The potential of DM199 to treat fetal growth restriction

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Synopsis: Fetal growth restriction is a condition of fetal undergrowth due to a poorly functioning placenta – the life support system of the unborn child. Fetal growth restriction is the leading cause of stillbirth. For those that survive the pregnancy, unhealthy fetal development in utero leaves a legacy of poor health echoing across the lifespan. **No treatment exists.**

A drug therapy that treats fetal growth restriction in utero could prevent stillbirths. But it could also improve lifelong health. Such a discovery would be an unequivocal breakthrough for human wellbeing.

There are two broad therapeutic strategies to tackle fetal growth restriction. The first are therapies acting on the placenta to improve its function. However, given the poor current understanding of placental biology and the lack of clear molecular targets, this approach seems remote from the clinic.

A more feasible target may be to widen blood vessels in the maternal circulation that supply the ailing placenta, increasing the flow of blood, oxygen & nutrients to the starved fetus. This includes opening the large blood vessels feeding the uterus itself (such as the uterine arteries) as well as dilating the many smaller arteries within the uterus supplying the placenta. Increasing maternal blood flow to the placenta could flush the fetus with much needed sustenance and reverse much of the pathology of growth restriction.

DM199 is a recombinant tissue kallikrein-1 (rhKLK1) protein made by DiaMedica Therapeutics. It switches on key molecular pathways to dilate blood vessels. Three decades of research has shown tissue kallikrein-1 can widen blood vessels to increase blood flow. Also, it is likely to have many molecular actions to promote blood vessel health. As such, it has potential to be an exciting drug treatment for fetal growth restriction.

A critical advantage of DM199 as a therapy for pregnancy is that it cannot penetrate the placental surface. This offers a unique safety profile in pregnancy over small molecule drugs (the most common drug type) that cross to the fetus.

This white paper discusses the potential of DM199 to treat fetal growth restriction.

SECTION 1: Overview of Fetal growth restriction

Fetal growth restriction is a pathological condition where a failing placenta cannot meet the oxygen and nutrimental needs of the growing fetus. The fetus starves, undergrows and sickens.

Growth restricted fetuses are at 10-15-fold increased risk of stillbirth. Those that survive are at high risk of serious multiple newborn complications, including developmental delay and cerebral palsy.

In addition, there are longer term adverse health outcomes arising from fetal growth restriction that remain a lifelong threat. Being growth restricted in utero increases the chance of major decrements in health and educational performance across all of childhood. It even increases the risk of conditions that occur in later adult life – stroke, cardiovascular disease and early death. Hence, the injury wrought by growth restriction remains with the fetus lifelong.

Fetal growth restriction is caused by fetal starvation due to poor placental function

Fetal growth restriction is a condition where the fetus falls short of growing to its genetically predetermined size[1]. The most common cause is poor placental function (or placental insufficiency). While the placenta still functions, it not working at full capacity in fulfilling its critical role of transporting oxygen and nutrients from the maternal circulation into the fetus. Starved, the fetus is unable to sustain healthy growth and development. Growth trajectory slows[1] because the fetus re-directs the limited supply of sustenance it does receive from healthy growth to survival. For instance, the limited oxygen it does receive is preferentially shunted to its brain. With such chronic starvation, the fetus undergrows and becomes smaller and less healthy, relative to its peers at the same gestation.

Fetal growth restriction is common

Fetal growth restriction complicates around 10% of all pregnancies, depending on how it is defined. Given there are 3.6 million births in the US a year, it is clearly a common condition. Fetal growth restriction is one of the 'big four' complications occupying most of the obstetrician's time (the remaining three being preeclampsia, spontaneous preterm birth and diabetes in pregnancy). All remaining obstetric conditions are comparatively rarer.

The immediate concern for fetal growth restriction is a high stillbirth risk.

Stillbirth – death of the baby prior to birth - is a catastrophe that prematurely ends 1 in 150 pregnancies in the US[2]. Impacting 21,000 US families a year, it occurs far more commonly than widely assumed. It disproportionately affects minorities, those living in rural communities, and those with the lowest incomes[2].

Fetal growth restriction is the leading cause of stillbirth – pregnancies where the placenta fails entirely. Fetuses diagnosed prenatally (via ultrasound estimation of fetal weight) to be <**10th centile weight** (relative to it gestational age matched peers) **incur a 3-4-fold increased risk of stillbirth (that is, a 300-400% rise)**, compared to peers in utero with a higher weight (that is, those over the 10th centile).

The risk of stillbirth sharply rises with each stepwise decrease in fetal weight centile below the 10th. The risk lifts exponentially for fetuses with a weight under the 3rd centile[3,4]. **Fetuses <3rd centile for weight incur a 10-15+ fold increased risk of stillbirth (that is, a 1000-1500% rise)**, compared to normally grown peers.

Growth restricted fetuses born alive are at risk of poor health across their lifespans

Fortunately, most growth fetuses are born alive. But the many weeks of starvation means unhealthy in-utero development. This rewires the fetus' physiology, leaving it at risk of a myriad of serious health conditions for life.

In the short term, growth restricted fetuses are at greater risk of many complications during the neonatal period (i.e. first 28 days of life), such as neonatal brain injury caused by low oxygenation (hypoxic ischemic encephalopathy), feeding difficulties, hypoglycemia and many other conditions[5]. Risks of fetal growth restriction persist across all of childhood, and they include reduced cardiovascular and metabolic health[6,7], and poorer neurodevelopmental[8] and childhood developmental outcomes[9].

Protracted substrate starvation in utero also gives rise to a legacy of adverse health outcomes across the life course[10,11]. Studies replicated around the world have strongly linked low birthweight with adult onset stroke[12], coronary artery disease[12-14], diabetes[15], obesity[16] and other metabolic conditions[17]. This has given rise to a new field of research, called the Developmental Origins of Health and Disease (DOHAD)[11]. The DOHAD phenomenon is exceptionally well characterized and can be readily recapitulated in animal models. *Finding a treatment for fetal growth restriction that prevents this cascade into lifelong ill-health would be an enormous breakthrough.*

Hence, a drug treatment that rescues poor placental function to support healthy fetal development might not just reduce the risk of stillbirth. It may also reduce the risk of numerous diseases across the lifespan, positioning the newborn for better lifelong health.

The clinical definition of fetal growth restriction

Most cases of fetal growth restriction are clustered among fetuses with a weight under the 10th centile. This is an arbitrary cut-off - some fetuses over the 10th centile weight be affected by placental insufficiency and are growth restricted[1]. Conversely some below the 10th weight centile are constitutionally small and healthy (they were predestined by their genes to be small – the placenta is working perfectly fine and they are not at increased stillbirth risk)[1]. The probability that a fetus is pathological growth restricted (not just constitutionally small) rises with progressive centile decline below the 10th centile[18]. Consequently, most fetuses <3rd centile weight are at the greatest risk of perinatal morbidity and mortality[19-21].

Hence, <10th centile or <3rd centile are common cut-offs used in the clinic to identify fetuses likely affected by fetal growth restriction. A <3rd cut-off enriches for the most serious cases and identifies those at highest risk of stillbirth. ***<3rd centile weight is therefore a well-accepted cut-off to define true fetal growth restriction.***

In the absence of a therapy, current management is close monitoring of the fetus and timed birth before stillbirth happens.

Fetal growth restriction is diagnosed by clinical examination at the prenatal clinic, followed by ultrasound confirmation of its size. Once diagnosed, clinical management is to **closely monitor the fetus** with regular tests of fetal wellbeing (because of the raised stillbirth risk), and to **time birth** (either by induced labor, or by cesarean section) at the most advanced gestational age possible, but **before a stillbirth happens.**

If the pregnancy has reached term gestation (37 weeks of gestation and beyond), management is straightforward. Delivery is planned to occur soon.

However, for fetal growth restriction diagnosed at a preterm gestation, management decisions are more complex. Clinicians do not immediately birth the baby due to the competing risks of prematurity: preterm birth itself can have poor health outcomes for the fetus, depending on how early the baby is born[22]. The earlier in gestation, the risker it is for the newborn. For instance, very preterm babies, such as those born between 22 to 30 weeks' gestation, are at high risk of cerebral palsy, intellectual deficits,

blindness, deafness, death soon after birth, and severe developmental delays[23]. The risks are particularly high for those that birth around 22-26 weeks' gestation. Importantly, all the risks of prematurity fall as the fetus advances in gestation.

Given the risks of preterm birth, the principle of clinical management is to allow the pregnancy to continue, to get the unborn baby to the most advanced gestation possible – ideally 37 weeks' gestation – as long as it is considered safe. But it is a high-risk situation. While the growth restricted fetus remains in utero, it is at elevated risk of stillbirth. Furthermore, placental function can often worsen, sometimes within weeks, and cause a stillbirth. The fetus is therefore closely monitored using various tests of fetal wellbeing, described below. If there are signs of advance 'deterioration' (for instance, bad ultrasound findings appear that suggest fetal low oxygenation and a high risk of imminent stillbirth) then the fetus is immediately delivered, irrespective of its gestation.

Tests of fetal wellbeing to monitor fetal health in fetal growth restriction

Umbilical artery Dopplers: A mainstay of fetal monitoring is measuring the degree of blood flow resistance in the umbilical artery (in the umbilical cord). As placental function gets worse, resistance to blood flow within it increases. The degree of blood flow resistance in any blood vessel can be measured using Doppler ultrasound (Figure 1).

In healthy pregnancies, there is continuous forward blood flow in the umbilical artery throughout every phase of the fetal cardiac cycle (each cycle being a single pump of the ventricles of the fetal heart). The fetal cardiac cycle is split into 'systole' and 'diastole'. Forward blood flow in the umbilical artery is at peak velocity during the squeeze of the heart muscle (**systole**). Forward flow then slows as the heart muscle relaxes (**diastolic** – the lowest pressure phase of the cardiac cycle) but there is still continuous forward flow of blood (Figure 1).

As the placental circulation shuts down with fetal growth restriction, resistance in blood flow increases in the placental circulation. As fetal growth restriction pathology deteriorates, there is typically stepwise deterioration: more and more blood flow resistance, where each step signifies worsening in placental health. Each deteriorating step puts the fetus in more jeopardy.

The **mildest** abnormality is **increased diastolic blood flow resistance,** where there is still forward flow during the diastolic phase of the cardiac cycle, but resistance is increased compared to normal pregnancy.

When placental insufficiency worsens, Doppler findings may progress to **absent end diastolic flow**, where there is no blood flow at all during diastole with each cardiac cycle (Figure 1). This already has a

strong association with fetal hypoxia (low oxygenation) and vastly increased risk of stillbirth or bad neonatal outcomes.

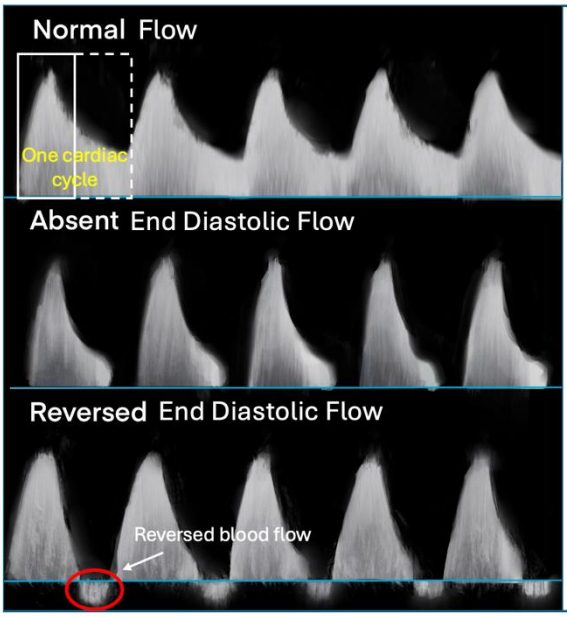


Figure 1: Umbilical artery Doppler Blood Flow patterns

Representative images of blood flow patterns seen on Doppler ultrasound

Normal Flow

Continuous forward flow of blood

Absent End Diastolic Flow

Forward flow of blood ceases for a short period during diastole, in every cardiac cycle (5 cycles shown)

Reversed End Diastolic Flow

Flow of blood reverses direction for a short period during diastolic, in every cardiac cycle (5 cycles shown)

The latter two patterns are abnormal. They can reflect poor placental function. They are strongly associated with a very high risk that the baby is receiving inadequate levels of oxygen. Both are associated with a high stillbirth risk

White box – Systole/Systolic phase of the cardiac cycle.

White dotted box – Diastole/Diastolic phase of the cardiac cycle (see text for explanation of systole & diastole)

When placental insufficiency is very severe, **reversed end diastolic flow** occurs. This arises when flow resistance to blood flow in the placenta is so high that during diastole, blood actually flows backwards (the wrong way) during diastolic (Figure 1). This only happens when large segments of the placenta have ceased to function entirely and it is a very sinister sign: - it is strongly associated with a high risk of imminent stillbirth. *When absent, or reversed diastolic flow are seen, serious consideration needs to be made for immediate delivery.*

Other tests of fetal wellbeing: Other tests clinicians use to monitor the fetal condition in the situation of early onset fetal growth restriction include the **cardiotocograph** (monitors fetal heart rate patterns, where particular abnormal patterns appear at advanced stages of fetal hypoxia); the **amniotic fluid index** (the amount of amniotic fluid around the fetus can fall if the placenta is not working well) and ultrasound Doppler of the **ductus venosus** (fetal blood vessels in the liver, close to the heart) or the **middle cerebral artery** (a vessel in the brain). It is particularly worrying if a number of these parameters become abnormal.

Uterine artery Dopplers: Strongly associated with preterm fetal growth restriction is **increased blood flow resistance in the uterine arteries**, also measured using Doppler ultrasound. Unlike the tests mentioned so far (which directly monitor the fetal compartment), this primarily reflects resistance in the maternal circulation that supplies nutrients to the placenta. Increased uterine artery resistance reflects high resistance in the maternal circulation and is strongly associated with fetal growth restriction (and

the related placental condition of preeclampsia). Once fetal growth restriction is diagnosed, it is not typically used to monitor fetal wellbeing. *However,* m*easuring uterine artery resistance may be an excellent way to monitor the response of therapeutic drugs that aim to increase maternal blood supply to the placenta, to rescue fetal growth restriction.*

International guidelines class preterm fetal growth restriction into two subtypes:

International guidelines further divide preterm fetal growth restriction into two types, according to gestation: **early onset fetal growth restriction** (diagnosed <32 weeks gestation) and **late onset fetal growth restriction** (32 weeks gestation, and over). These are worth noting, as they are sufficiently different clinical entities where clinical trials seeking to treat may need different designs.

Early onset preterm fetal growth restriction (diagnosed <32 weeks gestation)

Early onset preterm fetal growth restriction is uncommon, complicating around 0.5-1.0% of all pregnancies[24]. However, it is an extremely high-risk condition where the individual risk of stillbirth and prematurity is very high.

To avoid stillbirth, clinicians are often forced to deliver preterm. Yet prematurity and fetal growth restriction is a particularly risky combination- costly for the babies, their families and the health system. These infants are at highest risk of severe neonatal complications: lung disease (bronchopulmonary dysplasia), gut injury requiring surgery (necrotizing enterocolitis), retinal damage, prolonged ventilation and hospital stay[25].

Early onset preterm fetal growth restriction is typically (but not always) progressive, where placental deterioration occurs over weeks and the fetus gets progressively sicker. And for most cases, without carefully timed delivery by clinicians, stillbirth would be a common outcome. Hence, most will be delivered at a preterm gestation, where birth was timed by the clinical team to deliberately avoid stillbirth. Because deterioration usually occurs over weeks, we would still expect the baby to be born preterm.

Early onset preterm fetal growth restriction differs from late-onset fetal growth restriction because of its 1) **strong association with preeclampsia**, the 2) **severity of placental and fetal disease** and 3) **its largely predictable pattern of deterioration** (which can be captured on the fetal tests of wellbeing just described, mainly Doppler ultrasound of the umbilical artery)[24].

Association with preeclampsia: Early onset preterm fetal growth restriction is strongly associated with the development of preeclampsia. **Hence, a drug that can treat both preeclampsia and fetal growth restriction may be particularly useful.**

Severity of placental and fetal disease: Early onset preterm fetal growth restriction is the most severe variant of fetal growth restriction. There is an **extremely high risk of perinatal death** (either a stillbirth, or death during the neonatal period) **– an absolute risk of 15-20%**[26,27] (compared with baseline population rate of around 1 in 150, or 0.7%). The risk is higher the earlier in gestational age when it arises – for preterm fetal growth restriction diagnosed between 20-26 weeks' gestation, the risk of perinatal death is 31%[25]. *The risk of death falls sharply as gestation advances, so clinicians are singularly focused on gaining days and weeks during this critical period.*

Predictable, stepwise pattern of deterioration detectable on Doppler ultrasound: As the placenta deteriorates, the umbilical artery Dopplers parameters usually progresses in a stepwise fashion, from elevated diastolic blood flow resistance to absent end-diastolic flow, to reversed end-diastolic flow (as described above). This means it is feasible to closely monitor fetuses in early onset preterm fetal growth restriction, to try and advance gestation but time birth before stillbirth occurs. However, it is not always perfect and with expectant management, stillbirth remains a risk.

Late onset preterm fetal growth restriction (diagnosed 32-37 weeks' gestation).

Late onset preterm fetal growth restriction (32 weeks' gestation and above) is far more common than early onset fetal growth restriction, complicating around 2-5% of all pregnancies. The absolute risk of very poor outcomes such as stillbirth is considerably lower than for early onset fetal growth restriction. Also, while some might, many will not acutely deteriorate over weeks. This means the prospect of advancing for many weeks and reaching the desired target gestation of around 37 to 38 weeks gestation is higher than for early onset growth restriction.

However, compared to early onset fetal growth restriction, the steps to acute deterioration are less predictable in fetal growth restriction of later onset. Therefore, while we still use umbilical artery Dopplers to closely monitor affected cases, it is a less accurate tool to identify acute deterioration (it can miss some babies at high risk of stillbirth). Also, the chances of superimposed preeclampsia occurring in late onset fetal growth restriction are less, compared to early onset disease.

While late onset fetal growth restriction is a less severe subtype of fetal growth restriction than early onset fetal growth restriction, it is still a far riskier pregnancy compared to unaffected pregnancies. Compared to normally grown fetuses, the relative **stillbirth risk remains elevated at 3-10+ fold increase**, depending on the severity of the fetal growth restriction.

Also, these **fetuses are still at risk of significant morbidity soon after birth**, and the lifelong poor health outcomes described above. Critically, as it is simply **far more common** than early onset fetal growth

restriction, it **causes a far higher total burden of human disease,** compared to early onset fetal growth restriction. Late onset fetal growth restriction accounts for far more cases of stillbirth overall than early onset fetal growth restriction.

*A therapeutic treatment that can enhance placental function over many weeks could improve outcomes for **both** early onset, and late onset fetal growth restriction. Better placental function and fetal growth could reduce stillbirth risk, improve short term neonatal health prospects, but also offer better lifelong health.*

SECTION 2: Overview of biology, and conceptual therapeutic strategies to treat fetal growth restriction

The pathology causing fetal growth restriction starts in early pregnancy. early placenta fails in its task to properly embed into the wall of the uterus. It also fails to correctly remodel the maternal vascular system into a widened circulatory network that delivers plentiful oxygen nutrients for the rest of the pregnancy.

Poor placental embedding from the outset: the early origins of fetal growth restriction

As early as the first trimester of pregnancy, the developing placenta remodels the maternal vasculature inside the uterus so it can efficiently absorb nutrients from the maternal bloodstream during pregnancy. As the early placenta embeds in the uterus, columns of placental cells invade inwards through the inner third of the uterus. The columns of placental cells 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)[28-30]. The net result is significant remodeling of the maternal vascular architecture that directly borders the placenta. This remodeling leads to large volumes of maternal blood that flow gently along the placental surface. This configuration sets up ideal conditions for the efficient transfer of nutrients and oxygen from the maternal bloodstream to the placenta.

Without proper placental implantation, uterine vascular remodeling does not lead to a healthy exchange of nutrients from mother to baby[31,32]. During the early pathological steps that lead to fetal growth restriction in later pregnancy, the process of spiral arteriole remodeling of the muscular layer is incomplete, meaning the maternal **spiral arterioles** retain their ability to contract.

Contraction of the maternal blood vessels decreases how much blood can pass through to the 'intervillous' space. The intervillous space are large pools of maternal blood that bathe the placenta. It is this critical intervillous space where oxygen and nutrients are transported from the maternal circulation

to the placenta. *In fetal growth restriction, there is less maternal blood flow to the intervillous space, meaning less oxygen and nutrient delivery to the placenta.*

The growth restricted placenta itself then develops in a very abnormal way. There is a raft of pathological abnormalities that can be observed (for the interested, read our recent review[33]). On a macro level, the placentas are smaller than gestationally matched peers. The umbilical cord is thinner. At the microscopic level, the 'placental tree' has far less branching meaning there is less surface area for the placenta to soak up oxygen and nutrients from the maternal blood (sitting in the intervillous space) into the fetal circulation. At the molecular level, stressed placental cells are less healthy – they are far more prone to cell death and oxidative stress. Overall, the growth restricted placenta suffers types of tissue injury where the net effect is to compromise its ability to support healthy fetal development.

Preeclampsia and fetal growth restriction are related placental disorders

Many of these early pathological steps are very similar to the related placental condition of preeclampsia. In fact, many patterns of cellular and tissue injury in the placenta are very similar between fetal growth restriction and preeclampsia. They are also clinically related – those pregnancies diagnosed with one condition are at high risk of the other arising during the pregnancy.

The clinical difference is that **for preeclampsia**, **there is evidence of maternal injury**, such as hypertension and potentially injury to one of mum's vital organs (such as her brain, liver, kidneys, lungs, clotting system). Fetal growth restriction is often present with preeclampsia, but not always. In contrast, **fetal growth restriction is a diagnosis specific to the baby**. *However, given their shared pathology, it is conceivable that drugs could be found that treat both conditions.*

(A White Paper on the potential of DM199 to treat preeclampsia is available on this link:

https://www.diamedica.com/our-focus/literature-publications).

Conceptual strategies to identify a breakthrough treatment for fetal growth restriction.

A disease modifying drug for fetal growth restriction would be an enormous breakthrough for human health. There may be two conceptual approaches to developing a therapy.

Approach 1: Finding drugs that target the placenta

There are preclinical studies evaluating agents that act on the placenta itself to improve placental function. One example is to administer Insulin Growth Factor-1 (IGF-1), a protein that switches on a signaling pathway to facilitate cellular growth (and is not specific to the placenta). Older studies where

IGF-1 was infused in a sheep model of fetal growth restriction showed a variable response – some growth rescue of fetal organs but not overall fetal growth itself[34]. More recently, a group in Cincinatti targeted IGF-1 via nanoparticle delivery to the placenta in a guinea pig model of fetal growth restriction. They reported rescue of fetal weight[35]. Currently, there are no clinical trials of drugs that directly target the placenta to rescue fetal growth restriction (that we know of).

There may be significant challenges to developing drugs that directly target the placenta. First, our understanding of placental biology remains rudimentary. Second, rescuing placental function may be futile without also increasing maternal blood flow to supply the placenta (see approach 2). *For these reasons, targeting the placenta would appear to be a more distant therapeutic prospect compared to targeting the maternal vascular system itself.*

Approach 2: Finding drugs that maternal vascular system in the uterus to better nourish the placenta

As mentioned, there is high resistance to blood flow across the maternal vascular circulatory network within the uterus. This means less maternal blood flows into the intervillous space, the 'vats' of maternal blood that bathe the placental surface (where nutrients are passed off from the mother to the baby, through the placenta). Finding a drug that increase the flow of blood within the uterine vascular circulatory network could offer sufficient nutrients to the placenta to reduce the injury arising from fetal growth restriction.

Finding drugs that can dilate blood vessels to allow more blood flow seems achievable sooner, compared to drugs directly targeting the placenta for a few reasons. Firstly, drugs already exist that target blood vessels. Secondly, the molecular biology of blood vessels is far better characterized than placental biology - we already know of many molecular pathways that could be plausibly targeted. Lastly, it may be possible that drugs could be found that target the maternal vascular system **but do not cross the placenta**. Such drugs may be a particularly safe option in terms of minimizing fetal risk.

For these reasons, finding drugs that increase maternal blood flow to the placenta by targeting the maternal uterine vasculature may be the most promising approach to treat fetal growth restriction. Given this, the remainder of this white paper take a closer look at the biology of blood vessels, then drug strategies to target blood vessels as a path to treat fetal growth restriction.

Targeting maternal blood vessels in the uterus to treat fetal growth restriction.

There is a network of maternal blood vessels within the uterus that supplies the placenta and fetus with oxygen and nourishment

During pregnancy, there is a huge volume of blood within the uterus – in healthy pregnancies - at any one time, 20% of the mother's entire blood volume is flowing within the uterine vasculature. Blood first flows into the uterus via the **uterine arteries** – there are two, each running along each side of the uterus. As noted above, *significant resistance to blood flow within the uterine circulation can be measured by Doppler ultrasound of the uterine arteries.*

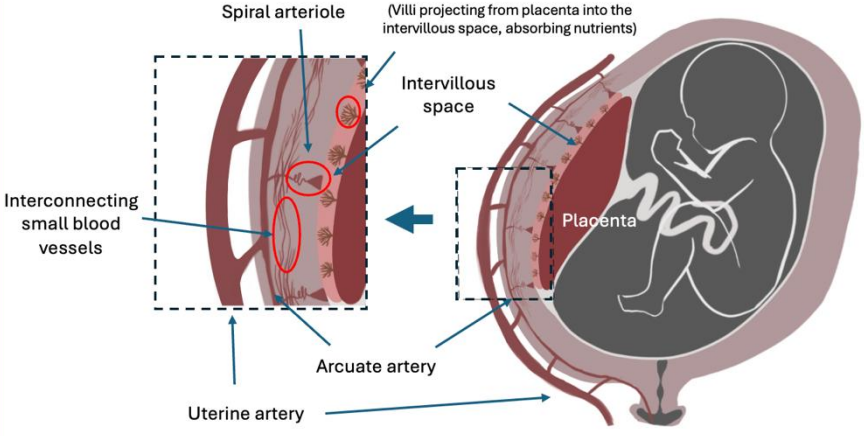


Figure 2: Vasculature of the pregnant uterus

The **uterine artery** is the main vessel supplying blood to the uterus. It branches into vessels that supply the **arcuate artery,** located within the uterine muscle.

Blood vessels arise from the arcuate artery, head towards the placenta and end as as **spiral arterioles.**

The spiral arterioles are terminal blood vessels that release blood into the **intervillous space**. The intervillous space is a pool of maternal blood where placental tissue (villi) absorb oxygen and nutrients from the maternal blood.

Like all solid organs, there many **interconnecting small blood vessels** within the uterine muscle.

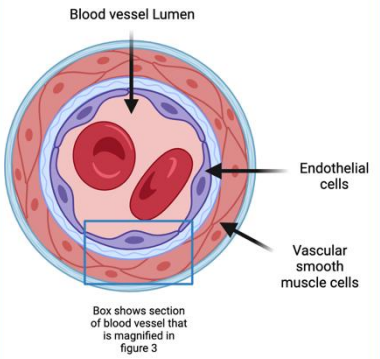
The uterine arteries then branch into blood vessels called **arcuate arteries** that run through the superficial surface of the uterus, deep within the muscle (Figure 2). Branching off arcuate arteries are **radial arteries** that bore through the uterine muscle towards the placenta. These end in a series of **spiral arterioles** (discussed above - the small vessels that open into the intervillous space). Like all solid organs, there are numerous small interconnecting blood vessels.

A closer look at the inner workings of blood vessels

We will next zoom into the anatomy of a blood vessel. This is important to understand how the drug DM199 works, which will be introduced soon.

An arterial blood vessel has two important layers: the inner endothelial cell layer and the outer vascular smooth muscle cell layer (Figure 3). The inner lining of blood vessels in contact with blood is the **endothelial layer**, compromised of a continuous sheet of **endothelial cells**. These endothelial cells are

Figure 3: Cross section of a blood vessel



bound together to form a sealed sheath. The outer layer of blood vessels is comprised of **vascular smooth muscle cells**. Unlike endothelial cells, which are a single cellular layer in depth, vascular smooth muscle cells create muscle through multiple layers of cells. Hence, the inner surface of the endothelial layer directly interacts with the blood moving through the vessel, and the outer surface of the endothelial cell layer binds to the vascular smooth muscle cell layer of the blood vessel. (Figure 3)

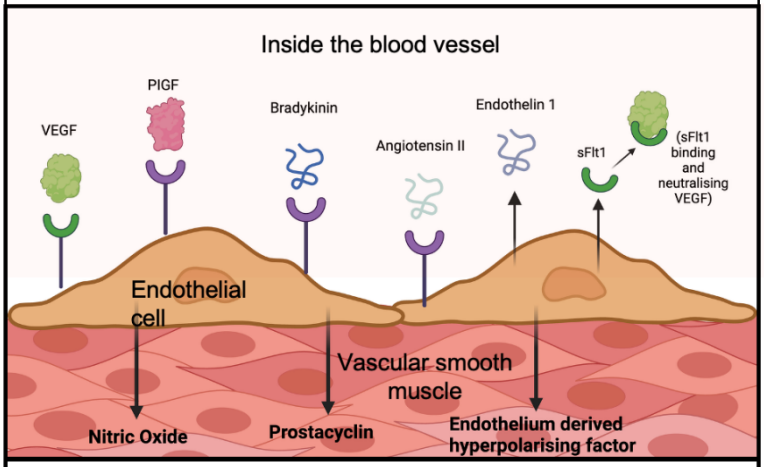
The primary role of the vascular smooth muscle cell layer is **to either contract**, which narrows the diameter of the blood vessel, and increases blood pressure (akin to 'squishing the tube'); **or relax**, which widens the diameter of the blood vessel and decreases blood pressure.

Signals received from the endothelial cells inside the arterial wall dictate whether vascular smooth muscle cells contract or relax. The **endothelial cells are therefore the master regulators of blood vessels**. They take the lead in coordinating blood pressure and general vascular health.

Endothelial cells: (Figure 4)

1) ***<u>Receive signals from molecules in the blood instructing them to contract or relax</u>***: Molecules travel through the bloodstream and bind to specific receptors on the surface of endothelial cells. The receptors will relay signals inside the endothelial cell to then issue further commands. The signaling molecules travel through the bloodstream and bind to corresponding receptors on the surface of endothelial cells (like a highly

Figure 4: 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 sFlt1 and others.

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

specific lock and key mechanism). Once the receptors receive the signal, the final instructions to 'contract' or 'relax' are relayed inside the endothelial cells through sophisticated molecular circuitry.

Examples of molecules that endothelial cells may receive signals from the bloodstream include vascular endothelial growth factor (**VEGF)**, placental growth factor (**PIGF**), or **bradykinin,** which dilated blood vessels. In contrast, angiotensin II (**ANG2**) is a molecule that contract blood vessels (and increase blood pressure).

2) ***Send signals locally to the vascular smooth muscle lying underneath****:* 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.

Finding drugs that act on the endothelium to instruct the underlying vascular smooth muscle to relax, dilate and widen (allowing more blood to flow within the uterus) may be a promising option to treat fetal growth restriction.

Clinical trials to treat fetal growth restriction so far

Fetal growth restriction is distinguished by the disappointing fact there have been so few trials of any drugs, whatsoever. And nearly no clinical trials of drugs currently registered that aim to rescue fetal growth restriction. *This does offer market opportunity to anyone with a first-in-class drug to treat fetal growth restriction.*

1. Sildenafil: This drug enhances **nitric oxide** release in endothelial cells (by blocking an enzyme that inhibits its production). It is therefore a vasodilator and postulated to widen maternal blood vessels that supply the placenta. Being a small molecule drug (tablet), it is likely to readily diffuse through the placenta and enter the fetal circulation.

There was a coordinated effort where concurrent global clinical trials were run to evaluate whether oral sildenafil could be used to treat preterm fetal growth restriction. Branded the 'Strider trials', individual trials were run in the UK, Netherlands, Canada and Australia/New Zealand. While each were standalone trials, the intent was to combine the results in a large individual participant meta-analysis to provide a clear answer whether sildenafil is effective as a treatment for fetal growth restriction

The UK[36] and Australia/New Zealand Strider[37] trials were completed, but findings for the individual trials were negative for the primary outcomes. Unfortunately, the Netherlands trial raised concerns that

sildenafil may cross into the placenta and cause serious harm to the fetal lungs (neonatal pulmonary hypertension) possibly increasing neonatal loss[38]. While the increase was not statistically significant, the trends were deemed sufficiently concerning that all ongoing Strider trials were ceased. Thus, **sildenafil cannot be further considered an agent** to be administered during pregnancy.

While small molecule drugs still offer excellent potential as a drug class, the Strider experience suggests researchers need to establish fetal safety for investigational agents that enter the placenta, as they may have untoward effects on the unborn baby. Optimally, biologic drugs should be found that do not cross to the fetal circulation.

2. Other therapeutic approaches:

Adenoviral delivery of Vascular Endothelial Growth Factor (VEGF): This is an interesting and bold concept of injecting Adenoviral VEGF into the uterine artery. The concept is that these adenoviral VEGF enter endothelial cells to switch on the production of VEGF. This VEGF is released locally, acting on endothelial receptors to dilate arteries. The team published preclinical studies in large animal models that support the concept[39,40]. While the concept of gene therapy is interesting, human trials have not started (as far as we can tell). As it was first proposed nearly two decades ago, it is uncertain whether clinical trials of this bold concept of adenoviral delivery of VEGF will happen soon.

Melatonin: Protect Me is a trial of melatonin to treat severe preterm fetal growth restriction that is ongoing (Registration number: ACTRN 1261700151381)[41]. Leaning into the anti-oxidant properties of melatonin, it does not aim to rescue fetal growth restriction in utero. Instead, melatonin aims to protect the fetal brain by quenching oxidative stress in fetal neurons, minimizing fetal brain injury. Excitingly, Protect Me has completed recruitment, but is still gathering 2-year outcomes of neurocognitive performance.

SECTION 3: THE POTENTIAL OF DM199 TO TREAT FETAL GROWTH RESTRICTION

A promising therapeutic strategy to treat fetal growth restriction is to identify drugs that open up blood vessels in the pregnant uterus, increasing maternal blood flow to the placenta. Ideally, the drug does not cross the placenta.

DM 199: a promising candidate treatment for fetal growth restriction

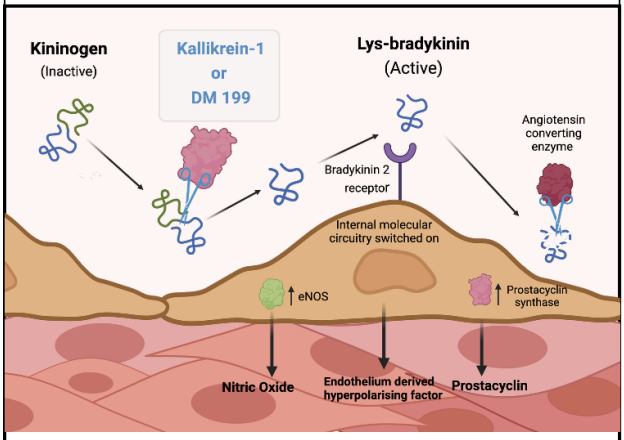
DiaMedica Therapeutics developed DM199, a manufactured, synthetic version of tissue Kallikrein-1 (rhKLK1) with small modifications that increase its stability. KLK1 is a natural protein made by, and secreted from healthy endothelial cells and other tissues, such as the kidneys, pancreas, and lungs.

DM199 has potent blood vessel dilation effects by switching on existing natural molecular machinery within cells.

The main role of KLK1 is to produce active bradykinins which dilates blood vessels

Bradykinin is a circulating peptide that powerfully vasodilates blood vessels (Figures 4 and 5). 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 5). Bradykinin is a short nine amino acid peptide that binds to and activates bradykinin 2 receptors studded on the surface of endothelial cells. Due to rapid degradation of bradykinin by inactivating enzymes, it exerts its biological action locally at the site of production. 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, prostacyclin** and **endothelium-derived hyperpolarizing factor.**

Figure 5: 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 **lys-bradykinin.** This happens in the bloodstream.

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;** increases the enzyme **prostacyclin synthase** which makes **prostacyclin,** and upregulates **endothelium derived hyperpolarising factor.**

Lys-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-inflammatory 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)[42]. Adding or removing a phosphate molecule acts as an on-off switch for many receptors on cells. In addition, binding the bradykinin 2 receptor sends a signal into the cell's nucleus to produce more of the protein enzyme itself, **eNOS**.

Nitric oxide and **prostacyclin** diffuse from endothelial cells into vascular smooth muscle cells and cause the muscle to relax. This dilates blood vessels, increasing blood flow[43]. 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 prostacyclin to relax smooth muscle cells, leading to increased vasodilation and blood flow.[44]

DM199 is synthetic version of KLK1. Hence, it can switch on existing molecular circuits to promote **significant relaxation of blood vessels.** As such, **DM199 is an excellent candidate to treat fetal growth restriction.**

Unlike other drugs that may switch on one molecular pathway to dilate blood vessels (for example, sildenafil only targets nitric oxide)**, DM199 can switch on all three of the major molecular pathways involved in blood vessel relaxation** - nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor. By targeting multiple pathways that regulate vascular tone and endothelial function, the combination of NO, prostacyclin and EDHF might synergistically enhance placental perfusion. If so, it offers a promising therapeutic strategy to improve fetal growth and healthy development.

DM199 may open interconnecting small blood vessels within the uterus

There is circumstantial evidence that DM199 may dilate interconnecting small blood vessels within the uterus that help supply the placenta with maternal blood.

To explain the place and likely importance of the **small interconnecting blood vessels within the uterus** (anastomotic circulation) an analogy that might be considered is a line of trucks supplying provisions to a sports stadium nestled deep within a suburb. The trucks first need to run along superhighways feeding into the suburbs themselves. These superhighways are analogous to the **uterine arteries**. Upon reaching the suburban roads, the line of trucks then needs to negotiate their way through a grid of smaller roads

that interconnect. **The small interconnecting blood vessels** might be then analogous to these smaller roads within the suburb.

If these smaller roads (aka the small interconnecting blood vessels) are jammed with suburban traffic, delivery of provisions to the hungry sports fans would slow. And if the suburban roads were gridlocked, the bank up of slow traffic would extend all the way back to the superhighways, where traffic flow will become sluggish (analogous to increased Doppler blood flow resistance seen on ultrasound).

Alternately, we can envisage the flow of provisions to the sports stadium would be quick and smooth if the grid of suburban roads were widely opened to facilitate truck traffic. This is what DM199 may be able to do – take control of all roads, opening them widely to facilitate the flow of traffic to the stadium (or, to the placenta).

There are several lines of evidence suggesting DM199 may indeed, dilate (open up) small interconnecting blood vessels within the uterus to increase blood supply to the placenta.

1. Biological plausibility

As already noted, it has been established that at the molecular level, bradykinin signals via its receptor on endothelial cells (bradykinin 2 receptor) to switch on the three key molecules that dilate blood vessels.

A search of the publicly available 'human vascular atlas' shows the blood vessels within the uterus are replete with bradykinin 2 receptors, the receptor that receives signals from the actions of DM199 to facilitate healthy blood vessel growth (ie production of the peptide, bradykinin). *This means the blood vessels **within the uterus** possess the molecular machinery to respond to the effects of DM199.*

2. Preclinical laboratory studies.

There is preclinical evidence suggesting DM199 should improve blood flow at the blood vessel level, including **stimulating new blood vessel growth.**

In a classic study, researchers injected 'adenoviral tissue kallikrein 1' into the adductor muscle of mice (limb muscle)[45]. This is a molecular technique where the tissues that the adenovirus is injected into produces tissue kallikrein 1, for around 1-3 weeks. The researchers reported injection of adenoviral tissue kallikrein 1 increased production new blood vessels: both arterioles (larger) and capillaries (smaller vessels)[45]. Significantly, the increased vessel number persisted for 8 weeks, long after the adenovirus stopped making tissue kallikrein 1 protein. This suggests the beneficial effects of tissue kallikrein-1 in stimulating new blood vessel growth can last a while.

New blood vessel growth is akin to constructing a network of new roads for trucks to smoothly run to a (somehow) rapidly expanding stadium to deliver provisions (returning to the traffic analogy from above). Pregnancy is a time of rapid placental and fetal growth. It can be envisaged that this rapid growth needs complimentary growth of the uterine vascular to support it. *It is possible DM199 could facilitate rapid growth in blood vessels to maximise placental blood flow.*

3. Human trials suggest tissue kallikrein 1 may increase blood flow of interconnecting vessels in the brain

Aside from stimulating new blood vessel growth, there is evidence in humans that tissue kallikrein 1 can dilate existing collateral vessels (akin to small interconnecting vessels present in the uterus). A Japanese group reporting a fascinating clinical trial in the 90's, where **tissue kallikrein 1** (the natural form of DM199) was **injected intravenously** to those who had previously suffered a **stroke** and still had old blood clots in the brain impeding cerebral circulation[46]. The tissue kallikrein 1 they gave was isolated from human urine.

The team reported **tissue kallikrein 1 increased blood flow velocity** (measured by Doppler ultrasound) **in the carotid artery** (the main arteries in the neck supplying blood to the brain). The paper shows an impressive cerebral angiography image which shows a **dramatic blood flow increase** within small **arteries in the brain** as **soon as 30 minutes after the administration of tissue kallikrein 1**[46].

4. Our ongoing trials suggests DM199 may decrease uterine artery Doppler blood flow resistance in pregnancy

In a dose escalation trial of DM199 the authors are running in South Africa, we have been administering DM199 to participants with preeclampsia. All cases are acutely hypertensive (BPs >150/100) and for planned medical stabilization, then delivery within 48-72 hours. Increasing doses of DM199 are being given to successive groups of 3 participants per dose. Sequential IV, then subcutaneous dosing of DM199 is being given. (the protocol for this ongoing investigator-initiated trial has been published and can be freely downloaded)[47].

Being a first in pregnant women trial of DM199, the primary outcomes are safety, tolerability and pharmacokinetics. However, we also obtaining a range of exploratory outcomes including blood flow resistance, measured using Doppler ultrasound. The Dopplers ultrasound of the uterine arteries are being done just before DM199 was given, and two hours after the drug is commenced. Results in an interim analysis (the study is unblinded and recruit is still ongoing), are shown in Figure 6:

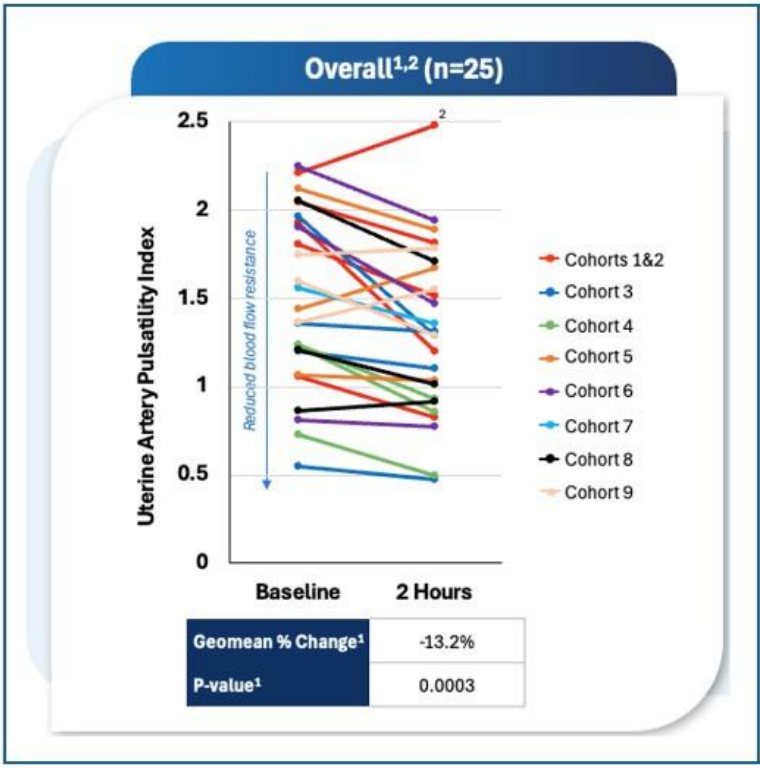


Figure 6: Uterine Artery Pulsatility Index after intravenous administration of DM199

Each cohort represents increasing doses of DM199:

eg

Cohort 1 had 0.1 ug/kg

Cohort 5 had 0.75 ug/kg

Cohort 9 had 2.0 ug/kg

- Mostly 3 participants per cohort
- All had preeclampsia and were acutely hypertensive
- Uterine Artery Pulsatility Index, a measure of blood flow resistance, was measured by Doppler ultrasound

These data provide strong early encouragement that DM199 has the potential to treat fetal growth restriction by reducing blood flow resistance within the uterine circulation. It suggests DM199 may indeed act on the uterine vascular to improve blood flow.

But these data should be considered exploratory data, or hypothesis generating. They require proper validation. There are important caveats to consider:

1. This early phase trial where these data were derived was (appropriately) designed to test the safety of DM199. Uterine artery Doppler findings were not regarded as a primary outcome for this trial.
2. This first in pregnant women trial has very small numbers and is not blinded.
3. The trial is focused on preeclampsia, not fetal growth restriction (although preeclampsia does have the shared pathology of reduced uterine circulation blood flow and reduced placental perfusion).
4. It is unclear whether the 13.2% reduction in resistance is clinically meaningful. It might be. Harking back to the analogy, a 13% increase of traffic flow in the superhighway may reflect vastly smoother traffic flow in the suburban roads (aka the small blood vessels within the uterus).
5. It is unclear whether a reduction in resistance can be sustained beyond the 2 hours after DM199 is first administered as we did not do further Doppler ultrasounds (since, whether the response is sustained needs evaluation in future trials).

For all these reasons, the exciting trends we have observed in our trial of reduced Doppler uterine artery resistance should be considered early evidence at this stage. They certainly require validation. But given biological plausibility (see points 1-3 above) plus the dearth of candidate therapies to tackle fetal growth restriction, trials of DM199 to treat fetal growth restriction are warranted.

Critical safety advantage: DM199 does not cross the placenta into the fetal circulation

An important competitive advantage of DM199 is that it does not cross the placenta into the fetal circulation. This vastly reduces concerns about fetal exposure to the drug.

Most drugs are **small molecule drugs** (such as the many drugs sitting on the pharmacy shelves) and overwhelming majority cross into the placenta. Being tiny chemicals, they simply pass through the placenta to enter the fetal circulation. Most won't cause harm to the fetus, but some drugs might. An example is the Strider trials discussed above where trials were stopped due to concerns that sildenafil (a small molecule) was entering the fetal circulation and potentially injuring the fetal lungs.

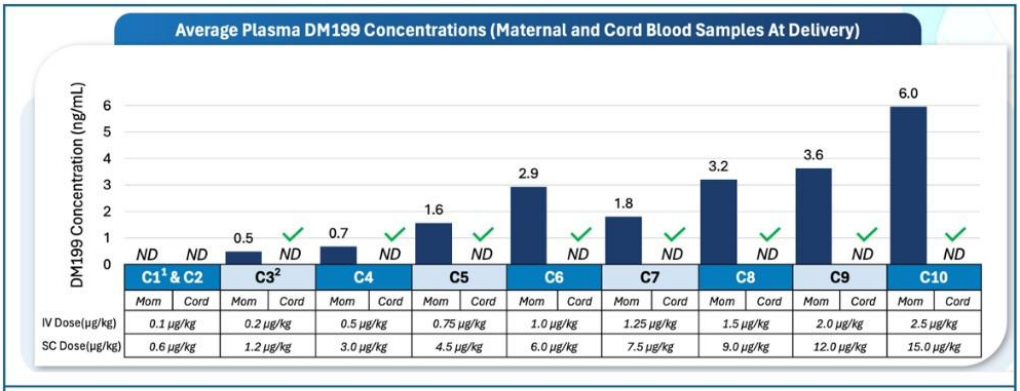


Figure 7: DM199 blood concentrations in the maternal and fetal circulation at the time of birth

Each cohort (C1-C10) represents increasing doses of DM199:. Doses are shown in the figure for each cohort

- Mom: DM199 concentrations in the maternal blood for that cohort (eg cohort 4 is C4).
- Cord: DM199 concentrations in the fetal blood around the time of birth, for that cohort (taken from a blood vessel in the umbilical cord on the placenta)
- **ND** means **'not detectable'**
- Dose of DM199 given for each cohort are shown

A dose dependent increase of DM199 in the maternal blood was seen. *In contrast, DM199 remained undetectable in the fetal blood for any dose of DM199*

Protein drugs, such as DM199, do *not* cross the placenta. They are too big to diffuse through the cell surface and there are no known active transport mechanisms.

In our current trial of DM199 to treat preeclampsia, we did pharmacokinetics studies where we confirmed DM199 does not cross into the fetal circulation (Figure 7).

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. Like DM199, 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 prostaglandins given to prime the cervix in preparation for labor, and the peptide oxytocin given to stimulate labor[48,49].

Hence, the concept of administering DM199, an analogue of a naturally occurring protein, should not be regarded as foreign to clinicians, patients and parents. If proven to treat fetal growth restriction, the fact it mimics a natural protein could enhance its acceptability and facilitate rapid clinical adoption.

DM199 Human Clinical Trials and Reproductive Toxicology Studies

Human Clinical Trials of DM199 so far

Including our early phase trial in preeclampsia (still recruiting), DM199 has been tested in over 300 humans across multiple dose ranges and for up to 95 days of administration. 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 with 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 ReMEDy1, the subgroup of patients who did not receive mechanical thrombectomy (n=46) showed better physical recovery in the DM199-treated group compared to the placebo-treated group. The improved endpoint of excellent outcomes was based on modified Rankin score of 0-1, a reduced number of deaths, while also preventing stroke-in-evolution or recurrent ischemic events.

Reproductive Toxicology Studies

DiaMedica has completed the pre-requisite developmental and reproductive toxicology (DART) studies which were necessary prior to dosing pregnant humans. These studies evaluated the potential of the drug to adversely affect 3 phases of reproduction, Segment I - fertility, Segment II - development of the embryo and fetus, and Segment III - on the fetus through sexual maturation of the offspring of treated mothers. These studies did not identify any reproductive risks.

In addition, DiaMedica completed a placental transfer study in rodents to assess whether DM199 crossed the placental barrier (Figure 8). Pregnant rats were administered large doses of DM199 and sacrificed at different time points after administration. DM199 (KLK1) was subsequently measured in the blood of both the dams (maternal, n=30) and the pups (fetal, n=24). As shown figure 8 below, a clear rise

and fall of drug levels in the blood was observed in the dams (maternal circulation) but reassuringly, no detectable amount of DM199 was found in the pups (fetal circulation) at any time point. The findings mirrored those in the preeclampsia trial (shown in Figure 7), which was done subsequent to the pregnant rat repro-toxicology studies shown in figure 8.

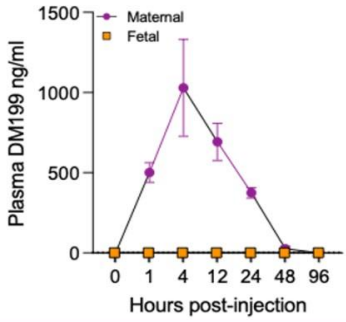


Figure 8: DM199 concentrations in the maternal and fetal circulations, in the rat.

Possible Adverse Events of DM199

Many of our potential safety concerns regarding giving DM199 in pregnancy (which we articulated in a prior white paper we published on preeclampsia) have been addressed via our ongoing clinical trials for preeclampsia. So far, the DM199 has been found to be highly safe and well tolerated.

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

Dropping the blood pressure too low may reduce the amount of maternal blood supplying the placenta (going back to road analogy, this will be akin to all the trucks on the road losing their forward thrush). The fetus will then become acutely distressed.

However, it should be possible to find an appropriate dose of DM199 that dilates blood vessels and increase blood flow to the placenta, without dropping blood pressure too low. In fact, this is the strategy for the current pivotal trial of DM199 to treat stroke (ReMEDy 2 trial, Clinical trial registration: NCT 05065216).

Small falls of maternal blood pressure would not cause fetal distress. Only drops of blood pressure to very low levels (hypotension) will cause fetal distress, such as blood pressure levels under 90/60 mmHg. Furthermore, if hypotension arises which leads to fetal distress, simple intravenous fluid replacement and/or the use of existing drugs are available options to restore blood pressures to fix the distress. But it is clearly better to avoid this in the first place.

Trials so far provide reassurance that hypotension as a side effect of DM199 could be very uncommon (unless it is an exceptionally high dose):

1. **DM199 Chronic Kidney Disease trial**. DiaMedica previously performed a DM199 trial to treat chronic kidney disease. Over a 95-day period, the trial showed DM199 delivered subcutaneously who were

hypertensive to begin with had reductions in blood pressure. In contrast, those with started the treatment with normal blood pressures (normotensive) did not have falls in blood pressure.

2. **DM199 preeclampsia trial:** Reassuringly, while higher doses of DM199 did efficiently reduce blood pressure (in this trial, all participants had preeclampsia and very high initial blood pressures), no one in the pregnant cohort experienced sustained hypotension that caused fetal distress.

In future trials of DM199 to treat fetal growth restriction, maternal blood pressure will need to be monitored as a safety outcome. However, it seems possible that an effective dose of DM199 may be found that dilates blood vessels to improve uterine blood flow but does not cause hypotension.

Planned clinical trials of DM199 as a treatment for preeclampsia and fetal growth restriction

First clinical trial of DM199 as a treatment for fetal growth restriction.

As noted, 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[47].

Part 1 of the clinical trial is a dose escalation study (Part 1). It is nearly completed and some of the findings have been described in this white paper.

Part 2, yet to commence, will test DM199 in three clinical cohorts, each recruiting 30 participants.

- **Cohort 2A:** 30 women with **preeclampsia** and acute hypertension and for planned delivery.
- **Cohort 2B:** 30 women with preterm **preeclampsia** for planned pregnancy prolongation.
- **Cohort 2C:** 30 women **with early onset preterm fetal growth restriction** for planned pregnancy prolongation.

Hence, Cohort 2C, will be the first ever trial of DM199 to treat fetal growth restriction.

Further details about Cohort 2C

This will be an open label, single arm phase II trial. Being the first in fetal growth restriction trial, the primary aim is appropriately to assess safety and tolerability. However, we have also designed the trial to explore early signs of efficacy (exploratory). It will be performed at Tygerberg Hospital in Cape Town, South Africa.

Inclusion criteria

- Pregnancies with the diagnosis of fetal growth restriction (a fetus <3rd centile weight, relative to its gestational age).
- Gestational age 27+0 weeks and 32+6 weeks gestation and they are deemed suitable for expectant management.

- Participants may have co-existing preeclampsia, but they do not need to have preeclampsia.

The planned intervention

- Subcutaneous doses of DM199 (three separate doses will be tested)
- Since safety is the main outcome of interest in this first early phase trial, there will not be a placebo arm.

Main safety outcomes

- Safety and tolerability
- Umbilical cord blood levels of DM199 at birth (fetal levels of DM199)

Main efficacy outcomes

Uterine artery vascular resistance

- We will obtain data on changes in the *Uterine artery vascular resistance* (via Doppler ultrasound), comparing vascular resistance before starting the medication with vascular resistance after the medication has commenced.

Other key efficacy signals

- Birthweight centile
- Changes in other fetal vessels (measured by ultrasound), notably the Umbilical Artery Doppler ultrasound
- Length of pregnancy prolongation

All efficacy data will be considered preliminary as numbers are too low to generate data to establish efficacy (in either direction).

We expect this first trial of DM199 to treat preterm fetal growth restriction, will establish safety. It may also offer early signs of efficacy. We hope the first trial will justify the launch of an ambitious pivotal trial – a multicenter, multi-international trial of DM199 to improve neonatal outcomes.

Summary:

DM199 is a promising 'first-in-class', breakthrough treatment for fetal growth restriction:

- May increase blood flow to the placenta, improving delivery of oxygen and nutrients to the growth restricted fetus. This could improve fetal health
- In multiple Phase 1 and 2 clinical trials of DM199 in non-pregnant populations, and in an ongoing dose escalation trial of pregnant women with preeclampsia, safety and tolerability endpoints have been confirmed
- Does not cross into the fetal circulation, implying a strong safety profile
- Animal developmental and reproductive toxicology studies support potential safety in pregnant mothers and fetuses.

DM199 is an attractive commercial candidate because:

- Clinical trials for fetal growth restriction can be done quite quickly because primary outcomes to show benefit are short-term (relative to other many other chronic adult diseases).
- Potential economic benefits include less neonatal intensive care costs if the baby is born less preterm, and shorter hospital admission costs. There may be numerous societal benefits if the newborn enjoys better lifelong health.

DiaMedica Therapeutics plans to evaluate DM199 as a treatment for fetal growth restriction (as well as preeclampsia) by partnering with the academic authors of this white paper. Professors Tong, Cluver and Walker will run the first ever clinical trial.

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