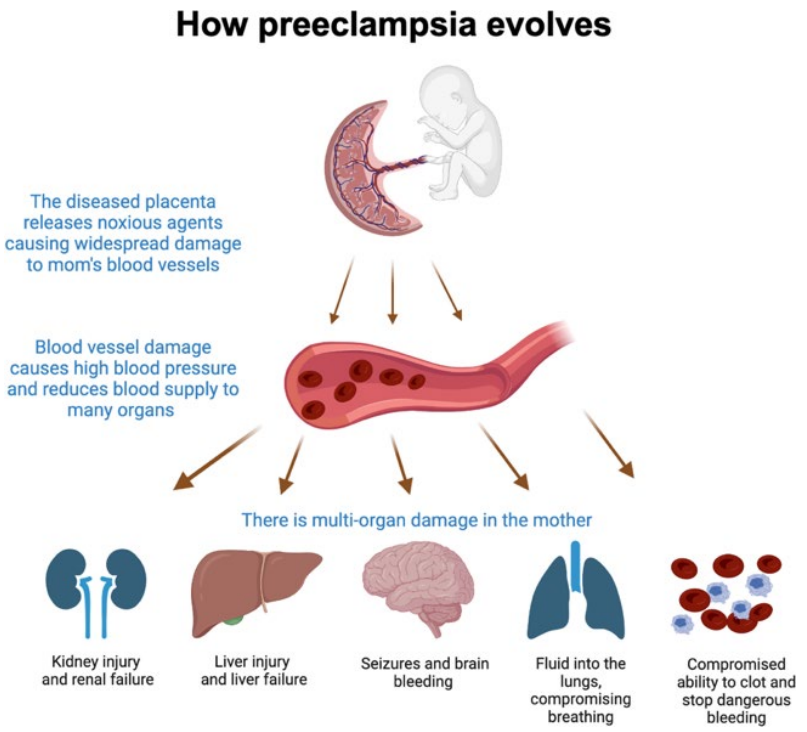
- Powerfully reduces blood pressure, a hallmark of preeclampsia
- May reduce endothelial dysfunction and maternal blood vessel injury
- May rescue the placental disease, improving fetal health but also break the pathological cycle of preeclampsia (placenta ↔ maternal blood vessels)
- In multiple Phase 1 and 2 clinical trials, safety and tolerability endpoints confirmed
- Too large to cross the placenta, confirmed in pre-clinical animal studies
- Animal developmental and reproductive toxicology studies support potential safety in pregnant mothers and fetuses.

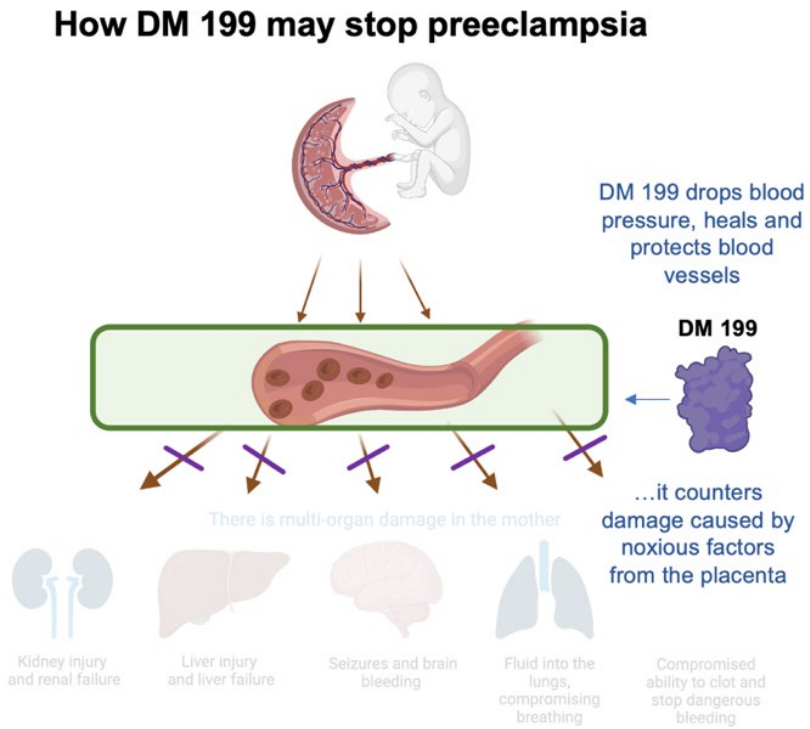
## DM199 is an attractive commercial candidate because:

- Clinical trials for preeclampsia can be done quite quickly because primary outcomes to show benefit are short-term.
- Potential economic benefits include less neonatal intensive care costs if the baby is born less preterm, and shorter hospital admission costs if the mother stays in better health.

**DiaMedica Therapeutics plan to evaluate DM199 as a treatment for preeclampsia by partnering with the academic authors of this white paper. Professors Tong, Cluver and Walker are arguably the top preeclampsia experts in the world in trialing new agents to treat preeclampsia. They run the only clinical trials unit in the world that has completed successive clinical trials on new agents to treat preeclampsia**

# PICTORIAL SUMMARY:



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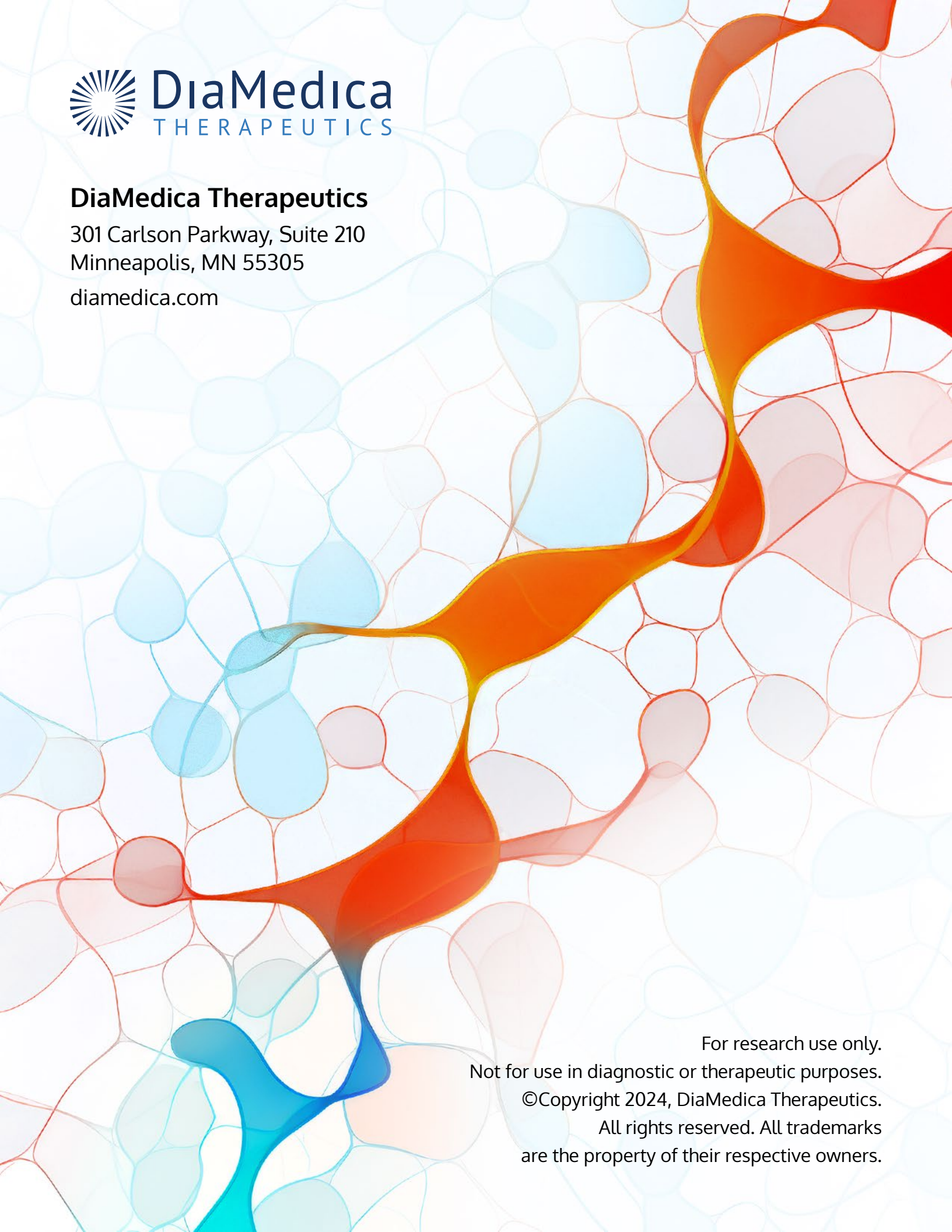

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