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Maternal uterine artery VEGF gene therapy for treatment of intrauterine growth restriction

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Abstract

Intrauterine growth restriction (IUGR) is a serious pregnancy complication affecting approximately 8% of all pregnancies. The aetiology is believed to be insufficient maternal uteroplacental perfusion which prevents adequate nutrient and oxygen availability for the fetus. There is no treatment that can improve uteroplacental perfusion and thereby increase fetal growth in the uterus.

Maternal uterine artery gene therapy presents a promising treatment strategy for IUGR, with the use of adenoviral vectors encoding for proteins such as Vascular Endothelial Growth Factor (VEGF) demonstrating improvements in fetal growth and neonatal outcome in preclinical studies. Mechanistically, maternal VEGF gene therapy delivered to the uterine arteries increases uterine blood flow and enhances vascular relaxation short term, while reducing vascular contractility long term. It also leads to vascular remodeling with increased endothelial cell proliferation in the perivascular adventitia of uterine arteries. Safety assessments suggest no vector spread to the fetus and no adverse risk to the mother or fetus; a clinical trial is in development. This article assesses research into VEGF maternal uterine artery directed gene therapy for IUGR, investigating the use of transgenes and vectors, their route of administration in obstetrics, and the steps that will be needed to take this treatment modality into the clinic.

Intrauterine growth restriction

Optimal fetal growth depends on normal functioning maternal, placental and fetal factors and the external environmental, on the background of a genetically pre-determined growth potential. Intrauterine growth restriction (IUGR) can occur due to a malfunction of one or a number of these factors. IUGR is potentially life threatening and affects 8% of all pregnancies, contributing to 50% of stillbirths (1). Of those diagnosed with IUGR, approximately 1 in 500 cases are classified as both severe and early onset, occurring before 28 weeks of gestation. Severe IUGR can be caused by structural abnormalities of the fetus, maternal medical disorders and congenital infections, but most commonly, it is impaired uteroplacental function that restricts delivery of nutrients to the fetus, resulting in slowing or even cessation of fetal growth, termed placental insufficiency.

In normal pregnancies, effective first-trimester infiltration of the trophoblast in the maternal spiral arteries leads to the creation of a high flow, low resistance maternal circulation. Angiogenesis and vasodilation in the placenta are enhanced by the production of factors such as placental growth factor (PIGF), vascular endothelial growth factor (VEGF) and insulin-growth factor (IGF) (2,3), which facilitates a reduction in placental resistance. The obstetric syndromes of pre-eclampsia and IUGR appear to be interrelated through VEGF biology. An increase in soluble fms-like tyrosine kinase 1 (sFlt1), which acts as a soluble receptor for VEGF in the maternal circulation, is observed in both conditions (4). High sFlt1 is a key pathological hallmark of pre-eclampsia and IUGR (5,6)(7). Increasing available VEGF through its local overexpression may be a good therapeutic approach in these conditions. Treatments based on the manipulation of VEGF and related angiogenic factors are therefore likely to be effective for IUGR and pre-eclampsia (**Figure 1**)(8).

When severe and early in onset, management of affected pregnancies involves prompt delivery of the fetus before death or irreversible organ damage occurs, particularly to the

brain. However, delivering the fetus in severe early onset IUGR adds additional risks to the baby from extreme prematurity (9). In this situation the question of viability also arises, and decision making with parents is challenging (10). Substantial improvements in morbidity and mortality can be seen if delivery of such pregnancies occurs even one week later (e.g. from 26 to 27 weeks) and if there are modest increases in birth weight (eg 100g). In an EU and US multi-centre observational study of babies born after severe early onset FGR, median survival gained per day *in utero* between 24 and 27 weeks of gestation was 2% (range 1.1 – 2.6) (11). It is in these severe early-onset cases of IUGR that novel therapeutics are initially being considered, where the benefit of gaining in gestation length or improved fetal weight might outweigh the potential risks of a novel therapy. If it is found to be safe and efficacious there is potential to use new therapies in more moderate FGR, which affects a larger number of pregnancies.

Gene therapy

Gene therapy allows for the transfer of genetic material into a target cell with the aim of achieving therapeutic benefit. Since the first gene therapy trials in the 1990s, the hope has been that gene therapy could improve the management and outcomes of genetic diseases, particularly single-gene disorders. There are currently over 1800 completed or on-going gene therapy clinical trials, of which over two-thirds are for cancer. In 2012 GlyberaTM, a treatment for familial lipoprotein lipase deficiency became the first gene therapy product to be approved for licensing in Europe (12). Gene therapies are increasingly reaching clinical use for treatment of a wide range of inherited single gene disorders such as haemophilia, thalassaemia, immunodeficiencies and metabolic storage disorders. Concerns about germline gene transfer and off-target effects in the fetus however, are holding back translation into the clinic of fetal-directed gene therapy (13) and currently, it is not considered ethical. Serious

maternal obstetric diseases such as pre-eclampsia and IUGR also affect the fetus and neonate long term. Targeting gene therapy to the mother and not the fetus, with a view to improve fetal outcome is considered acceptable from an ethical, legal and regulatory perspective (14). Appreciating the molecular basis of untreatable obstetric diseases has lead to an understanding of the potential role that gene therapy could play (8) through manipulation of angiogenic proteins such as VEGF. Results of pre-clinical studies are compelling and clinical trials are being planned.

The choice of vector is critical in gene therapy. Manufacture of the vector would ideally be simple and cost-effective. Clinical grade manufactured vectors need to be tested rigorously for replication competent viruses. Vectors should be capable of being targeted to the specific tissue or organ and generate a transgenic protein for the required length of time to have a therapeutic effect without causing side effects (13). For obstetric conditions such as IUGR and placental insufficiency, the therapeutic time frame would be short, limited by the length of gestation. When targeting specific organs such as the uteroplacental circulation, the method of delivery can have considerable impact on the level and site of genetic expression. For the above reasons short acting vectors such as adenovirus (Ad.) and non-viral vectors have been most often investigated in maternal gene therapy for obstetric conditions (15)(16)(17)(18,19)(20–25).

Adenoviral vectors efficiently transfect a wide range of cells, producing short-term protein expression, and are the most commonly used vectors in clinical trials of gene therapy (12). One of their well-recognised side-effects, however, is the potential to trigger both a B cell and T cell mediated immune reaction. This is being addressed by recent development of less immunogenic adenoviral vectors or to select serotypes to which fewer patients have pre-existing immunity (26). Adenoviral vectors enter cells through the binding of fibre proteins on their outer capsid to the coxsackie and adenovirus receptor (CAR). While this receptor is

found on a wide range of cell types, it has very limited expression on the syncytiotrophoblast. This could be an advantage for a gene therapy aiming to target the maternal uteroplacental circulation without transducing the placenta. An alternative is to inject vectors directly into the placenta (27) but this runs the risk of large amounts reaching the fetal circulation because of breaches to the fetal villous architecture, effectively becoming a fetal gene transfer technique.

Vascular Endothelial Growth Factor

Members of the Vascular Endothelial Growth Factor (VEGF) family and their receptors are key regulators in the growth and development of blood vessels within the placental villi (28). So far seven VEGF proteins have been identified, of which VEGF A, B, C, and D, and Placental Growth Factor (PlGF) are found in humans. Vasculogenesis, the formation of new blood vessels, and angiogenesis or blood vessel growth both result from the binding of VEGF-A, or the processed forms of VEGF-C and VEGF-D, to VEGF receptor 2 (VEGFR-2). Activation of VEGFR-2 causes endothelial cell proliferation and migration, increased endothelial cell survival, increased vascular permeability, and activation of endothelial nitric oxide synthase (eNOS). This last effect also vasodilates through increased nitric oxide (NO) synthesis. In contrast, the soluble form of VEGFR-1, soluble fms-like tyrosine kinase 1 (sFlt-1), binds VEGF-A and PlGF, inhibiting their actions.

In IUGR and placental insufficiency the normal balance of these factors shift towards an anti-angiogenic state, with increased sFlt-1 concentration and a reduction in the maternal bioavailable VEGF-A and PlGF (29)(4). Correcting this imbalance is therefore a potential strategy for treating FGR. Infusion of recombinant VEGF-A_{121} attenuated some features of pre-eclampsia and IUGR in pregnant mice and rats that were induced either by adenovirus vector overexpression of sFlt1 (30)(31) or by infusions of the antibody IgG from women with

pre-eclampsia (32). However, given the angiogenic and vasodilatory actions of VEGF, it may be preferable to target increased VEGF availability to the maternal uteroplacental circulation using locally delivered gene therapy, rather than increase systemic maternal VEGF levels. Therapeutic angiogenesis is the term given to the induction of new blood vessel formation by delivering angiogenic genes to ischemic tissues (30). Ad.VEGF gene therapy is being translated into the clinic for ischemic cardiovascular disorders, including acute myocardial infarction, chronic cardiac ischemia, peripheral artery disease and stroke. Therapeutic angiogenesis can also be applied in the maternal uteroplacental circulation as a way to improve fetal growth *in utero*, as described below.

Preclinical Studies on Maternal Uterine Artery VEGF Gene Therapy

Maternal application of VEGF gene therapy has been tested in a variety of pre-clinical studies using adenovirus vectors. The choice of VEGF protein is important because of their different effects mediated via VEGF receptors and neuropilins. The major VEGF-A isoform, VEGF-A_{165}, is the predominant and most potent form in humans and it binds to VEGF Receptors 1 and 2. VEGF-D acts via VEGF Receptors 2 and 3. Both are angiogenic, but VEGF-D appears to have a more favourable structural and functional profile than that of VEGF-A_{165}, eliciting a more restricted range of biological responses and may therefore have fewer side effects, making it the agent of choice for therapeutic angiogenesis.

Equally it is now known that the type of VEGF isoform can critically regulate VEGF function. This can be through the endothelial response, for example, the 165 and 121 isoforms of VEGF-A can elicit differential signal transduction and endothelial responses through programming VEGFR2 endocytosis, ubiquitylation and proteolysis (31). Alternatively different binding of VEGF-A isoforms to the co-receptors heparin sulphate and

neuropilin-1 differentiates the effects of VEGF-A_{165} that binds both and mediates angiogenic sprouting and endothelial cell organization, from VEGF-A_{121} that binds neither and therefore lacks these functions (32).

The impact of Ad.VEGF on uterine blood flow (UBF) was first examined at mid-gestation in uncompromised normal sheep pregnancies using the VEGF-A_{165} isoform. Results are summarised in **Table 1**. UBF was quantified at baseline and at 4-7 days following direct uterine artery (UtA) injection of Ad.VEGF-A_{165} at laparotomy. The artery was digitally occluded during vector injection and afterwards for up to 5 minutes total time to maximise transduction of the downstream endothelium (20). By 4-7 days, volume blood flow in the UtA was increased three-fold when compared to a contralateral UtA injection of a non-vasoactive control adenoviral vector encoding bacterial ß-galactosidase (Ad.LacZ). Ad.VEGF-A_{165} transduced vessels harvested at this short-term time point demonstrated an enhanced contractile response to phenylephrine and increased relaxation response to bradykinin when examined in an organ bath, as well as upregulation of endothelial nitric oxide synthase (eNOS) and VEGFR-2 (22). Using adenovirus containing the unprocessed VEGF-D isoform found no effect. Further experiments using the pre-processed short-form of Ad.VEGF-D (Ad.VEGF-$D^{\Delta N \Delta C}$) demonstrated similar effects on vasoreactivity, up-regulation of phosphorylated eNOS and enhanced UtA endothelial cell proliferation (22), but without the uterine artery inflammatory infiltrate that was observed after Ad.VEGF-A_{165} transduction (20).

The effects of Ad.VEGF- A_{165} on UBF long term were examined using indwelling ultrasonic flow probes in normal sheep pregnancies. Using an identical injection protocol, at 28 days post-injection, vessels treated with Ad.VEGF- A_{165} exhibited a 36.5% increase in UBF compared to just 20.1% in vessels treated with Ad.LacZ (21), which represents a virtual doubling of the normal gestational increase in UBF. A similar tendency was observed long-

term after injection of Ad.VEGF-$D^{\Delta N \Delta C}$ (22). In both long term studies, reduced phenylephrine-induced vasoconstriction continued to be observed but changes in vasorelaxation and VEGFR-2 expression were no longer evident. Nevertheless at approximately 30 days following treatment there was still evidence of neovascularisation within the perivascular adventitia despite undetectable levels of transgenic VEGF protein. This suggests that the vasoactive effects of Ad.VEGF persist beyond the period of transgenic protein expression, probably via angiogenesis mechanisms, an effect that has been seen in many other vascular beds.

The effect of Ad.VEGF-mediated changes in UBF on fetal growth has been examined in two preclinical animal models of IUGR (**Table 2).** High nutritional intake in pregnant adolescent dams at a time when they are still growing promotes maternal tissue growth at the expense of the pregnancy leading to marked IUGR in approximately half of pregnancies (33). This IUGR sheep paradigm replicates many of the key features of uteroplacental FGR in the human including early reductions in UBF (>40%), placental weight, vascularity, secretory function and mRNA expression of VEGF and VEGFR-1, followed by asymmetrical IUGR characterised by brain sparing (preserved head growth with reduced abdominal growth) and abnormal umbilical artery Doppler velocimetry (34). In two separate IUGR sheep cohorts, following bilateral UtA injections of Ad.VEGF-A_{165} in mid-gestation, ultrasound abdominal circumference measurements were increased by approximately 20% when examined at three and four weeks following treatment compared to animals with equivalent baseline measurements receiving control treatments (Ad.LacZ or saline only, **Table 2**) (24,25). There was evidence of an attenuated brain sparing effect (catch up of abdominal to head growth). Significantly fewer Ad.VEGF-A_{165} treated fetuses demonstrated marked FGR at term (24), lamb birthweight tended to be higher (25), and in both studies there was evidence of increased placental efficiency (g fetus/lamb per g placenta). Postnatally Ad.VEGF-A_{165}-

220 treated lambs continued to grow faster in absolute terms throughout the first 12 weeks of life
221 in the absence of any change in fractional growth velocity or markers of adiposity (**Table 2**).
222 DNA methylation studies found no evidence of altered epigenetic status in ten different genes
223 related to postnatal growth and metabolism.
224 In a second animal model of FGR induced by periconceptual nutrient deprivation of Dunkin
225 Hartley guinea pigs, complementary experiments have demonstrated similar efficacy, safety
226 and mechanism of action of Ad.VEGF gene therapy to improve fetal growth and neonatal
227 outcome. Placentation in this species more closely mimics the human, being haemochorial in
228 nature, and shares a similar process of trophoblast cell invasion and proliferation (35). In this
229 FGR model there is an approximate 40% reduction in fetal weight associated with
230 uteroplacental insufficiency and brain sparing (36). As direct injection of the UtA in the
231 guinea pig is associated with considerable morbidity and mortality, a less invasive technique
232 of administration has been developed using a thermosensitive Pluronic gel, which is applied
233 externally to the uterine and radial arteries at laparotomy to achieve transduction with
234 Ad.VEGF-A_{165} or Ad.LacZ at 30-34 days gestation (term = 65) (37). Using this technique,
235 Ad.VEGF-A_{165} treatment improved fetal growth at term and a lower brain: liver weight ratio,
236 implying that brain sparing had been mitigated (**Table 2**) (38). More recently in a second
237 delivered cohort, there are no adverse effects on perinatal morbidity or mortality, improved
238 postnatal growth and amelioration of the increase in adult blood pressure associated with
239 IUGR in this animal model (submitted, **Table 2**).
240 The improvements in fetal birth weight and uterine artery blood flow in IUGR-animal models
241 treated with Ad.VEGF establish VEGF as a transgenic factor with therapeutic benefit in
242 IUGR-affected pregnancies, making it a strong candidate for translation into human clinical
243 trials. Extrapolating from animal studies to humans is challenging particularly for pregnancy
244 conditions where animal models of disease may not completely recapitulate the disease (35)

and where they may be fundamental differences in VEGF biology and VEGF measurement techniques (39).

Risks of maternal uterine artery VEGF gene therapy

An important consideration for any prenatal therapy is safety, some data of which is presented in **Table 1 and 2.** This therapy aims to reduce or eliminate the adverse effects of poor growth in the fetus without exposing the fetus to significant amounts of the vector. Transfer of a gene therapy across the placenta to the fetus in significant quantities would be undesirable, as it would risk germline transmission and may have adverse effects on fetal development. Current evidence suggests that the extent of placental transfer depends on the vector, the animal, the route of administration, and the gestation age at which it is administered. In the pre-clinical studies of maternal Ad.VEGF gene transfer, local delivery to the uterine arteries has been achieved either by direct injection combined with proximal occlusion of the vessel or by external application of a thermolabile Pluronic gel to the vessel wall (37). These delivery methods do not lead to evidence of detectable vector in the sheep or guinea pig fetus. Exposure of human placental villous explants to high dose Ad. vector showed that, where the syncytiotrophoblast was deficient there was occasional transduction of the underlying cytotrophoblast, but no evidence of the vector crossing the basement membrane (40). In translation to clinical practice this could be replicated using a balloon catheter, introduced into each uterine artery in turn using x-ray guided interventional radiology. This technique has been used for over 30 years to treat fibroids and manage postpartum haemorrhage, and is now being used increasingly during pregnancy, with catheters and deflated balloons placed into the uterine arteries before Caesarean section when heavy bleeding is anticipated (41).

Vector modification of the fetal germ line could theoretically occur following maternal gene therapy. This would be dependent first on whether the vector reached the fetus, and then was able to access the germline. The gestational age of the fetus and route of injection are probably the determining factors for germline gene transfer risk. Early gestation direct fetal gene therapy leads to germline transmission when retroviral vectors are injected into the peritoneal cavity of first trimester fetal sheep (60), and after first trimester but not second trimester intraperitoneal injection of lentiviral vectors into fetal macaque monkeys (61). Compared with direct fetal gene therapy therefore, maternal uterine artery gene therapy for IUGR that is will be conducted after mid gestation should carry a very low risk of fetal germline transduction. This is an important safety consideration in any clinical trial protocol. Data derived from pre-clinical studies show no evidence of placental toxicity. T cells and macrophages were not markedly increased in the placenta of pregnant rabbits treated with an Ad. vector (42). *Ex vivo* studies assessing the effect of an Ad.VEGF vector on human placental villous explants showed no changes in the expression of enzymes associatd with placental dysfunction such as lactate dehydrogenase and human chorionic gonadotropin (40).

The EVERREST consortium of academic health science centres, universities and small medium enterprises was awarded European Commission funding to investigate the efficacy and safety of Ad.VEGF therapy in pregnant women diagnosed with severe early onset IUGR through phase I/IIa clinical trials (43). The consortium were awarded orphan drug status from the European Committee for Orphan Medicinal Products for FGR, the first time that a drug has been recognised for the treatment of FGR. In stakeholder and patient interviews conducted by the EVERREST consortium, maternal gene therapy was for the majority of stakeholders considered to be acceptable if there was clear fetal benefit (44). Most women felt they would be able to make informed decisions about taking part in such a trial whilst

pregnant, so long as they were provided with the information required to make an autonomous decision. The primary aim of the the phase I clinical trial is to determine safety. The planned route of administration is via interventional radiology guided uterine artery injection, with a temporary cessation of blood flow during vector injection of up to 5 minutes. There are concerns that systemic gene transfer with generalised transgenic VEGF expression would detrimentally lower maternal blood pressure and reduce uteroplacental perfusion, such as was seen after systemic administration of sildenafil citrate in FGR sheep pregnancy (45). Temporary cessation of uterine artery blood flow could potentially worsen fetal hypoxia. It is now appreciated from radiological studies however, that the the uterus is provided by a rich blood supply from the ovarian, cervical and vaginal arteries including a system of utero-ovarian communicating arteries (46)(47)(48). Careful monitoring of fetal wellbeing and prompt release of uterine artery occlusion will be needed in any clinical trial protocol. Other potential risks include vascular leak, which is less likely when using VEGF-D rather than VEGF-A isoforms, and the pro-inflammatory state from VEGF-induced macrophage activation. Reassuringly long term studies on adenovirus gene therapy show an excellent safety profile up to 10 years after clinical trials of local intracoronary and lower limb administration (49)(50), and Ad.VEGF-$D^{\Delta N \Delta C}$ is being tested in a phase III clinical trial for refractory angina pectoris “ReGenHeart” ClinicalTrials.gov Identifier:NCT03039751. The consortium has developed a prospective “natural history” cohort, to carefully define the characteristics and outcomes of pregnancies affected by severe early onset IUGR (51) which is also refining the inclusion criteria for a clinical trial. During this study the consortium has also studied which angiogenic markers may be most useful for defining the inclusion criteria in the trial. Monitoring the change in concentration of sFlt1 and its ratio with PlGF may be useful to predict the onset of pre-eclampsia and IUGR (52), and a similar approach is being investigated. This will need to ensure that only those women with the most severely affected

pregnancies take part, while achieving sufficient numbers of babies to allow long term to evaluate safety and efficacy.

Conclusion

Local expression of VEGF in the uterine arteries increases uterine blood flow, alters uterine artery vascular reactivity, increases angiogenesis and improves fetal growth in IUGR pregnancies without apparent maternal or fetal harm. Translation to the clinic will be complex as getting informed patient consent and demonstrating safety will be key. Findings from preclinical studies however suggest that maternal VEGF gene therapy is promising as a therapy for severe early onset IUGR.

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Conflict of Interest Statement

The author receives funding from UCLH NIHR Biomedical Research Centre and is a consultant for Magnus Growth Ltd, a company which is aiming to take to market a novel treatment for fetal growth restriction, for which she receives a token consultancy payment and shareholding in the company.

Figure Legends

Figure 1:

Sites of action in the uteroplacental circulation and blood, of the interventions currently under investigation as treatments for intrauterine growth restriction and pre-eclampsia. sFlt-1, soluble fms-like tyrosine kinase 1; VEGF, vascular endothelial growth factor; NOS, nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylate cyclase; GTP, guanosine-5′-triphosphate; cGMP, cyclic guanosine monophosphate; 5′ GMP, guanosine monophosphate; PDE5, phosphodiesterase type 5 inhibitor

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Effect	Short term (4-7 days after vector injection)		Long term (28-30 days after vector injection)	
	Ad.VEGF-A_{165}	Ad. VEGF-$D^{\Delta N \Delta C}$	Ad.VEGF-A_{165}	Ad. VEGF-$D^{\Delta N \Delta C}$
Uterine artery blood flow	↑*	-	↑*	tendency to ↑
Tissue VEGF expression by ELISA and Immunohistochemistry	Injected uterine artery Perivascular adventitia	Injected uterine artery Perivascular adventitia	Not detectable	Not detectable
Blood VEGF expression	Maternal, no fetal	Maternal, no fetal	Not detectable	Not detectable
Uterine artery adventitial angiogenesis	↑*	↑*	↑*	↑*
Vascular contractility	↓*	↓*	↓*	↓*
Vascular relaxation	↑*	↑*	→	→
Vector spread	Maternal / no fetal	-	-	-
Histological analysis	Oedema and macrophage infiltration	No abnormality	No abnormality	No abnormality
VEGF Receptors	↑VEGFR2	-	→	
eNOS Western blot	↑	↑	→	→
Maternal BP and HR	→	→	→	→
Fetal BP and HR	→	→	→	

Table 1: Results of Ad.VEGF delivery to the uterine arteries of normal sheep pregnancies (17,18,19). *$p < 0.05$. eNOS: endothelial nitric oxide synthase; BP: blood pressure; HR: heart rate

Model	**Over-nourished adolescent ewe (IUGR sheep)**		**Maternal nutrient restricted IUGR guinea pig**		
Time of assessment	**Term pregnancy**	**3 months postnatal**	**3-7 days post vector application**	**30 days post vector application**	**4 months postnatal**
Vector dose	1×10^{12}/ewe	1×10^{12}/ewe	$1x10^{10}$vps/animal	$1x10^{10}$vps/animal	$1x10^{10}$vps/animal
Uterine artery blood flow	n=18: No change detectable	Not done	Not applicable	Not applicable	Not applicable
Fetal and neonatal growth velocity	n=18: Fetal abdominal circumference ≈20% greater. Significantly fewer severe IUGR lambs	n=17: Fetal abdominal circumference ≈20% greater. Lamb birth weight tended to be ≈20% greater near term. Higher absolute neonatal growth rate.	Not applicable	n=45: Increased birthweight, increased brain, lung and liver weights	n=15: Increased birthweight, increased neonatal growth. Amelioration of adult hypertension
Maternal, fetal and neonatal health	No AEs (n=18)	No AEs (n=17)	No AEs (n=10)	No AEs (n=12)	No AEs (n=15)
Presence or expression of vector	RT-PCR: increased VEGF receptor (FLT1/KDR) mRNA expression in the maternal but not fetal placental compartments	not done	RT-PCR: fetal tissues (-), transduced Uterine and Radial Artery (+), all other maternal tissues (-). ELISA: transduced Uterine and Radial Artery (+)	RT-PCR: fetal (-) ELISA: (-)	Not done

Table 2: Efficacy and safety of Ad.VEGF-A_{165} in preclinical FGR animal studies (20-22).
AE: Adverse Event; RT-PCR=Real time polymerase chain reaction; FLT-1=Vegfr 1; KDR=Vegfr 2