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REVIEW

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Fetal gene therapy

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Abstract

Fetal gene therapy was first proposed toward the end of the 1990s when the field of gene therapy was, to quote the Gartner hype cycle, at its "peak of inflated expectations." Gene therapy was still an immature field but over the ensuing decade, it matured and is now a clinical and market reality. The trajectory of treatment for several genetic diseases is toward earlier intervention. The ability, capacity, and the will to diagnose genetic disease early—in utero—improves day by day. A confluence of clinical trials now signposts a trajectory toward fetal gene therapy. In this review, we recount the history of fetal gene therapy in the context of the broader field, discuss advances in fetal surgery and diagnosis, and explore the full ambit of preclinical gene therapy for inherited metabolic disease.

KEYWORDS
gene therapy, inherited metabolic disease

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1 | HISTORY OF GENE THERAPY AND FETAL GENE THERAPY

The concept of fetal gene therapy is as old as the field of gene therapy itself. In this review, we will provide an overarching historical perspective addressing the prospects of fetal gene therapy, specifically for inherited metabolic disease.

Advancements in recombinant DNA engineering in the 1970s provided the essential tools underpinning the advent of genetic engineering. Mammalian genetic engineering, as a purely scientific endeavor, drove the development of adenovirus, retrovirus, lentivirus, and adeno-associated virus (AAV) gene therapy vectors.* These enabled the delivery of genetic material, first into cells and then into mouse embryos (retrovirus[1]), rabbits (adenovirus[2]), and mouse lung (AAV[3]). These technologies underpinned the first experiments on fetal gene delivery in mice for the purpose of gene marking and cell lineage tracking.[1,4] In 1986, therapeutic human fetal gene therapy was proposed.[5] A year later, a vanguard study demonstrated the feasibility and attractiveness of fetal gene transfer as a therapy: Adult macaques and fetal sheep received gene-marked adult allogeneic stem cells transduced with retroviral vectors. The macaques engrafted poorly whereas the fetal sheep engrafted well. The authors concluded, "*in utero* transplantation/gene transfer may provide a viable adjunct to postnatal gene transfer or may even provide an alternative when the diagnosis of a severe genetic disease is made *in utero*."[6] In 1987, this preclinical data was presented to the United States National Institutes of Health Recombinant DNA Advisory Committee (NIH RAC) in an application to perform a human clinical trial for fetal gene therapy.[7,8]

Separately, on September 14, 1990 commenced a clinical trial to treat two children with adenosine deaminase severe combined immune deficiency (ADA-SCID) with retrovirus-mediated ex vivo gene therapy. By 1995, reference 9 and a second ADA-SCID trial[10] had provided evidence of the potential therapeutic benefit of ex vivo human gene therapy. By the end of the decade, the NIH RAC had approved 43 clinical trial protocols for postnatal gene therapy of inherited genetic disease.[11] Hopes for human gene therapy were high.

In July 1998, two "pre-protocols" for in utero gene therapy were submitted to the NIH RAC which convened a committee in September for their consideration. In early January 1999, the committee sponsored a Gene Therapy Policy Conference to consider the scientific, safety, legal, ethical, and societal implications of fetal gene transfer.[7,8] The report recognized that prenatal gene therapy presented great potential to treat disease. However, it emphasized that, before any clinical trials could be performed, a more substantial body of preclinical evidence was required, and technological advancements in gene therapy were necessary. In November 1997, the UK Gene Therapy Advisory Committee (GTAC) New Emerging Technologies subgroup was tasked with evaluating the potential of gene therapy in utero.[12]

In parallel with excitement being generated about in utero gene therapy, progress on in utero stem cell transplantation (IUSCT) garnered attention and supported the premise of prenatal cell and gene therapies for genetic diseases. The first in utero marrow transplantation was performed in 1967 for congenital hemolytic disease associated with Rh blood group alloimmunization.[13] From the late 1980s–1990s, increasing numbers of IUSCT procedures were being performed for a variety of non-immunological congenital hematological disorders such as beta-thalassemia major, bare lymphocyte syndrome and chronic granulomatous disease with either no or

*In this review, we use the term "gene therapy" as an umbrella term for all genetic therapy. Namely, viral and nonviral. For the latter, we include oligonucleotide therapies such as siRNA, antisense. Notably, they are categorized differently, by regulatory agencies, as ATMPs versus biologics.[175]

only very partial and transitory benefit (summarized in reference 14). By the late 1990s, there had been 26 attempts at in utero hematopoietic stem cell transplantation,[15] but only two patients, with X-linked severe combined immune deficiency, demonstrated therapeutic benefit of split chimerism leading to immune reconstitution.[16,17] The pre-protocols for fetal gene therapy submitted to the NIH stimulated a robust debate. In July 1999, Nature Medicine published arguments for[18] and against[19] fetal gene therapy. Unfortunately, these events coincided with the start of the most turbulent period in the history of gene therapy.

In 1997, a clinical trial of in vivo gene therapy in adults had commenced, to treat ornithine transcarbamylase deficiency using an adenovirus vector.[20] On September 13, 1999, 98 h after receiving gene transfer, the eighteenth patient, Jesse Gelsinger, died. Ultimately, it was disclosed that each of the 17 preceding patients suffered fevers, myalgias, nausea or vomiting 48 h after vector injection.[20] None showed evidence of therapeutic efficacy. An NIH RAC meeting was convened on December 8, 1999 to consider the trial and its many failings.[21] Across the Atlantic, clinical trials of ex vivo gene therapy for X-linked Severe Combined Immune Deficiency (X-SCID) had started at the Necker Institute in Paris in March 1999[22] and at Great Ormond Street Hospital for Children in London in July 2001.[23] Both trials yielded encouraging results. The first two Paris patients experienced full reconstitution of their immune system.[24] However, in October 2002, the journal *Nature* reported that one of the infants in the Paris trial had developed leukemia.[25] Ultimately, five of the twenty infants would develop leukemia (although four were treated successfully).[26] In August 2001, a trial was initiated to treat patients with hemophilia B by injection of AAV2 into the femoral artery.[27] In the high-dose group, factor IX expression of 11% of physiologic concentrations was achieved—sufficient to convert severe into mild hemophilia. However, immune response eliminated expression after 5 weeks.[28,29]

For the field of gene therapy, the ensuing 5 years was a slow walk back toward the light, metaphorically and literally. Inherited night blindness, Leber's Congenital Amaurosis, was treated by subretinal injection of AAV2 vector in young adults.[30,31] ADA-SCID was treated by ex vivo hematopoietic stem cell therapy[32] (this time without evidence of leukemia[26]). In 2010,[33,34] the first reports of gene therapy using chimeric antigen receptor T cells to treat lymphoma and chronic lymphocytic leukemia, respectively, were published. A milestone in the rehabilitation of the field was the partial correction of six hemophilia B patients following intravenous injection of AAV8 carrying human factor IX to hepatocytes.[35] Soon thereafter were encouraging trial results in metachromatic leukodystrophy,[36] Wiskott-Aldrich syndrome,[37] and aromatic acid decarboxylase deficiency.[38] All of these improvements were in postnatal recipients but were important to establish the potential safety of gene therapy and consideration of in utero gene therapy.

2 | VECTORS

No single vector is a panacea: each vector class has attributes which have been exploited for specific uses.

Vectors derived from adenovirus, reovirus, and herpes simplex virus have been used increasingly for oncolytic virotherapy[39] and as vaccines.[40,41] However, their pro-inflammatory characteristics make them unsuitable for durable treatment of inherited disease. In contrast, lentivirus and AAV vectors have become a mainstay of treatment of inherited genetic disease. Lentivirus vectors are usually configured to integrate into the host genome and are therefore suitable for genetic modification of stem cells or rapidly dividing cells where dilution of vector genomes would be problematic. However, further modified to disable their ability to integrate into the genome, they have been shown to mediate durable gene expression in mice after fetal intracranial injection[42] and have been used in adult rats to treat focal neocortical epilepsy.[43] Lentivirus vectors consist of a nucleocapsid surrounded by an envelope derived from the plasma membrane. Vector tropism, conferred by membrane glycoproteins, can be modified by exchanging the native glycoprotein for another virus' glycoproteins such as vesicular stomatitis virus glycoprotein ("VSV-G"). AAV vectors have been developed to be administered in vivo, topically, and systemically to target different cells and tissues. The single-stranded DNA payload is enclosed in a naked icosahedral capsid. Vector tropism is modified by configuration of the capsid. Historically, these were from alternative AAV serotypes "in the wild." More recently, novel synthetic capsid variants have been created using a range of capsid engineering techniques. The only remnant virus sequences in the vector payload encode the inverted terminal repeats (ITRs) which are critical for vector production and function. In most AAV vectors, the ITRs are usually derived from AAV serotype 2, hence the common designation AAV2/x where x is the capsid. This is usually shorted to AAVx to describe just the capsid.

For many years, gene therapy was either "viral" or "nonviral": Disassembly of viruses rendered them non-replicative and non-pathogenic yet retained their evolved gene delivery supremacy. Alternatively, conjugation of genetic material—usually DNA—with cationic polymers, liposomes created new, synthetic entities capable of packaging long stretches of genetic material which could be

delivered relatively benignly but at a cost of inefficiency and transience. Compared to nonviral methods, viral vectors were much more efficient at delivering DNA into the nucleus, achieving durable gene expression but at the cost of restriction in the lengths of genetic material (the viral packaging capacity) to be delivered and the potential to evoke an immune response or cause genotoxicity. In the 2000s and 2010s, viral vectors prevailed with continual wins in clinical trials.

Most gene therapy trials, particularly using viral vectors, are gene supplementation, in that they deliver additional working copies of the gene in a recessive disease or a haploinsufficiency. Gene delivery is unlikely to restore physiological expression to all target cells yet may achieve overexpression in some cell populations. Some indications tolerate this better than others. Adult gene therapy for hemophilia B likely resulted in supraphysiological expression of the factor IX protein, but only in a small proportion of hepatocytes. Nevertheless, these cells tolerate overexpression and contribute to sufficient circulating protein concentrations to provide therapeutic benefit.[44] 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.

Recently, nonviral technologies have proven their worth in delivering mRNA for applications where transient expression is acceptable or even preferable. For example, clinically, it has been used to deliver mRNA as a vaccine,[45,46] antisense RNA to treat spinal muscular atrophy[47] and, in separate studies, to treat hereditary transthyretin amyloidosis by delivery of siRNA[48] and gene editing machinery (template plus Cas9 mRNA).[49] Recently, Gao et al.[50] and Riley et al.[51] independently demonstrated delivery of mRNA predominantly to the liver after injection of lipid nanoparticles into the mouse fetus by injection into the liver and via the vitelline vessel, respectively.

3 | WHY PERFORM FETAL GENE THERAPY: WHAT ARE THE POTENTIAL BIOLOGICAL ADVANTAGES OF FETAL VERSUS POSTNATAL INTERVENTION?

The question "Why perform fetal gene therapy?" has two different meanings, and the emphasis has shifted as gene therapy has matured. At the turn of the millennium, consideration of potential biological advantages was the consensus interpretation of the question when the NIH RAC stated the need for more preclinical studies.

Potential advantages were considered to include (i) prevention of disease before irreversible pathological changes have occurred, (ii) exploitation of the tolerogenic nature of the fetal immune system, (iii) more efficient delivery of genetic material to biological compartments which may be less accessible postnatally, (iv) delivery to stem cells which are more abundant in the fetus, and (v) requirement for less vector since the fetal body mass is low. Each of these advantages have now been validated by more than two decades of research.

3.1 | Comparison of fetal and postnatal therapy

Comparison of the relative age and developmental stage of fetal and neonatal mice to human fetal and neonatal development is complex. Nevertheless, there are a small number of studies comparing fetal and neonatal mouse interventions, either directly or indirectly. In treating hypophosphatasia with gene therapy, Sugano et al. demonstrated that fetal treatment was superior to neonatal intervention, possibly because of improved chondrocyte transduction.[52] Neonatal gene therapy has revolutionized treatment of human type I spinal muscular atrophy with improved clinical results noted with earlier treatment. These clinical findings were supported by earlier landmark studies demonstrating therapeutic benefit after treatment in knockout mice with an AAV9 vector[53–55] although one was recently retracted for inconsistencies in reporting.[56] Rashnonejad et al. demonstrated the feasibility of fetal gene therapy by intraperitoneal AAV9 injection in knockout mice.[57] Interestingly, their treated mice did not exhibit the ear and tail necrosis seen after neonatal gene therapy. For Gaucher disease gene therapy, Massaro et al. compared fetal versus neonatal intracerebroventricular injection in mice. Only fetal intervention completely prevented neuronal loss. This is consistent with the observation that severe biochemical abnormalities and inflammation are already present at birth in neonatal knockouts.[58]

3.2 | Biodistribution and the nervous system

Numerous preclinical studies have demonstrated that vectors exhibit different tropisms when injected earlier versus later in life. A landmark mouse study demonstrated that neonatal intravenous AAV9-GFP resulted in mainly neuronal expression, whereas adult injections yielded predominantly astrocyte transduction.[59] In mice, injection of

AAV9-GFP into the fetal vitelline vessels at 16 days of gestation achieved stronger neuronal expression than injection into neonates via the superficial temporal vein.[60] This has often been attributed to tightening of the blood brain barrier soon after birth; however, the concept of the blood brain barrier is complex and often misunderstood. Assembly of components of the human blood brain barrier have been observed at 18 weeks of gestation.[61] Saunders et al. rebut the concept that the early-life blood brain barrier is immature or dysfunction, and propose "...that the specific barrier mechanisms present at any particular stage of brain development are ones that are appropriate for that stage of its development...."[62] Differences in expression after delivery at different ages may therefore represent different molecular barriers, viral receptor distribution and intracellular viral transport mechanisms, amongst others.

3.3 | Fetal immune system

A potential advantage of fetal gene therapy is exploitation of the tolerogenic nature of the fetal immune system. Fetal tolerance is multifactorial, involving many cell types including regulatory T cells,[63] natural killer cells,[64] and dendritic cells.[65] There is evidence that the fetal environment is not sterile[66] and the fetal immune system is capable of mounting an adaptive immune response (reviewed in[67]). Many studies, including those described herein, have demonstrated long-term gene expression after fetal delivery. In some, fetal gene delivery has been shown to avoid immune-mediated elimination of efficacy that has thwarted adult gene delivery.[68,69] However, only a few have explicitly challenged the tolerance or interrogated the underlying mechanisms. In 2003, a comparison was made between fetal and adult mouse gene delivery of human factor IX using adenovirus serotype 5 vector. Adenovirus serotype 5 vector is highly efficient at transducing liver via interaction with coagulation factor X.[70] Adult-injected mice expressed high plasma concentrations but, after 40 days, expression was eliminated, consistent with the production of high concentrations of anti-factor IX antibodies. Repeated injections with human factor IX protein generated an increasingly strong anti-factor IX antibody response. In contrast, mice injected in utero expressed factor IX for up to at least 250 days with no anti-factor IX antibodies. Repeated post-natal challenge injections with factor IX elicited no anti-factor IX antibody response: they were tolerized to human factor IX.[71,72] Further experiments provided evidence that tolerance was maintained by $CD4^+/CD25^+$ regulatory T cells.[73] Sabatino et al. performed similar experiments in mice using AAV1 vector and human factor IX, observing immune tolerance after fetal delivery. Importantly, they observed evidence of T cell immune tolerance.[74] More recently, fetal gene delivery has been shown to induce postnatal immune tolerance to the transgenic protein in sheep receiving AAV6.2- and AAV8-GFP[75] and macaques receiving AAV5-factor X.[76]

Interestingly, there have been publications reporting immune response following fetal gene delivery of vector in mice,[77] gene-modified hematopoietic stem cells in mice[78] and vector delivery in rats,[79] sheep,[80] and monkeys.[81] Possible explanations may be that certain gene products are less tolerogenic than others (considering that most genes in preclinical experiments are human and not conspecific to the preclinical model) and that some modes of delivery may be more tolerogenic or less pro-inflammatory.

As we continue to advance our fundamental knowledge regarding the biologic rationale for fetal gene therapy, postnatal gene therapy has been progressing well. For some indications, such as spinal muscular atrophy[82] and hemophilias A[83] and B,[84] immune suppression may be provided in response to elevated serum alanine transaminase. However, immune suppression has proven to be unnecessary in many cases of targeted delivery to the CNS, for example direct AAV2 infusion into the substantia nigra and ventral tegmental area.[85] Fewer reports of adverse immunological events have been reported following intraparenchymal delivery, possibly because vector doses are lower and leakage into the circulation is minimal.

As noted above, the potential for induced immune tolerization to vector components has been considered. On the one hand, this might allow repeated postnatal vector readministration. On the other, this might compromise immunity to the virus from which the vector is derived.[97] However, this fear may be allayed by several studies. Sheep receiving lacZ by adenovirus vector at 60 days of gestation (term 145 days) exhibited a robust anti-adenovirus antibody response when challenged by re-injection at 125 days of gestation.[98]

Fetal delivery of human factor IX by adenovirus vector to mice caused immune tolerization only to the human protein but not to the adenovirus vector. Subsequent adult administration of vector resulted in high factor IX expression but also induced anti-adenovirus antibodies. A second adenovirus injection did not increase factor IX expression further but evoked an even stronger antibody response.[71,72] Similarly, although tolerance to the transgene was induced following AAV vector-mediated fetal delivery, tolerance to AAV vector itself was not observed.[75] Presumably, in both cases, this was due to the persistent expression of the transgene protein but only transient exposure of the fetus to the viral vector

proteins. Therefore, fetal delivery is a "free shot" in that it does not evoke an anti-vector immune response but also does not induce tolerance. This is supported by a study where the investigators were able to give one injection of AAV1-factor IX in utero then a repeat with the same vector in the adult mice.[74] Similarly, studies if in utero CRISPR-mediated gene editing observed generation of anti-SpCas9 antibodies after adult, but not fetal injection of AAV9[96] or adenovirus[94] to deliver an SpCas9-base base editor.

It is also worth considering the timing of gene delivery itself, rather than the nature of the immune system. The adult immune system is likely to have encountered archetypal viruses and bacteria from which vectors and genetic editors have been developed. Preexisting immunity to the respective AAV serotypes likely caused loss of transgenic protein expression in one of the first clinical trials for hemophilia B.[29] In subsequent trials this has led to a strict exclusion criterion of patients with preexisting anti-virus/vector immunity.[35] Anti-vector neutralizing antibodies may limit efficient vector delivery and cytotoxic T-cell responses may eliminate vector transduced cells.

Although the fetal immune system will likely be naïve to these viruses, existing maternal IgG antibodies and even T cells are able to traffic the placental barrier which may be sufficient to eliminate any effective transgenic protein expression. This is important in AAV mediated gene transfer where a preexisting anti-AAV immune response has been shown to limit fetal gene editing in a serotype-specific manner[99] and preexisting maternal T-cell immunity to AAV has been recognized to eliminate cells which have been transduced with vector.[29]

3.4 | Potential risks

Implicit and always considered alongside was the question "*What are the potential biological risks of fetal gene therapy?*" A list of these risks was compiled by the NIH RAC and UK GTAC committees, though many were not exclusive to fetal gene therapy.

First was fear of cancer, borne from the recognition that the fetus could be readily transduced and that rapidly dividing fetal tissues might be susceptible to oncogenic integration events. Studies reported hepatocellular carcinoma formation after injection of fetal or neonatal mice with lentivirus vectors derived from equine infectious anemia virus[100,101] and feline immunodeficiency virus[102] but not HIV-based lentivirus.[103] Carcinomas have also been detected after mouse injection of certain AAV vectors.[104] The leukemias associated with gene-modified hematopoietic stem cell transplantation in X-SCID patients were caused, in several cases, by vector integration near and upregulation of the LMO2 proto-oncogene.[105] Therefore, risk of cancer is not exclusive to fetal therapy.

Second, germline transmission was of major concern. Gene therapy is restricted, legally, to somatic cells; germline gene modification is prohibited and not the goal of fetal gene therapy. The late 1990s pre-protocols for fetal gene therapy were met with criticism that they "are likely to result in genetic changes in fetal germline cells."[106] Porada and colleagues demonstrated low-level germline transmission after retroviral vector delivery to the fetal sheep.[107] Lee et al. found evidence of transgene expression in the ovarian epithelium but not male or female germ cells, following fetal intraperitoneal injection of lentivirus vector into fetal macaques.[108] Vector sequences could not be found in spermatozoa of mice injected in utero with lentivirus vector[71,72] or fetal sheep injected with adenovirus vector.[80] They have been found in the semen of rabbits injected as adults with AAV2 vector[109] and AAV5 sequence was detected in semen for up to 12 months following a postnatal human hemophilia A gene therapy trial, although no vector DNA was detected in spermatozoa.[110] Vector genome was detected in testis and genome and RNA transcript were detected in ovary, in two infants who received AAV9 gene therapy for spinal muscular atrophy. Therefore again, this risk is not exclusive to fetal gene therapy.[111]

4 | PRECLINICAL GENE TRANSFER OF METABOLIC GENES

Aside from immune deficiency, **ADA-SCID** exhibits metabolic disturbance which leads to neurodevelopmental delay, abnormal muscle tone and deafness. It is treated with donated hematopoietic stem cell transplantation, enzyme replacement therapy and, more recently, gene therapy. Despite being the first human disease to benefit from gene therapy,[9,10] and a pre-protocol being submitted to the NIH RAC to perform fetal gene therapy in ADA-SCID using hematopoietic stem cells corrected with a retrovirus vector,[7,8] there have been no studies on fetal gene therapy, even in animal models. However, there have been numerous studies describing fetal delivery in preclinical models for at least 10 different metabolic diseases (Table 1).

Tay-Sachs is a type of GM2 gangliosidosis and sphingolipidosis caused by mutations in hexosaminidase A. The infantile form manifests as neonatal neurodegeneration and early death. Probably, the first example of preclinical fetal gene therapy of a metabolic disease was in 1996 by Lacorazza et al., who injected neural progenitor cells into E14.5 mice. These cells had been genetically modified using ecotropic retrovirus to overexpress hexosaminidase A. The investigators reported enzyme

TABLE 1 Studies describing treatment of metabolic disease by fetal gene therapy.

Indication	Protein	Gene delivered	Organism	Vector	Promoter	Delivery route	Gestation age (days)	Reference
Tay-Sachs	β-hexosaminidase a-subunit	HEXA (human)	CD-1 outbred mouse	Ecotropic retrovirus		Ex vivo cells injected intra-cerebro-ventricular	14.5/20	[86] #11018}
Citrullinemia type I	Argininosuccinate synthetase	AS (human)	Knockout mouse	Adenovirus		Intrahepatic	15/20	[87] #955}
Mucopolysaccharidosis type VII	β-glucuronidase	*GUSB* (rat)	Knockout mouse	Amphotropic and ecotropic retrovirus		Intrahepatic	13.5/20	[88] #1786
Mucopolysaccharidosis type VII	β-glucuronidase	GUSB (human)	Knockout mouse	Retrovirus		Intraplacental	13.5/20	[89] #197
Mucopolysaccharidosis type VII	β-glucuronidase	GUSB (human)	Knockout mouse	AAV1 & 2		Intracerebro-ventricular	15.5/20	[90] #2993
Mucopolysaccharidosis type VII	β-glucuronidase	GUSB (human)	Knockout mouse	Adenovirus	CAG	Intracerebro-ventricular	12–17/20	[91] #1798
Glycogen storage disease type II (Pompe disease)	α-glucosidase	GAA (human)	Knockout mouse	AAV1 & 2	CAG	Intrahepatic & intraperitoneal	15/20	[77] #1518
Crigler–Najjar type 1	Bilirubin UDP-glucuronyltransferase	UGT1A1 (human)	Mutant rat (Gunn)	Lentivirus	PGK	Intrahepatic	17–19/22	[92] #771
Crigler–Najjar type 1	Bilirubin UDP-glucuronyltransferase	UGT1A1 (human)	Mutant rat (Gunn)	Lentivirus	PGK	Intrahepatic	17–19/22	[79] #2511
Human erythrocyte R-type pyruvate kinase deficiency (PKD)	Pyruvate kinase	PKLR (human)	Knockout mouse	γ-retrovirus	SFFV	Ex vivo stem cells injected intrahepatically	14.5/20	[93] #4706
Hypophosphatasia	Tissue-nonspecific alkaline phosphatase	TNALP with deca-aspartates	Knockout mouse	AAV9	CMV	Intraperitoneal	15/20	[52] #5494
Gauchers disease	Glucocerebrosidase	GBA (human)	Knockout mouse	AAV9	GUSB	Intracerebroventricular	16/20	[58] #9507
Hereditary tyrosinemia type 1 (HT1)	Fumarylacetoacetate hydrolase	Gene editing disruption of upstream gene	Knockout mouse	Adenovirus		Intravenous (vitelline vessel)	16/20	[94] #9569
Mucopolysaccharidosis type I (MPS I).	α-L-iduronidase	αID (canine)	Canine mutant model	Retrovirus (Mo-MuLV)	PGK	Ex vivo transduced cells injected into yolk sac	32–38/63	[68,69] #937
Mucopolysaccharidosis type I (MPS I).	α-L-iduronidase	αID (human)	Canine mutant model	Retrovirus	MPSV	Injection into yolk sac and peritoneal cavity	35/63	[95] #720
Mucopolysaccharidosis type I (MPS I).	α-L-iduronidase	Base-editing of point mutation	Mouse knockout	AAV9		Injection into yolk sac vessel	15.5/20	[96] #11118

Abbreviation: *TNALP*, tissue nonspecific alkaline phosphatase.

secretion by the neural progenitors, and cross-correction of neighboring cells, to concentrations which they estimated would be therapeutic in a relevant model.[86]

Citrullinemia type I (CTLN1) is a urea cycle disorder caused by mutations in argininosuccinate synthetase. In the severest form, accumulation of ammonia in the blood and cerebrospinal fluid causes seizures, brain damage, and liver failure. Adenovirus vector was used to deliver human argininosuccinate synthetase by intrahepatic injection into a mouse model of this disease at 15 days of gestation. Treatment extended survival from 3 days to up to 10 days.[87] Dilution of the non-integrating adenovirus vector, a consequence of rapid liver growth and cell division at this age,[112] is a likely cause of loss of expression.

Mucopolysaccharidosis type VII (Sly syndrome) is a lysosomal storage disease caused by deficiency of β-glucuronidase which is encoded by the *GUSB* gene. In its severest form, it can present in utero as fetal hydrops, placentomegaly, and polyhydramnios. Ecotropic or amphotropic retrovirus vectors were used to deliver *GUSB* to murine fetal liver cells, which are a source of hematopoietic stem cells. These cells were injected into the placentas at 13.5 days of gestation. Vector sequence was detected in neonatal tissues but therapeutic efficacy was not reported.[88] A similar, subsequent experiment resulted in detectable enzyme activity at 60 days and anecdotal phenotypic improvement; however, any therapeutic effect had disappeared by 214 days.[89] Switching to delivery of the gene by injection of AAV1 into the fetal cerebral brain ventricles resulted in vector delivery throughout the brain, and strong expression, secretion, and cross-correction of β-glucuronidase. Survival was significantly improved up to 1 year, although the absence of visceral expression explains the failure to correct bone abnormalities.[90] A second group delivered GUSB adenovirus serotype 5 to fetal mouse cerebral lateral ventricles. Despite histologically sparse enzyme distribution and modest brain enzyme activity (≈3–10% of normal), accumulation of brain lysosomal glycosaminoglycans was prevented at 120 days.[91]

Glycogen storage disease type II (Pompe disease) is a lysosomal storage disease caused by deficiency in acid α-glucosidase. It causes progressive muscle weakness and in severe forms can manifest as fetal hypertrophic cardiomyopathy.[113] Rucker et al. compared delivery of the human GAA coding region by fetal intrahepatic injection of two AAV serotypes. Expression was predominantly observed in the diaphragm, likely from leakage of vector into the fetal peritoneal cavity. AAV2-treated mice expressed supraphysiological expression of α-glucosidase in the diaphragm and partial restoration of contractile force. AAV1-treated mice achieved even higher expression.[77] AAV1 was also the vector of choice in delivery of human GAA to the peritoneal cavity of two late-first trimester rhesus macaques.[81]

Crigler–Najjar type 1 is caused by mutations in the UGT1A1 gene which encodes bilirubin UDP-glucuronyltransferase. Absence of the enzyme results in high concentrations of serum unconjugated bilirubin which leads to brain damage and death. Seppen et al. published two studies using a lentiviral vector to deliver human *UGT1A1* via in utero intrahepatic injection into a rat model of this disease, at 19 days of gestation. They observed partial correction of hyperbilirubinemia for more than a year.[79,92]

Human erythrocyte R-type pyruvate kinase deficiency is caused by mutations in the PKLR gene. It causes chronic non-spherotic hemolytic anemia. There is a wide heterogeneity of clinical presentation, but homozygous and compound heterozygous mutations can cause severe anemia.[114] Partial correction of the mouse model was achieved by fetal intrahepatic injection of hematopoietic stem cells transduced with a retroviral vector carrying human R-type pyruvate kinase gene.[93]

Hypophosphatasia is another clinically heterogeneous disease which, at its most severe, is lethal at birth from respiratory failure, hypercalcemia, and uncontrolled convulsions.[115] The underlying defect is in the gene tissue nonspecific alkaline phosphatase (*TNALP*) which contributes to the bone mineralization pathway. Sugano et al. compared intraperitoneal AAV9 vector injection to fetal (E15) and neonatal TNALP-knockout mice. Fetal injection extended median survival from 10 days to up to at least 8 weeks; seizures were abolished, and bone mineralization was restored.[52]

Gaucher disease, caused by mutations in the lysosomal enzyme glucocerebrosidase, exhibits a clinical spectrum. The milder adult form presents as hepatomegaly, splenomegaly, fatigue, and avascular bone crises. It is managed with enzyme replacement and substrate reduction therapy. Severe enzyme deficiency causes acute neuronopathic Gaucher Disease manifesting as dysphagia, seizures and developmental delay and, in extreme cases, fetal hydrops and ichthyosis.[116] Glucocerebrosidase-knockout mice develop tetraparesis and do not live beyond 15 days. AAV9 injected into the cerebral lateral ventricles of these mice at 16 days of gestation extended survival up to at least 18 weeks. Neurodegeneration was abolished and neuroinflammation ameliorated profoundly. Neonatal intracerebroventricular injection achieved similar results but failed to completely prevent neuronal loss.[58]

Tyrosinemia type I is caused by mutated fumarylacetoacetate hydrolase (*FAH*). Accumulation of upstream metabolites causes liver and kidney disease and neuropathy.

Patients may present from infancy to adulthood with neonates suffering acute liver failure, coagulopathy, and ascites. Medical management is oral Nitisinone plus a low tyrosine and phenylalanine diet. Nitisinone inhibits an enzyme, 4-hydroxyphenyl pyruvate dioxygenase (HPD) located upstream to *FAH*, preventing accumulation of the intermediary toxic metabolites.[117] Rossidis et al. performed proof-of concept fetal base-editing in the FAH knockout mouse model. The editor, designed to introduce a nonsense mutation in the same HPD gene, was delivered intravenously into the fetal vitelline vessels at 16 days of gestation using an adenovirus vector. Untreated mice do not survive beyond ≈3 weeks and have hyperbilirubinemia and elevated blood liver enzymes. In utero gene editing rescued the lethal phenotype with animals demonstrating weight gain and survival equal to unedited disease mice maintained on nitisinone.[94]

Attempts at treating **mucopolysaccharidosis type IH** (MPSIH, Hurler syndrome) first employed ex vivo gene therapy following the success of allogeneic bone marrow transplantation in this disorder. MPSIH is caused by mutations in α-L-iduronidase. Hematopoietic stem cells transduced with a retroviral vector encoding α-L-iduronidase were injected at mid-gestation (≈30 days) into the yolk sac of MPSI dogs. Genetically modified cells were detected in marrow (up to 12%) and circulation at 12 months; however, enzyme was undetectable.[68,69] Three years later, the investigators repeated these experiments but via direct retrovirus injection using the same fetal route. Enzyme activity was detected only in the liver and kidney of one pup that was stillborn but not in the remaining seven. Proviral copy number declined over the 4-month duration of the experiment.[95] More recently, base editors were delivered by a dual AAV split-intein approach, into the vitelline vessel at 15.5 days of gestation in an α-L-iduronidase mouse model carrying a G → A (tryptophan → stop) mutation recapitulating the common G → A (W402X) mutation present in many patients with MPSIH. This study demonstrated significant improvement in survival and amelioration of metabolic, musculoskeletal, and cardiac disease with improvement in some outcome measures following prenatal compared to postnatal base editing.[96]

5 | WHY PERFORM FETAL GENE THERAPY: IN WHICH CIRCUMSTANCES MIGHT HUMAN FETAL GENE THERAPY BE JUSTIFIED?

To translate fetal gene therapy to the clinic, several criteria must be met. It must be technically feasible, there must be sound and timely prenatal diagnosis, and there must be a clear benefit over postnatal intervention.

5.1 | The practical considerations of fetal gene delivery

As the character of Dr Marta Shearing says in the film "The Bourne Legacy" "getting [the gene therapy vector] where you want it, how you want it, is the nightmare."[118] Both vector configuration and route of administration are critical determinants of successful gene transfer. To some, delivery to the fetus may seem fantastical yet fetal surgery was established more than 60 years ago and is now a mature discipline.

In 1961, Albert Liley performed x-ray-guided intraperitoneal blood transfusion to a fetus diagnosed with Rhesus disease.[119] An excellent personal history and review of the field is provided by a pioneer, Harrison.[120] In 1981, direct intravascular transfusion into chorionic plate vessels was achieved using fetoscopic visualization[121] with refinements made to manage fetal bleeding disorders, parvovirus B19 infection and maternal alloimmunization.[122,123] Nowadays, fetal anemia is frequently treated safely with fetal blood transfusion via umbilical vein injection.[124] In mice, alternative routes of delivery such as via the maternal circulation or intraplacental injection have also been investigated; however, this would deliver substantial vector doses to the maternal tissues. Therefore, these approaches[125,126] will likely remain tools in the realm of scientific investigation and clinical prenatal gene therapy would likely occur via umbilical vein or intracardiac injection (systemic routes of delivery) or intracerebroventicular to specifically target the CNS.

Although intravascular or intracerebroventricular delivery to the fetus may initially appear unfeasible, it is important to consider it in the context of more advanced surgical interventions for fetuses with structural birth defects that are now commonplace at specialized, multidisciplinary fetal treatment centers. For example, a shunt can be placed between the bladder and amniotic cavity to treat lower urinary tract obstruction. Similarly, a shunt placed between the thorax into the amniotic fluid can drain pleural effusions such as chylothorax and hydrothorax or be used to drain large cystic lung lesions.[127] Balloon valvuloplasty is performed in selected cases of hypoplastic left-heart syndrome. Fetal endoscopic tracheal occlusion is performed for congenital diaphragmatic hernia.[128,129] Particularly impressive is the development of fetal surgery for myelomeningocele which is now in routine clinical practice for mothers and fetuses meeting specific criteria.[130] This is the most severe form of spina bifida where there is incomplete closure of the vertebral column with subsequent hindbrain herniation and exposure of the spinal cord to the detrimental effects of the amniotic fluid. Most cases of neural tube defect are sporadic, although there is a high risk of

recurrence for siblings of affected individuals. Variants in the homocysteine remethylation gene *MTHFR* is a risk factor in some human populations.[131] A randomized trial of prenatal versus postnatal surgery[132] provided level I evidence that fetal surgery had better outcomes than postnatal surgery.[133]

5.2 | Fetal therapy (but not gene therapy) for genetic disease

Increasingly, fetal blood transfusion is used to treat α-thalassemia major[134] and other rare anemias although transfusion of diagnosed metabolic disease (e.g., pyruvate Kinase deficiency[135]) is still uncommon. Fetal stem cell transplantation of fetal stem cells has been applied to a range of genetic diseases, including metabolic diseases such as acute neuronopathic (type II) Gaucher disease, Hurler syndrome, and Niemann Pick type A (reviewed extensively in reference 136).

There are two ongoing clinical trials to treat α-thalassemia major (NCT02986698) and osteogenesis imperfecta[137,138] (NCT03706482) using hematopoietic stem cells and mesenchymal stem cells, respectively.

5.3 | Prenatal gene therapy to treat pregnancy complications

Obstetric conditions are, by their nature, only treatable before birth.

Preterm birth is defined as delivery before 37 weeks of gestation; 40% of cases of preterm birth are associated with infection. Recently, Suff et al. described a protective benefit of cervico-vaginal AAV gene therapy in a mouse model of preterm birth induced by intravaginal delivery of bioluminescent *Escherichia coli* at 16.5 days of gestation.[†] Prophylactic cervical application of AAV8 vector carrying the antimicrobial peptide human β-defensin-3 reduced bacterial ascent from the vagina through the cervix and significantly increased the number of live-born pups although no effect on gestational age was observed.[139]

Impaired utero-placental perfusion causes fetal growth restriction. David et al. restored fetal growth and uterine artery blood flow in guinea pig[140,141] and sheep[142] models of fetal growth restriction by administration of adenovirus serotype 5 vector delivering VEGF-A to the uterine arteries. In a sheep model of fetal growth restriction where ewes were undernourished to impair placenta growth and restrict lamb birth weight, gene therapy improved fetal growth, as well as postnatal growth rate.[142] Ongoing work is refining the inclusion and exclusion criteria for the first-in-human EVERREST clinical trial which aims to deliver gene therapy to increase the levels of VEGF in the uterine arteries of pregnant women with severe early onset FGR.[143]

5.4 | Fetal diagnosis. The biggest hurdle

For a disease to be a suitable target for fetal gene therapy, accurate in utero diagnosis is essential. In 1980, Rodeck first described drawing of fetal blood by direct vision using fetoscopy.[144] Soon after, Daffos reported the technique used today, namely blood sampling from the umbilical cord under continuous ultrasound guidance. In probands, fetal anomalies are usually first detected by ultrasound. Such anomalies may result from a range of genetic aberrations including aneuploidy, deletions, or duplications of smaller stretches of DNA and single nucleotide variants. Once an anomaly is detected, an invasive prenatal diagnostic procedure may be offered: chorionic villus sampling, amniocentesis, or sampling of fetal blood. Enzymatic assay of chorionic villus samples for the diagnosis of lysosomal storage diseases is effective[145] if suspected. Mass spectrometry and gas chromatography/mass spectrometry provide valuable measurement of metabolites in diagnosis of metabolic disease. Analysis of amniotic fluid has been used to diagnose a range of diseases including propionic acidemia,[146] methylmalonic acidemia and ornithine transcarbamylase deficiency.[147] Targeted testing of specific genes can be performed by fluorescent in situ hybridization, quantitative PCR, multiplex ligation-dependent probe amplification, chromosomal microarray analysis and copy number variation sequencing. The vast reduction in cost and increase in speed has heralded the adoption of exome sequencing[148] and next generation sequencing (reviewed in references 149 and 150). Increasingly prenatal diagnosis is becoming performed noninvasively using circulating free fetal DNA.[151] Current examples of this noninvasive prenatal diagnosis (NIPD) include for mutations in the fibroblast growth factor receptor 2 (e.g., Apert syndrome) and 3 (e.g., thanatophoric dysplasia).[152] Fetal next generation has now been adopted by the UK National Health Service.[153] However, even when one or more causative genes are identified for a particular genetic disease, in many cases, mutations are distributed throughout the gene, in exons and introns. Some dominant disorders are not fully penetrant so genetic diagnosis

[†]The lacZ gene, encoding *E. coli* β-galactosidase, has been used in myriad preclinical gene transfer studies as a marker gene: it converts the chromogenic synthetic analogue of lactose, X-gal, into a deep blue insoluble product. It is entirely different to the mammalian GLB1 gene which encodes β-galactosidase. Mutations in the human gene cause an ultrarare G_{M1} gangliosidosis, mucopolysaccharidosis type IVB (also known as Morquio B).

does not always predict severity or even presence of disease. Therefore, these technologies are currently most useful in cases of known pathogenic variants. Consideration of these diagnostic options has been explored in detail recently.[154] One of the strongest indicators of a genetic disease is the diagnosis of one or more affected siblings and a family history.[155,156]

For some diseases, reliable and predictive genetic diagnosis is possible. Spinal muscular atrophy is caused by biallelic deficiency in the autosomal survival motor neuron 1 (*SMN1*) gene. The survival motor neuron 2 (*SMN2*) gene arose evolutionarily as a tandem chromosomal duplication and individuals may possess 0–8 copies. It can compensate partially for loss of *SMN1*. The number of *SMN2* copies determines the severity of disease. A recent neonatal trial for spinal muscular atrophy recruited patients carrying two copies; untreated, life expectancy usually does not exceed 2 years. Five of these patients were identified by prenatal testing.[157] Testing by NIPD is now being offered in the presence of known SMN1 mutations.[158]

6 | THE MOTHER

Fetal therapy must not only be advantageous to postnatal intervention and provide a favorable balance of benefit and risk to the fetus but must also minimize risk to the mother. This has been discussed more extensively in other reviews.[159] Recently, this was delineated in a position statement made, by the International Fetal Transplantation and Immunology Society. Complications of fetal therapy may include the need for emergency cesarean section due to fetal hemorrhage, fetal bradycardia, preterm premature rupture of the membranes, infection, and preterm birth.[160] Specific to gene therapy is the risk that vector may cross the placental barrier into the maternal circulation, leading to a maternal immunological response to the vector or transgenic protein that may compromise gene transfer and maternal health. Furthermore, in the era of gene editing, the risk of maternal gene editing resulting from leakage of the therapeutic into the maternal circulation must also be considered. Concerns regarding a maternal immune response to the fetal gene therapy as well as maternal editing may assuaged by the fact that the dose delivered to the fetus is considerably smaller on a per kilogram basis when considering the weight of the mother. Furthermore, studies in mouse models did not demonstrate any maternal gene editing following in utero deliver of CRISPR editing constructs via either an AAV or adenovirus to the fetus.[94,96] Another potential concern is maternal germline gene transfer. It is unlikely that fetal gene therapy would lead to maternal germline gene transfer as the oocytes are protected by the blood-follicle barrier and vector would be present in the maternal circulation in very low levels.[161] A recent study, however, demonstrated that ovarian microinjection of adenoviruses was able to penetrate the blood-follicle barrier and transduce granulosa cells.[162] In macaques, fetal delivery of AAV8 resulted in transplacental gene transfer in maternal tissues.[163] Ultrasound-guided intraperitoneal, intracerebroventricular, or intravenous injection of AAV9 vector caused premature delivery in fetal piglets of the domestic pig; saline injection did not.[164] In contrast, delivery of AAV9 to a different strain, the Yucatan minipig, to the umbilical vein following hysterotomy resulted in live births following cesarean section delivery.[165] There are maternal safety issues specific to metabolic disease as fetal metabolic disease may affect the mother adversely, manifesting as acute fatty liver, for example.[166–168] Therefore, there may be benefit for in utero therapy of some fetal metabolic diseases to the health of the mother. Other potential risks to the mother include cancer, although the mature adult tissues are likely to be far less susceptible to oncogenic integration events than the rapidly dividing fetal tissues. Fetal gene therapy presents a very challenging situation regarding how to inform the mother of all the potential risks and has been considered extensively.[169] The father/partner should also not be forgotten, as in most jurisdictions, they will share parental responsibility after the baby is born and therefore should be recognized as having an important contribution to decisions as to whether to proceed with fetal gene therapy.[170] The benefit of fetal therapy should be weighed against the alternative, either delivery at term with immediate neonatal treatment or elective early cesarean section and disease management at birth. The later case is exemplified by the management of large sacrococcygeal teratomas which place the fetus at risk of heart failure due to a vascular steal scenario. In select cases, these fetuses are electively delivered at 28–32 weeks gestation to allow for debulking of the large vascular component of the tumor as studies have demonstrated delaying the delivery to term results in increased fetal death.[171] Parental perceptions of fetal therapy will not be explored here but there have been several studies and reviews. Women generally expressed interest in enrolling for clinical trials which could benefit their unborn baby.[172] Schwab et al. reported favorable attitude to fetal enzyme replacement therapy for lysosomal storage diseases[173] and gene therapy for spinal muscular atrophy.[173] Ultimately, multidisciplinary, nondirective prenatal counseling, and informed consent will be critical to the implementation of in utero gene therapy.

7 | WHAT NEXT?

The submission of pre-protocols for human fetal gene therapy in 1998 was unfortunate timing, as the field entered a period of turmoil. Moreover, broader political forces were at work; members of the NIH RAC voiced concerns that, in the United States, "intervening in utero may ... impinge on politically sensitive abortion-related issues and decisions."[174] The pre-protocols were also ahead of their time. One public detractor wrote "So far, human gene therapy has failed, and it seems paradoxical that this failure should be used as a justification to extend genetic manipulation in humans to less-differentiated cells."[19] This is not true anymore: human gene therapy for inherited genetic disease is a clinical and commercial reality. As of February 2023, market approval has been granted for eight indications: Libmeldy (Atidarsagene autotemcel), Skysona (Elivaldogene autotemcel) and Zynteglo (Betibeglogene autotemcel) are ex vivo lentivirus therapies for metachromatic leukodystrophy, adrenoleukodystrophy and β-thalassemia, respectively. Hemgenix (Etranacogene dezaparvovec-drlb), Luxterna (Voretigene neparvovec), Roctavian (valoctocogene roxaparvovec), Upstaza (Eladocagene exuparvovec), and Zolgensma (Onasemnogene abeparvovec) are in vivo AAV therapies for hemophilia B, Leber's congenital amaurosis, hemophilia A, aromatic acid decarboxylase deficiency and spinal muscular atrophy. Many more are approved for clinical trial.[175,176]

A template for progression to human fetal gene therapy is now emerging from the trajectories and confluence of clinical trials for several genetic diseases...

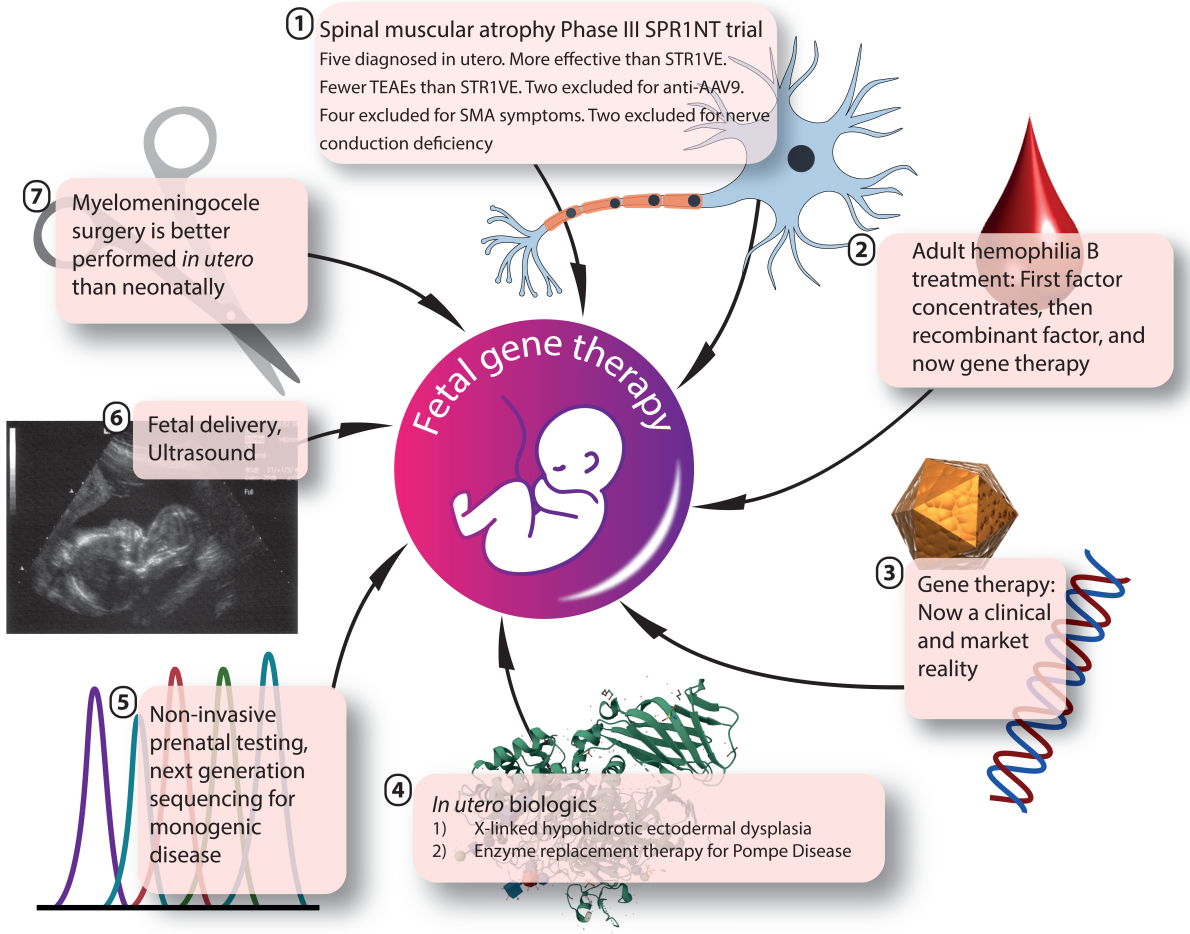

FIGURE 1 A depiction of seven technological advances which support a case for fetal gene therapy. Clockwise, from top: (1) The spinal muscular atrophy SPR1NT trial recruited five patients after fetal diagnosis. Four were excluded from the trial for already having SMA symptoms. Two already exhibited nerve conduction deficiency. There were fewer adverse events in this trial than those infants treated at an older age. (2) The trajectory of treating hemophilia was from concentrated factor from plasma to a biologic (recombinant factor) now to gene therapy which is (3) a clinical and market reality. (4) Recombinant proteins for treating inherited genetic disease are now being applied in utero. (5) Noninvasive prenatal testing and next generation sequencing are both improving genetic diagnosis. (6) Ultrasound-guided directly into the fetal circulation is performed routinely worldwide. (7) The outcome of fetal surgery for myelomeningocele has now been shown to be superior to postnatal intervention.

Historically, type I spinal muscular atrophy manifests as neuromuscular disease until death usually before 2 years of age. Treatment has been transformed by the development of two genetic therapies. The first, Nusinersen, marketed as Spinraza, is an oligonucleotide delivered intrathecally triannually after four loading doses. Onasemnogene abeparvovec-xioi, marketed as Zolgensma, delivers working copies of the *SMN1* gene by a single intravenous injection of AAV9 vector.[177] In a protocol-based, multicenter prospective observational study measuring motor score (CHOP INTEND) within 6 months of injection, children younger than 8 months saw a 13.8 point increase, those between 8 and 24 months a 7.7 point increase but those older than 24 months saw no significant improvement.[178] The phase III SPR1NT trial has now provided evidence of efficacy in that 14 infants diagnosed genetically who were treated prior to disease onset (between 8 and 34 days old) showed improved developmental outcomes and better functional independence than children who were treated at older ages. Further support for a beneficial risk–benefit profile was provided by the observation that no serious TEAEs related to treatment were observed in this trial. In contrast, three were observed in the STR1VE-US13 trial treating older infants.[179] The authors suggested that this might be a consequence of the relative non-responsiveness of the neonatal immune system to nonself antigens (including capsid proteins). Several aspects of this study support an even earlier, fetal approach: (1) diagnosis—five of the treated infants were diagnosed prenatally, (2) efficacy—treatment did not prevent motor deficit completely since five treated children fell below the normal range in the Bayley gross motor score, (3) disease onset—eight infants were excluded from the trial since disease was already detectable. Four infants displayed clinical signs at the time of diagnosis and four exhibited reduced nerve action potential. (4) Preexisting immunity—two infants were excluded from the trial since they had anti-AAV9 antibodies (though these may have been transmitted maternally).[157]

Adults with hemophilia A and hemophilia B were initially only treatable with transfusions of plasma-derived factor concentrate. In the 1990s, in developed countries, this was superseded by recombinant clotting factor injections. In 2022 the first "one-and-done" gene therapy products were approved.[180] A similar pattern can be seen for patients with lysosomal storage diseases: currently enzyme replacement therapy and substrate inhibition/depletion are first-line treatments but patients are now being recruited to gene therapy trials.[181] X-linked hypohidrotic ectodermal dysplasia, caused by mutation of the ectodysplasin A gene presents as failure of sweat glands and tooth formation. Being a developmental disease, neonatal treatment is too late. Three affected fetuses from two families with affected sons received genetic diagnosis, and ultrasound diagnosis of absence of mandibular tooth germs. Intra-amniotic delivery of recombinant fusion protein, consisting of the receptor-binding domain of ectodysplasin A and the constant domain of human IgG1, restored development of sweat glands and tooth germs.[182] In 2022, a phase I clinical trial of in utero enzyme replacement therapy was initiated for treatment of eight lysosomal storage diseases (NCT04532047). In utero enzyme replacement therapy for Pompe disease in a case report was safe and efficacious.[183]

8 | CONCLUSION

Several trials signpost a direction of travel from symptomatic management to biological therapy to genetic therapy. There is evidence of a clinical benefit in fetal versus postnatal intervention. Other trials, provide evidence that gene therapy, specifically, can be more efficacious the earlier in life it is delivered. Maturation of technology for genetic diagnosis in combination with imaging modalities and biomarkers at last enables effective prenatal diagnosis (Figure 1). All these support the notion that fetal gene therapy is soon to be realized and, that reviews which speculate when fetal gene therapy might happen, such as this one, may be a thing of the past.

AUTHOR CONTRIBUTIONS

All authors contributed to the drafting of this manuscript.

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CONFLICT OF INTEREST

Manju A. Kurian is a founder of and consultant to Bloomsbury Genetic Therapies. Honorarium from PTC for invited lecture (BPNA Jan 2023). Simon N. Waddington is a founder of and consultant for Bloomsbury Genetic Therapies and is a member of the SMAB of Forge Biologics. Anna L. David provides paid consulting services to Pierre Fabre Group and EspeRare Foundation. Paul Gissen is a founder of and consultant to Bloomsbury Genetic Therapies. Ahad A. Rahim is a founder of and consultant to Bloomsbury Genetic Therapies. Jerry K. Y. Chan and Ashley K. Boyle declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

There is no data within this article.

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REFERENCES

1. Stuhlmann H, Cone R, Mulligan RC, Jaenisch R. Introduction of a selectable gene into different animal tissue by a retrovirus recombinant vector. *Proc Natl Acad Sci U S A*. 1984;81:7151-7155.
2. Ballay A, Levrero M, Buendia MA, Tiollais P, Perricaudet M. In vitro and in vivo synthesis of the hepatitis B virus surface antigen and of the receptor for polymerized human serum albumin from recombinant human adenoviruses. *EMBO J*. 1985;4:3861-3865.
3. Flotte TR, Afione SA, Conrad C, et al. Stable in vivo expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. *Proc Natl Acad Sci U S A*. 1993;90:10613-10617.
4. Sanes JR, Rubenstein JL, Nicolas JF. Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. *EMBO J*. 1986;5:3133-3142.
5. Anderson WF. Prospects for human gene therapy in the born and unborn patient. *Clin Obstet Gynecol*. 1986;29:586-594.
6. Eglitis MA, Kantoff PW, McLachlin JR, et al. Gene therapy: efforts at developing large animal models for autologous bone marrow transplant and gene transfer with retroviral vectors. *Ciba Found Symp*. 1987;130:229-246.
7. U.S. National Institutes of Health. Recombinant DNA Advisory Committee. Minutes of meeting: September 24-25, 1998. *Hum Gene Ther*. 2000a;11:1083-1109.
8. U.S. National Institutes of Health. Recombinant DNA Advisory Committee. Prenatal gene tranfer: scientific, medical, and ethical issues: a report of the recombinant DNA advisory committee. *Hum Gene Ther*. 2000b;11:1211-1229.
9. Blaese RM, Culver KW, Miller AD, et al. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. *Science*. 1995;270:475-480.
10. Bordignon C, Notarangelo LD, Nobili N, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. *Science*. 1995;270:470-475.
11. Teichler Zallen D. US gene therapy in crisis. *Trends Genet*. 2000;16:272-275.
12. UK Gene Therapy Advisory Committee. Gene therapy advisory committee. Report on the potential use of gene therapy in utero. Health Departments of the United Kingdom, November 1998. *Hum Gene Ther*. 1999;10:689-692.
13. Clinicopathological Conference. A case of haemolytic disease with congenital rubella demonstrated at the royal postgraduate medical school. *Br Med J*. 1967;2:819-822.
14. Touraine JL, Raudrant D, Golfier F, et al. Reappraisal of in utero stem cell transplantation based on long-term results. *Fetal Diagn Ther*. 2004;19:305-312.
15. Flake AW, Zanjani ED. In utero hematopoietic stem cell transplantation: ontogenic opportunities and biologic barriers. *Blood*. 1999;94:2179-2191.
16. Flake AW, Roncarolo MG, Puck JM, et al. Treatment of X-linked severe combined immunodeficiency by in utero transplantation of paternal bone marrow. *N Engl J Med*. 1996;335:1806-1810.
17. Wengler GS, Lanfranchi A, Frusca T, et al. In-utero transplantation of parental CD34 haematopoietic progenitor cells in a patient with X-linked severe combined immunodeficiency (SCIDXI). *Lancet*. 1996;348:1484-1487.
18. Schneider H, Coutelle C. In utero gene therapy: the case for. *Nat Med*. 1999;5:256-257.
19. Billings PR. In utero gene therapy: the case against. *Nat Med*. 1999;5:255-256.
20. Raper SE, Yudkoff M, Chirmule N, 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;13:163-175.
21. Hollon T. Researchers and regulators reflect on first gene therapy death. *Nat Med*. 2000;6:6.
22. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med*. 2002;346:1185-1193.
23. Gaspar HB, Parsley KL, Howe S, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. *Lancet*. 2004;364:2181-2187.
24. Cavazzana-Calvo M, Hacein-Bey S, Basile GDS, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science*. 2000;288(5466):669-672.
25. Check E. Regulators split on gene therapy as patient shows signs of cancer. *Nature*. 2002;419:545-546.
26. Williams DA, Thrasher AJ. Concise review: lessons learned from clinical trials of gene therapy in monogenic immunodeficiency diseases. *Stem Cells Transl Med*. 2014;3:636-642.

27. High KA. Clinical gene transfer studies for hemophilia B. *Semin Thromb Hemost*. 2004;30:257-267.
28. Branca MA. Gene therapy: cursed or inching towards credibility? *Nat Biotechnol*. 2005;23:519-521.
29. Manno CS, Arruda VR, Pierce GF, et al. Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. *Nat Med*. 2006;12:342-347.
30. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2231-2239.
31. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2240-2248.
32. Aiuti A, Cattaneo F, Galimberti S, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med*. 2009;360:447-458.
33. Kochenderfer JN, Wilson WH, Janik JE, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood*. 2010;116:4099-4102.
34. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365:725-733.
35. Nathwani AC, Tuddenham EG, Rangarajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med*. 2011;365:2357-2365.
36. Biffi A, Montini E, Lorioli L, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science*. 2013;341:1233158.
37. Aiuti A, Biasco L, Scaramuzza S, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science*. 2013;341(6148):1233151.
38. Hwu WL, Muramatsu S, Tseng SH, et al. Gene therapy for aromatic L-amino acid decarboxylase deficiency. *Sci Transl Med*. 2012;4:134ra161.
39. Baker AT, Aguirre-Hernandez C, Hallden G, et al. Designer oncolytic adenovirus: coming of age. *Cancers (Basel)*. 2018;10(6):201.
40. Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet*. 2020;396:467-478.
41. Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. *N Engl J Med*. 2021;384:2187-2201.
42. Rahim AA, Wong AMS, Howe SJ, et al. Efficient gene delivery to the adult and fetal CNS using pseudotyped non-integrating lentiviral vectors. *Gene Ther*. 2009;16:509-520.
43. Snowball A, Chabrol E, Wykes RC, et al. Epilepsy gene therapy using an engineered potassium channel. *J Neurosci*. 2019;39:3159-3169.
44. Nathwani AC, Reiss UM, Tuddenham EG, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med*. 2014;371:1994-2004.
45. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384:403-416.
46. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;383:2603-2615.
47. Baranello G, Darras BT, Day JW, et al. Risdiplam in type 1 spinal muscular atrophy. *N Engl J Med*. 2021;384:915-923.
48. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med*. 2018;379:11-21.
49. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med*. 2021;385:493-502.
50. Gao K, Li J, Song H, et al. In utero delivery of mRNA to the heart, diaphragm and muscle with lipid nanoparticles. *Bioact Mater*. 2023;25:387-398.
51. Riley RS, Kashyap MV, Billingsley MM, et al. Ionizable lipid nanoparticles for in utero mRNA delivery. *Sci Adv*. 2021;7:eaba1028.
52. Sugano H, Matsumoto T, Miyake K, et al. Successful gene therapy *in utero* for lethal murine hypophosphatasia. *Hum Gene Ther*. 2012;23:399-406.
53. Armbruster N, Lattanzi A, Jeavons M, et al. Efficacy and biodistribution analysis of intracerebroventricular administration of an optimized scAAV9-SMN1 vector in a mouse model of spinal muscular atrophy. *Mol Ther Methods Clin Dev*. 2016;3:16060.
54. Dominguez E, Marais T, Chatauret N, et al. Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice. *Hum Mol Genet*. 2011;20(4):681-693.
55. Valori CF, Ning K, Wyles M, et al. Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy. *Sci Transl Med*. 2010;2:35ra42.
56. Foust KD, Wang X, McGovern VL, et al. Retraction note: rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. *Nat Biotechnol*. 2022;40(11):1692. doi:10.1038/s41587-022-01497-7
57. Rashnonejad A, Amini Chermahini G, Gunduz C, et al. Fetal gene therapy using a single injection of recombinant AAV9 rescued SMA phenotype in mice. *Mol Ther*. 2019;27:2123-2133.
58. Massaro G, Mattar CNZ, Wong AMS, et al. Fetal gene therapy for neurodegenerative disease of infants. *Nat Med*. 2018;24:1317-1323.
59. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol*. 2009;27:59-65.
60. Rahim AA, Wong AMS, Hoefer K, et al. Intravenous administration of AAV2/9 to the fetal and neonatal mouse leads to differential targeting of CNS cell types and extensive transduction of the nervous system. *FASEB J*. 2011;25:3505-3518.
61. Virgintino D, Errede M, Robertson D, et al. Immunolocalization of tight junction proteins in the adult and developing human brain. *Histochem Cell Biol*. 2004;122:51-59.
62. Saunders NR, Dziegielewska KM, Mollgard K, Habgood MD. Physiology and molecular biology of barrier mechanisms in the fetal and neonatal brain. *J Physiol*. 2018;596:5723-5756.
63. Mold JE, Michaëlsson J, Burt TD, et al. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science*. 2008;322:1562-1565.
64. Ivarsson MA, Loh L, Marquardt N, et al. Differentiation and functional regulation of human fetal NK cells. *J Clin Invest*. 2013;123:3889-3901.

65. McGovern N, Shin A, Low G, et al. Human fetal dendritic cells promote prenatal T-cell immune suppression through arginase-2. *Nature*. 2017;546:662-666.
66. Mishra A, Lai GC, Yao LJ, et al. Microbial exposure during early human development primes fetal immune cells. *Cell*. 2021;184(3394–3409):e3320.
67. Rackaityte E, Halkias J. Mechanisms of fetal T cell tolerance and immune regulation. *Front Immunol*. 2020;11:588.
68. Lutzko C, Kruth S, Abrams-Ogg AC, et al. Genetically corrected autologous stem cells engraft, but host immune responses limit their utility in canine alpha-L-iduronidase deficiency. *Blood*. 1999a;93:1895-1905.
69. Lutzko C, Omori F, Abrams-Ogg AC, et al. Gene therapy for canine alpha-L-iduronidase deficiency: in utero adoptive transfer of genetically corrected hematopoietic progenitors results in engraftment but not amelioration of disease. *Hum Gene Ther*. 1999b;10:1521-1532.
70. Waddington SN, McVey JH, Bhella D, et al. Adenovirus serotype 5 hexon mediates liver gene transfer. *Cell*. 2008;132: 397-409.
71. Waddington SN, Buckley SM, Nivsarkar M, et al. In utero gene transfer of human factor IX to fetal mice can induce postnatal tolerance of the exogenous clotting factor. *Blood*. 2003a;101:1359-1366.
72. Waddington SN, Mitrophanous KA, Ellard F, et al. Long-term transgene expression by administration of a lentivirus-based vector to the fetal circulation of immuno-competent mice. *Gene Ther*. 2003b;10:1234-1240.
73. Nivsarkar MS, Buckley SM, Parker AL, et al. Evidence for contribution of CD4+ CD25+ regulatory T cells in maintaining immune tolerance to human factor IX following perinatal adenovirus vector delivery. *J Immunol Res*. 2015;2015: 397879.
74. Sabatino DE, Mackenzie TC, Peranteau W, 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;12:12.
75. Davey MG, Riley JS, Andrews A, et al. Induction of immune tolerance to foreign protein via adeno-associated viral vector gene transfer in mid-gestation fetal sheep. *PLoS One*. 2017;12: e0171132.
76. Chan JKY, Gil-Farina I, Johana N, et al. Therapeutic expression of human clotting factors IX and X following adeno-associated viral vector-mediated intrauterine gene transfer in early-gestation fetal macaques. *FASEB J*. 2019; 33:3954-3967.
77. Rucker M, Fraites TJ Jr, Porvasnik SL, et al. Rescue of enzyme deficiency in embryonic diaphragm in a mouse model of metabolic myopathy: Pompe disease. *Development*. 2004;131:3007-3019.
78. Alonso-Ferrero ME, Valeri A, Yanez R, et al. Immunoresponse against the transgene limits hematopoietic engraftment of mice transplanted in utero with virally transduced fetal liver. *Gene Ther*. 2011;18(5):469-478.
79. Seppen J, van Til NP, van der Rijt R, Hiralall JK, Kunne C, Elferink RP. Immune response to lentiviral bilirubin UDP-glucuronosyltransferase gene transfer in fetal and neonatal rats. *Gene Ther*. 2006;13:672-677.
80. David A, Cook T, Waddington S, 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;14:353-364.
81. Conlon TJ, Mah CS, Pacak CA, et al. Transfer of therapeutic genes into fetal rhesus monkeys using recombinant adeno-associated type I viral vectors. *Hum Gene Ther Clin Dev*. 2016; 27:152-159.
82. Chand D, Mohr F, McMillan H, et al. Hepatotoxicity following administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal muscular atrophy. *J Hepatol*. 2021;74:560-566.
83. Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-year outcomes of valoctocogene roxaparvovec therapy for hemophilia A. *N Engl J Med*. 2023;388:694-705.
84. Pipe SW, Leebeek FWG, Recht M, et al. Gene therapy with etranacogene dezaparvovec for hemophilia B. *N Engl J Med*. 2023;388:706-718.
85. Pearson TS, Gupta N, San Sebastian W, et al. Gene therapy for aromatic L-amino acid decarboxylase deficiency by MR-guided direct delivery of AAV2-AADC to midbrain dopaminergic neurons. *Nat Commun*. 2021;12:4251.
86. Lacorazza HD, Flax JD, Snyder EY, Jendoubi M. Expression of human β–hexosaminidase α–subunit gene (the gene defect of Tay–Sachs disease) in mouse brains upon engraftment of transduced progenitor cells. *Nat Med*. 1996;2:424-429.
87. Ye X, Mitchell M, Newman K, Batshaw ML. Prospects for prenatal gene therapy in disorders causing mental retardation. *Ment Retard Dev Disabil Res Rev*. 2001;7:65-72.
88. Casal ML, Wolfe JH. Amphotropic and ecotropic retroviral vector viruses transduce midgestational murine fetal liver cells in a dual-chambered cocultivation system. *Gene Ther*. 1997;4:39-44.
89. Casal ML, Wolfe JH. In utero transplantation of fetal liver cells in the mucopolysaccharidosis type VII mouse results in low-level chimerism, but overexpression of beta-glucuronidase can delay onset of clinical signs. *Blood*. 2001;97:1625-1634.
90. Karolewski BA, Wolfe JH. Genetic correction of the fetal brain increases the lifespan of mice with the severe multisystemic disease mucopolysaccharidosis type VII. *Mol Ther*. 2006;14:14-24.
91. Shen JS, Meng XL, Maeda H, Ohashi T, Eto Y. Widespread gene transduction to the central nervous system by adenovirus in utero: implication for prenatal gene therapy to brain involvement of lysosomal storage disease. *J Gene Med*. 2004;6: 1206-1215.
92. Seppen J, van der Rijt R, Looije N, van Til NP, Lamers WH, Oude Elferink RP. Long-term correction of bilirubin UDP-glucuronyltransferase deficiency in rats by in utero lentiviral gene transfer. *Mol Ther*. 2003;8:593-599.
93. Meza NW, Alonso-Ferrero ME, Navarro S, et al. Rescue of pyruvate kinase deficiency in mice by gene therapy using the human isoenzyme. *Mol Ther*. 2009;17:2000-2009.
94. Rossidis AC, Stratigis JD, Chadwick AC, et al. In utero CRISPR-mediated therapeutic editing of metabolic genes. *Nat Med*. 2018;24:1513-1518.
95. Meertens L, Zhao Y, Rosic-Kablar S, et al. In utero injection of alpha-L-iduronidase-carrying retrovirus in canine mucopolysaccharidosis type I: infection of multiple tissues and neonatal gene expression. *Hum Gene Ther*. 2002;13:1809-1820.
96. Bose SK, White BM, Kashyap MV, et al. In utero adenine base editing corrects multi-organ pathology in a lethal lysosomal storage disease. *Nat Commun*. 2021;12:4291.

97. Senut MC, Gage FH. Prenatal gene therapy: can the technical hurdles be overcome? *Mol Med Today*. 1999;5:152-156.
98. Yang EY, Cass DL, Sylvester KG, Wilson JM, Adzick NS. Fetal gene therapy: efficacy, toxicity, and immunologic effects of early gestation recombinant adenovirus. *J Pediatr Surg*. 1999;34:235-241.
99. Riley J, Dave A, Zoltick P, Peranteau W. Pre-existing maternal humoral immunity to adeno-associated virus impairs fetal gene editing in a serotype-specific fashion. *Mol Ther*. 2022;30:1-592.
100. Themis M. Monitoring for potential adverse effects of prenatal gene therapy: genotoxicity analysis in vitro and on small animal models ex vivo and in vivo. *Methods Mol Biol*. 2012;891:341-370.
101. Themis M, Waddington SN, Schmitt M, et al. Oncogenesis following delivery of a non-primate lentiviral gene therapy vector to fetal and neonatal mice. *Mol Ther*. 2005;12:763-771.
102. Condiotti R, Goldenberg D, Giladi H, et al. Transduction of fetal mice with a feline lentiviral vector induces liver tumors which exhibit an E2F activation signature. *Mol Ther*. 2014;22:59-68.
103. Waddington SN, Nivsarkar MS, Mistry AR, et al. Permanent phenotypic correction of hemophilia B in immunocompetent mice by prenatal gene therapy. *Blood*. 2004;104:2714-2721.
104. Chandler RJ, LaFave MC, Varshney GK, et al. Vector design influences hepatic genotoxicity after adeno-associated virus gene therapy. *J Clin Invest*. 2015;125:870-880.
105. Howe SJ, Mansour MR, Schwarzwaelder K, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest*. 2008;118:3143-3150.
106. King D, Shakespeare T, Nicholson R, Clarke A, McLean S. Risks inherent in fetal gene therapy. *Nature*. 1999;397:383.
107. Park PJ, Colletti E, Ozturk F, et al. Factors determining the risk of inadvertent retroviral transduction of male germ cells after in utero gene transfer in sheep. *Hum Gene Ther*. 2009;20:201-215.
108. Lee CC, Jimenez DF, Kohn DB, Tarantal AF. Fetal gene transfer using lentiviral vectors and the potential for germ cell transduction in rhesus monkeys (*Macaca mulatta*). *Hum Gene Ther*. 2005;16:417-425.
109. Schuettrumpf J, Liu JH, Couto LB, et al. Inadvertent germline transmission of AAV2 vector: findings in a rabbit model correlate with those in a human clinical trial. *Mol Ther*. 2006;13:1064-1073.
110. Rangarajan S, Walsh L, Lester W, et al. AAV5-factor VIII gene transfer in severe hemophilia A. *N Engl J Med*. 2017;377:2519-2530.
111. Thomsen G, Burghes AHM, Hsieh C, et al. Biodistribution of onasemnogene abeparvovec DNA, mRNA and SMN protein in human tissue. *Nat Med*. 2021;27(10):1701-1711.
112. McKay TR, Rahim AA, Buckley SM, et al. Perinatal gene transfer to the liver. *Curr Pharm Des*. 2011;17:2528-2541.
113. Hamdan MA, El-Zoabi BA, Begam MA, Mirghani HM, Almalik MH. Antenatal diagnosis of pompe disease by fetal echocardiography: impact on outcome after early initiation of enzyme replacement therapy. *J Inherit Metab Dis*. 2010;33-(Suppl 3):S333-S339.
114. Yozgat AK, Erdem AY, Kaçar D, Özbek N, Yaralı N. Pyruvate kinase deficiency misdiagnosed as congenital dyserythropoietic anemia type i. *Hematol Transfus Cell Ther*. 2020;42:75-76.
115. Nakamura-Utsunomiya A, Okada S, Hara K, et al. Clinical characteristics of perinatal lethal hypophosphatasia: a report of 6 cases. *Clin Pediatr Endocrinol*. 2010;19:7-13.
116. Daykin EC, Ryan E, Sidransky E. Diagnosing neuronopathic Gaucher disease: new considerations and challenges in assigning Gaucher phenotypes. *Mol Genet Metabol*. 2021;132:49-58.
117. de Laet C, Dionisi-Vici C, Leonard JV, et al. Recommendations for the management of tyrosinaemia type 1. *Orphanet J Rare Dis*. 2013;8:8.
118. Gilroy T. *The bourne legacy*. Universal Pictures; 2012.
119. Liley AW. Intrauterine transfusion of foetus in haemolytic disease. *Br Med J*. 1963;2:1107-1109.
120. Evans LL, Harrison MR. Modern fetal surgery-a historical review of the happenings that shaped modern fetal surgery and its practices. *Transl Pediatr*. 2021;10:1401-1417.
121. Rodeck CH, Kemp JR, Holman CA, Whitmore DN, Karnicki J, Austin MA. Direct intravascular fetal blood transfusion by fetoscopy in severe rhesus isoimmunisation. *Lancet*. 1981;1:625-627.
122. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling via the umbilical cord using a needle guided by ultrasound report of 66 cases. *Prenat Diag*. 1983;3:271-277.
123. Daffos F, Forestier F, Kaplan C, Cox W. Prenatal diagnosis and management of bleeding disorders with fetal blood sampling. *Am J Obstet Gynecol*. 1988;158:939-946.
124. Prefumo F, Fichera A, Fratelli N, Sartori E. Fetal anemia: diagnosis and management. *Best Pract Res Clin Obstet Gynaecol*. 2019;58:2-14.
125. Nakamura S, Ando N, Watanabe S, Akasaka E, Ishihara M, Sato M. Hydrodynamics-based transplacental delivery as a useful noninvasive tool for manipulating fetal genome. *Cells*. 2020;9(7). doi:10.3390/cells9071744
126. Nakamura S, Watanabe S, Ando N, Ishihara M, Sato M. Transplacental gene delivery (TPGD) as a noninvasive tool for fetal gene manipulation in mice. *Int J Mol Sci*. 2019;20(23). doi:10.3390/ijms20235926
127. Peranteau WH, Adzick NS, Boelig MM, et al. Thoracoamniotic shunts for the management of fetal lung lesions and pleural effusions: a single-institution review and predictors of survival in 75 cases. *J Pediatr Surg*. 2015;50:301-305.
128. Deprest JA, Benachi A, Gratacos E, et al. Randomized trial of fetal surgery for moderate left diaphragmatic hernia. *N Engl J Med*. 2021a;385:119-129.
129. Deprest JA, Nicolaides KH, Benachi A, et al. Randomized trial of fetal surgery for severe left diaphragmatic hernia. *N Engl J Med*. 2021b;385:107-118.
130. Sacco A, Simpson L, Deprest J, David AL. A study to assess global availability of fetal surgery for myelomeningocele. *Prenat Diagn*. 2018;38:1020-1027.
131. Greene NDE, Stanier P, Copp AJ. Genetics of human neural tube defects. *Hum Mol Genet*. 2009;18:R113-R129.
132. Adzick NS, Thom EA, Spong CY, et al. A randomized trial of prenatal versus postnatal repair of myelomeningocele. *N Engl J Med*. 2011;364(11):993-1004.
133. Iskandar BJ, Finnell RH. Spina bifida. *N Engl J Med*. 2022;387:444-450.
134. Horvei P, MacKenzie T, Kharbanda S. Advances in the management of α-thalassemia major: reasons to be optimistic. *Hematol Am Soc Hematol Educ Program*. 2021;2021:592-599.

135. Maisonneuve E, Sohier Lepine M, Maurice P, et al. Prenatal management of fetal anemia due to pyruvate kinase deficiency: a case report. *Transfusion*. 2023;63(1):257-262.
136. de Coppi P, Loukogeorgakis S, Götherström C, et al. Regenerative medicine: prenatal approaches. *Lancet Child Adolesc Health*. 2022;6:643-653.
137. Götherström C, David AL, Walther-Jallow L, Åström E, Westgren M. Mesenchymal stem cell therapy for osteogenesis imperfecta. *Clin Obstet Gynecol*. 2021;64:898-903.
138. Gotherstrom C, Westgren M, Shaw SW, et al. Pre- and postnatal transplantation of fetal mesenchymal stem cells in osteogenesis imperfecta: a two-center experience. *Stem Cells Transl Med*. 2014;3:255-264.
139. Suff N, Karda R, Diaz JA, et al. Cervical gene delivery of the antimicrobial peptide, human beta-defensin (HBD)-3, in a mouse model of ascending infection-related preterm birth. *Front Immunol*. 2020;11:106.
140. Swanson AM, Rossi CA, Ofir K, et al. Maternal therapy with Ad.VEGF-A(165) increases fetal weight at term in a Guinea-pig model of fetal growth restriction. *Hum Gene Ther*. 2016;27:997-1007.
141. Vaughan OR, Rossi CA, Ginsberg Y, 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:R344-r353.
142. Carr DJ, Wallace JM, Aitken RP, et al. Uteroplacental adenovirus vascular endothelial growth factor gene therapy increases fetal growth velocity in growth-restricted sheep pregnancies. *Hum Gene Ther*. 2014;25(4):375-384.
143. Spencer R, Ambler G, Brodszki J, 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. *BMC Pregnancy Childbirth*. 2017;17:43.
144. Rodeck CH. Fetoscopy guided by real-time ultrasound for pure fetal blood samples, fetal skin samples, and examination of the fetus in utero. *Br J Obstet Gynaecol*. 1980;87:449-456.
145. Verma J, Thomas DC, Sharma S, et al. Inherited metabolic disorders: prenatal diagnosis of lysosomal storage disorders. *Prenat Diagn*. 2015;35:1137-1147.
146. Dai M, Xiao B, Zhang H, et al. Biochemical and genetic approaches to the prenatal diagnosis of propionic acidemia in 78 pregnancies. *Orphanet J Rare Dis*. 2020;15:276.
147. Shi C, Li S, Gao Y, Deng Z, Hao H, Xiao X. Prenatal diagnosis of two common inborn errors of metabolism by genetic and mass spectrometric analysis of amniotic fluid. *Front Pediatr*. 2022;10:824399.
148. Fu F, Li R, Yu Q, et al. Application of exome sequencing for prenatal diagnosis of fetal structural anomalies: clinical experience and lessons learned from a cohort of 1618 fetuses. *Genome Med*. 2022;14:123.
149. Hillman SC, Willams D, Carss KJ, McMullan DJ, Hurles ME, Kilby MD. Prenatal exome sequencing for fetuses with structural abnormalities: the next step. *Ultrasound Obstet Gynecol*. 2015;45:4-9.
150. van den Veyver IB, Eng CM. Genome-wide sequencing for prenatal detection of fetal single-gene disorders. *Cold Spring Harb Perspect Med*. 2015;5(10). doi:10.1101/cshperspect.a023077
151. Camunas-Soler J, Lee H, Hudgins L, et al. Noninvasive prenatal diagnosis of single-gene disorders by use of droplet digital PCR. *Clin Chem*. 2018;64:336-345.
152. NHS England. National genomic test directory testing criteria for rare and inherited disease V4 October 2022 (official). 2022.
153. Emms A, Castleman J, Allen S, Williams D, Kinning E, Kilby M. Next generation sequencing after invasive prenatal testing in fetuses with congenital malformations: prenatal or neonatal investigation. *Genes (Basel)*. 2022;13(9). doi:10.3390/genes13091517
154. Sparks TN, Dugoff L. How to choose a test for prenatal genetic diagnosis: a practical overview. *Am J Obstet Gynecol*. 2023;228:178-186.
155. Wright CF, Campbell P, Eberhardt RY, et al. Genomic diagnosis of rare pediatric disease in the United Kingdom and Ireland. *N Engl J Med*. 2023;388(7):1559-1571.
156. Wu W, Zhou X, Jiang Z, et al. Noninvasive fetal genotyping of single nucleotide variants and linkage analysis for prenatal diagnosis of monogenic disorders. *Hum Genomics*. 2022;16:28.
157. Strauss KA, Farrar MA, Muntoni F, et al. Onasemnogene abeparvovec for presymptomatic infants with two copies of SMN2 at risk for spinal muscular atrophy type 1: the phase III SPR1NT trial. *Nat Med*. 2022;28(7):1381-1389.
158. Parks M, Court S, Bowns B, et al. Non-invasive prenatal diagnosis of spinal muscular atrophy by relative haplotype dosage. *Eur J Hum Genet*. 2017;25:416-422.
159. Rousseau AC, Riggan KA, Schenone MH, Whitford KJ, Pittock ST, Allyse MA. Ethical considerations of maternal-fetal surgery. *J Perinat Med*. 2022;50:519-527.
160. Sagar R, Almeida-Porada G, Blakemore K, et al. Fetal and maternal safety considerations for in utero therapy clinical trials: iFeTiS consensus statement. *Mol Ther*. 2020;28:2316-2319.
161. Siu MK, Cheng CY. The blood-follicle barrier (BFB) in disease and in ovarian function. *Adv Exp Med Biol*. 2012;763:186-192.
162. Kanatsu-Shinohara M, Lee J, Miyazaki T, Morimoto H, Shinohara T. Adenovirus-mediated gene delivery restores fertility in congenitally infertile female mice. *J Reprod Dev*. 2022;68:369-376.
163. Mattar CN, Nathwani AC, Waddington SN, 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;19:1950-1960.
164. Rich KA, Wier CG, Russo J, et al. Premature delivery in the domestic sow in response to in utero delivery of AAV9 to fetal piglets. *Gene Ther*. 2022;29(9):513-519.
165. Dave A, Berkowitz CL, Luks VL, et al. Comment on: premature delivery in the domestic sow in response to in utero delivery of AAV9 to fetal piglets. *Gene Ther*. 2023;30(3-4):232-235.
166. Innes AM, Seargeant LE, Balachandra K, et al. Hepatic carnitine palmitoyltransferase I deficiency presenting as maternal illness in pregnancy. *Pediatr Res*. 2000;47:43-45.
167. Ramanathan R, Ibdah JA. Mitochondrial dysfunction and acute fatty liver of pregnancy. *Int J Mol Sci*. 2022;23(7). doi:10.3390/ijms23073595
168. Spronsen FJ, Smit GP, Erwich JJ. Inherited metabolic diseases and pregnancy. *BJOG*. 2005;112:2-11.
169. Strong C. Regulatory and ethical issues for phase I in utero gene transfer studies. *Hum Gene Ther*. 2011;22:1323-1330.

170. Johansson M, Hermerén G, Sahlin NE. Paternal consent in prenatal research: ethical aspects. *Med Health Care Philos*. 2020;23:325-331.
171. Roybal JL, Moldenhauer JS, Khalek N, et al. Early delivery as an alternative management strategy for selected high-risk fetal sacrococcygeal teratomas. *J Pediatr Surg*. 2011;46:1325-1332.
172. Sheppard M, Spencer RN, Ashcroft R, et 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;47(4):484-491.
173. Schwab ME, Brown JEH, Lianoglou B, et al. Fetal therapies and trials for lysosomal storage diseases: a survey of attitudes of parents and patients. *Orphanet J Rare Dis*. 2022;17(1):25.
174. Fox JL. Future tense for in utero gene therapy. *Nat Biotechnol*. 1998;16:1002-1003.
175. Iglesias-Lopez C, Agusti A, Vallano A, Obach M. Current landscape of clinical development and approval of advanced therapies. *Mol Ther Methods Clin Dev*. 2021;23:606-618.
176. Lee S, Lee JH. Cell and gene therapy regulatory, pricing, and reimbursement framework: with a focus on South Korea and the EU. *Front Public Health*. 2023;11:1109873.
177. Chilcott EM, Muiruri EW, Hirst TC, Yanez-Munoz RJ. Systematic review and meta-analysis determining the benefits of in vivo genetic therapy in spinal muscular atrophy rodent models. *Gene Ther*. 2022;29(9):498-512.
178. Weiss C, Ziegler A, Becker LL, et al. Gene replacement therapy with onasemnogene abeparvovec in children with spinal muscular atrophy aged 24 months or younger and bodyweight up to 15 kg: an observational cohort study. *Lancet Child Adolesc Health*. 2022;6:17-27.
179. Day JW, Finkel RS, Chiriboga CA, et al. Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of SMN2 (STR1VE): an open-label, single-arm, multicentre, phase 3 trial. *Lancet Neurol*. 2021;20:284-293.
180. Sheridan C. For hemophilia and thalassemia, a new era of 'one-and-done' gene therapies has arrived. *Nat Biotechnol*. 2022;40:1531-1533.
181. Sevin C, Deiva K. Clinical trials for gene therapy in lysosomal diseases with CNS involvement. *Front Mol Biosci*. 2021;8:624988.
182. Schneider H, Faschingbauer F, Schuepbach-Mallepell S, et al. Prenatal correction of X-linked hypohidrotic ectodermal dysplasia. *N Engl J Med*. 2018;378:1604-1610.
183. Cohen JL, Chakraborty P, Fung-Kee-Fung K, et al. In utero enzyme-replacement therapy for infantile-onset Pompe's disease. *N Engl J Med*. 2022;387(23):2150-2158.

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