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Fetal therapies – (*Stem cell transplantation; enzyme replacement therapy;* in utero *genetic therapies*)

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ABSTRACT

Advances in ultrasound and prenatal diagnosis are leading an expansion in the options for parents whose fetus is identified with a congenital disease. Obstetric diseases such as pre-eclampsia and fetal growth restriction may also be amenable to intervention to improve maternal and neonatal outcomes. Advanced Medicinal Therapeutic Products such as stem cell, gene, enzyme and protein therapies are most commonly being investigated as the trajectory of treatment for severe genetic diseases moves toward earlier intervention. Theoretical benefits include prevention of in utero damage, smaller treatment doses compared to postnatal intervention, use of fetal circulatory shunts and induction of immune tolerance. New systematic terminology can capture adverse maternal and fetal adverse events to improve safe trial conduct. First-in-human clinical trials are now beginning to generate results with a focus on safety first and efficacy second. If successful, these trials will transform the care of fetuses with severe early-onset congenital disease.

1. Introduction

An ever-expanding knowledge base, along with advances in high resolution ultrasound technology and prenatal diagnostic techniques, means that single gene disorders are increasingly diagnosed prenatally. Additionally, obstetric conditions such as pre-eclampsia and early-onset fetal growth restriction are increasingly predictable. Alongside standard options, this presents the opportunity for in utero treatment with Advanced Medicinal Therapeutic Products such as stem cell, gene, enzyme and protein therapies. Theoretical benefits include the amelioration of in utero damage, requirement of smaller treatment doses compared to postnatal intervention, making use of circulatory shunts permitted by the fetal circulation, possible induction of immune tolerance, and improved outcome. Indeed, for some very specific single gene disorders, carefully timed in utero protein exposure during the critical developmental stage may correct the effects of an inherited condition that is not possible after birth. Recently completed clinical trials have investigated in utero stem cell transplantation for osteogenesis imperfecta and alpha thalassaemia. Two current clinical trials are studying protein and enzyme replacement for genetic disease. However, in utero intervention is not without risks, and maternal and fetal wellbeing must always be prioritised, as well as consideration of the potential for generational effects on both the mother's other potential offspring and her treated fetus. Internationally agreed terminology is now available to capture adverse events for the mother and her fetus which will increase the safety of genomic medicinal trials. This review considers the latest clinical developments in the development of fetal therapies.

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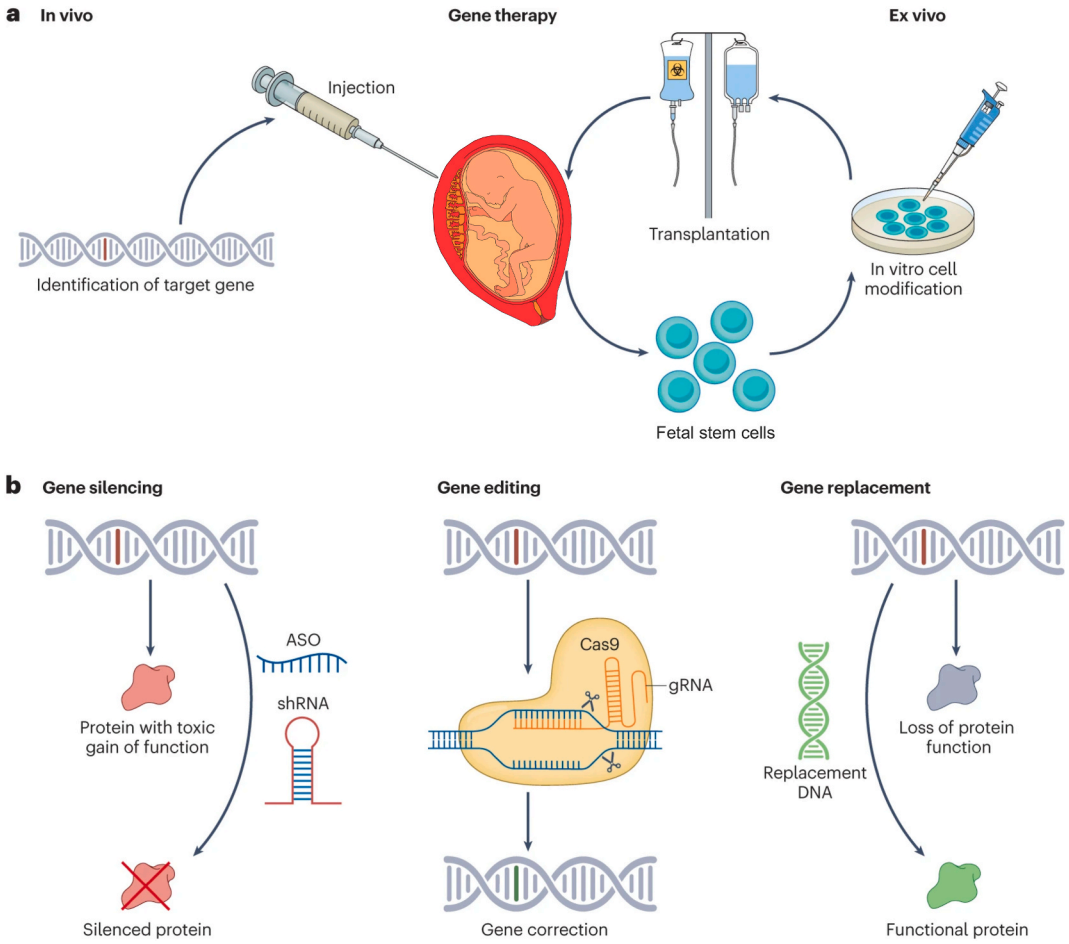

Fig. 1. Types of fetal genetic therapies. a, In vivo methodology (left) involves direct delivery of the gene therapy agent into the fetus. Ex vivo strategies (right) involve isolation of cells from the fetus, in vitro manipulation of the cells to correct the genetic abnormality and, finally, re-infusion of the modified cells back into the donor fetus. b, Genetic therapies can also be classified into silencing, editing or replacement approaches depending on the type of correction required. Gene silencing (left) aims to suppress or reduce the expression of a protein that has acquired a toxic gain of function. A commonly used gene-silencing technology employs antisense oligonucleotides (ASOs), which target mRNAs and alter their translation. Alternatively, short hairpin RNAs (shRNAs) can be used to initiate RNA interference. Gene editing (middle) involves correction of a genetic mutation using engineered nucleases such as CRISPR–Cas9. A guide RNA (gRNA), which is complementary to a specific target sequence that needs to be modified, is combined with the nuclease Cas9, which introduces DNA double-strand breaks that are repaired via non-homologous end joining. Gene replacement therapy (right) is mostly used to correct loss-of-function mutations and consists of delivery of genetic material to replace the defective gene and restore expression of the normal protein. Modified from Ref. [94].

2. Advanced therapy medicinal products as fetal therapies

Advanced therapy medicinal products, or ATMPs, are medicines for human use that are based on genes, tissues or cells. The first ATMPs to be used in utero were stem cells, of which there are broadly two main groups; haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs were the first stem cell type to be discovered and are the most extensively investigated stem cell population, both experimentally and clinically. HSC transplantation is the mainstay of treatment of haematological conditions such as leukaemias and immune disorders, with over 40,000 HSC transplantations performed annually in Europe alone [1,2]. However, there remain significant immunological barriers to postnatal allogeneic HSC transplantation, meaning that myeloablation, along with its associated safety concerns, is required for successful transplantation.

MSCs are multipotent stem cells with the potential to differentiate along the osteogenic, chondrogenic and adipogenic lineages. Their good safety profile and low immunogenic risk means they can be transplanted across major histocompatibility barriers without immunosuppression [3,4]. The first clinical infusion of MSCs was performed over 20 years ago and MSCs have since been used in a diverse range of conditions, from osteoarthritis to Graft versus Host Disease [3,5,6]. Two meta-analyses of randomised controlled trials investigating administration of MSCs to 321 and 2696 subjects respectively, found the only significant association with MSC administration was transient fever, with no increase in thrombotic events, infection, death or malignancy [5,7].

Gene therapy modifies a person's genes to treat or cure disease (Fig. 1). Often this works via gene supplementation, where a vector

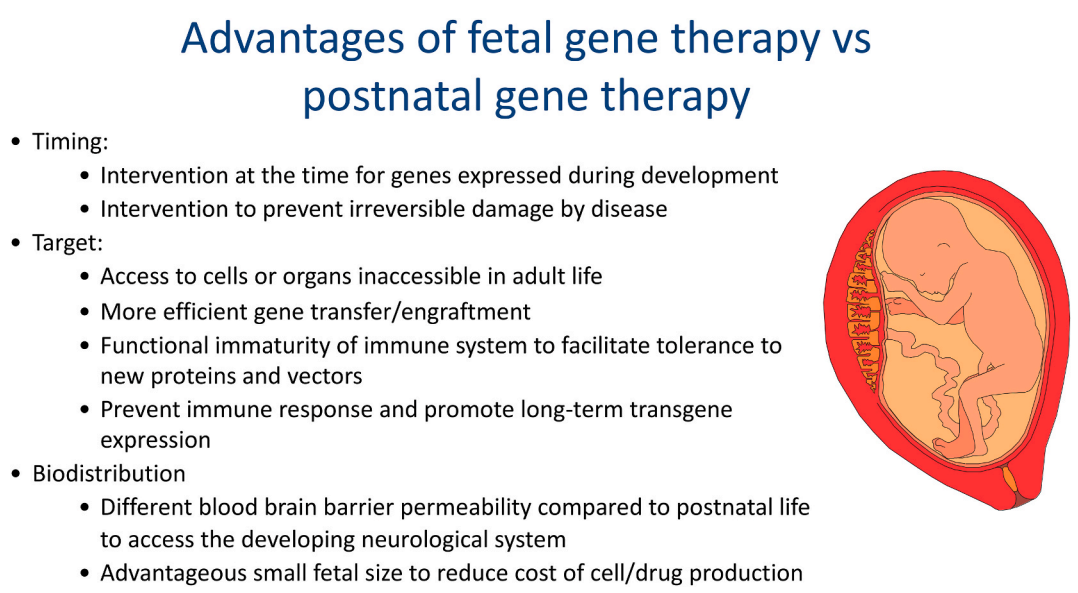

Fig. 2. Advantages of a fetal versus a postnatal gene therapy approach.

delivers additional working copies of a gene to produce a therapeutic transgenic protein. The vector is often coupled with a specific promoter that only activates transgenic protein expression in target cells. Physiological expression to all target cells is uncommon but overexpression in some cell populations is usually sufficient to have a beneficial effect. This is seen in adult gene therapy for haemophilia B where supraphysiological expression of the factor IX protein in a small proportion of hepatocytes creates sufficient circulating protein concentrations for therapeutic benefit [8]. Most often a viral vector is used, the most common being adeno-associated virus (AAV) and lentivirus [9]. The virus is modified to prevent its ability to cause infectious disease, with all viral protein-coding sequences removed so that it cannot replicate. Alternatively, plasmid DNA or non-viral vectors have been used.

Gene silencing can also be used to suppress or reduce expression of a protein that has acquired a toxic gain of function. A commonly used gene-silencing technology employs antisense oligonucleotides (ASOs), which target mRNAs and alter their translation. Gene editing involves correction of a genetic mutation using engineered nucleases such as CRISPR–Cas9 which break the DNA at the site of the mutation, allowing the correct DNA to be inserted. In this way, only the gene of interest should be amended.

In addition to in vivo gene therapy, where genetic material is delivered directly into the patient, ex vivo gene therapy is an alternative option in which a patient's cells are removed, modified with gene therapy or editing and then replaced. This is most commonly performed with a patient's own haematopoietic stem cells, such as in treatment of severe combined immune deficiencies (SCID). In the fetus, this can be performed using amniotic fluid stem cells which have haematopoietic characteristics and stable multilineage haematopoietic engraftment [10].

Finally, the simplest technique may be to provide the missing protein itself, with for example in utero fetal vascular enzyme replacement or intra-amniotic protein therapy.

3. Rationale

The fetus possesses a number of unique characteristics which theoretically make it an ideal recipient for advanced therapies (Fig. 2). The small size of the fetus allows one to maximize the product dose per recipient weight. The average fetal weight at 20 weeks is 300 g, compared to a 3.5 kg newborn or a 70 kg adult (1:12:233 wt ratio). The ability to give a 200 fold lower number of cells or vector particles to achieve the same concentration is important from a practical point of view, as, for example, expansion of stem cells to obtain larger numbers whilst maintaining 'stem-ness' is a major difficulty. Physiological conditions for systemic distribution of therapeutic products are better in the fetus due to circulatory shunts including the foramen ovale and ductus arteriosus, which reduce distribution of product to the pulmonary circulation, avoiding sequestration of stem cells in the lungs [11]. Systemic expression of viral vector-derived transgenic protein has been achieved in preclinical models using intraperitoneal or intravenous umbilical vein injection [12]. If delivery to the airways is required, this is possible via intra-amniotic injection making use of fetal breathing movements, or via tracheal injection using fetoscopic or ultrasound-guided delivery [13]. Direct injection of fetal organs may also be useful for vector targeting for example to the gut via intra-gastric injection or the diaphragm via thoracic injection [14,15].

Another anatomical advantage of prenatal treatment is the immaturity of the blood-brain-barrier, which permits transfer of product across to the brain and may be beneficial in conditions such as lysosomal storage diseases where neurodegeneration is of great significance [16]. Fetal life is a time of stem cell proliferation and migration to different anatomic compartments. Transplantation of stem cells at this time may permit migration alongside affected recipient cells. Higher levels of engraftment during fetal life have also been reported, especially when a fetal-to-fetal transplantation approach is applied [17]. The fetal immune system is relatively naïve,

Table 1
Candidate single gene disorders for fetal genetic therapy.

Disease	Gene (s)	Prenatal diagnosis	Current treatments	Target cells/ organ	Incidence	Benefit to treat in utero
Homozygous α-thalassaemia	*HBA1 and HBA2*	Ultrasound (hydrops) Family history/carrier parents	IUT IUSCT clinical trial in analysis: ClinicalTrials.gov Identifier: NCT02986698	HSCs in liver	1:10,000, higher in SE Asian populations	Prenatal onset/Lethal in utero
Severe osteogenesis imperfecta	*COL1A1, COL1A2*	Prenatal ultrasound/ NIPD Family history/carrier parents	Supportive/ bisphosphonates IUSCT clinical trial in analysis: ClinicalTrials.gov ID NCT03706482	Bone	1:100,000	Prenatal onset/Lethal in utero
Hurler syndrome (MPS1)	*IDUA*	Family history/carrier parents	ERT/HSCT IUERT in clinical trial: Clinicaltrials.gov Identifier: NCT04532047	CNS, liver, heart	1:100,000	Permeable BBB/need to access the developing brain
Pompe disease (infantile onset)	*GAA*	Ultrasound (cardiomyopathy) Family history/carrier parents	ERT IUERT in clinical trial: Clinicaltrials.gov Identifier: NCT04532047	Liver, heart, skeletal muscles	1:40,000	Outcomes are improved with earlier treatment suggesting that in utero treatment is optimal
Neuronopathic Gaucher	*GBA*	Family history/carrier parents	Supportive IUERT in clinical trial: Clinicaltrials.gov Identifier: NCT04532047	CNS, liver, heart	1:1,000,000	Permeable BBB/need to access the developing brain
X-linked hypohydriotic epidermolysis bullosa	*EDA-A1*	Absent dentition on ultrasound/MRI Family history/carrier parents	Supportive In utero protein replacement therapy in clinical trial: ClinicalTrials.gov ID NCT04980638	Sweat glands, teeth	4:100,000 males	Prenatal onset/access to skin and oral cavity via amniotic fluid
Factor VII deficiency	*FVII*	Family history/carrier parents	FVII replacement	CNS	1:500,000	Prenatal onset
Congenital myotonic dystrophy type 1	*DMPK*	Polyhydramnios, reduced fetal movements Family history/carrier parents	Supportive	CNS, heart, muscles	1:50,000	Prenatal onset
Spinal muscular atrophy type 0/1	*SMN1/ SMN2*	Family history/carrier parents	Supportive	Nervous system	1:20,000	Prenatal onset/Lethal in utero
Niemann-Pick C disease	*NPC1// NPC2*	Ultrasound (hydrops) Family history/carrier parents	Supportive	CNS, liver, spleen, lungs	3: 1,000,000	Prenatal onset
Rett syndrome	*MECP2*	Family history/carrier parents	Supportive	CNS	1:10,000 females	Permeable BBB/need to access the developing brain
Angelman syndrome	*UBE3A*	Family history/carrier parents	Supportive	CNS	1:15,000	Permeable BBB/need to access the developing brain
Tay-Sachs disease	*HEXA*	Family history/carrier parents	Supportive	CNS	1:3500 in Ashkenazi population	Permeable BBB/need to access the developing brain
X-linked myotubular myopathy	*MTM1*	Family history/carrier parents	Supportive	Skeletal muscles	1:50,000 males	Prenatal onset
Infantile metachromatic leukodystrophy	*ARSA*	Family history/carrier parents	Supportive	CNS	1:40,000	Permeable BBB/need to access the developing brain
Dystrophic epidermolysis bullosa	*COL7A1*	Polyhydramnios, skin desquamation, Family history/carrier parents	Supportive	keratinocytes	1:120,000	Prenatal onset/access to skin via amniotic fluid

IUT: Intra-uterine transfusion; IUSCT: in utero stem cell transplantation; HSC: haematopoietic stem cells; MPS: mucopolysaccharidosis; ERT: enzyme replacement therapy; HSCT: haematopoietic stem cell transplantation; BBB: blood brain barrier; IUERT: in utero enzyme replacement therapy; NIPD: non-invasive prenatal diagnosis; MRI: magnetic resonance imaging.

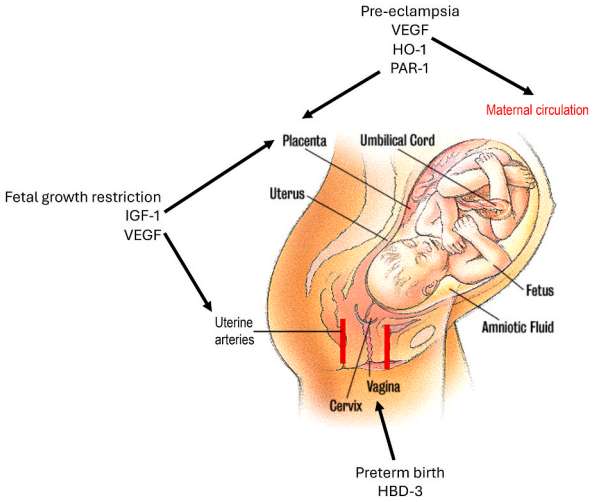

Fig. 3. Summary of gene targets being developed for the prevention and treatment of obstetric conditions. For FGR and pre-eclampsia, the aim is to reduce the fetoplacental pathology in both disorders; either to enhance uteroplacental perfusion, angiogenesis (VEGF) and/or to improve nutrient transport across the placenta (IGF-1). Targets include the systemic circulation, placenta and uterine arteries. For preterm birth, the antimicrobial peptide HBD3 has been delivered to the cervix to enhance cervical immunity and prevent bacteria in the vagina from accessing the uterine cavity. VEGF: vascular endothelial growth factor; IGF-1: insulin-like growth factor 1; HO-1: heme oxygenase 1; PAR-1: Protease-activated receptor-1; HBD3: human beta defensin 3.

meaning there may be no need for myeloablation prior to stem cell transplantation, and also a decreased likelihood of rejection. A significant benefit is the potential for development of immune tolerance towards the therapeutic product, meaning that further postnatal booster doses may be given without immune reaction [18–21]. Similarly, prenatal gene therapy may access proliferating progenitors, rather than quiescent cells in adult patients, providing a significant advantage to increase the efficiency of CRISPR-Cas9 mediated homology directed repair [22]. In diseases with perinatal onset, prenatal treatment may ameliorate in utero damage, or the possibility of treating before the development of irreversible pathology. This may offer significant psychological benefit to carefully counselled parents who do not wish to terminate the pregnancy or to adopt a 'watch and wait' mentality but prefer a proactive in utero approach [23]. Finally, from an economic point of view, giving smaller product doses to a more responsive recipient with a potentially larger beneficial effect would have significant implications.

3.1. Target diseases

Table 1 summarises the potential candidate single gene disorders for fetal genetic therapy, including those conditions for which there are currently clinical trials ongoing. For a disease to be amenable to in utero treatment with an advanced therapeutic product, it must be possible to make a reliable diagnosis prenatally. For some women, a family history of a recessive or X-linked condition may mean they are aware their fetus is at higher risk of a condition, and they will have decided if they wish to proceed with invasive testing to obtain confirmation of the diagnosis. For others, a suspected diagnosis may be made at the mid-trimester anomaly scan and counselling regarding molecular diagnosis, pregnancy options and potential trial participation will need to take place within a much shorter period of time. Currently, most disorders amenable to in utero clinical trials with ATMPs will need to be confirmed molecularly via invasive testing. Whole exome or genome sequencing is increasingly available, giving the option of making a diagnosis of a single gene disorder sufficiently early in gestation for feasible genetic correction of the disorder. The field of free fetal DNA testing for monogenic disorders is also advancing rapidly, with reliable non-invasive prenatal diagnosis available for conditions such as achondroplasia and thanatophoric dysplasia, and likely to be available for more conditions shortly [24].

The natural history of the condition should be known, so that fetuses receiving in utero products can be compared with the untreated population, and it should be considered that the fetus will obtain benefit from prenatal administration. Single gene disorders that are most likely to be suitable for in utero treatment are those with prenatal effects or perinatal lethality such as severe osteogenesis imperfecta or alpha thalassaemia major, and those with critical in utero developmental stages such as XLHED or metabolic storage disorders.

There is also great opportunity in treating severe obstetric pathologies using genetic therapies such as viral vectors, nanoparticles and siRNA (Fig. 3). As these conditions are more prevalent and often as life-threatening as many single gene disorders it is surprising that more progress has not been made in therapeutic development. This is likely due to the inherent problems in defining the underlying pathogenesis of pre-eclampsia, fetal growth restriction and spontaneous preterm labour which commonly have a mixed aetiology of a syndrome rather than a close phenotype/genotype relationship that is necessary for genetic therapies. Research to define the natural history of severe obstetric pathologies to inform tight inclusion and exclusion criteria for clinical trials is required such as that developed for early-onset fetal growth restriction [25,26].

4. History of fetal advanced therapies

Initial research into the possibility of fetal genetic therapies focussed on the transplantation of haematopoietic stem cells. The first report of successful in utero haematopoietic transplantation for a genetic condition was published in the late 1980s detailing a patient whose first child died of Bare Lymphocyte Syndrome, a form of Severe Combined Immunodeficiency. Cordocentesis confirmed that her second child was also affected by the condition, thus at 30 weeks of pregnancy she underwent an in utero infusion of fetal liver haematopoietic cells and thymic cells into the placental umbilical vein cord insertion. There were no adverse effects and the remainder of the pregnancy was uneventful until normal vaginal birth at term. The diagnosis was confirmed at birth. Subsequently engraftment was confirmed, with T cells expressing donor HLA class 1, but the child had no B cell development and had seven further stem cell infusions within the first few months of life [27]. Following this seminal case, a number of in utero transplantations for fetuses with SCID were performed [28–33]. Eight out of nine fetuses who underwent HSC transfusion were born alive and appeared well, without evidence of graft versus host disease. These successful cases opened the doors for in utero stem cell transplantation to be considered a possible treatment for myriad conditions. In the 1990s and 2000s, IUHSCT was attempted for the haemoglobinopathies, but whilst tolerance to donor antigens was found in some of the cases, thus suggesting a postnatal stem cell transplant from the same donor without pre-conditioning may be possible, all surviving children were transfusion dependent [18]. The storage diseases were another potential target group for IUSCT, but all published case reports show no alteration to the expected clinical course, and it was concluded this was not an appropriate treatment for this group of conditions [18].

The possibility of in utero stem cell transplantation with mesenchymal cells then arose. A trial of postnatal MSC administration to a group of infants with severe osteogenesis imperfecta showed that five of six patients demonstrated engraftment of cells and accelerated growth velocity; compared with an average of 20% of the growth rate of age and sex matched unaffected infants pre transplant, growth velocity reached 70% over the 6 months immediately following the infusion [34]. In utero administration of MSCs was then performed in two fetuses with severe OI with some evidence of benefit [35,36]. Further details can be found below. There are no published reports of in utero transplantation of MSC for conditions other than OI.

Similarly, research into the field of gene therapy exploded in the 1990s, with 43 clinical trial protocols for postnatal gene therapy of genetic disease approved by the NIH RAC by the end of the decade [37]. However, the death of a participant in a clinical trial of in vivo gene therapy for ornithine transcarbamylase (OTC) deficiency, along with the development of leukaemia in 5 of 20 infants who received ex vivo gene therapy for X-linked Severe Combined Immune Deficiency meant that the safety of the field as a whole was called into question [38,39]. Following safety improvements, there have since been promising results, for example in patients with haemophilia. Current treatment is replacement therapy with human FVIII (hFVIII) or hFIX, with benefit seen when only 1% of the normal levels of clotting factor are reached. Therapy is expensive but mostly effective, yet a proportion of patients develop antibodies to treatment. Postnatal gene therapy in six haemophilia B patients was found to be safe, and patients showed partial correction following intravenous injection of AAV8 carrying human factor IX to hepatocytes [40]. Prenatal animal studies have shown that permanent phenotypic correction and tolerance can be induced in immune-competent haemophilia B mice by fetal intra-vascular injection of lentiviral or AAV-1 vectors encoding the hFIX protein [41,42]. This has been translated to large animals with successful transduction of sheep and non-human primates; injection of an AAV system in late gestation produced clinically relevant levels of hFIX sustained for more than 6 years without toxicity [43–46]. However, as haemophilia only manifests after birth, and postnatal gene therapy appears effective, it is unlikely that human fetal gene therapy for haemophilia will be justified.

In comparison, congenital FVII deficiency, the most common autosomal bleeding disorder, can lead to life-threatening neonatal central nervous system hemorrhage prior to birth. Increasing FVII expression above 1% substantially reduces the risk and incidence of spontaneous hemorrhage. Neonatal intravenous delivery of an AAV8 vector to a mouse FVII knockout model protected against fatal hemorrhage, significantly improving survival in these animals [47]. The same study also demonstrated significant human FVII (hFVII) expression (20.4% $\pm$ 3.7%) in fetal monkeys after in utero vector delivery. A fetal gene therapy to prevent catastrophic perinatal bleeding in patients with FVII deficiency is therefore a more likely prospect for fetal gene therapy. A summary of the history of fetal gene therapy is available in this review [37].

5. Current clinical trials of fetal advanced therapies

Four clinical trials of in utero stem cell, enzyme and protein administration are currently recruiting or in follow up. In addition to investigating replacement of stem cells or genes, attention is also now being placed on in utero delivery of the faulty proteins or enzymes directly, with ongoing studies detailed below. The most developed product to date is protein replacement therapy for XLHED which is in Phase 2 trials, whilst there are three phase 1 trials investigating in utero transplantation of HSC for alpha thalassaemia or MSC for osteogenesis imperfecta, and in utero enzyme replacement therapy for lysosomal storage diseases.

5.1. Protein therapy for XLHED

Edelife, an open label international multicentre phase 2 clinical trial, is now in progress to investigate the prenatal treatment of X-linked hypohidrotic ectodermal dysplasia (XLHED) (https://pro.edelifeclinicaltrial.com). In the trial, recombinant ectodysplasin A protein, termed Fc-EDA or investigational treatment ER004, is delivered via three intra-amniotic injections to affected male fetuses between 26 and 32 weeks of pregnancy. XLHED is a rare developmental disorder of ectodermal derivatives including hair, sweat glands, and teeth caused by a genetic deficiency of ectodysplasin A1 (EDA1). Life threatening hyperthermia can result due to the absence of sweat glands and perspiration. In affected families, prenatal diagnosis can be made non-invasively based on ultrasound scan

findings of oligodontia and mandibular hypoplasia [48]. Results from a previous clinical trial and compassionate cases are extremely promising [49,50]. Nine male patients with a diagnosis of XLHED were treated with Fc-EDA; three shortly after birth and six prenatally. Prenatal patients received the recombinant protein via intra-amniotic injection at 26 week's gestation, with some participants receiving multiple doses. In patients who received Fc-EDA postnatally, neither sweat glands nor sweating ability were detected at the age of 12–60 months. In contrast, prenatal replacement resulted in appropriate sweat gland development and pilocarpine-inducible sweating in all treated subjects, who also attained more permanent teeth than their untreated affected relatives. Long term follow up of these patients has now reached 6 years (n = 2), with all prenatally treated boys continuing to sweat normally. It appears that provision of the recombinant protein at the appropriate developmental stage is sufficient to allow normal sweat gland development, whilst postnatal administration is ineffective. The phase 2 trial, which aims to determine the effectiveness of the treatment in a further 20 participants, is currently recruiting and results are awaited.

5.2. Enzyme replacement therapy for lysosomal storage diseases

In Utero Enzyme replacement therapy for fetuses with lysosomal storage diseases (LSDs) is being investigated in a Phase 1 Clinical Trial at UCSF (https://fetus.ucsf.edu/in-utero-enzyme-replacement-therapy/#educational_information).

Lysosomal storage diseases (LSDs) are a group of inborn errors of metabolism caused by genetic mutations coding for critical enzymes. Abnormal build-up of toxic materials throughout the body results in severe multi-organ damage, including neurodegeneration, cardiac problems, and impaired growth. While each LSD is a rare disease, collectively LSDs affect 1 in 5000 births. The standard of care is for postnatal enzyme replacement therapy (ERT), however, infants often develop antibodies against the enzyme, the blood brain barrier prevents delivery to the brain, and some infants do not survive into the postnatal period to receive therapy. It is hoped that in utero ERT may permit development of immune tolerance, allow delivery to the brain during fetal development of the blood brain barrier and ameliorate in utero damage. Treated neonates would need to continue receiving ERT or could if available, receive a postnatal stem cell transplant. A preclinical study has demonstrated increased survival, improved immune tolerance and decreased morbidity for mice treated pre-rather than postnatally [51].

A female infant with infantile-onset Pompe disease (IOPD) and two previously affected siblings received six infusions of Alglucosidase alfa (20 mg per kilogram of estimated fetal weight) under ultrasonic guided umbilical vein injection between 24 weeks 5 days and 34 weeks 5 days of gestation. The infant received Rituximab for immune tolerance induction postnatally. Unlike both her siblings, who had left ventricular hypertrophy and abnormal motor development, and who succumbed to the condition in infancy, the recipient had normal cardiac and musculoskeletal findings and was doing well aged one year. Importantly, she had persistently low antibody titres against Alglucosidase alfa, suggesting immune tolerance [52].

A phase 1 clinical trial of in utero enzyme replacement (ERT) for fetuses with LSDs (Mucopolysaccharidosis types 1,2,4a, 6 and 7, IOPD, Gaucher disease types 2 and 3 and Wolman disease) is currently recruiting in the US ("PEARL trial" Clinicaltrials.gov Identifier: NCT04532047). The trial aims to recruit 10 pregnant women carrying fetuses with these disorders to investigate the safety and feasibility of in utero ERT. Diagnosis must be made on chorionic villus sampling (CVS) or amniocentesis prior to inclusion in the trial. ERT will be given every 2–4 weeks between 18 and 35 week's gestation, by umbilical vein injection. After birth, the baby will receive standard care, including ERT, induction of immune tolerance and possibly stem cell transplantation with clinical trial follow up until age 5.

5.3. HSC transplantation for alpha thalassaemia

A trial of in utero blood transfusion and haematopoietic stem cell (HSC) transplantation for fetuses with alpha thalassaemia major has reported the birth of the first two participating infants [53]. (https://fetus.ucsf.edu/intrauterine-therapy-alpha-thalassemia-major/#educational_information).

Alpha thalassaemia major was previously thought to be perinatally lethal, with progressive fetal anemia leading to heart failure and fetal hydrops, and the potential for development of maternal mirror syndrome. Successful neonatal outcomes have been reported with serial in-utero blood transfusions, with survival to birth and subsequent normal neurological outcomes [54]. However, children require myeloablation and stem cell transplantation to enable long term survival without being transfusion dependent. The investigators hypothesized that fetuses would tolerate a maternal HSC transplant without conditioning and would develop low level engraftment with sustained tolerance to maternal alloantigens; and that this would enable them to receive a postnatal booster maternal transplant with minimal immunoablative conditioning, thus achieving a cure without the requirement for full myeloablation. This hypothesis is being tested in a phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT02986698). Here, HSCs retrieved from the maternal bone marrow during pregnancy are used for transplantation; these are delivered to the fetus once during the pregnancy, at a gestation of 18–26 weeks. The HSC transplant is given at the same time as an ultrasound-guided intrauterine blood transfusion for fetal anemia, meaning that no additional needling procedures are required for the purpose of the transplant. In utero blood transfusions are then repeated every 3 weeks until delivery [53]. Two cases have been reported to date. Both were diagnosed with severe hydrops on ultrasound and received an initial blood transfusion prior to the HSC transplant. After transplantation at 23 and 25 weeks respectively, both fetuses received four more IUTs prior to birth. Prenatal hydrops resolved in both and neonatal hospitalizations were 16 and 13 days, respectively. Maternal chimerism was detected in both patients, but levels only persisted throughout the year in the first patient. The parents of the first patient have decided against a further maternal bone marrow transplant. Both patients receive monthly transfusions and have had normal development and Bayley neurologic testing at 1 year. There were no unanticipated safety events in either mother/child pair. These results have led the study team to confirm that a two-step strategy of prenatal blood transfusions with a

maternal HSC transplant, followed by a postnatal booster transplant, may be required for a definite cure. The trial is currently closed for analysis and further results are awaited.

5.4. MSC transplantation for osteogenesis imperfecta

The BOOSTB4 trial aims to investigate the safety and efficacy of pre- and postnatal MSC transplantation for fetuses with osteogenesis imperfecta (https://www.boostb4.eu). ClinicalTrials.gov Identifier: NCT03706482.

Osteogenesis imperfecta (OI) is a rare clinically and genetically heterogenous group of heritable disorders of connective tissue characterised by osteopenia, growth restriction, bone deformities, fractures and chronic pain [55]. Around 90% of people with OI will have one of the four classical types, where type 1 is mild, type 2 perinatally lethal, type 3 the most severe form compatible with life, and type 4 an intermediate severity [56]. The condition is caused by $>$ 1500 different sequence variations in the genes coding for type I collagen (COL1A1 or COL1A2), whilst rarer types of OI are caused by mutations in other associated genes [55–57]. Diagnosis is suspected when there are in utero fractures and deformities, and a femur length more than 2 standard deviations below the mean, and can be confirmed with amniocentesis. There is currently no curative treatment. Bisphosphonates are used to reduce bone resorption and increase bone mass, but have no effect on fracture rates, reduction of pain or improved functional mobility[58–61].

There are two published cases of fetuses with type 3 and 4 OI who received prenatal and postnatal transplantation of MSCs [35,62]. The limited data show that the procedures were safe and clinically promising when comparing the clinical course to other individuals with identical OI sequence variants. The patient with type 3 OI has a COL1A2 variant identical to three other reported cases; one of whom died from OI despite bisphosphonate treatment, and two individuals who are described clinically as having type 2/3 OI [63,64]. She received one dose of MSCs prenatally and four postnatal booster doses. Bone biopsies showed the presence of donor cells at 9 m of age. She has continued to grow along her growth chart at around 5SD below the mean, and had decreased fracture frequency following booster does (from 2/year to 0/year over two years) [35,62]. It is difficult to determine the definitive impact of MSC transplantation in these two heterogeneous cases, but the findings suggest a potential clinical benefit, and there has been no evidence of any early or late adverse reactions in over 21 and 14 years of follow up respectively.

The BOOSTB4 trial was thus set up to investigate pre- and postnatal MSC transplantation for severe OI and received regulatory and ethical approval in Sweden and the UK [65]. The clinical trial has completed recruitment. First trimester human fetal liver-derived MSCs were administered once under ultrasound guidance into the umbilical vein for prenatal participants, with three postnatal intravenous booster doses given every 3 months after birth. Participants are being compared with historical and untreated prospective controls. Infants under 18 months of age and pregnant women whose singleton fetus had severe OI with a confirmed glycine substitution in COL1A1 or COL1A2 were potential candidates. Short term follow up has been completed, and long term follow up until participants are aged 10 years is underway. The primary outcome measures are safety and tolerability of repeated BOOST cell administration. The secondary outcome measures are number of fractures from baseline to primary and long-term follow-up, growth, change in bone mineral density, clinical OI status and biochemical bone turnover.

6. Clinical trials in development for obstetric pathologies

There are no current clinical trials of prenatal gene therapy but two potential genetic therapies are being taken towards clinical trial for severe obstetric conditions. A recent review of the field is here [66].

6.1. RNAi modulation of placental sFlt1 for pre-eclampsia

An effective treatment of preterm pre-eclampsia is urgently required to improve maternal and fetal/neonatal outcomes of this complex and life-threatening disorder. The clinical manifestation of the disease result from excess circulating soluble receptor FLT1 (sFLT1 or sVEGFR1) of placental origin. Apheresis, which filters maternal blood through a dextran sulfate column, can lower sFLT1 and other circulating factors, controlling both blood pressure and proteinuria to extend preeclamptic pregnancies short term [67].

A potential genomic approach uses short interfering RNAs (siRNAs) that target nucleic acids to selectively silence the three sFLT1 mRNA isoforms primarily responsible for placental overexpression of sFLT1. In a baboon pre-eclampsia model, a single dose of siRNAs suppressed sFLT1 overexpression and clinical signs of preeclampsia, with results suggesting that a single injection might suppress sFLT1 levels for several weeks, potentially extending pregnancy duration with likely beneficial effects [68]. Dosing will need to be carefully titrated to modify sFLT1 silencing to the desired level, as excessive silencing could potentially be disadvantageous, reducing blood pressure and thereby reduced uterine blood flow.

A randomised placebo-controlled Phase I trial (ClinicalTrials.gov NCT05881993) in healthy non-pregnant volunteers has completed, and the intervention is now being taken forward into clinical trial in pregnant women with pre-eclampsia and elevated sFlt1 levels.

6.2. Maternal gene therapy for severe early onset growth restriction

The EVERREST consortium is planning a phase I/IIa clinical trial to examine the safety and efficacy of maternal VEGF gene therapy as a treatment for severe early-onset fetal growth restriction. Preclinical studies have shown that maternal VEGF gene therapy, delivered locally via an adenoviral vector into the maternal uterine arteries, increases uterine artery volume blood flow and vasodilates the uterine arteries in pregnant sheep through increased expression of endothelial nitric oxide synthase and perivascular adventitial

angiogenesis [69–71]. In two animal models of FGR, this intervention has also been shown to safely increase fetal growth velocity and neonatal outcome [72–74]. The EVERREST clinical trial would involve vector administration, via interventional radiology, into the maternal uterine arteries in pregnancies affected by severe early-onset FGR between 22 and 26 weeks' gestation. This therapeutic approach was, in 2015, the first to be granted European Medicines Agency orphan status for placental insufficiency leading to fetal growth restriction. The scheme offers incentives and benefits to encourage the development of therapies for rare conditions [75]. Ethical review with multinational stakeholder patient input did not identify any fundamental or insurmountable objections to a trial of maternal gene therapy for severe early-onset FGR [25,76] In order to proceed to a phase I/IIa clinical trial to evaluate the safety and efficacy of maternal VEGF gene delivery, the multicentre EVERREST Prospective Study (NCT02097667) appraised various strategies to identify which FGR pregnancies would be most suitable for a clinical trial [25]. At diagnosis of severe, early-onset FGR, ultrasound measurements, such as abnormal umbilical artery (UmA) Doppler velocimetry, and maternal serum placental growth factor (PlGF) concentration demonstrated robust prediction of fetal or neonatal death and delivery at or prior to 28 + 0 weeks of gestation, allowing the selection of a population with at least a 50% perinatal loss rate and 50% severe neonatal morbidity [26].

7. Risks of the procedure

In utero therapies carry known and theoretical risks, relating to both the products themselves and the way in which they are administered. The International Fetal Transplantation and Immunology Society (IFeTIS; https://www.fetaltherapies.org) facilitated a panel discussion of international experts in 2019 to define the key safety aspects of in utero clinical trials [77].

The risks of an in utero injection procedure itself are likely to be similar to those of amniocentesis and fetal blood transfusion. Ultrasound guided injection into the umbilical vein is technically very similar to fetal blood transfusion, a procedure performed worldwide for decades. Zwiers et al. reported a large series of 937 fetal blood transfusions for fetal anaemia performed in a single national referral centre between 2001 and 2015, with a 1.2% complication rate and 0.6% fetal loss rate per procedure [78]. Recognised complications of fetal blood transfusion include emergency Caesarean section (0.4%), fetal bradycardia (0.3%), preterm prelabour rupture of the membranes (PPROM, 0.1%), infection (0.1%), and preterm birth (0.1%) [78]. Having experienced operators perform the procedure, administering fetal paralysis, and avoiding administration into free umbilical cord loops reduces the complication rate [78]. The risk of fetal bleeding falls almost 40-fold by accessing the intrahepatic umbilical vein rather than the placental cord insertion [79]. Avoiding transplacental needling decreases the risk of fetal-maternal bleeding 4-fold, which is an important consideration to reduce maternal exposure to an Advanced Therapy Medicinal Product (ATMP). In the trial of fetal blood transfusion plus HSC transplantation for alpha major thalassaemia, the HSCs are given at the time of blood transfusion. However, for other trials such as the BOOSTB4 trial, the cell product is given in a much smaller volume than a blood transfusion, into a non-anaemic fetus, and thus the changes in circulatory volume are likely to be less pronounced and the risk of complications may be even lower. Intra-amniotic administration of medication, as is performed in the XLHED trial, is likely to have similar procedural complications to those of ultrasound-guided amniocentesis. A recent meta-analysis found the risk of pregnancy loss following amniocentesis at 15–24 weeks to be around 0.11% [80].

All invasive procedures carry the risk of materno-fetal transfer of infectious disease. Whilst transmission of hepatitis B, hepatitis C, or HIV in mothers on highly active antiretroviral therapy (HAART) via amniocentesis is minimal, there is little data on the risk of vertical transmission for other invasive procedures, and excluding seropositive women from current fetal therapy trials is sensible [81,82]. Maternal exposure to fetal or donor antigens may cause sensitization, placing future pregnancies at risk of haemolytic disease of the newborn or affecting maternal blood transfusion. Rho(D) globulin should be given to the 15% of pregnant women who are Rhesus blood group-negative to reduce sensitization risk to 0.35% [83]. Sensitization to other red cell antigens, especially outside of the context of women undergoing fetal transfusion for Rh incompatibility who are already sensitized to one antigen, is rare. Maternal venous thromboembolism rates are unlikely to be increased by short in utero procedures with minimal recovery times. Care should be taken to avoid follow up protocols which prevent women from having adequate time to mobilise whilst inpatient after the trial procedure, as immobility is far more likely to be a risk factor for VTE than the procedure itself. Performing procedures under strict aseptic technique minimises the risk of infection, which is of importance as the majority of maternal deaths reported following amniocentesis have been due to *Escherichia coli* infection. Finally, ultrasound guidance will minimise the possibility of maternal bowel injury, which is quoted to occur in <0.1% of amniocentesis procedures [84,85].

All of the therapeutic products described above may cause an allergic or immune reaction to the product itself or a component part involved in its manufacture. Additionally, despite extensive microbiological testing and production in accordance with good manufacturing practice, an advanced therapy drug product could theoretically transmit an infectious disease from the donor or itself could be a source of microbiological contamination. Additional theoretical concerns are that an ATMP could disrupt normal fetal development in a way not predicted by preclinical studies, it may have off target effects in other fetal organs or even cross the placenta and have an effect upon the mother, with potential to affect her future offspring via the germline.

Ectopic tissue formation or oncogenesis is a concern often raised with stem cell and gene therapies. A recent meta-analysis of randomised controlled trials of adult and childhood MSC transplantation did not observe acute toxicity, organ system complications, infection, death, or malignancy; the only significant association detected was transient fever [5]. Postnatal HSC transplantation is

associated with increased risk of cancer, but this is related to the pre-transplant ablative conditioning and treatments that the recipient may have had for their condition prior to transplant, including chemotherapy and radiotherapy. None of the published cases of IUSCT with HSC, or the current alpha thalassaemia trial have used myeloablation prior to HSC transplant, thus this risk is minimised. Gene therapy was associated with oncogenesis in early clinical trials of gammaretroviral vectors. This was thought to occur due to their tendency to integrate within the vicinity of transcriptional start sites in the host genome resulting in oncogene activation or tumour-suppressor gene inactivation [86]. Since then, clinical trials of integrating vectors have focussed on lentivirus vectors that have a better oncogenic profile, and non-integrating lentivirus vectors have been developed. In vivo gene therapy also carries the potential risk of germline transfer of genetic modification causing transgenerational effects. Conventional gene therapy is still a blunt tool; since the genetic payload often remains episomal or integrated semi-randomly, native regulation of expression is lost. Therefore, not only can inappropriate expression occur in the wrong tissues, but it may also occur at the wrong time in development, if delivered early. Furthermore, the semi-random integration, which has recently also been demonstrated to occur with AAVs, raises safety concerns.

Postnatal HSC transplantation may result in development of Graft versus Host Disease. This remains a potential complication after in utero transplantation, but the tolerogenic environment of the fetal immune system is believed to increase the threshold for this complication.

8. Monitoring strategies

A great deal of discussion between clinical trial investigators and national regulatory authorities has taken place on the most appropriate monitoring strategies surrounding in utero administration of ATMPs. The timescales outlined below were formed during the development of the BOOSTB4 Clinical Trial and feedback from the UK MHRA and Swedish Läkemedelsverket, and were agreed at the iFeTiS meeting of international fetal therapy experts [77].

As adverse events are most likely to occur in the short-term following fetal injection, an inpatient monitoring period of both mother and fetus of 24–48 h is recommended. This will enable detection of both procedure-related complications and toxic or allergic reactions to the product administered. Fetal monitoring should be similar to that recommended after standard fetal needling procedures. This will include the procedure taking place under ultrasound guidance, which permits not only technical accuracy but also timely action for possible fetal resuscitation or delivery by emergency Caesarean if a suitable gestational age has been reached. Fetal ultrasound assessment should also assess for fetal hemorrhage, effusions, and hydrops, with Doppler evaluation of fetal middle cerebral and umbilical arteries for vascular perfusion and fetal anaemia. Fetal monitoring plans will need to be individualised depending upon the gestational age, underlying condition and parental preference, but are likely to involve cardiotocography (CTG) monitoring to confirm fetal wellbeing if carried out in the third trimester. Maternal observations should also be regularly recorded during this initial monitoring period, with blood sampling likely to be required according to trial protocols.

Regular follow-up with ultrasound examination to assess fetal growth and wellbeing, fetal Doppler blood flow, organ-specific growth and echotexture is indicated for the remainder of the pregnancy. At each visit the mother should be asked about development of any side effects. The timing and mode of delivery will likely depend upon the underlying fetal congenital disorder. Regardless of mode of delivery, umbilical cord blood can be collected at birth for fetal biochemistry, immune reaction to the therapeutic products, cell engraftment, donor-specific immune tolerance, or expression of the target protein.

Long-term follow-up of the baby will depend upon the underlying congenital condition and should be planned carefully. Maternal health should also be monitored postpartum to collect information on any adverse events. Current clinical trials require long-term follow-up of both mother and infant, for example the BOOSTB4 Clinical Trial Protocol specifies a long term follow up period of 10 years. Maternal blood testing will enable exclusion of maternal engraftment or donor-specific antibody formation. In the same way that subsequent pregnancies do not appear to be affected by amniocentesis or cord blood transfusion, it is not expected that future pregnancies will be affected by the administration of in utero advanced therapies. Even in cases of fetal surgery, high live birth rates in subsequent pregnancies are seen [87].

In recent years, grading systems for maternal and fetal, and neonatal, adverse events have been developed and these should be used for adverse event reporting [88,89]. Admission for routine maternal and fetal monitoring in the absence of pathology is common in pregnancy and should not be documented per se as an adverse event. Likewise, there is often an increased likelihood of preterm delivery in pregnancies where the baby is affected by a severe congenital disorder, and this will need to be anticipated during in utero trials.

9. The patient perspective

Patient groups have provided input during protocol development of the clinical trials detailed above. Additionally, investigators involved in the BOOSTB4 trial carried out research into stakeholder views on the acceptability of in utero stem cell treatment for babies with osteogenesis imperfecta [23]. Participants underwent 30–40 min semi structured interviews where they discussed their experiences of living or working with the condition, their views on current treatments and their views on in utero stem cell treatment. 27 patients, parents of patients and patient advocates were interviewed, along with 29 health professionals. Three common themes

emerged during the interviews. Firstly, participants felt there were potential benefits to in utero treatment, with early interventions viewed positively from both a clinical and psychosocial point of view. Common concerns were that research during pregnancy was complicated, with potential risks to both mother and child, that there was uncertainty and potential for false hope and disappointments. Finally, participants discussed decision making, explaining that parents face a difficult decision when it comes to prenatal treatment, especially in families without prior history of the condition. They felt it was difficult to 'do nothing' and so wanted to ensure there would be support for informed decision making and no potential pressure to choose prenatal treatment.

10. Ethics of fetal genetic therapies

A number of ethical issues must be considered in the field of experimental advanced prenatal therapies. Firstly, non-directive counselling must be carefully provided by experienced independent practitioners who ensure there is no therapeutic misconception. It should be made clear that taking part in a trial is experimental rather than necessarily therapeutic, and all potential risks and benefits explained. As products pass through clinical trial phases and become licensed as treatments, with risks and benefits increasingly understood, this will become simpler. Consent for prenatal interventions is also important from an ethical point of view. Whilst only the pregnant mother is required to consent for prenatal procedures, if postnatal 'booster' doses are required, it would seem good practise to confirm the consent of the non-birthing partner for the ongoing participation of their child [76,90,91]. It is theoretically possible that for some fetuses, inclusion within a trial may not be in their best interests. For example, a fetus who would otherwise have died from a perinatally lethal condition may receive the prenatal experimental treatment, and be rescued from the perinatal lethality of the condition, but survive with a very impacted quality of life. This should be accounted for in the design of prenatal trials, with appropriate inclusion and exclusion criteria, but will also form a cornerstone of discussion with potential participants prior to enrolment in the trial. Finally, regulations surrounding termination of pregnancy in different countries should also be considered within clinical trials of ATMPs. Participants travelling from countries without access to legal termination of pregnancy may well view participation in a trial differently from women who have access in their home country. For example, if a woman is found to have a fetus with a very severe form of the condition where trial participation may not be in their best interests, and termination of pregnancy would be an alternative option, the team will need to be very clear on whether termination of pregnancy is possible at the trial site, knowing it is not possible in the woman's home country.

11. Outstanding issues

A number of issues remain outstanding in terms of moving advanced therapies into clinical practice, from both knowledge base and practical perspectives. Current clinical trials, including prospective control arms, are ideally placed to discover and solve these issues. For example, previously, no maternal and fetal adverse events reporting criteria were available in the MedDRA dictionary. The EVERREST International Adverse Event Consensus Group of fetal therapy, obstetric, neonatal, and pharmaceutical industry experts developed the Maternal Fetal Adverse Event Terminology (MFAET) which is now mapped with the Medical Dictionary of Regulatory Activities (MedDRA) [89,92]. Other gaps in our knowledge include what the effect on the fetal heart rate is of intrauterine intravascular administration of medication and how best to categorise fetal heart rate monitoring prior to the third trimester.

A neonatal adverse event severity scale has been developed to help researchers use a common approach when reporting AEs in neonates after trial interventions [88]. Finally a gap in AE terminology for lactation and breast/chestfeeding has been filled by an international group of experts, based on the generic Common Regulatory Criteria for Adverse Events (CTCAE) structure. Three new definitions were developed and mapped to MedDRA (March 2023) to improve assessment of postpartum complications after fetal therapeutic trials [93].

Often with rare diseases, recruitment needs to be multinational in order to reach sufficient patient numbers. This is challenging from a practical perspective for parents, not least with language barriers, funding and the practicalities of travelling with an unwell child. It is also challenging practically for trial organisers who will need to mitigate for differing standards of care and follow up policies in different countries, and to form agreements with local sites to provide specific parts of care required for the clinical trial. Insurance provision for advanced therapy trials in rare diseases, particularly prenatally, can often be difficult, with regulations country dependent. Indeed for trials with multiple sites, obtaining insurance which can cover all sites can be a major stumbling block. Regulatory and ethical approval can also be challenging to obtain for prenatal trials, with requirements varying wildly even across different European countries. Finally the costs of clinical trials for prenatal treatments for rare diseases are often significant and routes to market are not well paved. Obtaining orphan disease designation can be very helpful in obtaining funding provision for trials in rare diseases.

12. Summary

Four clinical trials of in utero therapies are currently in progress. For specified rare single gene diseases these therapies hold promise for in utero correction of disease and improved postnatal outcome. However, the potential benefits must be carefully balanced

against the risks to both mother and child and compared with the option of postnatal treatment. Current clinical trials will demonstrate whether the benefits sufficiently outweigh the risks in order to justify introducing in utero administration as standard treatments and will inform on appropriate monitoring strategies.

MCQs with answers and explanations

1) X-linked hypohidrotic ectodermal dysplasia (XLHED):
   a. Is a rare developmental disorder of ectodermal derivatives including hair, sweat glands, and teeth
   b. Is caused by a genetic deficiency of ectodysplasin A1 (EDA1).
   c. Can only be diagnosed in utero by invasive molecular diagnostic techniques
   d. Is currently being investigated in a phase 2 clinical trial in which serial intra-amniotic injections of Fc-EDA are given to affected fetuses
   e. Is treated equally successfully prenatally and postnatally

   a.T b.T c.F d.T e.F

   c. In families affected by XLHED, diagnosis can be made by ultrasound or magnetic resonance imaging findings of oligodontia and mandibular hypoplasia.

   e. There is a specific developmental window in which replacement of the missing protein results in appropriate sweat gland development, and postnatal administration is not effective.

2) Alpha thalassaemia:
   a. Is an autosomal dominant condition found mostly in people of Chinese and southeast Asian descent
   b. Presents on ultrasound with findings of hydrops fetalis
   c. Is always perinatally lethal
   d. Is currently being investigated in a phase 1 clinical trial of in utero blood transfusion and HSC transplantation
   e. Is amenable to treatment with postnatal bone marrow transplantation

   a.F b.T c.F d.T e.T

   a. Alpha thalassaemia is an autosomal recessive condition caused by deletion of the alpha globin genes. With a single gene deletion, no abnormalities are detected. Two deleted genes result in alpha thalassaemia minor in which patients experience mild anaemia. Three deleted genes, Haemoglobin H disease, result in a neonate which may appear well at birth but will go on to develop haemolytic anaemia. When all four genes are deleted, known as alpha thalassaemia major, the fetus presents with severe hydrops and other associated abnormalities.

   c. Successful neonatal outcomes have been reported with serial in-utero blood transfusions, with survival to birth and subsequent normal neurological outcomes. Definitive postnatal treatment can be achieved using stem cell transplantation.

3) Osteogenesis imperfecta:
   a) Is always caused by mutations in the COL1A1 gene
   b) Presents with in utero fractures, deformities and short long bones
   c) Postnatal bisphosphonate administration increases bone mass, decreases fracture rates and reduces pain
   d) Is being investigated in a phase 1 clinical trial of prenatal and postnatal MSC administration
   e) Is never perinatally lethal

   a.F, b.T, c. F, d.T, e. F

   a. OI is most commonly caused by mutations in the COL1A1 and COL1A2 genes, but rarer types have been found and are caused by other associated genes.

   c. Bisphosphonates have only been shown to increase bone mass, with no improvement in fracture rates, pain or mobility.

   e. Type II OI is perinatally lethal.

4) Stem cell transplantation:
   a. The stem cells are classed as an advanced therapy medicinal product
   b. With haematopoietic stem cells can be administered to immune competent postnatal patients without prior myeloablation
   c. Is under investigation in a clinical trial as a prenatal treatment for alpha thalassaemia
   d. Is under investigation in a clinical trial as a prenatal treatment for osteogenesis imperfecta
   e. Is under investigation in a clinical trial as a prenatal treatment for XLHED

a.T, b.F, c.T,d.T, e.F
b. Myeloablation is required for postnatal HSC transplantation and is associated with significant morbidity and mortality
e. A clinical trial is investigating prenatal intra amniotic injection of recombinant protein for XLHED.

5) Fetal gene therapy/gene editing:
   a. Has already been applied in humans clinically
   b. Does not have a risk of fetal germline gene transfer
   c. Gene therapy or gene editing of autologous fetal stem cells may potentially provide a more targeted therapeutic approach than systemic fetal gene therapy
   d. Is designed to target genetic disorders that manifest as severe and life-threatening before birth.
   e. Has no ethical concerns

a.F, b.F, c.T, d.T, e.F
b. Preclinical studies suggest that the germline may be transduced if gene therapy viral vectors are injected into the peritoneal cavity or umbilical vein. Transduction of both male and female germline has been reported.
c. Correcting autologous fetal stem cells such as those present in the amniotic fluid has been tested as a therapeutical approach pre-clinically. It may avoid the concerns about germline gene transfer/gene editing as the genetic correction of the fetal stem cells would occur in the laboratory before transplantation into the fetus.
f. Ethical concerns about fetal gene therapy/gene editing include ensuring non-directive counselling and that patients have sufficient time to make an informed decision about participation in a clinical trial; concerns about the intervention affecting fetal development, maternal safety and the maternal and fetal germline. Nevertheless, if parents with fetuses affected by severe, life-threatening congenital diseases are asked about whether they would participate in a clinical trial of a fetal gene therapy, many express positive support.

CRediT authorship contribution statement

Rachel Sagar: Writing – original draft. **Anna L. David:** Conceptualization, Writing – review & editing.

Declaration of competing interest

ALD is paid as a consultant by Pierre Fabre Medicamente to be the chair of a Data Safety Monitoring Committee for their "Edelife" first in human clinical trial of intraamniotic protein therapy for a congenital skin disease. https://edelifeclinicaltrial.com/
RS has no conflicts of interest to declare.

Practice Points

- Improvement in ultrasound and genetic prenatal diagnosis technologies are permitting the testing of fetal genetic treatments for severe life-threatening single gene disorders such as osteogenesis imperfecta, metabolic storage disorders and inherited skin conditions in clinical trials.
- Currently there are as yet no clinically approved therapies and pregnant people with an affected fetus should be referred to clinical trial sites to consider trial recruitment.
- New terminology to define and grade safety signals for the mother, fetus, neonatal and lactation are improving the assessment of safety in clinical trials during pregnancy.

Research Agenda

- Improvements in prenatal diagnosis of single gene disorders using non-invasive prenatal diagnosis, whole exome and whole genome sequencing.
- Assessment of the safety, and short and long-term efficacy for both mother and fetus/neonate of in utero protein/enzyme replacement approaches to treat single gene disorders.
- Preclinical development and clinical translation of novel genomic strategies to prevent or to treat severe life-threatening obstetric diseases such as preterm pre-eclampsia and/or severe early-onset fetal growth restriction.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpobgyn.2024.102542.

References

[1] Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, et al. Hematopoietic stem cell transplantation in Europe 2014: more than 40 000 transplants annually. Bone Marrow Transplant 2016 Jun 22;51(6):786–92.
[2] Lafarge X. What compatibility in 2017 for the haematopoietic stem cell transplantation? Transfus Clin Biol 2017 Sep 1;24(3):124–30.
[3] Squillaro T, Peluso G, Galderisi U. Review clinical trials with mesenchymal stem cells: an update. Cell Transplant 2016;25:829–48.
[4] Rodríguez-Fuentes DE, Fernández-Garza LE, Samia-Meza JA, Barrera-Barrera SA, Caplan AI, Barrera-Saldaña HA. Mesenchymal stem cells current clinical applications: a systematic review. Arch Med Res 2021 Jan;52(1):93–101.
[5] Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. Beltrami ap. PLoS One 2012 Oct 25;7(10):e47559.
[6] Kadri N, Amu S, Iacobaeus E, Boberg E, Le Blanc K. Current perspectives on mesenchymal stromal cell therapy for graft versus host disease. Cell Mol Immunol 2023 May 10;20(6):613–25.
[7] Thompson M, Mei SHJ, Wolfe D, Champagne J, Fergusson D, Stewart DJ, et al. Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: an updated systematic review and meta-analysis. EClinicalMedicine 2020 Feb;19:100249.
[8] Nathwani AC, Reiss UM, Tuddenham EGD, Rosales C, Chowdary P, McIntosh J, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. N Engl J Med 2014 Nov 20;371(21):1994–2004.
[9] Keeler AM, ElMallah MK, Flotte TR. Gene therapy 2017: progress and future directions. Clin Transl Sci. 2017 Jul;10(4):242–8.
[10] Loukogeorgakis SP, Shangaris P, Bertin E, Franzin C, Piccoli M, Pozzobon M, et al. In utero transplantation of expanded autologous amniotic fluid stem cells results in long-term hematopoietic engraftment. Stem Cell 2019;37(9):1176–88.
[11] Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. Transplant Proc 2007 Mar;39(2):573–6.
[12] David A, Cook T, Waddington S, Peebles D, Nivsarkar M, Knapton H, et al. Ultrasound-guided percutaneous delivery of adenoviral vectors encoding the β-galactosidase and human factor IX genes to early gestation fetal sheep *in utero*. Hum Gene Ther 2003 Mar 7;14(4):353–64.
[13] Peebles D, Gregory LG, David A, Themis M, Waddington SN, Knapton HJ, et al. Widespread and efficient marker gene expression in the airway epithelia of fetal sheep after minimally invasive tracheal application of recombinant adenovirus in utero. Gene Ther 2004 Jan;11(1):70–8.
[14] David AL, Peebles DM, Gregory L, Waddington SN, Themis M, Weisz B, et al. Clinically applicable procedure for gene delivery to fetal gut by ultrasound-guided gastric injection: toward prenatal prevention of early-onset intestinal diseases. Hum Gene Ther 2006 Jul 13;17(7):767–79.
[15] Weisz B, David AL, Gregory LG, Perocheau D, Ruthe A, Waddington SN, et al. Targeting the respiratory muscles of fetal sheep for prenatal gene therapy for Duchenne muscular dystrophy. Am J Obstet Gynecol 2005 Sep;193(3 Pt 2):1105–9.
[16] Massaro G, Mattar CNZ, Wong AMS, Sirka E, Buckley SMK, Herbert BR, et al. Fetal gene therapy for neurodegenerative disease of infants. Nat Med 2018;24(9):1317–23.
[17] Taylor PA, McElmurry RT, Lees CJ, Harrison DE, Blazar BR. Allogenic fetal liver cells have a distinct competitive engraftment advantage over adult bone marrow cells when infused into fetal as compared with adult severe combined immunodeficient recipients. Blood 2002 Mar 1;99(5):1870 LP–1872.
[18] Tiblad E, Westgren M. Fetal stem-cell transplantation. Best Pract Res Clin Obstet Gynaecol 2008;22(1):189–201.
[19] Mattar CN, Biswas A, Choolani M, Chan JKY. The case for intrauterine stem cell transplantation. Best Pract Res Clin Obstet Gynaecol 2012;26(5):683–95.
[20] Choolani M, Chan J, Fisk NM. Fetal therapy: 2020 and beyond. Prenat Diagn 2010 Jul 22;30(7):699–701.
[21] Roybal JL, Santore MT, Flake AW. Stem cell and genetic therapies for the fetus. Semin Fetal Neonatal Med 2010 Feb;15(1):46–51.
[22] Peranteau WH, Flake AW. The future of in utero gene therapy. Mol Diagn Ther 2020 Apr;24(2):135–42.
[23] Hill M, Lewis C, Riddington M, Crowe B, DeVile C, David AL, et al. Stakeholder views and attitudes towards prenatal and postnatal transplantation of fetal mesenchymal stem cells to treat Osteogenesis Imperfecta. Eur J Hum Genet 2019 Aug;27(8):1244–53 [*].
[24] Chitty LS, Mason S, Barrett AN, McKay F, Lench N, Daley R, et al. Non-invasive prenatal diagnosis of achondroplasia and thanatophoric dysplasia: next-generation sequencing allows for a safer, more accurate, and comprehensive approach. Prenat Diagn 2015 Jul;35(7):656–62.
[25] Spencer R, Ambler G, Brodszki J, Diemert A, Figueras F, Gratacós E, et al. EVERREST prospective study: a 6-year prospective study to define the clinical and biological characteristics of pregnancies affected by severe early onset fetal growth restriction 2017 Dec 23;17(1):43.
[26] Spencer R, Maksym K, Hecher K, Maršál K, Figueras F, Ambler G, Whitwell H, Nené NR, Sebire NJ, Hansson SR, Diemert A, Brodszki J, Gratacós E, Ginsberg Y, Weissbach T, Peebles DM, Zachary I, Marlow N, Huertas-Ceballos A, David AL. Maternal PlGF and umbilical Dopplers predict pregnancy outcomes at diagnosis of early-onset fetal growth restriction. J Clin Invest 2023 Sep 15;133(18).
[27] Touraine JL, Raudrant D, Royo C, Rebaud A, Roncarolo MG, Souillet G, et al. IN-UTERO transplantation of stem cells in bare lymphocyte syndrome. Lancet 1989;333:1382.
[28] Touraine J-LL, Raudrant D, Golfier F, Rebaud A, Sembeil R, Roncarolo M-GG, et al. Reappraisal of in utero stem cell transplantation based on long-term results. Fetal Diagn Ther 2004;19(4):305–12.
[29] Flake AW, Roncarolo M-G, Puck JM, Almeida-Porada G, Evans MI, Johnson MP, et al. Treatment of X-linked severe combined immunodeficiency BY IN utero transplantation of paternal bone marrow. N Engl J Med 1996 Dec 12;335(24):1806 [*].
[30] Pirovano S, Notarangelo LD, Malacarne F, Mazzolari E, Porta F, Lanfranchi A, et al. Reconstitution of T-cell compartment after in utero stem cell transplantation: analysis of T-cell repertoire and thymic output. Haematologica 2004 Apr 1;89(4):450–61.
[31] Diukman R, Golbus MS. In utero stem cell therapy. Journal of reproductive medicine for the obstetrician and gynecologist jun. 1992. p. 515–20.
[32] Westgren M, Ringdén O, Bartmann P, Bui T-H, Lindton B, Mattsson J, et al. Prenatal T-cell reconstitution after in utero transplantation with fetal liver cells in a patient with X-linked severe combined immunodeficiency. Am J Obstet Gynecol 2002 Aug 1;187(2):475–82.
[33] Wengler GS, Lanfranchi A, Frusca T, Verardi R, Neva A, Brugnoni D, et al. In-utero transplantation of parental CD34 haematopoietic progenitor cells in a patient with X-linked severe combined immunodeficiency (SCIDX1). Lancet 1996 Nov 30;348(9040):1484–7.
[34] Horwitz EM, Gordon PL, Koo WKK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci USA 2002;99(13):8932–7.
[35] Le Blanc K, Gotherstrom C, Ringdén O, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. Transplantation 2005;79(11):1607–14. *.
[36] Götherström C, Westgren M, Shaw SWS, Åström E, Biswas A, Byers PH, et al. Pre- and postnatal transplantation of fetal mesenchymal stem cells in osteogenesis imperfecta: a two-center experience. Stem Cells Transl Med 2014 Feb;3(2):255–64 [*].
[37] Waddington SN, Peranteau WH, Rahim AA, Boyle AK, Kurian MA, Gissen P, et al. Fetal gene therapy. J Inherit Metab Dis 2024 Jan 7;47(1):192–210. *.

[38] Raper SE, Yudkoff M, Chirmule N, Gao G-P, Nunes F, Haskal ZJ, et al. A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. Hum Gene Ther 2002 Jan 1;13(1):163–75.
[39] Williams DA, Thrasher AJ. Concise review: lessons learned from clinical trials of gene therapy in monogenic immunodeficiency diseases. Stem Cells Transl Med 2014 May;3(5):636–42.
[40] Nathwani AC, Tuddenham EGD, Rangarajan S, Rosales C, McIntosh J, Linch DC, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med 2011 Dec 22;365(25):2357–65.
[41] Waddington SN, Nivsarkar MS, Mistry AR, Buckley SMK, Kemball-Cook G, Mosley KL, et al. Permanent phenotypic correction of hemophilia B in immunocompetent mice by prenatal gene therapy. Blood 2004 Nov 1;104(9):2714–21. *.
[42] Sabatino DE, Mackenzie TC, Peranteau W, Edmonson S, Campagnoli C, Liu Y-L, et al. Persistent expression of hF.IX after tolerance induction by in utero or neonatal administration of AAV-1-F.IX in hemophilia B mice. Mol Ther 2007 Sep 1;15(9):1677–85.
[43] David A, Cook T, Waddington S, Peebles D, Nivsarkar M, Knapton H, et al. Ultrasound-guided percutaneous delivery of adenoviral vectors encoding the beta-galactosidase and human factor IX genes to early gestation fetal sheep in utero. Hum Gene Ther 2003 Mar 1;14(4):353–64.
[44] David AL, McIntosh J, Peebles DM, Cook T, Waddington S, Weisz B, et al. Recombinant adeno-associated virus-mediated in utero gene transfer gives therapeutic transgene expression in the sheep. Hum Gene Ther 2011 Apr;22(4):419–26.
[45] Mattar CNZ, Nathwani AC, Waddington SN, Dighe N, Kaeppel C, Nowrouzi A, et al. Stable human FIX expression after 0.9G intrauterine gene transfer of self-complementary adeno-associated viral vector 5 and 8 in macaques. Mol Ther 2011 Nov 1;19(11):1950–60 [*].
[46] Mattar CNZ, Gil-Farina I, Rosales C, Johana N, Tan YYW, McIntosh J, et al. In utero transfer of adeno-associated viral vectors produces long-term factor IX levels in a cynomolgus macaque model. Mol Ther 2017 Aug 2;25(8):1843–53.
[47] Binny C, Mcintosh J, Peruta M Della, Kymalainen H, Tuddenham EGD, Buckley SMK, et al. AAV-mediated gene transfer in the perinatal period results in expression of FVII at levels that protect against fatal spontaneous hemorrhage. 2012.
[48] Wünsche S, Jüngert J, Faschingbauer F, Mommsen H, Goecke T, Schwanitz K, et al. Noninvasive prenatal diagnosis of hypohidrotic ectodermal dysplasia by tooth germ sonography. Ultraschall der Med 2015 Aug;36(4):381–5.
[49] Schneider H, Faschingbauer F, Schuepbach-Mallepell S, Körber I, Wohlfart S, Dick A, et al. Prenatal correction of X-linked hypohidrotic ectodermal dysplasia. N Engl J Med 2018 Apr 26;378(17):1604–10 [*].
[50] Schneider H, Schweikl C, Faschingbauer F, Hadj-Rabia S, Schneider P. A causal treatment for X-linked hypohidrotic ectodermal dysplasia: long-term results of short-term perinatal ectodysplasin A1 replacement. Int J Mol Sci 2023 Apr 12;24(8):7155.
[51] Nguyen Q-H, Witt RG, Wang B, Eikani C, Shea J, Smith LK, et al. Tolerance induction and microglial engraftment after fetal therapy without conditioning in mice with mucopolysaccharidosis type VII. Sci Transl Med 2020 Feb 26;12(532).
[52] Cohen JL, Chakraborty P, Fung-Kee-Fung K, Schwab ME, Bali D, Young SP, et al. In utero enzyme-replacement therapy for infantile-onset pompe's disease. N Engl J Med 2022 Dec 8;387(23):2150–8.
[53] MacKenzie TC, Frascoli M, Sper R, Lianoglou BR, Gonzalez Velez J, Dvorak CC, et al. Utero stem cell transplantation in patients with alpha thalassemia major: interim results of a phase 1 clinical trial. Blood 2020 Nov 5;136(Supplement 1):1. *.
[54] Kreger EM, Singer ST, Witt RG, Sweeters N, Lianoglou B, Lal A, et al. Favorable outcomes after in utero transfusion in fetuses with alpha thalassemia major: a case series and review of the literature. Prenat Diagn 2016 Dec;36(13):1242–9.
[55] Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet 2014 Jun;164(6):1470–81.
[56] Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet 1979 Apr;16(2):101–16.
[57] Van Dijk FS, Pals G, Van Rijn RR, Nikkels PGJ, Cobben JM. Classification of osteogenesis imperfecta revisited. Eur J Med Genet 2010;53(1):1–5.
[58] Thomas IH, DiMeglio LA. Advances in the classification and treatment of osteogenesis imperfecta. Curr Osteoporos Rep 2016 Feb 9;14(1):1–9.
[59] Glorieux FH, Bishop NJ, Plotkin H, Chabot G, Lanoue G, Travers R. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. N Engl J Med 1998 Oct 15;339(14):947–52.
[60] Dwan K, Phillipi CA, Steiner RD, Basel D. Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev 2014 Oct 19;7(10).
[61] Hald JD, Evangelou E, Langdahl BL, Ralston SH. Bisphosphonates for the prevention of fractures in osteogenesis imperfecta: meta-analysis of placebo-controlled trials. J Bone Miner Res 2015 May 1;30(5):929–33.
[62] Gotherstrom C, Westgren M, Shaw SWS, Astrom E, Biswas A, Byers PH, et al. Pre- and postnatal transplantation of fetal mesenchymal stem cells in osteogenesis imperfecta: a two-center experience. Stem Cells Transl Med 2014;3(2):255–64 [*].
[63] Pollitt R, McMahon R, Nunn J, Bamford R, Afifi A, Bishop N, et al. Mutation analysis of COL1A1 and COL1A2 in patients diagnosed with osteogenesis imperfecta type I-IV. Hum Mutat 2006;27(7):716.
[64] Kidszun A, Linebarger J, Walter JK, Paul NW, Fruth A, Mildenberger E, et al. What if the prenatal diagnosis of a lethal anomaly turns out to Be wrong? Pediatrics 2016 May 1;137(5):e20154514.
[65] Sagar RL, Åström E, Chitty LS, Crowe B, David AL, Devile C, et al. An exploratory open-label multicentre phase I/II trial evaluating the safety and efficacy of postnatal or prenatal and postnatal administration of allogeneic expanded fetal mesenchymal stem cells for the treatment of severe osteogenesis imperfecta in infants and fetuses: the BOOSTB4 trial protocol. BMJ Open 2024;14:79767.
[66] D'Alessandro SC, Boyle AK. Maternal gene delivery for the prevention and treatment of obstetric conditions. Curr Stem Cell Reports 2024 Jun 6:1–8. *.
[67] Thadhani R, Kisner T, Hagmann H, Bossung V, Noack S, Schaarschmidt W, et al. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. Circulation 2011 Aug 23;124(8):940–50.
[68] Turanov AA, Lo A, Hassler MR, Makris A, Ashar-Patel A, Alterman JF, et al. RNAi modulation of placental sFLT1 for the treatment of preeclampsia. Nat Biotechnol 2018 Nov 19.
[69] David AL, Torondel B, Zachary I, Wigley V, Nader KA, Mehta V, et al. Local delivery of VEGF adenovirus to the uterine artery increases vasorelaxation and uterine blood flow in the pregnant sheep. Gene Ther 2008 Oct 19;15(19):1344–50.
[70] Mehta V, Abi-Nader KN, Shangaris P, Shaw SWS, Filippi E, Benjamin E, et al. Local over-expression of VEGF-dδnδc in the uterine arteries of pregnant sheep results in long-term changes in uterine artery contractility and angiogenesisUshio-Fukai M, editor. PLoS One 2014 Jun 30;9(6):e100021.
[71] Mehta V, Abi-Nader KN, Peebles DM, Benjamin E, Wigley V, Torondel B, et al. Long-term increase in uterine blood flow is achieved by local overexpression of VEGF-A165 in the uterine arteries of pregnant sheep. Gene Ther 2012 Sep 20;19(9):925–35.
[72] Carr DJ, Wallace JM, Aitken RP, Milne JS, Mehta V, Martin JF, et al. Uteroplacental adenovirus vascular endothelial growth factor gene therapy increases fetal growth velocity in growth-restricted sheep pregnancies. Hum Gene Ther 2014 Apr 4;25(4):375–84.
[73] Swanson AM, Rossi CA, Ofir K, Mehta V, Boyd M, Barker H, et al. Maternal therapy with Ad.VEGF-a165 increases fetal weight at term in a Guinea-pig model of fetal growth restriction. Hum Gene Ther 2016 Dec;27(12):997–1007.
[74] Vaughan XO, Rossi CA, Ginsberg Y, White A, Hristova M, Sebire NJ, et al. Perinatal and long-term effects of maternal uterine artery adenoviral VEGF-A165 gene therapy in the growth-restricted Guinea pig fetus. Am J Physiol Regul Integr Comp Physiol 2018;315:344–53.
[75] Spencer R, Rossi C, Lees M, Peebles D, Brocklehurst P, Martin J, et al. Achieving orphan designation for placental insufficiency: annual incidence estimations in Europe. BJOG An Int J Obstet Gynaecol 2019 Aug 6;126(9):1157–67.
[76] Sheppard M, Spencer RN, Ashcroft R, David AL. Ethics and social acceptability of a proposed clinical trial using maternal gene therapy to treat severe early-onset fetal growth restriction. Ultrasound Obstet Gynecol 2016 Apr 1;47(4):484–91. *.
[77] Sagar R, Almeida-Porada G, Blakemore K, Chan JKY, Choolani M, Götherström C, et al. Fetal and maternal safety considerations for in utero therapy clinical trials: iFeTiS consensus statement. Mol Ther 2020;28(11) [*].
[78] Zwiers C, Lindenburg ITM, Klumper FJ, de Haas M, Oepkes D, Van Kamp IL. Complications of intrauterine intravascular blood transfusion: lessons learned after 1678 procedures. Ultrasound Obstet Gynecol 2017 Aug 1;50(2):180–6.
[79] Aina-Mumuney AJ, Holcroft CJ, Blakemore KJ, Bienstock JL, Hueppchen NA, Milio LA, et al. Intrahepatic vein for fetal blood sampling: one center's experience. Am J Obstet Gynecol 2008 Apr;198(4):387.e1–6.

[80] Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015 Jan 2;45(1):16–26.
[81] Somigliana E, Bucceri AM, Tibaldi C, Alberico S, Ravizza M, Savasi V, et al. Early invasive diagnostic techniques in pregnant women who are infected with the HIV: a multicenter case series. Am J Obstet Gynecol 2005 Aug 1;193(2):437–42.
[82] Gagnon A, Davies G, Wilson RD, Genetics Committee RD, Audibert F, Brock J-A, et al. Prenatal invasive procedures in women with hepatitis B, hepatitis C, and/or human immunodeficiency virus infections. J Obstet Gynaecol Can 2014 Jul 1;36(7):648–53.
[83] Pilgrim H, Lloyd-Jones M, Rees A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technol Assess 2009 Feb;13(10):1–103.
[84] RCOG. Green Top Guideline. Amniocentesis and chorionic villus sampling. 2010.
[85] RCOG. RCOG consent advice 6: amniocentesis. 2006.
[86] Coutelle C, Ashcroft R. Risks, benefits and ethical, legal, and societal considerations for translation of prenatal gene therapy to human application. In: Prenatal gene therapy. Totowa, NJ: Humana Press; 2012. p. 371–87.
[87] Goodnight WH, Bahtiyar O, Bennett KA, Emery SP, Lillegard JB, Fisher A, et al. Subsequent pregnancy outcomes after open maternal-fetal surgery for myelomeningocele. Am J Obstet Gynecol 2019 May;220(5):494.e1–7.
[88] Salaets T, Turner MA, Short M, Ward RM, Hokuto I, Ariagno RL, et al. Development of a neonatal adverse event severity scale through a Delphi consensus approach on behalf of the International Neonatal Consortium. Arch Dis Child 2019;0:1–7.
[89] Spencer RN, Hecher K, Norman G, Marsal K, Deprest J, Flake A, et al. Development of standard definitions and grading for maternal and fetal adverse event terminology. Prenat Diagn 2022 Jan 12;42(1):15–26 [*].
[90] Hill M, Lewis C, Riddington M, Crowe B, DeVile C, David AL, et al. Stakeholder views and attitudes towards prenatal and postnatal transplantation of fetal mesenchymal stem cells to treat Osteogenesis Imperfecta. Eur J Hum Genet 2019;27(8):1244–53 [*].
[91] Johansson M, Hermerén G, Sahlin N-E. Paternal consent in prenatal research: ethical aspects. Med Heal Care Philos 2019 Aug;10.
[92] David AL, Spencer RN. Clinical assessment of fetal well-being and fetal safety indicators. J Clin Pharmacol 2022 Sep;62(Suppl 1):S67–78. *.
[93] Maksym K, Morze K, Kalita–Kurzynska K, Boss M, David A, Spatz D, et al. 8 ABM/EABM European regional conference may 11–13, 2023 split, Croatia. Breastfeed Med 2023 May 1;18(5):A1.
[94] Giovannelli I, Higginbottom A, Kirby J, Azzouz M, Shaw PJ. Prospects for gene replacement therapies in amyotrophic lateral sclerosis. Nat Rev Neurol 2023 Jan 8;19(1):39–52.