GENE THERAPY FOR OBSTETRIC CONDITIONS

INTRODUCTION

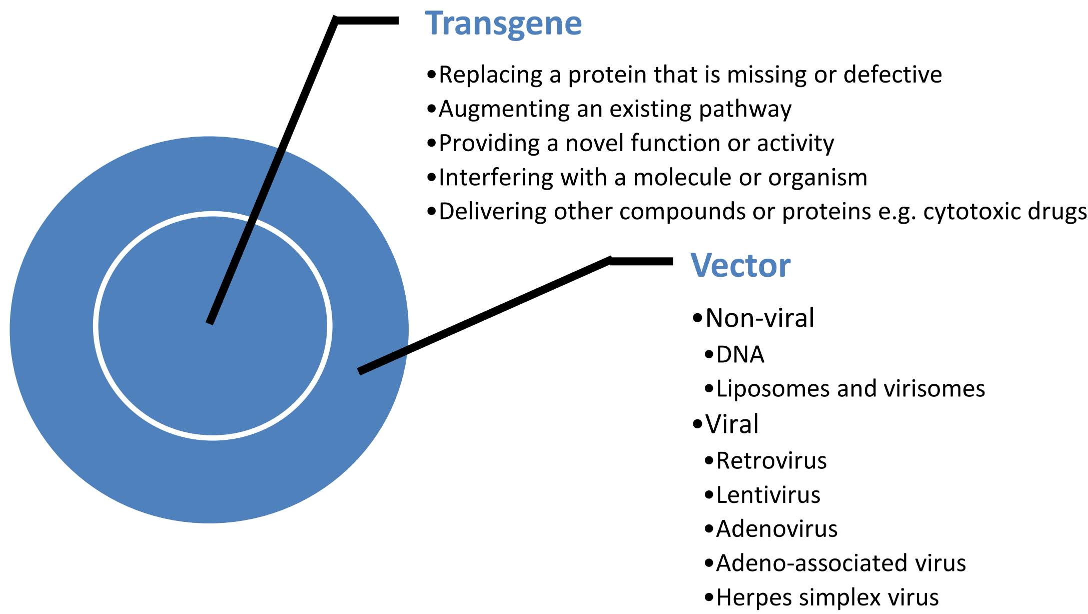
The first clinical trials of gene therapy in the 1990s offered the promise of a new paradigm for the treatment of genetic diseases. Over the decades that followed the challenges and setbacks which gene therapy faced often overshadowed any successes. Despite this, recent years have seen cause for renewed optimism. In 2012 Glybera™, an adeno-associated viral vector expressing lipoprotein lipase, became the first gene therapy product to receive marketing authorization in Europe, with a licence to treat familial lipoprotein lipase deficiency (1). This followed the earlier licensing in China of two gene therapies: Gendicine™ for head and neck squamous cell carcinoma and Oncorine™ for late-stage nasopharyngeal cancer. By this stage over 1800 clinical trials had been, or were being, conducted worldwide, and the therapeutic targets had expanded far beyond purely genetic disorders (2). So far no trials of gene therapy have been carried out in pregnancy, but an increasing understanding of the molecular mechanisms underlying obstetric diseases means that it is likely to have a role to play in the future. This review will discuss how gene therapy works, its potential application in obstetric conditions and the risks and limitations associated with its use in this setting. It will also address the ethical and regulatory issues that will be faced by any potential clinical trial of gene therapy during pregnancy.

WHAT IS GENE THERAPY?

Gene therapy is the introduction of genetic material into a cell so that it produces a therapeutic protein product. A gene therapy must therefore consist of genetic information, the transgene, and a way of introducing it into the cell, the vector. The genetic information contained in a transgene can include the code for the desired protein and promoters to regulate its expression. Depending on the therapeutic aim, the protein, regulation and vector may be designed to act in various ways.

25 **Types of transgene**

26 The actions of proteins generated by gene therapy, like all therapeutic proteins, can be divided into
27 several broad categories (3). The most simple and intuitive strategy is to introduce the gene for a
28 protein that is missing or defective, for example the β-haemoglobin chain for treatment of β-
29 thalassemia. There are numerous other single gene disorders that would be amenable to this
30 strategy, including cystic fibrosis, haemophilia, Duchenne muscular dystrophy, and X-linked severe
31 combined immunodeficiency (SCID-X). However, while monogenic disorders have been targeted
32 since the first clinical trials of gene therapy, achieving long term, safe, therapeutic expression has
33 proven difficult (2). In other fields, most notably oncology, a variety of therapeutic actions have been
34 exploited. Many of these involve interfering with existing pathways that contribute to tumour
35 growth, such as the formation of new blood vessels. Vascular endothelial growth factors (VEGFs) are
36 important mediators of angiogenesis and their overexpression has been associated with poor
37 prognosis in ovarian cancer (4). Anti-angiogenic gene therapy, which produces soluble VEGF
38 receptors to bind excess VEGF, has improved survival in a mouse model of ovarian cancer and is
39 planned to undergo a phase I clinical trial (5). Proteins with novel functions can also be produced, for
40 example enzymes that convert a nontoxic pro-drug into a cytotoxic metabolite, known as suicide
41 gene therapy. One example is herpes simplex virus 1 thymidine kinase (HSV1TK), which
42 phosphorylates ganciclovir (CGV) and converts it into the toxic CGV-triphosphate. Combination of
43 HSV1TK gene transfer using adenovirus vectors followed by CGV therapy has also been studied in
44 animal models as a potential treatment for uterine leiomyomata (fibroids) (6). Given the wide range
45 of actions of protein messengers, receptors and enzymes, there are many obstetric conditions in
46 which manipulation of protein pathways, particularly in relation to growth factors and angiogenesis,
47 could prove beneficial.



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49 Figure 1: Gene therapy involves using a vector, either viral or non-viral, to introduce a transgene, which in turn
50 codes for a therapeutic protein.

51

52 **Types of vector**

53 An ideal vector would introduce the transgene efficiently and specifically to the target cells, both
54 dividing and non-dividing, and cause it to be expressed for as long as desired, with no adverse
55 effects. For obstetric conditions the length of time would most likely be short, limited by gestational
56 length. The vector would also be able to carry a large amount of genetic material and be simple and
57 cheap to manufacture (7). In reality the non-viral and viral vectors currently available each have their
58 own advantages and limitations (8).

59 Non-viral methods involve introducing the transgene either physically or chemically into the target
60 cell(s). The most widely used non-viral vectors in clinical trials are liposomes, which are artificial lipid
61 vesicles containing the transgene that introduce it into the cell by binding with the cell membrane.
62 Membrane binding can be enhanced by incorporating viral envelope proteins into the lipid vesicle,

63 creating a kind of hybrid vector known as a virosome. Other methods to enhance gene transfer
64 include: microinjection of DNA directly into the nucleus, which can be used in the research setting
65 but is not feasible clinically; hyperdynamic injection using a high volume over a short period of time;
66 a gene gun, which penetrates cells with microparticles coated in DNA; and electroporation or
67 ultrasound, which uses electrical pulses or high frequency sound waves to make the cell membrane
68 temporarily permeable to genetic material. Non-viral vectors are associated with fewer side-effects
69 than viral vectors but also with reduced efficacy and only short-term expression (9, 10).

70 Viruses are a natural choice of vector because, as obligate intracellular parasites, they have evolved
71 to introduce their own DNA or RNA into host cells and use the cell machinery to replicate (Figure 2).
72 To produce a viral vector, the genes governing viral replication and protein production are removed
73 and replaced by the chosen transgene, incorporating a promoter to regulate its expression. Viral
74 vectors can be divided into two main categories: those where the transgene forms an episome
75 within the cytoplasm of the cell, such as adenoviruses, and those that integrate the transgene into
76 the host DNA, such as retroviruses and lentiviruses. Adeno-associated virus (AAV) vectors share
77 characteristics of both groups, mainly forming episomes, but over time have the potential to
78 integrate as well (11). The choice of viral vector, therefore, depends on the therapeutic aim.

79 Adenoviruses, which normally cause respiratory and gastrointestinal infections, infect a wide range
80 of dividing and non-dividing cells. They also trigger a host immune response, with B cells generating
81 antibodies to their surface proteins and T cells targeting infected cells by recognising the presence of
82 functional virus proteins (8). Adenoviral vectors are efficient, have widespread action, and can carry
83 large transgenes. Their usefulness in treating monogenic disorders is limited by their short-term
84 expression, however this makes them of particular interest for use in obstetric conditions, where
85 short-term expression may be desirable.

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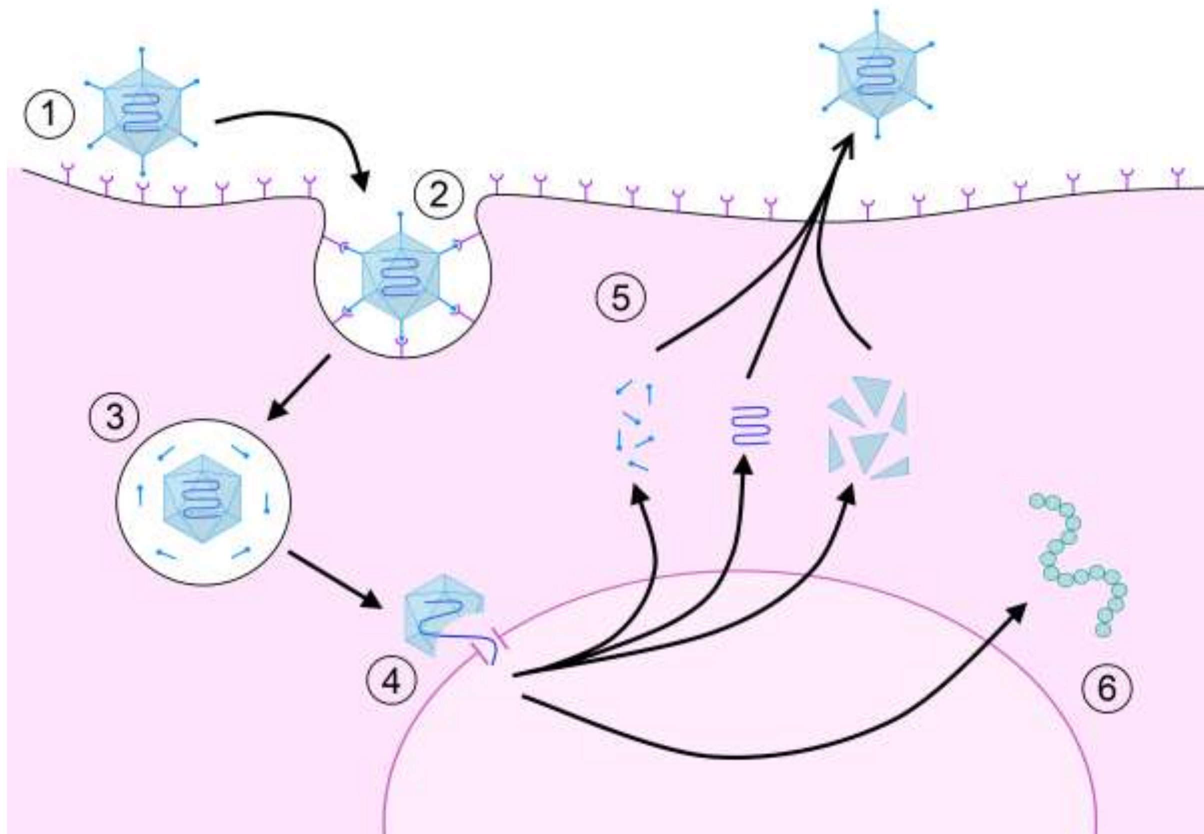


Figure 2: Similarities and differences between wild-type adenoviral replication and replication-deficient adenoviral vector action. (1) Fibre proteins on the viral capsid bind to coxsackievirus adenovirus receptors (CARs) on the cell surface (2) The virus is taken up via endocytosis (3) Within the endosome the fibres are shed and the viral core enters the cytosol (4) Microtubules transport the viral core to the nucleus, where DNA enters via a nuclear pore (5) Infection with a wild-type adenovirus results in DNA replication and synthesis of new viral structural and function proteins. These are assembled into new viral particles, which are then released from the cell (6) Transfection with a replication-deficient adenoviral vector results in transgenic protein expression.

Immunogenicity has been a problem, especially with early vector backbones. In 1999 a systemic inflammatory response to a high dose of adenovirus vector resulted in multi-organ failure and the death of an 18 year-old participant in a clinical trial of adenoviral gene therapy for ornithine transcarbamylase deficiency, an inborn error of urea synthesis (12). The participant's enrolment in

101 the trial was subsequently criticised, as the inclusion criteria specified an ammonia level of <70μM,
102 while his ammonia level had fluctuated above and below this cut off before enrolment.
103 Furthermore, there was criticism that adverse events in previous participants in the trial had not
104 been properly reported. Detailed investigations suggested that the participant had an exaggerated
105 vector-induced activation of innate immunity, which was not predicted based on the preclinical and
106 clinical data available at the time (13). By removing more of the viral genes, newer generation
107 helper-dependent adenoviral vectors have been developed that produce less of an immune
108 response (14). Even so, their expression is limited to weeks or months, in part because episomes are
109 not replicated and so are gradually lost from dividing cell populations.

110 Retroviruses and lentiviruses generate little immune response and integrate into the host genome.
111 Consequently they have the potential to provide long-term expression(15). This is important for the
112 correction of monogenic diseases, but also carries with it the risk of insertional mutagenesis.
113 Transgenes can include their own promoters, to improve and regulate expression after they have
114 been introduced. Integration of a transgene into the host genome has the potential to influence the
115 expression of nearby genes and if inserted upstream of a proto-oncogene, this can lead to
116 malignancy. There are concerns that the fetal genome may be more susceptible to insertional
117 mutagenesis due to the higher proliferation that takes place in utero (16). Insertional mutagenesis
118 and subsequent leukemia has been seen during some haematopoietic stem cell gene therapy trials,
119 such as for SCID-X (17). Both retroviruses and lentiviruses have a tendency to integrate in actively
120 transcribed genes, with retroviruses favouring the transcription start regions (8). Retroviruses but
121 not lentiviruses are limited by the fact that they can only transduce dividing cells because the
122 preintegration complex cannot cross the nuclear membrane (18). This could be an advantage for
123 targeting the placenta for example, which is a highly replicating organ.

124 Like adenoviruses, AAV infects a wide range of cells, but without causing any disease in humans. The
125 natural virus has a tendency to integrate into a safe region of chromosome 19. This tendency is lost

126 when the viral genes are removed to convert it into a vector, but the theoretical possibility of
127 insertional mutagenesis remains (8, 10). Insertion occurs much less frequently than with
128 retroviruses, however, and the transgene mainly forms episomes. This means that long-term
129 expression is best achieved in non-dividing cells (14). Although AAV vectors do not produce the
130 immune response associated with adenoviral vectors, they are limited in the size of transgene that
131 they can carry and the presence of neutralizing antibodies from previous natural infection can limit
132 their gene transfer.

133 **Targeting delivery with viral vectors**

134 One of the advantages of viral vectors is that they generate more efficient and widespread
135 introduction of the transgene than non-viral vectors. On the other hand it can be harder to target
136 delivery into specific cells, increasing the risk of harmful production of the protein in inappropriate
137 organs and the introduction of the transgene into germ cells, both of which are particular concerns
138 when considering gene therapy for obstetric conditions. Germline gene therapy, deliberate gene
139 transfer to oocytes or sperm cells to produce changes in future generations, was not considered to
140 be acceptable for ethical and safety reasons by the Gene Therapy Advisory Council in 1998 (19).
141 More recently mitochondrial DNA replacement (20) has been achieved in human oocytes using
142 spindle transfer and in January 2015 the UK government approved regulations to govern application
143 of these techniques to eradicate the transmission of serious mitochondrial disease from mother to
144 child (21). Inadvertent germline transmission remains a risk with some types of vector, either to the
145 pregnant woman, or, if there were a significant chance of the vector crossing the placenta, to the
146 fetus. In reality this risk if very low and is discussed in detail later. The targeting of viral vectors to
147 specific cells can be improved by using *ex vivo* gene transfer, by modifying the vector or transgene,
148 and by altering the route of administration.

149 With *ex vivo* gene transfer some of the target cell population, commonly stem cells, are removed,
150 transduced outside the body and then returned to the patient (10, 17). This avoids the risk of

151 inappropriate vector spread and allows higher multiplicities of infection of the viral vector to be used
152 without the risk of systemic side-effects. It is particularly suited to congenital haematological
153 conditions, for example the primary immunodeficiency SCID-X, where the stem cell population is
154 easily accessible from the blood or bone marrow (22). However, it has also been used to generate
155 skin grafts from epidermal stem cells in xeroderma pigmentosum and to modify hepatocytes for
156 autologous transplantation in familial hypercholesterolaemia. In the case of obstetric disorders
157 arising from the placenta, chorionic villi could be sampled under ultrasound guidance, with stem
158 cells isolated and transduced *ex vivo* (23). The amniotic fluid provides an alternative source of
159 multipotent stem cells that would be matched to the placenta (24-27).

160 Alternative ways of targeting gene therapies include: using vectors created from viruses that
161 naturally target certain cell types, such as herpes simplex viral vectors to transduce the central
162 nervous system (28); modifying the virus vector envelope proteins to target certain cell types
163 (pseudotyping); and using tissue-specific promoters (8, 14). In the case of adenoviruses, the fibre
164 proteins that form part of their capsid normally allow them to enter cells by interacting with the
165 coxsackievirus and adenovirus receptor (CAR). This receptor is found on a range of cells, making
166 delivery with adenoviral vectors normally quite non-specific. The fibre-mutant adenovirus vector
167 carrying the Arg-Gly-Asp peptide sequence (Ad-RGD) has altered fibre proteins which allow it to
168 enter cells via integrin receptors. This increases its tropism for certain cell types/organs, including
169 the placenta (29), endometriotic cells (30), and human leiomyoma cells, suggesting it could be used
170 to deliver targeted treatment for uterine fibroids (31). Protein expression could also be increased in
171 endometriotic cells lines by using promoters that are specific to endometriosis cells: secretory
172 leukocyte protease inhibitor (SLPI) and heparanase.

173 In obstetric disorders, targeting may also be achievable by altering the route of administration. The
174 therapeutic targets during pregnancy can be broadly considered to be the fetus, the placenta, or the
175 mother. Fetal gene therapy generally relies on direct administration, for example to the fetal

176 peritoneal cavity, umbilical vein, or amniotic fluid. However, attempts have been made to deliver
177 viral vectors to the fetus via intraplacental injection in pre-clinical studies (32-36) with variable
178 success, depending in part on the stage of gestation (32) (See Table 2). These studies have shown
179 that direct intraplacental injection results in effective local gene transfer to the placenta. However,
180 in situations where the target was the placenta rather than the fetus, unwanted spread to the fetus
181 and the mother could be an issue. An alternative method is to deliver gene therapy to the placenta
182 by injection into the uterine arteries, targeting the maternal side of the uteroplacental circulation. In
183 rabbits, low levels of gene transfer to the placenta were achieved using angiographically guided
184 injection of non-viral vectors into the uterine artery (37). Targeted gene transfer to the uterine
185 artery can also be achieved using direct external vascular application of the vector in combination
186 with Pluronic gel (38). This is a thermolabile gel which is liquid when cold and solidifies upon
187 warming, allowing the vector to coat the external vessel wall.

188 **FETAL GENE THERAPY**

189 When prenatal diagnostic methods identify a fetus with a life-threatening genetic disorder that
190 manifests *in utero*, fetal gene therapy is an appealing idea with several potential advantages over
191 postnatal gene therapy, including: the ability to target cells and tissues that are inaccessible
192 postnatally; the relative immaturity of the fetal immune system, allowing tolerance of the transgene
193 product; the possibility of preventing or limiting the complications of diseases that develop before or
194 shortly after birth; and the need for lower doses of the vector, thus reducing production costs (7, 14,
195 39-41) . For these reasons fetal gene therapy has been the focus of much research, including many *in*
196 *vivo* pre-clinical animal studies that have shown efficacy to cure disease such as in mice haemophilia
197 (42) metabolic storage disorders (43, 44) and muscular dystrophy (45), retinal blindness in mice (46)
198 and birds (47), and in mice to prevent the development of fetal anomalies such as cleft palate (48).
199 There are no studies performed in large animal models of disease but plasma levels of therapeutic
200 transgenic factor IX protein expression have been achieved in normal sheep (49) and non-human

201 primates (50) after fetal gene therapy using adeno-associated virus factor IX vector injection, and
202 neuronal transduction of the central and peripheral nervous systems is seen after systemic AAV
203 vector injection in non-human primates (51). Target diseases are considered to be those that are
204 life-threatening, that have pathology beginning *in utero* where accurate prenatal diagnosis is
205 feasible, a postnatal treatment would be too late or is not available, and a disease where there is a
206 close correlation between the genotype and the phenotype (52). At present no disease fits the
207 criteria and with current vector systems still being developed for postnatal treatment, it may be
208 some years before a fetal gene therapy may be translated into the clinic. An alternative strategy is
209 to gene correct fetal stem cells that are available in the amniotic fluid or placenta for example, which
210 can then be delivered back into the fetus as an autologous transplant (53). This avoids the fetal
211 immune system which is responsible for the failure of *in utero* stem cell transplantation for non-
212 immune deficiency congenital disease such as thalassaemia and metabolic storage disorders (54).
213 Pilot studies in sheep demonstrate the feasibility of this approach (24, 55) whereby CD34+ stem cells
214 with hematopoietic potential were derived from fetal sheep amniotic fluid, transduced and
215 underwent autologous transplantation into fetal sheep and immunodeficient mice. It has advantages
216 over systemic fetal gene transfer since transduction is limited to selected fetal cells, is performed
217 outside the fetus in well controlled conditions and it allows for detailed safety testing such as
218 insertion site analysis.

219 **MATERNAL GENE THERAPY**

220 The use of transgenic proteins in cell cultures and pregnant animals has helped to elucidate the
221 molecular mechanisms underlying normal pregnancy and obstetric diseases. This has the potential
222 for translation into gene therapies given to the pregnant woman rather than the fetus. Due to the
223 inherent interdependence that occurs in pregnancy, maternal gene therapy has the prospect of
224 providing both maternal and fetal benefit for a number of conditions. Furthermore, because
225 pregnancy represents a limited time period, the challenges of producing long-term gene expression

226 would not apply. Maternal gene therapy has not been explored as widely as fetal gene therapy, but
227 may have a role in both obstetric and non-obstetric conditions.

228 **Fetal growth restriction**

229 Fetal growth restriction (FGR) is a serious condition affecting 8% of all pregnancies and contributing
230 to 50% of stillbirths (56). In this condition, the fetus fails to achieve its growth potential and is born
231 smaller than anticipated. The causes are heterogeneous and can include structural abnormalities of
232 the fetus, aneuploidy, congenital viral infection or maternal medical disorders. More commonly,
233 impaired uteroplacental function restricts delivery of nutrients to the fetus, resulting in slowing or
234 even cessation of fetal growth. In about 1 in 500 cases, FGR is severe and early in onset, occurring
235 before 28 weeks gestation. Current management is to deliver the fetus before death or irreversible
236 organ damage occurs, particularly to the brain. However, early delivery in severe early onset FGR
237 adds additional risks to the baby from extremely preterm birth, with its own attendant short- and
238 long-term complications (57). Furthermore FGR may be detected while the fetal weight is far below
239 500g, a situation considered by many to be non-viable. Even modest increases in birthweight (e.g.
240 from 500 to 600g) and gestation at delivery (e.g. from 26 to 27 weeks) are associated with significant
241 improvements in mortality and morbidity (58). It is in these severe early-onset cases of FGR that
242 maternal gene therapy is initially being considered, where the benefit of gaining gestation or fetal
243 weight outweighs any potential risks of a novel therapy. If it is found to be safe and efficacious there
244 is potential to use maternal gene therapy in more moderate FGR, which affects a larger number of
245 pregnancies.

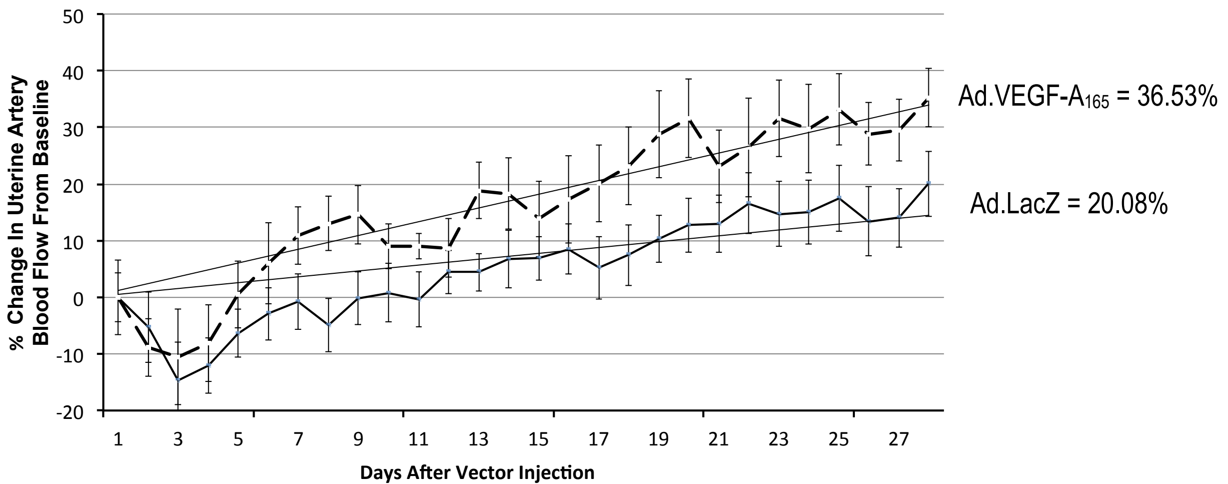
246 In normal pregnancy, trophoblast invasion of the maternal spiral arteries produces a low resistance,
247 high flow maternal uterine circulation. These changes are enhanced by the placental production of
248 vasoactive substances such as VEGF, which causes vasodilation and angiogenesis. In uteroplacental
249 FGR, trophoblast invasion is impaired, resulting in a relative reduction in uterine artery blood flow
250 (59, 60). There is also a reduction in available maternal VEGF and an increase in its soluble receptor,

251 soluble fms-like tyrosine kinase 1 (sFlt1) (61, 62). Our research over the last eight years has
252 demonstrated that adenovirus vector (Ad) mediated manipulation of VEGF expression in the uterine
253 arteries produces an increase in uterine artery blood flow (63-65) and ameliorates the effects of FGR
254 in animal models (66, 67).

255 There are currently seven proteins within the VEGF family, of which five occur naturally in humans:
256 VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). These proteins bind to various
257 combinations of the three VEGF receptors to produce different angiogenic and lymphangiogenic
258 effects (68). VEGF-A occurs in a variety of isoforms depending on pre-RNA splicing, including VEGF-
259 A_{165}. In contrast VEGF-D is naturally produced in an inactive long form, and has to be shortened to
260 the active form, VEGF-$D^{\Delta N\Delta C}$. With a viral vector, however, the pre-processed active form can be
261 produced directly. Both VEGF-A_{165} and VEGF-$D^{\Delta N\Delta C}$ have angiogenic effects, although the effect of
262 VEGF-$D^{\Delta N\Delta C}$ appears to be more diffuse (69).

263 The short and long-term effects of adenovirus vectors containing the A_{165} (Ad.VEGF-A_{165}) or pre-
264 processed D isoform (Ad.VEGF-$D^{\Delta N\Delta C}$) of VEGF were initially studied in normal sheep pregnancy. This
265 involved performing a laparotomy in mid-gestation dams to inject the VEGF adenoviral vector into
266 one uterine artery and a non-vasoactive control adenoviral vector coding for bacterial ß-
267 galactosidase (Ad.LacZ) into the contralateral uterine artery. Short-term studies comparing Ad.VEGF-
268 A_{165} with Ad.LacZ found that uterine artery blood flow, as assessed by Doppler ultrasound, increased
269 in both arteries 4-7 days after administration, as expected with increasing gestation. However, while
270 the increased flow was not significant in the Ad.LacZ treated arteries, there was a significant three-
271 fold increase in blood flow in the Ad.VEGF-A_{165} treated vessels. This was associated with a reduced
272 contractile response and increased relaxation response of Ad.VEGF-A_{165} vs. Ad.LacZ treated vessels
273 (63). Levels of endothelial nitric oxide synthase (eNOS) and VEGF receptor 2 (VEGFR-2) were also up-
274 regulated at day 4-7 in vessels transduced with Ad.VEGF-A_{165} compared with those transduced with
275 Ad.LacZ (64). Similar results were achieved in short-term studies comparing Ad.VEGF-$D^{\Delta N\Delta C}$ with

276 Ad.LacZ, in which vessels administered Ad.VEGF-$D^{\Delta N\Delta C}$ had a significantly reduced contractile
277 response, a significantly increased relaxation response, and a significant increase in phosphorylated
278 eNOS (64). Furthermore, compared with non-transduced vessels at the same gestational age, there
279 was a significant increase in endothelial cell proliferation in the main branch of the uterine artery 4-7
280 days after transduction with Ad.VEGF-$D^{\Delta N\Delta C}$.



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282 Figure 3: Injection of Ad.VEGF-A_{165} ($5x10^{11}$ vector particles) into the uterine artery of a pregnant ewe (day 98±2
283 of 140 day gestation) produces a significantly greater increase in uterine artery volume blood flow from
284 baseline as measured by implanted flow probes than a control vector (Ad.LacZ) injected into the contralateral
285 uterine artery (p=0.02). Change in uterine artery volume blood flow was calculated as a percentage change
286 from baseline (measured over 3 days before administration; adjusted to 0) and presented as mean ± SE (64).

287

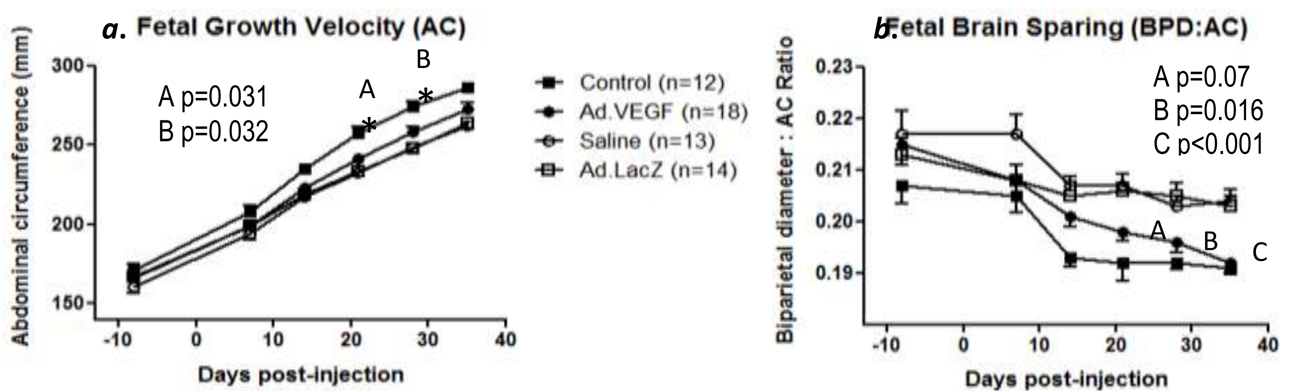
288 Longer-term effects on uterine artery volume blood flow were assessed using implanted flow
289 probes. In vessels injected with Ad.VEGF-A_{165}, a significant increase in uterine artery volume blood
290 flow persisted at 28 days after administration compared with vessels injected with Ad.LacZ (Figure 3)
291 (64). The same trend was seen after injection with Ad.VEGF-$D^{\Delta N\Delta C}$ (65). In both cases this was
292 associated with a persistent reduction in vascular contractile responses in the uterine arteries, as
293 well as a significantly increased number of adventitial blood vessels at 30-45 days after
294 administration (64, 65). At this stage, human VEGF expression was no longer detectable by enzyme-

295 linked immunosorbent assay (ELISA) in the uterine arteries or uterine wall and there was no on-
296 going up-regulation of VEGFR-2. These findings show that the beneficial effects of Ad.VEGF
297 administration persist even after transgene expression has ceased, which is probably related to the
298 formation of new vessels in the uterine artery adventitia (63-65).

299 Subsequent experiments were carried out using a sheep model of FGR, whereby adolescent ewes
300 conceive through single sire donor embryo transfer (to minimise genetic heterogeneity and ensure
301 optimise pregnancy rates) and are subsequently overnourished. The high dietary intake in these still-
302 growing adolescent dams results in nutrient partitioning away from the gravid uterus, causing
303 accelerated maternal tissue growth at the expense of the fetus (70). This is associated with a ≈42%
304 reduction in uterine artery volume blood flow from mid-gestation, followed by placental and fetal
305 growth restriction relative to the normally growing fetuses of adolescent dams fed a moderate
306 (control) diet. In this situation approximately 52% of high-intake pregnancies demonstrate "marked"
307 FGR, an accepted definition of which is a birth weight more than two standard deviations (SD) below
308 the genetic potential (71), which can be estimated from contemporaneous control-intake
309 pregnancies on a study-by-study basis. In these pregnancies fetal and placental weights are reduced
310 by ≈48%, whilst the remaining pregnancies (48%) are considered "non-FGR" (72). This experimental
311 paradigm replicates key clinical features of human uteroplacental insufficiency without requiring any
312 surgical interference to the uteroplacental circulation (73). An asymmetrical pattern of growth
313 restriction is observed, with prioritisation of brain growth (brain sparing) and increased umbilical
314 artery Doppler indices (74, 75). There is also impaired placental vascularisation and secretory
315 function, and reduced placental expression of VEGF (76-78).

316 Overnourished adolescent ewes were randomised to receive Ad.VEGF-A_{165} or a control treatment
317 (Ad.LacZ or saline) injected into both uterine arteries in mid-pregnancy. The fetal growth velocity, as
318 determined by serial ultrasound measurements of fetal abdominal circumference by a single masked
319 assessor, was significantly increased in the Ad. VEGF-A_{165} group compared with the Ad.LacZ or saline

320 groups at 3-4 weeks after injection, and there appeared to be amelioration of fetal brain sparing (66,
321 79). At necropsy at 131 days gestation (term = 145 days), significantly fewer fetuses demonstrated
322 marked FGR in the Ad.VEGF-A_{165} group than in the Ad.LacZ or saline groups. Increased fetal growth
323 velocity was also demonstrated in a second experimental group, in which overnourished adolescent
324 dams were similarly randomised to receive uterine artery injections of either Ad.VEGF-A_{165} or saline,
325 however the pregnancies were allowed to continue until spontaneous delivery at term (Figure
326 4)(80). At birth Ad.VEGF-A_{165}-treated lambs tended to be heavier (p=0.081) and demonstrated
327 increased absolute postnatal growth velocity during the first three months of life (81). Fractional
328 growth rates were not different, indicating appropriate growth relative to initial birth weight, and
329 body composition analysis revealed greater lean tissue mass. No impact on the epigenetic status of
330 key somatotrophic axis genes was observed.



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332 Figure 4: Serial ultrasound measurements of: *a.* fetal abdominal circumference (AC); and *b.* fetal biparietal
333 diameter to AC ratio (a marker of fetal brain sparing) in 57 singleton-bearing adolescent ewes receiving a
334 control (n=12) or high dietary intake (n=45) to promote normal or compromised fetal growth, respectively
335 (80). Overnourished ewes were randomised to receive bilateral uterine artery injections of Ad.VEGF-A_{165} (n-
336 18), Ad.LacZ (n=14) or saline (n=13) in mid-pregnancy. Control-fed ewes all received saline (n=12). Those time
337 points at which there were significant differences between Ad.VEGF-A_{165} and Ad.LacZ/saline-treated
338 overnourished groups are indicated.

339

340 An alternative animal model of FGR is periconceptual nutrient deprivation in the guinea pig, which
341 impairs placental functional development, reduces the placental exchange and trophoblastic
342 surface, increases the thickness of the exchange barrier and causes a 40% reduction in fetal weight
343 with brain sparing (82), all of which are features of human FGR. A further advantage of this model is
344 the fact that guinea pig placentation is the most similar to human placentation with a haemochorial
345 type of placenta, and a homologous process of trophoblast invasion (83) and trophoblast cell
346 proliferation (84). Mid-gestation nutrient-restricted Dunkin Hartley guinea pigs underwent
347 laparotomy at 30-34 days gestation (term = 65) and the uterine and radial arteries were transduced
348 with Ad.VEGF-A_{165} or Ad.LacZ, using a thermosensitive Pluronic gel (67). At necropsy, 31-34 days
349 later, administration of Ad.VEGF-A_{165} was associated with a significantly lower brain:liver weight
350 ratio, suggesting an attenuation of brain sparing.

351 In 2013 the European Union awarded a Framework Programme 7 grant to the EVERREST consortium
352 (85) which aims to conduct a phase I/IIa clinical trial to assess the safety and efficacy of Ad.VEGF
353 gene therapy given into the uterine arteries of pregnant women with severe early onset FGR. The
354 project is being undertaken by the EVERREST Consortium, a multinational, multidisciplinary group,
355 including experts in bioethics, fetal medicine, fetal therapy, obstetrics and neonatology. The clinical
356 trial protocol is currently under development, requiring input from a wide range of healthcare
357 experts within the consortium to consider inclusion, exclusion criteria and adverse event reporting.
358 The ethical and regulatory issues which are raised by a trial of this nature and the development of
359 the clinical trial protocol will be discussed later in the article.

360 Another therapeutic strategy in FGR is to try and improve placental function instead of increasing
361 uteroplacental blood supply. In normal pregnancy insulin-like growth factors (IGFs) I and II act within
362 the placenta to promote fibroblast proliferation and survival, and increase placental uptake and
363 transfer of glucose and amino acids (86). IGF-I also regulates cytotrophoblast and
364 syncytiotrophoblast differentiation (86). In sheep, direct administration of IGF-I, via a continuous

365 maternal infusion or regular injections into the amniotic cavity, has been shown to increase fetal
366 growth rate in a placental embolisation model of FGR (87, 88). Translating these routes of
367 administration into clinical practice would prove challenging, making IGF gene therapy an attractive
368 alternative.

369 The potential for IGF gene therapy to alter placental growth and function has been demonstrated *in*
370 *vitro* in cell cultures. Human primary placental fibroblasts transduced with adenoviral vectors
371 expressing either IGF-I (Ad.IGF-I) or IGF-II (Ad.IGF-II) showed significantly increased proliferation and
372 cell migration compared with non-transduced controls (89). Transduction of the BeWo
373 choriocarcinoma cell line by Ad.IGF-I also resulted in significantly increased amino acid uptake and
374 significant increases in mRNA and protein expression of amino acid (90) and glucose transporters
375 (91).

376 The effects of intraplacental IGF gene therapy have been studied *in vivo* using murine and rabbit
377 models of FGR, resulting from reduced uteroplacental blood supply (90-92). In the mouse model,
378 one of the two mesenteric branches of the uterine artery (MUAL) is ligated at laparotomy at day 18
379 of gestation (term = 19-20 days) (93). This results in reduced pup birthweight and a significant
380 reduction in placental expression of IGF-I, IGF-II, large neutral amino acid transporters (LAT) 1 and 2,
381 and glucose transporter 8 (GLUT8) compared with sham surgery (90, 91, 93). Intraplacental
382 administration of Ad.IGF-I at the time of MUAL resulted in significantly increased transporter
383 expression compared with MUAL alone, with levels comparable to a sham surgery control group (90,
384 91). The rabbit model harnesses the natural vascular watershed along each horn of the bicornuate
385 uterus, which affects the third pup from the ovarian end (the runt). Dams underwent laparotomy at
386 day 21, with PBS, Ad.IGF-I, or Ad.LacZ injected into the placenta of the runt in one horn (92). Where
387 runts were administered PBS, the pups closest to the ovarian end of the same horn also received
388 intraplacental PBS, to act as a control group. Fetal, placental, liver, lung, and musculoskeletal weight
389 were significantly lower in runts administered PBS compared with controls. However, runts

390 administered intraplacental Ad.IGF-I had significantly higher fetal, liver, and musculoskeletal weights
391 than runts receiving PBS or Ad.LacZ. In Ad.IGF-I runts these weights were comparable to the control
392 group, indicating normalisation of fetal growth. A note of caution is raised, however, by the loss of
393 all pups in four of the 14 dams administered intraplacental adenoviral vector.

394 **Pre-eclampsia**

395 Pre-eclampsia is a multisystem disorder, which manifests as maternal hypertension and proteinuria
396 after 20 weeks gestation. It affects 2-8% of pregnancies and in its severe form can lead to eclampsia,
397 multi-organ failure, and maternal and fetal mortality (94). Although the manifestations of pre-
398 eclampsia can often be managed in the short term, the only cure is to deliver the fetus and placenta,
399 sometimes very prematurely. Pre-eclampsia and FGR often co-exist, especially onset is early in
400 gestation (before 28 weeks), which reflects an overlap in the pathophysiology of the two conditions.
401 In pre-eclampsia, as in FGR, there is a relatively anti-angiogenic state, with an increase in sFlt-1 and a
402 reduction in available maternal VEGF (95, 96). Excess sFlt-1 production may be caused by placental
403 hypoperfusion and hypoxia secondary to inadequate trophoblast invasion, by inflammatory
404 cytokines such as tumour necrosis factor α (TNF-α) secondary to oxidative stress, or by a
405 combination of these factors. Either way, placental damage leads to the production of
406 proinflammatory microparticles, cytokines, sFlt-1 and other factors which in turn cause maternal
407 endothelial dysfunction and the clinical manifestations of the disease.

408 The role of sFlt-1 in the pathophysiology of pre-eclampsia has been examined in animal experiments
409 using pregnant rats (97, 98) and mice (99). Increases in maternal sFlt-1 levels, achieved either using
410 sFlt-1 infusion (98) or by systemic delivery of an adenoviral vector expressing sFlt-1 (Ad.sFlt-1) (97,
411 99) results in hypertension, proteinuria, renal pathology, and a significant reduction in fetal and
412 placental weight. These changes can be ameliorated by the simultaneous administration of an
413 adenoviral vector expressing VEGF-A_{165} (Ad.VEGF-A_{165}), which significantly reduces maternal plasma
414 sFlt-1 levels compared with Ad.sFlt-1 alone (99). When pregnant mice were administered Ad.VEGF-

415 A_{165} concurrently with Ad.sFlt-1, there was no evidence of the renal pathology or heavy albuminuria
416 that resulted from injection of Ad.sFlt-1 alone. Furthermore, in contrast to the significant rise in
417 blood pressure produced by Ad.sFlt-1 compared to a control vector, simultaneous administration of
418 Ad.sFlt-1 and Ad.VEGF-A_{165} resulted in a fall in blood pressure over the subsequent two days. A
419 similar short-term fall in maternal blood pressure and reduction in sFlt-1 levels was seen in healthy
420 pregnant mice administered a haemaglutinating virus of Japan envelope (HVJ-E) vector containing
421 the transgene for VEGF-A_{165} directly into the extraamniotic cavity on day 14.5 of gestation (100).

422 For more long-term expression, application of adenovirus vectors containing VEGF-A_{121} (Ad.VEGF-
423 A_{121}) has been investigated in the BPH/5 inbred mouse strain, which develops a pre-eclampsia-like
424 syndrome (101). Untreated pregnant BPH/5 mice demonstrate defective trophoblast invasion,
425 decreased uterine artery end-diastolic flow, increased fetal resorption, and late-gestational
426 hypertension and proteinuria. Serum VEGF and placental VEGF expression is lower in the strain
427 compared with 'normal' mice but without an increase in sFlt-1. Systemic intravenous delivery of
428 Ad.VEGF-A_{121} at 7.5 dpc in BPH/5 mice resulted in higher levels of VEGF when compared to mice
429 receiving control Ad.LacZ vector, that were comparable to untreated C57 mice at the same
430 gestation. Treatment also ameliorated the late gestation blood pressure rise, prevented the
431 development of proteinuria, and reduced fetal resorption by 50% compared with BPH/5 mice given
432 Ad.LacZ. These studies demonstrate the potential for manipulating VEGF to prevent the
433 development of or to treat pre-eclampsia.

434 Another potential therapeutic protein system is heme oxygenase (HO), an enzyme that consists of
435 two functional isoforms: the inducible form HO-1 and the constitutively active form HO-2, which is
436 important in the maintenance of healthy pregnancy. Through production of both carbon monoxide
437 and bilirubin, HO has the ability to block the production of both sFlt1 and reactive oxygen species,
438 and thus could be a novel therapeutic agent in the management of preeclampsia (96). In an
439 established mouse model of spontaneous miscarriage due to immunological mismatching (CBA/J x

440 DBA/2J combination) intraperitoneal injection of an adenovirus containing the HO-1 gene at 5 dpc
441 reduced fetal loss with evidence of diminished placental apoptosis (102). This suggests a protective
442 effect of systemic HO-1 up-regulation on pregnancy outcome and the prevention of oxidative
443 damage in the placenta.

444 Gene therapy may also have a role in developing novel therapeutics to manage pre-eclampsia.
445 Calcitonin gene related peptide (CGRP), a neuropeptide and vasodilator, has increased levels in
446 normal pregnancy, but plasma levels are lower in women with pre-eclampsia. Plasma CGRP levels
447 rise in response to magnesium sulphate, a drug used clinically to prevent eclamptic fits in severe pre-
448 eclampsia. Experiments in rat models of pre-eclampsia induced using L-NAME to chronically inhibit
449 nitric oxide production found that CGRP co-administration with L-NAME prevented the development
450 of gestational hypertension and reduced fetal mortality. Its potential for direct clinical application is
451 limited by its short half-life of 10 minutes, but this may be surmountable with the use of short-term
452 gene therapy (103).

453 **Preterm birth**

454 Preterm delivery is the last major complication of pregnancy to be considered, affecting at least 10%
455 of pregnancies worldwide, and is increasing in prevalence. There have, as yet, been no studies
456 published on the use of gene therapies to delay delivery in women who are in threatened preterm
457 labour, or to prevent preterm labour in high risk women. This is probably due to the multifactorial
458 nature of the condition for which some authors have described a “preterm parturition syndrome”
459 (104). Known triggers to parturition that could be amenable to gene therapy interventions include
460 intrauterine infection/inflammation, uterine ischaemia and progesterone.

461 **Pre-pregnancy and early pregnancy**

462 Gene therapy is also being explored in a number of conditions that have a direct effect on fertility
463 and early pregnancy, including endometriosis (105), ovarian failure (106), uterine fibroids (9), and
464 miscarriage (102, 107).

465 Uterine fibroids, or leiomyomas, are benign tumours of the myometrial smooth muscle, which can
466 increase the risk of pre-term labour, fetal malposition, obstructed labour and post-partum
467 haemorrhage. Two potential gene therapies have been developed to treat uterine fibroids, both
468 based on adenoviral vectors. The first is a suicide gene therapy using HSV1TK/GCV, where herpes
469 simplex virus 1 thymidine kinase is used to convert ganciclovir into a toxic metabolite (6). The second
470 uses a transgene for a dominant negative estrogen receptor (DNER), which inhibits the action of
471 'normal' wild-type estrogen receptors (108). In a rat model that is characterised by a mutation in the
472 tuberous sclerosis 2 tumour suppressor gene, injection of either gene therapy directly into the
473 uterine fibroids significantly reduced their volume over 30 days compared with control injections.
474 Because of the potential impact on a fetus, either from the direct effects of these transgenes or from
475 extensive fibroid degeneration, such gene therapies would need to be carried out before pregnancy.

476 Gene therapy has been used in early pregnancy in a mouse model of spontaneous miscarriage (102,
477 107). Successful pregnancy is dependent on changes in the maternal immune system, which allow it
478 to tolerate the presence of the fetus, specifically up-regulation of Th2 cytokines relative to Th1
479 cytokines. Two groups have used maternal intraperitoneal adenoviral gene therapy at 5 dpc,
480 resulting in beneficial effects on the Th2/Th1 cytokine ratio and significant reductions in fetal
481 resorption. As described above, Zenclussen et al. showed that overexpressing the HO-1 enzyme in
482 the maternal peritoneal cavity protected locally against rejection in transplantation models probably
483 due to its anti-oxidant, anti-inflammatory and cytoprotective functions (102). A separate group
484 investigated the effect of adenovirus vector containing the anti-inflammatory fusion protein CTLA4Ig
485 transgene. Vector injection into the peritoneal cavity reduced fetal loss rate in a mouse model of
486 miscarriage (CBA/J x DBA/2 cross) by skewing the Th2/Th1 cytokine balance, expanding peripheral

487 CD4(+) CD25(+) regulatory T cell populations and inducing indoleamine 2,3 dioxygenase (IDO) mRNA
488 and Foxp3 expression at the maternal-fetal interface, molecules that are key regulatory factors for
489 feto-maternal tolerance (107). The mechanism of action appears to be related to inhibition of
490 maternal spleen lymphocyte proliferation and regulation of apoptosis in the feto-placental unit
491 (109).

492 **Non-obstetric conditions during pregnancy**

493 Oncology accounts for over two thirds of gene therapy trials, and in the future maternal cancer may
494 become an indication for gene therapy during pregnancy (2). Pregnant women diagnosed with
495 cancer can face a painful choice between ending the pregnancy early to start treatment, through
496 termination or iatrogenic preterm delivery, undergoing treatment known to have adverse fetal
497 effects, or risking their own health by delaying intervention.

498 The field of cancer gene therapy is too wide to examine in detail but it is worth considering one
499 study on the effect of the antioxidant manganese superoxide dismutase (MnSOD) in pregnant mice
500 (110). MnSOD can protect against the side-effects of radiotherapy, but must be present for 24-48
501 hours after radiation exposure to be effective against on-going apoptosis. Administration of the
502 protein, even on multiple occasions, has not been effective at achieving this. Liposome mediated
503 gene transfer of MnSOD to mid-gestation pregnant mice 24 hours before total body irradiation
504 significantly increased pup survival over 220 days compared with pups from untreated irradiated
505 mothers. However, there was still a significant reduction in litter size and pup weight compared with
506 non-irradiated mice. This may be in part because whole body irradiation exposed the fetuses directly
507 to ionising radiation, rather than just the indirect effect of cytokines crossing the placenta. With the
508 use of targeted radiotherapy and shielding, fetal outcome may be further improved.

509 **RISKS AND LIMITATIONS OF MATERNAL AND PLACENTAL GENE THERAPY**

510 Some of the risks associated with gene therapy have already been discussed, and are currently being
511 ameliorated with developments in vector design. The risk of insertional mutagenesis with retroviral
512 vectors may be reduced by switching from retroviruses to lentiviruses, which are less likely to
513 integrate near actively transcribed genes, the development of self-inactivating vectors, and the use
514 of tissue-specific promoters rather than viral promoters (17). The immune reactions seen with
515 adenoviruses are being addressed using helper-dependent adenoviral vectors, which provoke less of
516 a T-cell response, using serotypes to which patients are unlikely to have pre-existing immunity, and
517 coating vectors with polymers like PEG to protect them from neutralising antibodies (111).

518 With maternal gene therapy, as with many obstetric or fetal interventions, an important risk is that
519 the intervention will be ineffective or only partially effective. In conditions such as recurrent
520 miscarriage, severe early onset FGR, and early onset pre-eclampsia this could mean that a pregnancy
521 which may have otherwise ended in miscarriage, stillbirth or termination instead leads to the
522 livebirth of a child with chronic health problems (52). Whether parents perceive this as a risk or a
523 benefit will be an individual matter, but it would need to be discussed as part of any pre-procedure
524 counselling. With the use of adenoviral vectors, which are well suited to the relatively short-term
525 nature of most obstetric conditions, there is also a risk that the generation of antibodies may mean
526 that an intervention could only be given once. This could have implications for future pregnancies
527 and for the woman’s long-term health if she were later to require adenoviral gene therapy for
528 another indication.

529 **Placental transmission**

530 As maternal gene therapy would target the maternal system, and possibly the maternal side of the
531 placenta, placental transmission to the fetus would generally be undesirable. If significant placental
532 transmission were to occur it could potentially lead to fetal gene transfer with the attendant risks of
533 fetal germline transmission and insertional mutagenesis, as well as the adverse effects of the
534 expressed protein on fetal development. This may be particularly important in the case of growth

535 factors (112, 113). Even in the case of placental gene therapy with direct intraplacental
536 administration, excessive spread to the fetus may not be desirable. The findings of pre-clinical
537 studies using maternal and placental administration are summarised in Tables 1 and 2 respectively.

538 The majority of studies in pregnant animals have been performed using adenoviral vectors. Wildtype
539 adenovirus is able to cross the placenta, as demonstrated by the presence of viral DNA in amniotic
540 fluid at amniocentesis (114). Whether this ability is retained by adenoviral vectors may depend upon
541 the animal used, the dose given, and the route of administration. Intravascular administration to
542 pregnant sheep (63, 64), mice (29), and guinea pigs (38) produced transgenic protein expression in
543 the placenta but not in the fetuses. The same pattern was seen when adenoviral vector was applied
544 to the outer surface of the uterine and radial arteries in guinea pigs using a thermolabile Pluronic gel
545 (38). In contrast, after intravascular administration in rabbits (37) and multiple dose intravascular
546 administration in rats (33), transgenic protein expression was detected in both the placenta and fetal
547 organs. Following maternal intraperitoneal administration, microscopy only demonstrated transgene
548 expression in the maternal side of the placenta, and not in fetal tissue (102, 107). Using PCR,
549 however, the transgene and adenovirus were detected in fetal homogenate (102). Direct
550 intraplacental administration of adenoviral vectors has almost always been found to result in
551 transgenic protein expression in the fetus and dam (32-36, 92), with the site of highest fetal
552 expression depending on the gestation of administration (32).

553 In the case of hybrid and non-viral vectors, the evidence is more limited and variable. Following
554 administration of plasmid/PEI or plasmid/liposome complexes into the uterine arteries of pregnant
555 rabbits, gene transfer was detected in the placenta and fetuses (37). Injection of virosomes into the
556 extraamniotic space in pregnant mice, however, produced expression in the decidua, basal lamina of
557 the placenta, and amnion, but not in the fetus (115). In contrast, after intravenous administration of
558 liposomes in mice, the vector was not detected in either placental or fetal tissue (110).

559 Given the variation in placental structure between animals, however, these findings may not be
560 directly applicable to human pregnancy. Studies exposing human placental villous explants to high
561 dose adenoviral vector found occasional transduction of the underlying cytotrophoblast when the
562 overlying syncytiotrophoblast was deficient, but no evidence of the vector crossing the basement
563 membrane (116). Given the importance of placental transmission to the safety of maternal gene
564 therapy more evidence is required, and further studies in the *ex vivo* perfused human placenta are
565 currently underway.

566 **Germline transmission**

567 The risk to the woman of oocyte transduction is probably no higher during pregnancy than at any
568 other time. The risk of fetal germline gene transfer after maternal gene therapy, however, will
569 depend on the degree of placental transmission and the gestation at which gene therapy is given.
570 Germline transmission has been observed after *in utero* fetal gene therapy, particularly in early
571 gestation. Intraperitoneal administration of a retroviral vector to fetal sheep resulted in low-level
572 transduction of sperm cells, with lower levels at later gestations (117). However, unlike humans,
573 where germ cell compartmentalisation is complete by 7 weeks gestation, germ cells in fetal sheep
574 continue to migrate to the testes after birth. In non-human primates, oocyte transduction has been
575 observed after fetal intraperitoneal administration of a lentiviral vector in the late first trimester, but
576 this route of administration has not been studied at later gestations (118). Although, theoretically,
577 maternal gene therapy in the second or third trimester should carry a very low risk of fetal germline
578 modification, this will be an important safety consideration for clinical translation.

579 **Placental toxicity**

580 The future use of gene therapy in pregnancy would not be possible if it resulted in significant
581 placental toxicity. So far, however, such toxicity has not been demonstrated. Administration of an
582 adenoviral vector into the uterine arteries of pregnant rabbits did not produce any evidence of a

583 placental inflammatory response, reflected by the number of placental macrophages and T cells (37).
584 Similarly when human placental villous explants were exposed to $5x10^{10}$ vp/ml of adenoviral vector
585 expressing VEGF or LacZ for 60 minutes there was no increase in human chorionic gonadotrophin or
586 lactate dehydrogenase, both of which can be released by placental cell damage (116). Further work
587 to study the effect of viral vectors on perfused human placental tissue is on-going.

588 **ETHICAL AND REGULATORY ISSUES**

589 Medical research involving pregnant women raises specific ethical and regulatory issues, particularly
590 in relation to early phase trials. While maternal gene therapy has yet to be considered by national
591 ethical and regulatory bodies, parallels may be drawn with regulatory reviews of hypothetical fetal
592 gene therapy proposals.

593 **Clinical research in pregnancy**

594 Clinical research in pregnancy remains limited, despite recognition that the physiological and
595 metabolic changes of pregnancy mean the results of clinical trials in non-pregnant subjects (often
596 predominantly male) may not be transferrable. The exclusion of pregnant women from many clinical
597 trials has been criticised as putting them at an unfair disadvantage by restricting their access to
598 evidence-based medicine (119, 120). Despite the 2002 Council for International Organizations of
599 Medical Sciences (CIOMS) recommendation that pregnant women be presumed eligible to
600 participate in research, progress has been slow. It has been argued that the reluctance to conduct
601 clinical trials during pregnancy may arise from a residing reaction to the thalidomide tragedy,
602 coupled with the categorisation of pregnant women as a vulnerable group (121).

603 The categorisation of all pregnant women as vulnerable to coercion or undue influence, as laid out
604 by the United States Code of Federal Regulations (122), has been challenged over recent years (121,
605 123). However, in clinical trials which involve risks to the mother for potential benefit to the fetus, it
606 could be argued that the internal and external pressures a pregnant woman faces affect her ability

to freely decide about participation (121, 124). While this must be a consideration for clinical researchers, and careful thought should be given to the consent procedures in any obstetric trial, it should not be seen as an insurmountable obstacle to conducting research in pregnancy. There are many occasions in routine obstetric care where an intervention that has a risk to the pregnant woman is performed for the benefit of the fetus, including antenatal corticosteroids for fetal lung maturation, antiarrhythmics for fetal tachyarrhythmias, and Caesarean section for fetal acidaemia. There is also a precedent for carrying out clinical trials of such interventions, including laser ablation for twin-twin transfusion syndrome (125) or prenatal open repair of myelomeningocele (126).

In semi-structured qualitative interviews, 24 women in four European countries who had experienced a pregnancy complicated by severe early onset fetal growth restriction were asked about their attitudes towards a future clinical trial of maternal gene therapy for this condition (127). The majority of women had felt capable of making treatment decisions during their pregnancies, and while they recognised the challenges of deciding whether or not to participate in such a trial, felt that with time and information it would have been their decision to make. Similarly CIOMS recommends that, even where these risks are uncertain, it is up to the pregnant woman to decide whether she considers them acceptable (124).

Early phase clinical trials in pregnancy

The development of entirely novel obstetric therapies, such as gene therapies, requires phase I safety trials, where the risks and benefits are inherently uncertain. Furthermore, because the primary outcome is safety, phase I trials often use a dose-escalation design, where the starting dose may be so low that no beneficial effect is anticipated. The Council of Europe “Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research” states that in research involving competent adults which does not produce a direct benefit, the risks should be acceptable to the participant (128). In pregnancy, however, it requires that such research should have only minimal risks. Similarly, the United States Code of Federal Regulations states that research

632 without direct benefit to the mother of fetus should involve only minimal fetal risks (122). It has
633 been argued that while maternal and fetal risks should be minimised, they should not have to be
634 minimal (129), as this constitutes an unreasonable barrier to obstetric research. Under the current
635 regulations, however, the future of early phase trials for those interventions that can only be carried
636 out in pregnancy may depend on the regulatory interpretation of potential benefit.

637 **Implications from national reviews of *in utero* gene therapy**

638 In February 1998 the UK Gene Therapy Advisory Committee (GTAC) New and Emerging Technologies
639 group presented their report on the potential uses of gene therapy *in utero* (19). This was followed,
640 in March 1999, by the US National Institute for Health (NIH) Recombinant DNA Advisory Committee
641 report on the scientific, medical, and ethical issues relating to prenatal gene therapy (52). At the
642 time both reports concluded that there was insufficient data to support a trial of fetal gene therapy.
643 They recommended that the risk benefit analysis and informed decision-making process would be
644 key elements of any future trials, and highlighted the psychosocial and emotional risks to pregnant
645 women, including the risk of coercion. They also advised that informed consent for participation
646 should include a clear explanation that the intervention would be experimental and that long-term
647 follow-up would be needed. Gene therapy to the mother intended to benefit the fetus was not
648 considered at the time, but any such application would need to weigh up the potential risks to the
649 mother as with any other gene therapy treatment, but also the risk to the fetus if significant
650 placental gene transfer were to occur.

651 **CONCLUSION**

652 An increasing understanding of the molecular basis of disease (allowing identification of potentially
653 therapeutic transgenes) together with on-going developments in vector design (allowing targeted
654 and regulated delivery) mean that gene therapy offers an ever-expanding range of future
655 therapeutic possibilities. In order to harness these advances for the benefit of pregnant women it is

656 vital that investigation continues into the pathophysiology of obstetric diseases, and that this is
657 translated into clinical research. Pregnancy offers unique ethical and practical challenges for the
658 conduct of clinical trials, which would only be intensified in a trial of maternal gene therapy. It is only
659 by addressing these challenges, however, that we can hope to provide evidence-based therapeutics
660 to future generations of pregnant women.

661

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1006

1007

1008 Table 1: Summary of vector spread and transgene expression following maternally administered gene therapy, pfu = plaque-forming units, vp = viral

1009 particles, PCR = polymerase chain reaction, ELISA = enzyme-linked immunosorbent assay.

Author	Route of delivery	Animal	Vector(s)	Dose	Day of administration (term)	Days until harvesting	Methods of detection	Detected in placenta?	Detected in fetus?
Heikkila 2001	Uterine artery	Rabbit	Ad.LacZ	$1x10^{10}$ pfu	14-28 (30)	3	X-gal staining and PCR	Yes	Yes
			Plasmid/PEI	250 μg					
			Plasmid/liposome	500 μg					
David 2008	Uterine artery	Sheep	Ad.VEGF-A_{165} or Ad.LacZ	$5x10^{11}$ particles	88-102 (145)	4-7	ELISA and PCR	Yes	No
Mehta 2012	Uterine artery	Sheep	Ad.VEGF-A_{165} or Ad.LacZ	$5x10^{11}$ particles	80-102 (145)	30-45	ELISA and PCR	No	No
Mehta 2011	Uterine artery	Guinea pig	Ad.LacZ	$1x10^{10}$ vp	45 (65)	2-7	ELISA, X-gal staining and PCR	Yes	No
	Internal iliac artery								
	Pluronic gel to outer surface of uterine and radial								

	arteries								
Xing 2000	Jugular vein	Rat	Ad.GFP-GT3 or Ad.CMV-GT3	2.3x10^{10} pfu	14 (22)	6	Immunofluorescence, PCR and Southern blot	No	Unclear if tested
				3 doses, total 5.4x10^{10} pfu	14-16 (22)	6 days from first dose		Yes	Yes
Katayama 2011	Tail vein	Mouse	Ad-RGD.Luc or WT-Ad.Luc	5x10^{8} pfu	10.5 (20)	2	Immunofluorescence and PCR	Yes	No
Epperly 2011	Intravenous	Mouse	MnSOD plasmid	100 μg	13 (20)	1	Immunohistochemistry and PCR	No	No
Zenclussen 2006	Maternal peritoneal cavity	Mouse	Ad.HO-1 or Ad.GFP	1x10^{5} and 1x10^{8} pfu	5 (20)	9	Fluorescent microscopy	Yes	No
							PCR	Yes	Yes
Li 2009	Maternal peritoneal cavity	Mouse	Ad.CTLA41g or Ad.GFP	1x10^{5} pfu	5 (20)	9	Confocal laser scanning microscopy	Yes	No

1010 Vectors: Ad.CMV-GT3 = adenoviral vector expressing human glucose transporter 3 gene under control of a cytomegalovirus promoter, Ad.GFP = adenoviral vector
1011 expressing green fluorescent protein, Ad.CTLA41g = adenoviral vector expressing green fluorescent protein and cytotoxic T lymphocyte-associated antigen 4, Ad.GFP-GT3 =
1012 adenoviral vector expressing green fluorescent protein and human glucose transporter 3 gene under control of a cytomegalovirus promoter, Ad.HO-1 = adenoviral vector

1013 expressing heme oxygenase 1, Ad.LacZ =adenoviral vector expressing β-galactosidase, Ad-RGD.Luc = modified adenoviral vector expressing firefly luciferase, Ad.VEGF-A_{165}

1014 = adenoviral vector expressing vascular endothelial growth factor A_{165} isoform, MnSOD = manganese superoxide dismutase, WT-Ad.Luc = wildtype adenoviral vector

1015 expressing firefly luciferase.

1016

1017 Table 2: Summary of vector spread and transgene expression following intraplacental administration of gene therapy, pfu = plaque-forming units, cfu =

1018 colony-forming units, PCR = polymerase chain reaction.

Author	Animal	Vector(s)	Dose	Volume of injection	Day of administration (term)	Days until harvesting	Methods of detection	Detected in placenta?	Detected in fetus?	Detected in dam?
Woo 1997	Mouse	Ad.RSVLuc	$5x10^{7}$ pfu per placenta	3 µL per placenta	12.5 (19.5-20.5)	3	Luciferase activity	Yes	Yes	Yes
		Ad.RSVLacZ					X-gal staining	Yes	Yes	Yes
Türkay 1999	Mouse	Ad.CMVntLacZ or Ad.RSVntLacZ	$5x10^{5}$ to $1x10^{9}$ pfu per placenta	500 nl per placenta	9.5 (20)	4	X-gal staining	Not examined	Yes	Yes
		Ad.CMVntLacZ or Ad.RSVntLacZ	$2.5x10^{6}$ to $1x10^{9}$ pfu per placenta	500 nl per placenta	11.5 (20)	2	X-gal staining	Not examined	Yes	Yes
		Ad.CMVntLacZ	$2x10^{8}$ pfu per placenta	1µl	14.5 (20)	4	X-gal staining	Not examined	Yes	Unclear if tested

		LZ.LacZ	9x10^{5} cfu per placenta	500 nl per placenta	9.5 (20)	4	X-gal staining	Not examined	Yes	No
		LZ.LacZ	9x10^{5} cfu per placenta	500 nl per placenta	11.5 (20)	2	X-gal staining	Not examined	Yes	No
Xing 2000	Rat	Ad.GFP-GT3 or Ad.CMV-GT3	0.75 to 1.125x10^{8} pfu per placenta	5-7.5µL per placenta	14 (22)	5-6	PCR	Yes	Yes	Yes
Senoo 2000	Mouse	Ad.CALacZ	3x10^{6} pfu per placenta	Not stated	14 (20)	3	X-gal staining	Not examined	No	Unclear if tested
Katz 2009	Mouse	Ad.LacZ	1x10^{8} pfu per placenta	5 µL per placenta	14 (20)	3	X-gal staining	Yes	Yes (1 of 9)	Not examined
Keswani 2015	Rabbit	Ad.LacZ	1x10^{9} pfu per placenta	40 µl per placenta	21 (30)	2	X-gal staining	Yes	Equivocal	Yes
							PCR	Yes	Yes	Not examined

1019 Vectors: Ad.LacZ =adenoviral vector expressing β-galactosidase, Ad.CMV-GT3 = adenoviral vector expressing human glucose transporter 3 gene under control of a
1020 cytomegalovirus promoter, Ad.GFP-GT3 = adenoviral vector expressing green fluorescent protein and human glucose transporter 3 gene under control of a
1021 cytomegalovirus promoter, Ad.RSVLuc = adenoviral vector expressing firefly luciferase under a respiratory syncytial virus promoter, Ad.RSVLacZ = adenoviral vector

1022 expressing β-galactosidase under a respiratory syncytial virus promoter, Ad.CMVntLacZ = adenoviral vector expressing β-galactosidase under a cytomegalovirus promoter,

1023 Ad.RSVntLacZ = adenoviral vector expressing β-galactosidase under a respiratory syncytial virus promoter, LZ.RSLacZ = lentiviral vector expressing β-galactosidase,

1024 Ad.CALacZ = adenoviral vector expressing β-galactosidase under a synthetic CAG promoter

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