BRAIN
REVIEW ARTICLE

In utero therapy for spinal muscular atrophy: closer to clinical translation

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5q-Spinal muscular atrophy (SMA) has been a trailblazer in the development of advanced therapies for inherited diseases. SMA is an autosomal recessive disorder affecting mainly motor neurons in the anterior horn of the spinal cord and brainstem motor nucle but currently considered a systemic disease. Advances in understanding the genetics of SMA led to the development of disease-modifying therapies, either transferring a healthy version of *SMN1*, the causative gene absent or altered in SMA, or modulating *SMN2*, a highly homologous but less functional version of *SMN1*, present in all patients. After successful clinical trials, these approaches have resulted in three marketed therapies. Severe SMA, 'type I', is the most common type and is considered both a developmental arrest and neurodegenerative disorder. As pathology starts during fetal life in type I patients, a cure is unlikely even when treatment is started shortly after birth in the pre- or mildly symptomatic state. *In utero* fetal therapy offers the opportunity to mitigate further or possibly prevent manifestations of the disease.

This review discusses clinical and developmental aspects of SMA, the advanced therapies approved (gene therapy, antisense oligonucleotide and small molecule compounds), and the rationale, options and challenges, including ethical and safety issues, to initiate *in utero* therapy. Looking beyond sporadic case reports of prenatal intervention, clinical trials of *in utero* SMA therapy can be envisaged and should be carefully designed and evaluated to move closer to clinical translation.

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Spinal muscular atrophy overview

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder mainly characterized by degeneration and loss of function of alpha motor neurons (MN) in the anterior horn of the spinal cord and brain stem motor nuclei. SMA is the second most common autosomal recessive inherited disorder with a global incidence of approximately 1/11 000.[1] In addition, untreated SMA is the leading cause of death from monogenic disease in infancy. The carrier frequency lies between 1/32 and 1/72 globally.[2] Symptoms in SMA patients derive from an insufficient amount of survival motor neuron protein (SMN), caused by loss-of-function pathogenic variants within or deletions of *SMN1*. SMN is encoded by two highly similar genes, *SMN1* and *SMN2*, located on chromosome 5. A single nucleotide variant (C → T at position c.840) in exon 7 results in the preferential exclusion of exon 7 in ∼90% of *SMN2* transcripts, which generates a truncated and unstable protein known as SMNΔ7.[3] Approximately 10% of functional full-length SMN, identical to that produced by *SMN1*, is produced from each *SMN2* copy, and this can partially compensate for *SMN1* loss and rescue what would otherwise be a lethal disorder. *SMN2* copy number correlates inversely with disease severity.[4]

The SMN protein is considered multifunctional and impacts various aspects of RNA metabolism. It is highly associated with the ribonucleoprotein (RNP) complex, regulating the biogenesis of small nuclear RNPs. Besides multiple interactions reported,[5] SMN also has a role in axonal transport,[6,7] RNA trafficking and the maintenance of neuromuscular junctions (NMJs).[8] Despite thorough research, it is not yet well understood why MNs are the most sensitive cells to decreased amounts of SMN.

SMA classification is based on age of onset of symptoms and the highest degree of motor function achieved.[9] The disease can be classified as types 0–IV, with type 0 being the most severe form. It should be noted that regardless of classification, manifestations and symptoms can vary in patients and the distinctions among the types are not absolute. However, the scheme remains relevant in the genetic era and provides useful clinical and prognostic information.

Type 0 SMA is the most severe and rarest of SMA forms.[10] It is associated with prenatal onset of symptoms such as decreased fetal movements. Patients suffer from arthrogryposis, as well as profound hypotonia, bulbar weakness (poor suck and swallow) and respiratory compromise at or soon after birth. Congenital heart defects and skin necrosis are also features of many type 0 cases, indicating systemic pathology from SMN deficiency. Their life expectancy is the lowest of all SMA types, typically with <1 month survival.[10] Type I SMA ('Werdnig-Hoffmann Disease') is the most common form, with more than 50% of SMA cases. Disease onset is before 6 months of age and by definition these individuals fail to gain independent sitting due to severe muscle weakness. Median survival in these children is less than 2 years of age, usually due to respiratory muscle dysfunction and respiratory failure.[11-13] The type II SMA phenotype (intermediate or 'Dubowitz' form) presents usually between 6 and 18 months of age with developmental arrest after sitting has been achieved, followed by a plateau, then a decline in motor skills.[14] While being able to sit upright and sometimes even stand using leg braces, affected children fail to achieve independent walking and are wheelchair-bound. They usually suffer from kyphoscoliosis and, if untreated, also from restrictive lung disease. Their cough and airway secretion clearing are progressively compromised. Even though the majority may survive into adulthood, they may require highly supportive management as gastrointestinal and respiratory complications increase.[15,16] Type III SMA ('Kugelberg–Welander' form) patients are able to walk independently during their early life. They show profound symptom heterogeneity, and patients are often misdiagnosed with myopathy or muscular dystrophy. The distribution pattern of muscle weakness is similar to type I and II SMA—with proximal > distal, lower > upper limb predominance—however, disease progression is much slower. As muscle weakness progressively increases, particularly of leg muscles, the use of a wheelchair may be required.[17] Type IV SMA is the mildest form of SMA, with symptoms usually arising around the third decade of life. The comorbidities resemble type III SMA, mostly with slowly progressive weakness of lower extremities. People affected have an average lifespan and retain the ability to walk throughout adulthood.[17]

While severity-based classification has clinical advantages, it is not always sufficient to provide prognostic information. Combining clinical features and *SMN2* copy number allows for a more precise prognosis. For example, infants with SMA type I and two copies of *SMN2* have less heterogeneity and follow a more predictable decline in survival (median of 10.5 months) than the overall group of type I patients.[13] Indeed, it is possible to correlate *SMN2* copy number with clinical characteristics of SMA, whereby copy number increases with decreasing disease severity. *SMN2* copy number variation is due to the plastic nature of this region of the genome. Patients with type 0 disease typically have only one *SMN2* copy, most type I cases show two *SMN2* copies, type II generally have three *SMN2* copies, whilst long-term walkers usually have four copies.[4] Determination of *SMN2* copy number is widely implemented to study SMA patients, provide some level of prognosis and is frequently used by payers to determine eligibility for SMN-directed therapy. However, not all *SMN2* copies are identical, and the actual structures and genomic sequences of *SMN2* copies are usually not considered for routine study.[18] In this regard, the detection of positive variants such as c.859G>C and c.835–44A>G as well as the effect of possible *SMN2-SMN1* hybrids may help to better determine the phenotype.[19,20] Exploration of the structure and quality of *SMN2* copies and equivalence in different patients may provide clues to the diversity in genotype-phenotype correlation.

Developmental aspects of spinal muscular atrophy: a rationale for why and when to treat the disease

Several studies have shown that SMA pathology in the neuromuscular system begins during prenatal development. In the typically developing fetus the neuromuscular system develops from the first interneural connections between motor neurons (MNs) and muscles, formed around 7–8 weeks of gestation and leading to flexion and trunk movements.[21] As neuronal connections are formed during development through axonal outgrowth, increased fetal movements can be seen even from 12 weeks. At around 15-weeks gestation, most neuromuscular junctions have been formed, with more specialized gross movement. At around 20-weeks gestation the fetus shows increased bilateral movements.[22] During fetal development, polyneuronal innervation of the muscle is present.[23] Until term, regression from polyneuronal into mononeuronal innervation in muscles occurs, which however differs among various muscles, with the diaphragm and intercostal muscles having the fastest regression.[24] Maturation of the motor unit (motor neuron–axon–neuromuscular junction–muscle) continues postnatally and is completed in early childhood.

SMN is ubiquitously produced in all tissues and throughout fetal development and postnatal life. The spatial and temporal requirements for this protein, however, differ throughout development. Motor neurons have the greatest need for SMN protein for proper development[25] and maintenance during fetal and early postnatal life,[26] less so in older children and adults. Immunoblot studies for SMN protein production have been performed on skeletal muscle, heart, kidney and brain tissues from postnatal and fetal controls and compared to fetal samples with SMA (predicted to be type I with two *SMN2* copies; mainly at approximately 14 weeks of gestation). It was shown that SMN protein levels were reduced in the SMA samples compared to control tissues, both pre- and postnatally, with the decrease correlating with severity.[27]

During the second and third trimester of gestation and the first 3 months after birth, the amount of SMN protein does not increase in SMA spinal cord samples, contrasting with the increase of SMN seen in age-matched controls, remaining 4-fold lower prenatally and 6-fold lower early postnatally.[26] It is likely that these periods of marked reduction in production of SMN protein in SMA during fetal and neonatal stages occur when developing motor neurons are most vulnerable and reliant upon this critical protein. The lack of sufficient SMN during early development appears to be the primary driver of MN dysfunction and death, although it has been observed that the most notable reduction of SMN protein levels happens in SMA skeletal muscle.[27,28] In control samples, the relative amounts of SMN in kidney, brain and heart were similar to skeletal muscle. However, the drop in postnatal tissues was not as severe, with kidney showing the smallest reduction.[27] Older patients with SMA appear to have a lesser need for SMN protein to sustain MNs, as evidenced by a much slower rate of decline in function and by neurophysiological testing. This is also supported by the observations that the disease-modifying therapies (DMTs) appear to have a lesser effect in older children and adults with milder impairment than in infants with more severe disease.[22] Biomarkers of disease activity, for example neurofilament levels in blood, are much higher in newborns (even if pre-symptomatic) and infants than in older individuals with more indolent disease.[29-31]

SMA also affects sensory-motor connectivity in mice and zebrafish models.[32,33] MN development has a direct influence on the development of dorsal root ganglion (DRG) and Schwann cells.[25] In the severe SMA mouse model, impaired radial growth of motor axons and Schwann cell ensheathment identified during embryogenesis hindered neonatal motor axon function and caused fast degeneration of unsheathed axons.[25] Neonatal treatment with *SMN2* splice modifiers increased radial growth of myelinated axons; however, *in utero* treatment was required to restore axonal growth and associated maturation, preventing subsequent neonatal axon degeneration and enhancing motor axon function.[25] In zebrafish *Smn* mutants, Schwann cells do not wrap axons tightly and had expanded nodes of Ranvier. DRG neurons showed abnormally short peripheral axons and failure to divide.[33] Increased SMN production in MNs rescued both cell types, highlighting the cell-autonomous effect of SMN, secondary to the developmental MN defects. Thus, these observations in animal models may be relevant for human pre/early postnatal MN network development—both cell-autonomous and non-cell autonomous.

Multiple lines of study have investigated nerve and muscle development and pathologic changes in tissues from fetuses with SMA. Abnormal nuclei shape, high nucleoplasm density, an increase in DNA fragmentation and lower choline acetyltransferase (ChAT) levels have been reported in SMA MNs from fetuses with two *SMN2* copies, indicating abnormal apoptosis and impaired motor neuron development[28,34] Furthermore, axon size was reduced and varicosities could be detected. In neuromuscular junctions, acetylcholine receptor (AChR) disaggregation and an increase in presynaptic vesicles could be seen, indicating synaptic defects in SMA development.[8] In skeletal muscle, myotube diameter size was significantly decreased and slow myosin heavy chain (MHC) levels were decreased, while fast MHC production increased, reflecting a possible delay in muscle maturation.[8,35,36] In addition, the early studies of muscle histopathology in SMA type I infants identified fetal-like muscle fibres.[37] The combination of all these neuropathologies suggests an element of developmental arrest even before denervation atrophy changes are evident.[34] These findings, summarized in Pérez-García *et al.*[22] and Tizzano and Zafeiriou,[38] point to prenatal effects of SMA on the whole neuromuscular system.

Numerous studies have shown that in humans, the higher the *SMN2* copy number, the more full-length SMN protein is produced and, in general, the milder the associated SMA phenotype. Some variants in *SMN2* have been identified that further alter SMN production, e.g. c.859G > C, which increases SMN production and is associated with a milder course of disease.[39] Thus, *SMN2* copy number may serve as a more general prognostic biomarker for disease severity that reflects pathological implications of SMN deficiency (Fig. 1), in addition to motor function impairment. Importantly, there could be a threshold for SMN levels below which the pathology in the SMA fetus emerges and above which the disease process is held in check. Individuals with one copy have cardiac malformation and arthrogryposis, congenital anomalies that are potentially detectable by ultrasound assessment during the prenatal period.[10] Fetuses with one and two *SMN2* copies present with early neuropathological findings[22] with early symptom onset within the first 3 months of life.[13] On the other hand, fetuses with three and four copies are predicted to produce SMN above the threshold to initiate fetal pathology and instead disease manifests solely in the postnatal period, usually after 6 months of life in most patients with three copies. There is no evidence currently that demonstrates prenatal MN loss or abnormal axonal/NMJ development at birth in individuals with three or more copies of *SMN2*. Indeed, in contrast with fetuses with two *SMN2* copies, fetuses with three *SMN2* copies do not show a detectable alteration in the developmental pattern, suggesting a wider therapeutic window to intervene.[8] However, these observations should be taken carefully until more data on neurofilaments, CMAP and *SMN2* variants are available from SMA neonates with three *SMN2* copies. In general, patients with four copies may remain without symptoms for years, but some cases may present earlier.[40]

The regulation of SMN production in different tissues and organs is more complex in humans and species with two *SMN* genes than in rodents and other sub-primate species that have a single copy of the *SMN* gene. In humans, it has been demonstrated that most of the complete messenger RNA and protein in the spinal cord during development originate from *SMN1*, whereas in other tissues such as kidney and muscle, the proportion is more balanced between both genes.[28] SMN production in the spinal cord is dramatically reduced upon loss of *SMN1*, and *SMN2* is not able to provide a sufficient compensatory source of full-length protein due to the biased expression towards *SMN1* in this tissue. Thus, regulatory mechanisms of *SMN2* expression (including transcript processing) during fetal development should be considered if an early therapeutic intervention such as gene addition therapy is to be administered.

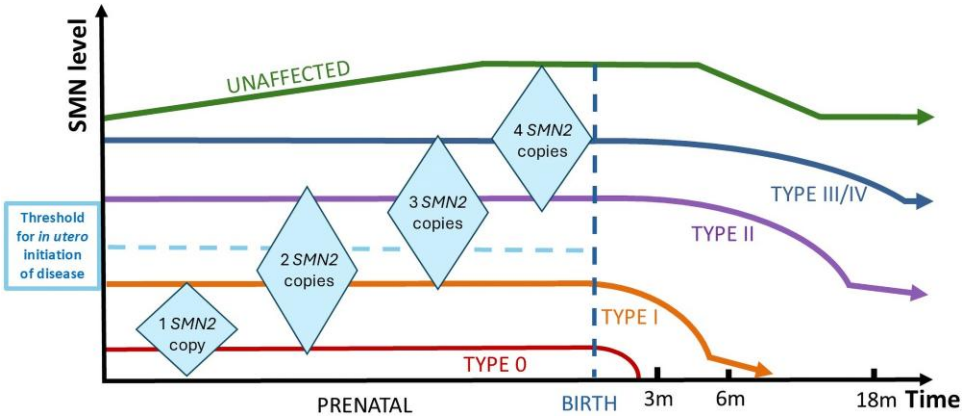

Figure 1 Possible threshold for development of prenatal spinal muscular atrophy neuropathology and extraneuronal manifestations. The amount of SMN protein present in spinal muscular atrophy (SMA) correlates with *SMN2* copy number, a key factor in disease severity. Generic SMN levels are presented in the figure in two ways in SMA: as straight lines for average levels over time, and as diamonds to account for person-to-person variation, which in some cases, mainly with two or three *SMN2* copies, may overlap considerably. SMN requirements are highest in the third trimester of fetal development and approximately the first 3 months after birth, then reduce (see green curve, based on Pérez-García *et al.*[22] and Ramos *et al.*[26]) Manifestations in individuals with SMA appear depending on the motor neuron pool, generally inversely related to *SMN2* copy number. Cases with one *SMN2* copy usually develop type 0 SMA, with cardiac malformation and arthrogryposis, anomalies potentially detectable during the prenatal period. Other problems, such as severe hypotonia, weakness, respiratory insufficiency and vascular issues, are noted at birth as part of congenital SMA. In fetuses with two *SMN2* copies, predicted to have type I disease, it has been demonstrated that pathology is present in the prenatal period, although the consequences usually manifest after birth (<3 months). Fetuses with three and four copies may produce SMN above the threshold for prenatal pathology (horizontal dotted blue line), and thus disease may develop and manifest just in the postnatal period, usually after several months of life (>6 months in type II, >18 months in type III disease). Patients with four copies may remain without symptoms for several months or years. Predicted prenatal levels of SMN are represented according to *SMN2* copy number.[4] Levels after birth represent the evolution according to natural history.[11,13] m = months; SMN = survival motor neuron protein.

Defining pre-symptomatic spinal muscular atrophy

SMA can be identified pre-symptomatically by using prenatal or newborn-specific genetic testing. Most of the cases diagnosed show *SMN1* bi-allelic absence. Rare variants in the SMN1 gene have been described in SMA patients with compound heterozygous pathogenic variants.[41,42] Non-invasive prenatal screening methods have emerged that use haplotyping of couples at risk.[43,44] Parents identified as carriers can choose to undergo *in vitro* fertilization and pre-implantation genetic diagnosis, or intrauterine insemination with sperm of a donor who is not an SMA carrier to avoid the risk of SMA.[45] In pregnancies from couples with previous family history, the fetus can be screened by chorionic villus sampling (CVS) at 10–14 weeks or amniocentesis at 16–20 weeks to determine whether SMA is likely. Pregnancy interruption was usually the outcome after diagnosis of SMA; however, with the current effective therapies, there might be a shift in decision-making towards pregnancy continuation.[46] A confirmed diagnosis of SMA in prenatal screening could create further patient demand for treatment, even prenatally (see later).

Genetic, electrophysiological and biochemical tests can serve as prognostic biomarkers that predict the future phenotype of groups of individuals with SMA upon diagnosis, both symptomatic and pre-symptomatic.[47,48] Genetic tests include *SMN1* deletion (for diagnosis) and *SMN2* copy number, which can also be performed *in utero* from DNA derived from chrorionic villi or an amniocentesis specimen, but in isolation is not fully predictive of the phenotype in an individual patient. For example, two copies of *SMN2* are approximately 90% predictive of a type 1 phenotype.[4] But, as mentioned, uncommon variants in the SMN2 gene, such as c.859G > C and c.835-44A > G, predict a milder phenotype. Electrophysiological tests include measurement of the compound motor action potential, motor unit number estimate and electrical impedance myography, which can serve as predictive, prognostic and pharmacodynamic biomarkers in postnatal individuals, but cannot be performed *in utero*.[48] Biochemical prognostic and pharmacodynamic biomarkers include SMN protein level (blood or CSF), neurofilament levels (blood or CSF) and creatine kinase levels (blood) and could be tested in a fetal blood sample.[49] *In utero* sampling of umbilical cord blood could be used to test for fetal drug level and for safety monitoring, but it involves a small but not negligible risk[49] and would need careful medical and ethical consideration in a protocol for prenatal therapy.

In newborn children with an asymptomatic phenotype, it is important to assess their disease characteristics if an absence of *SMN1* has been detected by screening. Traits associated with the typical SMA phenotype should be considered, e.g. manifestations in the neuromuscular system (such as hypotonia, decreased movement, reduced/absent tendon reflexes, feeding difficulty) and respiratory system (hypoxaemia, hypercapnia or abnormal breathing pattern). Even without such clinical findings, electrophysiological defects [reduced motor unit number estimation (MUNE) or compound muscle action potential (CMAP)][38] or a fluid biomarker (such as an elevated neurofilament level)[44] could indicate loss or non-functional MNs undergoing active but rather silent denervation. Collateral sprouting and reinnervation have been identified in symptomatic patients with SMA and may be present during fetal development.[50-52] We propose that this mechanism, in addition to polyneuronal innervation, could compensate for MN loss or dysfunctional neurons during this clinically silent period. As newborn screening for SMA is implemented more widely, data collection from structured patient registries will allow a thorough assessment of asymptomatic SMA and its baseline characteristics.[53,54] Eventually, by combining clinical and biomarker assessments, it may be possible to predict whether a neonate is truly asymptomatic

or pre-symptomatic and without evidence of silent burden of disease (see later and Fig. 2).

There is growing evidence that some babies identified shortly after birth with genetically confirmed SMA and two copies of *SMN2* may not be completely asymptomatic in the first weeks of life. Indeed, early areflexia may be present as well as hypoxaemia or hypercapnia, before further signs such as hypotonia, diaphragmatic breathing and tongue fasciculations appear.[56,57] Results of a pilot trial of newborn screening in Italy showed that ~40% of such newborns may already have manifestations at first assessment, before treatment is initiated.[58] Indeed, prodromal symptoms should be investigated and properly documented in all neonates with genetically confirmed SMA. It is possible that biomarkers such as CMAP/MUNE, neurofilament proteins[48,59] and *SMN2* variants[19] may inform on clinically inapparent disease progression when a neonate with SMA is detected by newborn screening. This information may be very important to initiate early treatment of newborns in SMA. Finkel and Benatar[55] proposed a classification of infants with SMA identified by newborn screening, recognizing an initial clinically silent stage followed by a pauci-symptomatic phase with a later phenoconversion in which the patient becomes truly symptomatic (summarized and further elaborated in Fig. 2).

Brief overview of spinal muscular atrophy therapeutics

Improvements in our understanding of the underlying defects in SMA remain critical for the development of therapeutic approaches. It is now appreciated that SMA is more than a disease of MNs. The model proposed by Mentis[32] includes sensory neurons and interneurons of the motor unit circuit. This is consistent with the observation that individuals with SMA type 0 have impaired sensory nerves and proprioceptive sensory neurons in the dorsal root ganglia (DRG) and may be vulnerable to overexpression of SMN.[60] Considerable evidence has emerged that SMN deficiency can create pathological changes and inflammation in neurons, but also glial cells throughout the CNS, as well as peripheral organs and non-neuronal cells.[8,34,35,61] Non-cell autonomous pathology is an increasingly studied topic, the clinical relevance of which is still not fully understood.[62] Therapies for SMA, which can be categorized into SMN-dependent and SMN-independent, vary in their proficiency to target different tissues. The absence or alteration of *SMN1* can be compensated to some extent by SMN protein produced from existing *SMN2* copies, which therefore provides a target for therapeutic approaches. SMN-dependent therapeutic approaches have demonstrated efficacy in several types of SMA, particularly in type I SMA patients, with impressive motor achievements. In other SMA types, the outcome of treatment is often stability and non-progression of motor dysfunction, which is very much appreciated by those affected.[63] Other promising treatment approaches, focused on non-SMN-targets in tissues outside the CNS, such as muscle, are currently in clinical trials.

The first marketed therapeutic for SMA was nusinersen (Spinraza®), an antisense oligonucleotide (ASO) which specifically inhibits the ISS-N1 motif in intron 7 of *SMN2* pre-mRNA to promote exon 7 inclusion and hence increased production of full-length SMN protein. Preclinical studies indicated that nusinersen had to be injected very early in neonatal development for maximal efficacy. Nusinersen is unable to penetrate the blood–brain barrier (BBB), and therefore periodic intrathecal injections need to be performed. Such treatment may not address non-CNS aspects of SMA pathology, as leakage of the ASO outside the CNS is very scarce. Promotion of exon 7 inclusion in *SMN2* transcripts can also be achieved with an oral compound, risdiplam (Evrysdi®), more recently approved for marketing. Risdiplam has the potential benefit of systemic delivery of drug to non-neuronal organs that may benefit from an increase in SMN protein production, e.g. muscle.

An alternative to *SMN2* upregulation is *SMN1* gene replacement, implemented in the clinic by using onasemnogene abeparvovec (OA; Zolgensma®), an adeno-associated virus serotype 9 (AAV9) vector driven by the ubiquitous and stable CBA promotor, in a single intravenous infusion. AAV9 is able to cross the BBB during early development, allowing transgene delivery to both peripheral tissues and the CNS, where it shows an avidity for neurons. Given that SMN is ubiquitously produced, this is expected to provide additional benefit. Concerns about long-term efficacy, safety, durability of effect and toxicity are still present.[64,65]

There are currently only limited data on the treatment of premature babies. A case report on a 30-week-old infant (two *SMN2* copies), who was treated with nusinersen as a bridge-therapy until appropriate use of OA, demonstrates the feasibility of premature therapy-delivery.[66] A German study also reported successful risdiplam implementation as a bridge before OA therapy in premature infants.[67] Another case of 30-week-old twins (one SMN2 copy) reported the favourable outcome of OA delivery enabled by premature delivery.[68] It is worth noting that in the last case, the single *SMN2* copy had a positive modifier c.835-44A > G that should have influenced the phenotype.

Combination therapy has been explored in clinical trials (Table 1) and clinical practice, and it is anticipated that there will be further scope for it as more information becomes available.[69] Possible ways in which this could be attempted are outlined in Fig. 3. A proposed classification for different therapeutic combinatorial approaches has recently been published, including switch therapies, bridge therapy, added on or combined.[70]

There is published evidence of combined SMN-dependent therapies in SMA. A retrospective report on dual treatment of five children with type I SMA who received nusinersen and OA resulted in overall improvement with no adverse effects, showing that combination therapy was tolerated in these patients.[71] Seven SMA type I patients who received both treatments (mostly nusinersen before OA) were assessed for motor function trajectories, ventilation hours and cough assist sessions. The second therapy improved ventilation, but there was no improvement in motor function trajectory compared to single therapy, pointing to the importance of early treatment.[72] A report of four cases of type I SMA initially treated with OA (one of them also with nusinersen) who later received risdiplam treatment showed increased therapeutic benefits from the combination therapy, with no significant adverse effects.[73] The multinational RESTORE registry captures 15-year real-world data on patients treated with the three DMTs, focusing mainly on OA.[74] The majority of these patients are treated with a splicing modifier prior to receiving OA, or the former is added on after OA administration. Informative safety and efficacy data are expected from this registry. The SMArtCARE registry in Germany, Austria and Switzerland will serve a similar function.[54]

While the vast majority of DMTs are indicated in patients with two or three *SMN2* copies, treatments for one- and four-copy individuals have also been reported, although they are often excluded by payer coverage policies.[75,76] One patient with SMA type 0 was treated with both nusinersen and OA, and although modest motor improvements were achieved, with continued motor gain at age

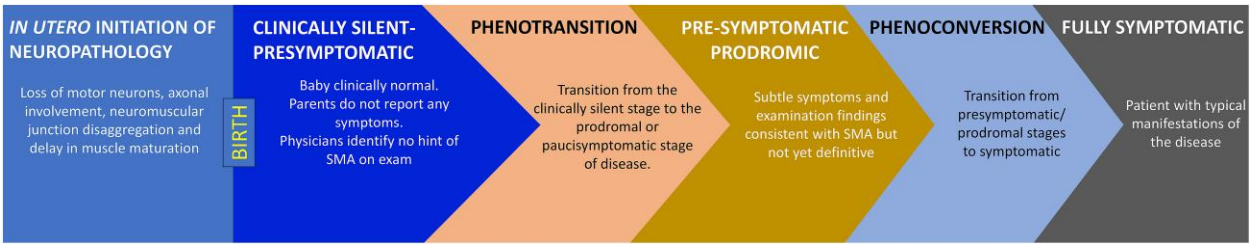

Figure 2 Evolution of clinical stages in spinal muscular atrophy babies with two *SMN2* copies from fetal stage to postnatal period. Most fetuses with spinal muscular atrophy (SMA) and two copies of *SMN2* are predicted to have severe type I disease with prenatal initiation of neuropathology. In cases detected by newborn screening, the three main stages observable are clinically silent—presymptomatic, prodromic and fully symptomatic. The disease evolution between stages occurs by phenotransition in the first case and phenoconversion in the second. In patients with three *SMN2* copies, the clinically silent phase varies but is typically at >3 months in duration and may be extended over years in four-copy individuals. Based on Finkel and Benatar[55] and Tizzano and Zafeiriou.[38]

13 months without regression of function, she remained profoundly weak with continued systemic complications from SMA, including chronic respiratory failure, dysphagia, congenital heart malformation, digit necrosis and diffuse macular rash.[75] Another patient with one copy of *SMN2* received early treatment with nusinersen at the age of 13 days and although mild motor improvement 2 months after treatment as well as minimal respiratory enhancement were achieved, tracheostomy at the age of 4 months was still required with increasing cardiac and autonomic dysfunction, ultimately leading to exitus at 5 months.[76]

Given that present SMN-dependent therapies are not able to provide a full cure, therapeutics that target SMN-independent pathways are also under investigation. While neuroprotective agents have shown no clear and convincing results in clinical trials,[77] it has been demonstrated in animal models that MNs in SMA show loss of excitatory input from primary sensory afferents and intermediate neurons, pointing to a neural circuit dysfunction.[32] A possible therapy is under investigation with the implant of epidural electrodes targeting sensory axons over the lumbosacral spinal cord (NCT054301139).[78] On the other hand, therapy in muscle, particularly with myostatin inhibitors, has become promising. Myostatin is a hormone that inhibits skeletal muscle growth. Serum myostatin levels are reduced in patients with SMA and may serve as an informative biomarker for progression of disease and response to an intervention.[79] Three clinical trials are currently in progress to examine the possible added benefit from drugs that inhibit myostatin (Table 1).

General perspective of *in utero* (gene) therapy

Advancements in maternal–fetal medicine and gene sequencing have greatly improved early detection and accurate diagnosis of genetic disorders during pregnancy, paving the way for a new therapeutic field, *in utero* molecular and cellular therapeutics. There are currently several approaches to *in utero* therapy, with the potential to treat single-gene pathogenic variants, such as blood transfusion, enzyme replacement therapy (ERT) and stem cell transplantation, offering the opportunity to intervene before irreversible disease onset. For anatomical conditions detected prenatally, fetal surgery can have real impact to improve outcomes[80]: open spina bifida disorders and congenital diaphragmatic hernia have been treated successfully.[81]

Anecdotal evidence of treatment during pregnancy with biologics for maternal atopic diseases seems encouraging in terms of lack of detrimental effects on maternal and fetal outcomes.[82] On-going clinical trials of fetal treatment with protein therapy (enzyme replacement for lysosomal diseases, recombinant ectodysplasin protein receptor–binding domain for X-linked hypohidrotic ectodermal dysplasia) and cell therapy [mesenchymal stem cells for osteogenesis imperfecta (BoostB4), maternal bone marrow haematopoietic stem cells for alpha thalassaemia major] will be highly relevant for clinical translation of any *in utero* therapy for SMA.[83,84] The PEARL clinical trial (NCT04532047) aims to treat lysosomal storage diseases that already have US Food and Drug Administration (FDA)-approved postnatal treatments, with *in utero* intervention. Eight diseases are included in this trial, including Mucopolysaccharidosis types 1, 2, 4a, 6 and 7, as well as infantile-onset Pompe disease, neuronopathic Gaucher disease and lysosomal acid lipase deficiency.

Recently, *in utero* ERT for a fetus with Pompe disease has been reported. Six umbilical cord injections at 2-week intervals were administered between 24 weeks 5 days gestation through to 36 weeks 5 days gestation. The benefit of prenatal therapy was reportedly superior in this patient in comparison with treatment after birth in their siblings. The child did not develop cardiac hypertrophy, had normal creatine kinase levels and no evidence of glycogen build-up in the placenta, suggesting that fetal pathophysiology had been prevented by the prenatal therapeutic approach.[85]

Successful *in utero* protein treatment for X-linked hipohydrotic dysplasia has been reported for six patients.[86,87] A recombinant form of ectodysplasin A1 (EDA1), Fc-EDA, that includes the receptor-binding domain, was administered at gestational week 26 and later by injection in the amniotic fluid. Treated children demonstrate sweat gland development and pilocarpine-inducible sweating and have not had hyperthermic episodes during the summer nor any significant eye, nose, throat or respiratory issues. They also have more permanent teeth than untreated siblings, even though oligodontia is not fully corrected. The follow-up has reached 6 years for the first children treated and shows possible dose-dependency. These results are in stark contrast with postnatal treatment with the same protein, which did not lead to patient improvements. To confirm and extend these prenatal treatment data, the phase 2 clinical trial EDELIFE has been initiated (NCT04980638).[88]

Furthermore, progress in gene therapy has been steady. Currently, over 700 active gene therapy investigational drug applications have been reported (https://www.nhlbi.nih.gov/health-topics/genetic-therapies). While postnatal applications of gene therapy are well developed, *in utero* gene therapy (IUGT), the delivery of relevant genes to the developing fetus to correct genetic

Table 1 Clinical trials in which combination therapies are currently investigated in spinal muscular atrophy

Clinical trial (NTC number)	Trial phase	Baseline therapy	Intervention	Mechanism of action	Patient criteria
RESPOND (NCT04488133)	Phase 4	Onasemnogene abeparvovec	Nusinersen	*SMN1* gene transfer + ASO to modulate *SMN2*	2–36 months
ASCEND (NCT05067790)	Phase 3b	Risdiplam (for at least 6months)	High-dose nusinersen	oral *SMN2* modifier + ASO	15–50 years
TOPAZ (NCT03921528)	Phase 2	Nusinersen treatment initiated in different cohorts with different ages	Apitegromab (IV) every 4 weeks	ASO + anti-promyostatin antibody	>2 years
SAPPHIRE (NCT05156320)	Phase 3	Nusinersen or risdiplam	Apitegromab (IV) every 4 weeks for 1 year	ASO or oral *SMN2* modifier + anti-promyostatin antibody	2–21 years
ONYX (NCT05626855)	Phase 3 (open-label extension)	Nusinersen or risdiplam	Apitegromab (IV) every 4 weeks for 2 years	ASO or oral *SMN2* modifier + anti-promyostatin antibody	>2 years + Completion of TOPAZ or SAPPHIRE
JEWELFISH (NCT03032172)	Phase 2	RG7800, olesoxime, nusinersen or onasemnogene abeparvovec	Risdiplam for 2 years	Any prior therapy + oral *SMN2* modifier	6 months–60 years
MANATEE (NCT03032172)	Phase 3	Risdiplam	Investigational anti-myostatin antibody	Oral *SMN2* modifier + anti-promyostatin antibody	2–25 years
RESILIENT (NCT05337553)	Phase 3	Nusinersen, risdiplam or previously treated with onasemnogene abeparvovec	Taldefgrobep alfa	Any SMN-dependent therapy + anti-promyostatin antibody	4–21 years

OA = onasemnogene abeparvovec; ASO = antisense oligonucleotide; NCT = National Clinical Trial.

defects, is yet to be applied clinically.[89] Prevention of disease onset, immune tolerance to the transgene product due to an immature immune system, increased vector biodistribution due to small body size and the possibility of transducing stem cells leading to permanent genetic correction are all potential beneficial features of this treatment modality.[90,91] However, proposals for the clinical application of *in utero* therapy in humans must be firmly rooted in established experience of therapeutic delivery to relevant animal models, including traditional drugs, gene therapies and cell therapies. Animal work should extend to large animals, with gestational age when administered, size, physiology, immune responses and delivery challenges more akin to humans. Risks to the developing fetus and the mother must be thoroughly assessed, as well as germline transmission. Both gene therapy and genome editing should be considered as therapeutic options. An initial path towards clinical application has been recently outlined.[83]

IUGT has been explored in a variety of animal models, including mice, guinea pigs, sheep, pigs and macaques. Diseases under study have included both inherited and idiotypic disorders: X-Linked hypohidrotic ectodermal dysplasia,[92] hereditary tyrosinemia type I,[92,93] thalassaemia,[94,95] haemophilia,[96-98] Gaucher disease,[99] Angelman syndrome[100] and fetal growth restriction.[101-103]

As an example, neuropathic Gaucher disease (nGD) is characterized by a mutation in the *GBA* gene, which leads to glucocerebrosidase enzyme deficiency. The lethal type II form affects newborn children, beginning with prenatal pathology and symptoms starting to develop around 3–6 months of age, with no currently available therapy. A study in nGD mice using AAV9 vector to deliver the *GBA* gene resulted in increased expression of neuronal glucocerebrosidase, which eliminated neurodegeneration and led to a large decrease in neuroinflammation and improved survival rate of mice.[104] Importantly, prenatal administration was more effective at improving the phenotype than neonatal delivery. In the search for less invasive ways of treating genetic disorders before birth, scientists working in mice with Angelman syndrome have found that delivering ASO to the fetal brain through amniotic fluid is as effective as delivering it via CSF.[99]

Currently, one of the biggest limitations of postnatal gene therapy is the natural immune response of the patient to the viral vector and/or the transgene product. A significant benefit of IUGT is the tolerogenic immune system of the fetus, but pre-existing maternal antibodies crossing the placenta may limit IUGT. However, this can be avoided either by conducting maternal antibody screening or carrying out IUGT at the beginning of the second trimester of pregnancy, before significant maternal antibody transfer.[105] This could, for instance, be of benefit to avoid anti-AAV antibodies, which have been detected in newborns.[106] Haemophilia B can be complicated by the presence of antibodies to human factor IX (hF.IX). A study in which IUGT was carried out using intramuscular injection of AAV1-hF.IX vector in haemophilia B fetal mice and subsequent postnatal challenge showed preserved expression of hF.IX with absence of hF.IX antibodies. In comparison, mice treated with AAV postnatally and receiving the challenge developed hF.IX antibodies, therefore showing the advantage of preempting the immune response.[107] In a more recent study, IUGT was used to achieve immune tolerance to foreign protein using AAV-*GFP* vectors in fetal sheep.[108] The treated sheep developed postnatal immune tolerance to GFP; however, such tolerance did not extend to AAV vector capsid proteins, with serotype-specific neutralizing antibodies being detected. Durable hepatic transgene expression was also serotype-dependent. Another study also used AAV9-*GFP* vector delivery in fetal sheep, whereby widespread distribution of vector genomes was present in all tissues harvested at term. Transduction in maternal ewes and also germline transduction were observed.[109]

IUGT may also enhance the chance of transducing stem and progenitor cells, as their presence is increased in a multitude of

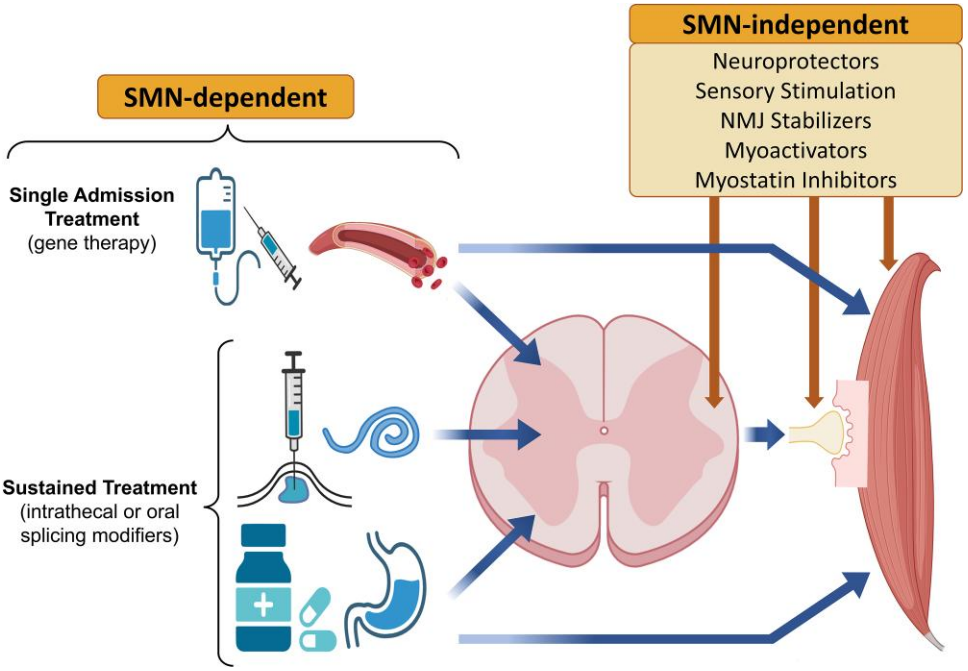

Figure 3 Various approaches to treat spinal muscular atrophy (SMN-dependent and SMN-independent) could be complementary. Data from clinical trials and clinical practice will be used to assess whether a multi-pronged approach results in beneficial effects. See Table 1: Clinical trials, for combination therapies currently investigated in spinal muscular atrophy (SMA). At present, combination therapy has yet to be experimentally investigated in the prenatal stage in terms of clinical research. SMN = survival motor neuron protein. Created in BioRender. Tizzano, E. F. Lindner, G. (2025) https://BioRender.com/o5kmjq7.

developing fetal organs. To maximize efficiency, the vector serotype and vector delivery route should be extensively explored. In a study on BALB/c mice fetuses at 14–15 days gestation, lentiviral vectors with different pseudotypes were used. The comparison of intramuscular and intrahepatic injection with the various vectors showed different patterns of expression of the marker gene.[110] Intrahepatic injection mostly led to marker gene expression in liver and heart, while most organs were negative; however, distinct positive cells could be seen in the lung and muscles near the injection site. Intramuscular injection led to primary expression in the targeted muscle groups and the heart, with isolated cells being visible in the liver.[110]

While fetal gene therapy offers opportunities for the actual prevention of genetic disorders, there are potential risks involved. First, gene therapy might disrupt normal fetal development as prenatal production of a particular transgenic protein might have unknown side effects, even if postnatal therapeutic function has been established.[111] Furthermore, gene therapy might increase the risk of unintentional germline transfer, even if animal studies to date have not demonstrated significant germline effects.[108] Nonetheless, if prenatal gene therapy is carried out after the 7th week of gestation, when germ cells are already compartmentalized, this risk may be reduced,[111] although not discarded. Gene therapy can also cause insertional mutagenesis, observed, for instance, with an early, non-self-inactivating gamma-retroviral vector used to treat SCID-X1 immune deficiency, in which 5 of 21 children developed malignant tumours.[112] Insertion of the vector into or near active tumour-promoting genes caused transcriptional activation and oncogenesis.[112] If those specific genes are particularly active during fetal development, there may be an enhanced risk of insertional mutagenesis from prenatal vector delivery.

Feasibility of *in utero* therapy in spinal muscular atrophy

Despite the current licensed and pipeline therapies, no strategies address the genesis of SMA *in utero*. It is recognized that the translation of fetal gene therapy to humans is challenging, but the technology is becoming more common and *in utero* gene therapy remains a very promising avenue for the treatment of genetic diseases arising during gestation. This statement is supported by the conclusions of a recent panel discussion held by the International Fetal Transplantation and Immunology Society (IFeTIS) regarding the scientific, clinical and ethical issues related to prenatal gene therapy.[113] Pre-symptomatic delivery of treatment may prevent development of the SMA phenotype and the irreversible damage that accompanies this, perhaps due to the deficiency being corrected during the period of motor unit maturation.[114] *In utero* treatment could lessen disease symptoms in the early years of life, slow disease progression, or perhaps even prevent disease onset and complications. Combining prenatal diagnostic screening for SMA with *in utero* delivery could have the potential to reduce the severity and number of SMA cases presenting to the clinic, thus reducing clinical burden. In light of this, *in utero* delivery is an attractive opportunity to prevent development of symptoms and potentially allow healthy offspring to be born. The cases of SMA that initially may be considered for *in utero* treatment are those with few *SMN2* copies (one or two) and with a previous family history of severe SMA.[115]

Mice are a common model used for prenatal vector delivery due to ease of use and handling and the presence of between 6 and 12 fetuses per pregnancy, each surrounded by their own interior gestation sac, allowing each fetus to receive different vector injections. ASO administration by intra-embryonic injection in mice can lead to effective distribution and improved phenotypic functions in

Table 2 Ethical aspects of *in utero* therapy

Risk	Ethical issues
Treatment limitations or failure to meet expectations	*In utero* therapy could lessen disease symptoms, slow disease progression or perhaps even prevent disease onset. However, treatment might be sub-optimal or have variable levels of success or fail to meet parental expectations of a cure.
Risk-benefit considerations/safety concerns	The disease under consideration for IUGT must have significant pathology that occurs during gestation, whereby waiting to initiate treatment after birth has clear limitations in efficacy, to support equipoise of benefit/risk. Risks can be present for the mother (fetal loss, preterm birth, infection, immune response, maternal-fetal surgery, insertional mutagenesis), the child (disrupted development, insertional mutagenesis) and future generations (unintentional germline transfer).
Restricted access	Cost may vary significantly based on several factors, including the phenotype, severity of the condition, therapeutic drug and selected treatment approach. These variables may determine whether cost can be reimbursed or access be denied. Equitable access may not be possible.

See text for further explanation. IUGT = *in utero* gene therapy.

pups. Transuterine microinjection of a metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*)-targeted ASO resulted in significantly reduced *MALAT1* RNA in multiple tissues of neonatal mice, which persisted throughout 30 days after birth.[116] Transuterine injection of a splice-switching ASO into the amniotic cavity immediately surrounding the embryo at embryonic Day (E)13.5 partially rescued hearing in juvenile Usher syndrome type 1c (Ush1c) mouse mutants, while direct injection into the E12.5 inner ear led to improved therapeutic outcomes, which were sustained well into adulthood, showing that *in utero* ASO administrations can preemptively correct disease phenotype.[117] Such studies could pave the way for *in utero* administration of nusinersen ASO. Indeed, in a recent study,[118] administration of intra-amniotic ASO treatment improved the phenotypes of preclinical models of SMA. On the other hand, intravenous administration of nusinersen may be feasible in the fetus, similar to enzyme replacement therapy, as the porous BBB in the fetus may allow penetration into the CNS. In addition, the *SMN2* modulator risdiplam could potentially be administered orally to the mother with the aim of crossing the placenta (see later). Experience of successful treatment of cystic fibrosis *in utero* with modulators has been reported.[119] A fetus with ultrasound findings suggestive of meconium ileus was diagnosed with cystic fibrosis by amniocentesis at 26 weeks, and oral maternal therapy with modulators was initiated at 31 weeks. No dilated bowel was observed in the fetus at 39 weeks, and no signs of bowel obstruction were observed after birth. Maternal treatment was continued during breastfeeding.[119]

These results encouraged the application of DMTs to fetuses with SMA. Indeed, preliminary successful results of a single case of prenatal treatment with risdiplam to a fetus with two copies of *SMN2* expected to develop severe SMA has been recently reported.[120] Drug was administered to the mother during the last 6 weeks of the pregnancy. Pharmacokinetic and pharmacodynamic data supported target engagement and a favorable effect on motor neuron development. Other cases of prenatal treatment with risdiplam have occurred in USA and Europe as a compassionate use effort. Resuls from these experiencies have not yet been released.

While administration in the last months of gestation could be possible, it should be stressed that labels for all currently marketed treatments note a lack of data and warn about their use in pregnant women. Furthermore, the postnatal observed adverse effects of the type of therapy administered (e.g. hepatotoxicity, off-target effects) should also be considered when treating a fetus with SMA.[69] Idiosyncratic liver injury is common during pregnancy, therefore SMA fetal liver may be more prone to hepatotoxicity or other adverse effects.[121]

Integration-deficient lentiviral vector (IDLV) transduction of the spinal cord in rodents *in vivo*[122] and *in utero* IDLV delivery[123] have been previously described. We have also reported *in utero* technology with IDLVs, of potential application in SMA. Intraspinal injection of IDLV expressing *eGFP* at E16 into CD1 wild-type mouse embryos led to complete transduction of the spinal cord at all levels, with *eGFP* expression sustained for at least 7 months post administration. ChAT+ MNs showed 100% transduction efficiency. IDLVs were more efficient at transducing MNs after *in vivo* injection than AAV vectors.[124] More recently, a laboratory has published the first use of IUGT in SMA mice to investigate the efficacy of fetal gene therapy for this disease.[104] This study compared intracerebroventricular (ICV) and intraplacental routes of administration, as well as both single-stranded (ss) and self-complementary (sc) AAV9-*SMN1* vectors, concluding that ICV injection of scAAV9 vectors was optimal. SMNΔ7 mice fetuses injected in this manner at E15 resulted in 43.8% full-term gestation births, with these pups going on to survive for a median of 105 days, compared to 12 days in untreated pups.[104] Atrophied muscle present in untreated SMA mice was rescued in IUGT-treated animals, which also showed increased numbers of MNs in spinal cord sections.[104] This study highlighted three issues of concern. First, supraphysiological levels of SMN protein were found in CNS samples (but not in muscle). This may need to be addressed, as it appears that overproduction of SMN protein from AAV9 may have long-term toxic neuronal effects in an SMA mouse model.[32] Second, low survival to full gestation was observed, with the authors citing a possible inflammatory response to the AAV capsid as a factor. It is tempting to speculate that vector engineering could rectify these issues, but no studies have been completed to address this. Third, the rescued mice still had a shorter median survival, about half of that in wild-type mice. While this study provided proof of principle that IUGT for SMA may be a viable option and should be explored further, a comparison between the phenotypic benefits of *in utero* and postnatal gene therapy for SMA should be thoroughly evaluated. Another study investigated *in utero* viral delivery in the domestic sow, showing AAV9-related fetal rejection, which suggests that in this model preclinical *in utero* AAV9 gene therapy studies may not be successful.[125]

Combinatorial *in utero* therapy (IUT) in SMA also remains a topic of future experimental investigation when more data are available from treating postnatal SMA patients under the aforementioned circumstances.

Ethical issues

The goal of fetal therapy is not only to improve outcomes for the fetus but also to minimize risks and complications for the mother during pregnancy. A summary of possible ethical risks involved in IUT is provided in Table 2. Complications of fetal therapy can include fetal loss, preterm birth, infection, maternal immune response or the need for maternal-fetal surgery.[83]

Multiple levels of risk need to be addressed when IUT is considered. The presence of two patients—mother and fetus—requires special precautions to be included in the protocol. Diagnostic and therapeutic monitoring of the fetus may involve added risk, e.g. percutaneous umbilical cord blood sampling (PUBS).[49] The mother's health also needs to be carefully monitored for adverse events. IUT, therefore, should be conducted in a centre with expertise in the care of the mother and fetus, to include maternal-fetal medicine and neonatologists, where such monitoring can be performed and adverse events addressed promptly. From the three gene-targeted medications approved for postnatal treatment of individuals with SMA, nusinersen is currently administered via lumbar puncture into the CSF and would be a challenge and risk to perform in the fetus. Other means of delivery of an ASO to the fetus are being considered. Risdiplam can be administered to the mother orally in a safe dosage, as recommended for adult patients with SMA. It is known that this drug crosses the placenta in animals and has been demonstrated in one prenatal therapy case.[119] OA carries risk of adverse immune response to capsid proteins or the transgene, which may be tempered in the relatively tolerant fetus but not necessarily in the mother.[80] In addition, animal studies have demonstrated concerns over gene integration into germ cells of the fetus[108] and even fetal loss.[126]

Regarding maternal-fetal surgical interventions, which have become relatively common in prenatal care, particularly in neural tube defects or diaphragmatic hernia, there might be uncertainty in predicting the seriousness of disease severity and its consequences; this makes discussions around treatment choices difficult and complex.[127] Gene therapy and genome editing carry the additional risk of the therapeutic vector crossing the placental barrier and causing maternal genotoxic damage or an immune response, or even maternal genome editing. Potential risks to the mother from gene therapy thus include insertional mutagenesis. Studies in mice have shown no evidence of maternal genome editing after *in utero* CRISPR nuclease delivery.[92,128] Germline modification following genetic therapy to the fetus would be an additional risk to future generations, and it would have to be assessed in suitable animal models.

Maternal–fetal surgery, gene therapy or pharmacological intervention can save the life of a fetus that would otherwise die but may result in a child with severe disabilities. One could argue against investing medical and financial resources for sub-optimal treatment. IUT can also raise ethical issues regarding the role of healthcare professionals in relation to maternal decision-making and the fetus' life. In a case of prenatal diagnosis of myelomeningocele, the parents decided to refuse resuscitation measures of a 25-week fetus if emergency delivery was necessary during the open uterine procedure.[129] The pressure to intervene, for instance in the case of SMA, could be increased by the rapid evolution of disease. If IUT were to be carried out in SMA fetuses, it would be imperative for healthcare professionals to carefully navigate this delicate environment by engaging in meaningful discussions with parents, weighing the potential risks and benefits of various treatment options, respecting parental autonomy while safeguarding the best interests of the fetus/child and clinical obligations.[130] Prenatal decision-making is therefore complex, emphasizing the need for early engagement and counselling, and it is crucial that informed decisions be made in a supportive environment. In the US, the FDA has special requirements for investigational testing of drugs in a pregnant woman, whether directed to the mother or the fetus as referred to in FDA 45 CFR 46: 'subpart B—additional protections for pregnant women, human fetuses and neonates involved in research' (https://www.ecfr.gov/current/title-45/subtitle-A/subchapter-A/part-46). The European Medicines Agency also has a policy for investigational drugs in pregnant women. In all cases, surveillance should follow, and efficacy and safety information from studies with predefined outcomes should be collected. Adverse outcome data of fetal exposure comprise both structural malformations that may be detected at birth or in early childhood, and non-structural or long-term functional effects that can be potentially important but also difficult to detect or define (https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/guideline-exposure-medicinal-products-during-pregnancy-need-post-authorisation-data_en.pdf). Most of these guidelines were developed in relation to classic pharmacological compounds. However, the recent booming of advanced therapies has created a thin red line between experimental research success and the possibility of application in a translational way to clinical use.[131]

Importantly, genetic therapies are the most expensive drugs currently available. Access to them can be restricted by reimbursement agreements between manufacturers and insurance companies or national health services. In addition to the cost of the drug, it is likely that fetal interventions will carry a significant price tag from clinical services. Access to this medical care could thus be particularly constrained financially in the case of fetal therapies.

The clinical and therapeutic experience in SMA so far is that the earlier intervention occurs, the better the outcome for the patient. However, there are still shortcomings of present therapies, particularly in severe SMA patients. Neurodevelopmental problems are currently observed in a proportion of treated patients with two *SMN2* copies that need further investigation.[132] Parents are demanding information about long-term effects and prognosis of the disease when treatment is offered. Facing a pregnancy with SMA diagnosis (either by previous family history or prenatal screening) for a fetus with one or two *SMN2* copies, parents may request information about premature delivery to initiate postnatal therapy as early as possible[68] or even request the alternative of initiating IUT.[119] This represents a challenge for physicians in communicating the diagnosis and shared decision of treatment.[130]

Despite the risks and ethical concerns, women still express interest in enrolling in clinical trials for fetal gene therapy to achieve a more favourable pregnancy outcome,[133] with Schwab et al.[134] reporting favourable attitudes to fetal enzyme replacement therapy for lysosomal storage diseases and SMA gene therapies (ASO, small molecule and AAV). Nonetheless, multidisciplinary approach, prenatal counseling and thorough informed consent will be critical for successful implementation of IUT.

Conclusions

There have been impressive advances in SMA therapy during the last decades, especially after the initiation of the investigational clinical trials in 2011–2012. However, none of the three currently approved therapies offer a complete cure. Early detection by newborn

screening followed by pre-symptomatic treatment ameliorates manifestations and delays the initiation of disease in most treated babies. Given that SMA with one or two *SMN2* copies presents with low SMN levels and pathology during prenatal stages, *in utero* therapy for SMA should be explored as a possible alternative to optimize response to a gene-directed treatment. Therefore, the development of pre-clinical investigations and well-designed clinical trials of IUT in SMA (balancing risk, benefit and ethical issues) should be regarded as an opportunity to approach treatment of the disease as early as possible, maximizing therapeutic benefit.

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