EXPERT OPINION

Update on gene and stem cell therapy approaches for spinal muscular atrophy

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Introduction: Spinal muscular atrophy (SMA) is the leading genetic cause of pediatric death to which at present there is no effective therapeutic. The genetic defect is well characterized as a mutation in exon 7 of the survival of motor neuron (*SMN*) gene. The current gene therapy approach focuses on two main methodologies, the replacement of *SMN1* or augmentation of *SMN2* readthrough. The most promising of the current work focuses on the delivery of *SMN* via AAV9 vectors via intravenous delivery.

Areas covered: In the review the authors examine the current research in the field of stem cell and gene therapy approaches for SMA. Also focusing on delivery methods, timing of administration and general caveats that must be considered with translational work for SMA.

Expert opinion: Gene therapy currently offers the most promising avenue of research for a successful therapeutic for SMA. There are many important practical and ethical considerations which must be carefully considered when dealing with clinical trial in infants such as the invasiveness of the surgery, the correct patient cohort and the potential risks.

Keywords: gene therapy, spinal muscular atrophy, stem cells, translational therapy

1. Introduction

Spinal muscular atrophy (SMA) in an autosomal recessive neurodegenerative disease. The disorder is the leading genetic cause of pediatric death with an incidence of 1 in 6000 live births [1-3]. SMA is characterized by muscle loss, atrophy and paralysis brought about by the loss of spinal cord motor neurons [2]. SMA has three major clinical classifications type I, II and III also known as Werdnig–Hoffmann disease, Dubowitz disease and Kugelberg–Welander disease, respectively. There is also an adult onset type IV SMA. Type I SMA patients are diagnosed by 6 months of age, having presented with severe proximal muscle wasting and hypertonia. These children are never able to sit upright unaided and death usually occurs by 2 years of age due to loss of function of respiratory muscles. Type II patients present similarly to type I patients with a later onset of symptoms at 18 months of age. Although type II patients can sit unaided, they never stand or walk. Type III SMA is a much milder form of the disease with onset after 18 months of age, patients often live into adulthood.

The determinant mutation has been well characterized as a mutation in the survival of motor neuron (*SMN1)* gene [2]. Mutation of *SMN1* results in reduced levels of full-length SMN protein. In humans *SMN1* has a highly homologous duplicate copy *SMN2*. *SMN1* and *SMN2* differ by a C to T transition in exon 7 [4,5]. This mutation in exon 7 of *SMN2* results in a truncated and unstable SMN protein which is rapidly degraded [6,7]. A patient's *SMN2* copy number determines the severity of his/her disease. Patients with a low copy number of 2 *SMN2* copies or less present with SMN type I, whereas those with 3 – 4 copies present with SMN

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Article highlights.

- Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease which is the leading genetic cause of pediatric death.
- As the genetic defect involved in SMA and the target cells, motor neurons, are known both gene therapy and stem cell therapy present themselves as promising therapeutic approaches.
- Though there are many murine models for SMA which have provided important insights into the mechanisms of the disease and important preclinical work, a more suitable model is an important focus for researchers.
- From the present preclinical data, gene therapy replacement of SMN offers the more promising approach for translating a successful therapeutic.

This box summarizes key points contained in the article.

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type III. Therefore, the presence of a mutation in *SMN1* determines the disease and the copy number of *SMN2* modulates the disease severity.

This review looks at the aspects of SMA that make it an ideal candidate for both gene and cell therapies. The authors will examine the research that is approaching a therapy for this orphan disease. They will also discuss the current understanding of the genetic basis of the disease and animal models that increase the understanding of SMA, and aid in the efforts to produce an effective therapy.

2. Genetic basis of the disease

The genetic basis of SMA has been well characterized as a reduction in SMN protein levels due to a mutation in *SMN1*. The severity of the disease is dictated by the amount of SMN protein produced by *SMN2*. The mutation of *SMN2* produces splice variants of SMN lacking exon 7, often referred to as SMN Δ7, which results in an unstable protein; therefore, the efficiency of splicing determines the level of protein produced. As will be discussed later in more detail, investigators have explored the potential for drug and gene therapies which increase *SMN2* readthrough.

The SMN protein is a ubiquitous 38 kDa housekeeping protein. Though found in low levels throughout the cytoplasm, SMN is mainly located in the nucleus. SMN protein is localized with other proteins, Gemin2-8 and Unrip, in a large multiprotein complex called gems or SMN complexes in the nucleus [3,8-14]. To date, the predominant function of this complex is to assemble small nuclear ribonucleoproteins (snRNPs) and pre-mRNA splicing [9,15,16]. This pathway was recently reviewed comprehensively by Coady and Lorson [17]. Briefly, SMN is involved in the assembly of the Sm proteins on snRNPs in the cytoplasm and the formation of the heptameric ring, a ring-like complex consisting of seven Sm proteins and additional snRNA-specific proteins [18]. This complex subsequently interacts with importin-β, and is transported into the nucleus. In the nucleus, the SMN complex is associated with the Cajal bodies. Reduced levels of snRNP have been observed in mouse models of SMA, and the reversal of the SMA phenotype can be brought about with normal snRNP levels [19-22].

Though SMN is ubiquitously expressed in all cells types, motor neurons are selectively vulnerable to reduced levels of SMN protein. Ruggiu *et al.* recently presented a hypothesis for motor neurons susceptibility to lower SMN protein levels [23]. This work showed that under normal conditions motor neurons produced less full-length SMN mRNA from *SMN2* than non-motor neurons. This is due to poor efficiency of exon 7 inclusion in *SMN2* mRNA in the motor neurons compared with other cells types of the spinal cord. As exon 7 is essential for protein stability, this would correlate with reduced protein levels. This finding is reinforced by work showing that though two copies of *SMN2* can produce sufficient protein for most cell types, eight copies are required to rescue motor neurons and the resultant phenotype [24]. There is also evidence of a negative feedback loop where reduced levels of SMN protein reduce the efficiency of exon 7 inclusion [23].

There is much debate as to whether SMA is actually a neurodegenerative disease or whether it is a developmental one. Severe type I SMA is believed to begin during the period when the neuromuscular system is still in a phase of maturation. A mouse model with inducible expression of SMN protein was developed in order to assess the importance of SMN levels during development. They showed that high levels of SMN are critical in early development and that subsequent removal of SMN at 28 days of age does not result in any adverse phenotype [25]. This work suggests that there is a critical point in development where high levels of SMN are necessary. Further research and debate are necessary to conclude whether SMA is indeed a developmental disease or neurodegenerative.

As researchers have extensive knowledge of the genetic defect involved in SMA, and they have also identified the target cell type, SMA presents as a prime target disease for successful gene and cell therapies.

3. Animal models of SMA

Though researchers have a great insight into the genetics of SMA, the complexities of these genetics make the development of a high fidelity model a challenge that still faces researchers in the field of SMA. The gene for SMN protein is highly conserved across many species including *Caenorhabditis elgans*, fruit fly, zebrafish and mice [24,26-29]. Humans are the only species which possess the *SMN2* gene, which gives low levels of full-length functional SMN in the absence of *SMN1*. The complete knockout of the *SMN* gene in other species, which do not have *SMN2* gene to compensate, resulted in embryonic lethality [24,30-32]. Due to the extensive knowledge of the mouse genome, the mouse presents

a suitable platform for the development of models of SMA. Numerous mouse models of SMA have been developed using different approaches and have made a considerable contribution to the understanding of the disease. They have also provided researchers with models where potential therapeutic approaches can be assessed. The first challenge of producing a faithful model of the human disease state is to obtain suitable levels of SMN protein. Low levels of SMA results in neonatal lethality allowing little time for observation and a small therapeutic window. High levels of SMN protein result in animals with no detectible phenotype. The 'severe' or 'SMN2' mouse models are murine *SMN* null, but are engineered to carry varying copy numbers of human *SMN2*. Dependent on the copy number of *SMN2,* these animals have a widely varying phenotype; animals with 2 or less copies died at approximately P7, animals with 4 or more copies had complete rescue of the phenotype [24,29]. Though this model has a small window for therapeutic intervention, it does demonstrate that the phenotype can be rescued by increased copies of human *SMN2* and it also emphasizes that significance of varying levels of SMN protein. From this 'severe' model many researchers have tried to increase the life span of the model by the addition of a third *SMN1* transgene with point mutations. The two most widely used of these models are the *SMNΔ7* and A2G models. The *SMNΔ7* mouse model contains the *SMNΔ7* in addition to *SMN2* and results in a milder phenotype with survival up to 13 days [6]. This finding was surprising as *SMNΔ7 in vitro* appeared to be pro-apoptotic [33]. The A2G model is another modification to the *SMN2* model which is based on an A to G missense mutation observed in *SMN* of type II and III SMA patients [34]. This mutation results in a much milder type III-like phenotype, with survival up to P227 with 1 copy of *SMN2* and 1 copy of A2G [35]. Is it noteworthy that neither of these additional transgenes can rescue embryonic lethality in the absence of *SMN2* [6,35]. As reduced levels of SMN protein have a significant effect on motor neurons, other researchers chose to examine SMA using conditional knockout models. The *Cre/lox* model of SMA involved the conditional knockout of murine *SMN* exon 7 under the control of a neuronal-specific promoter [36]. This results in a milder type II-like phenotype with survival up to approximately P30 [36,37].

No animal models of any human disease can recreate the human disease state with 100% fidelity, we have to use the best models which are available to us and fit the therapeutic approach being investigated. That said, all of these models have contributed considerably to the understanding of SMA and are of the upmost importance in providing a platform where potential therapeutics can be developed. Some challenges with the present models still remain to be solved in order to progress translation. As there are three main different types of SMA, researchers must carefully chose the most suitable mouse model to reflect the disease type they wish to examine. For example, the milder A2G model maybe the most suitable model for type III SMA, whereas the

Δ7 model maybe a more appropriate model for type I SMA. Though an important caveat here is to remember models in which mice die within the first 30 days would be comparable with *in utero* in human. With this point taken into account, rescue of the Δ7 model represents a prenatal rescue which may not be feasible in humans. The lack of a suitable large animal SMA model for proof-of-principle work may slow the translational process. Though translation can occur with small animal proof-of-principle studies and large animal safety work, the development of a large animal SMA model would greatly aid in the understanding of the disease. Also larger animals would allow to foresee any potential pitfalls which may occur.

4. Gene therapy approaches

4.1 SMN augmentation

As outlined above, SMA is a monogenic disorder with a well-characterized affected cell type, presenting gene replacement of *SMN* as a clear choice for the development of therapy for SMA. Numerous viral vectors exist which have been shown to have a tropism for cells of the central nervous system (CNS), such as lentiviral vectors (LV) and adeno-associated viral vectors (AAV) [38-41]. These vectors have the ability to transduce non-dividing cells and also give long-term stable gene expression.

As mentioned above, the most direct and obvious approach is gene replacement with full-length *SMN* to increase levels of SMN protein. Three main routes of viral vector administration have been examined for this approach: intramuscular administration of a viral vector capable of retrograde transport to the motor neurons of the CNS, systemic delivery using a vector which can cross the blood–brain barrier (BBB) or direct delivery to the CNS either via direct injection into spinal cord or into the cerebrospinal fluid (CSF) via the intraventricular or intrathecal spaces.

The first noteworthy gene therapy approach by Azzouz *et al.* for *SMN* replacement was an intramuscular delivery approach. For this work, researchers chose a LV-based vector system for its ability to transduce non-dividing cells. The equine infectious anemia virus (EIAV) vector was chosen over classic HIV-1-based LV system. The EIAV vector was pseudotyped with the glycoprotein for rabies virus (Rabies G), conferring the ability to be retrogradely transported from muscle to motor neurons within the CNS [42]. One of the advantages of using a vector capable of retrograde transport is that it is a less invasive approach then direct injection into the spinal cord. When injected into the gastrocnemius or facial muscles of P2 mice, EIAV lac-Z gives robust expression of motor neurons in the spinal cord and brain stem, respectively [43]. This system was used to deliver *SMN* to a Δ7 model of SMA. Vector was delivered to the gastroconemius, facial, intercostal and tongue muscles of P2 SMA mice. Increased levels of SMN protein in motor neurons, and increased motor neurons survival were observed. This EIAV retrograde delivery approach of

SMN increased the survival of the Δ7 SMA model by up to 38% [43]. This work presented very promising results and encouraged more research into the use of gene therapy approaches for SMA.

The discovery that AAV9 vector is capable of crossing the BBB opened up a new potential vector for gene therapy for SMA. Three separate groups have shown that intravenous delivery of self-complementary AAV9 (scAAV9) can rescue the phenotype of SMA mice [44-46]. Foust *et al.* delivered scAAV9 via the facial vein of P1 Δ7 SMA mice [44]. Increased levels of SMN protein were observed, though it was still lower than that of control animals. scAAV9 SMN-treated animals showed improvement in motor testing such as righting time and open field testing when compared with untreated animals and green fluorescent protein (GFP) control animals. The most compelling finding of this work was the dramatic improvement in survival, the average survival of this model untreated is approximately 15 days, GFP-treated animal did not survive past 22 days, scAAV9SMN-treated animals survived past 250 days. Two other groups have shown similar results with scAAV9SMN in Δ7 mice, both of these studies used codon-optimized *SMN* sequences [46,47]. As codon-optimization has been reported to increase gene expression, both groups showed high levels of SMN protein expression with a 10-fold increase of SMN protein from the codon-optimized *SMN* [46,48]. Dominguez *et al.* reported increased survival in all treated animals after a single injection into the temporal vein of P1 pups with scAAV9 codon-optimized *SMN*. The AAV9 vector approach for gene delivery is a very exciting avenue of research due to its minimally invasive intravenous administration, and its ability to transduce the CNS following systemic delivery. Preclinical work has shown that AAV9 can give robust motor neuron expression following delivery to the CSF in a large animal model [49]. This is promising for the translation of AAV9 to the clinic, both in regards to efficacy of transduction and the feasibility of the route of administration and dose ranging.

Direct delivery of viral vectors to the CNS is another popular administration route utilized by SMA researchers. Direct injection of AAV8SMN into both the spinal cord and cerebral lateral ventricles of newborn Δ7 pups resulted in widespread SMN protein expression [50]. The SMN expression observed in this work showed expression on all levels of the spinal cord and in spinal cord motor neurons, suggesting that the SMN protein is being expressed in the appropriate areas to bring about a therapeutic effect. These AAV8 SMN-treated animals showed improved skeletal muscle size, improved neuromuscular junction structure and improved motor function. AAV8SMN was shown to increase survival to an average of 50 days, correlating to 233% increase. However, this increase in survival is modest when compared with the rescue provided by scAAV9 or scAAV8 (250 and 157 days, respectively) [44,50]. As self-complementary AAV produces gene expression more rapidly than AAV and higher levels of transduction [51], the difference in survival between AAV8 and scAAV8 may

again demonstrate that early up-regulation of SMN protein levels is critical.

Though scAAV9 intravenous injection does give robust SMN expression and a significant increase in survival, in a head-to-head comparison with direct intracerebroventricular (ICV) injection into the CSF, injection into the CSF gives significant increase in weight gain and lifespan over intravenous injection [52]. This work demonstrates the dramatic and significant difference that the route of administration can make.

4.2 Up-regulation of SMN2 activity

As outlined above, an increase in *SMN2* copy number can rescue the SMN phenotype in SMA mice. Strategies which improve *SMN2* readthrough to increase the amount of full-length SMN protein produced are a viable alternative to *SMN1* gene replacement. The use of antisense oligonucleotides or bifunctional RNAs to redirect splicing has shown promising results in increasing exon 7 inclusion [53]. These antisense oligonucleotides function by targeting regulatory elements. Bifunctional RNA can increase *SMN2* readthrough by binding to cellular splicing factors and by inhibiting intronic repressors, resulting in correct splicing of exon 7 [54,55]. Direct delivery via ICV injection of bifunctional RNAs has shown increased levels of full length of SMN protein in the brain [54]. *Trans*-splicing RNA is an approach that involves at its most simple, combining functional exons from *SMN1* and *SMN2* to produce a chimeric *SMN1-SMN2* mRNA which can produce functional full-length SMN protein [56]. This approach can increase SMN protein levels and improve survival in the Δ7 SMA mouse, but requires co-expression of antisense oligonucleotides to boost expression *in vivo*. These strategies are all promising but require further refinement and *in vivo* work before being considered for translational work.

5. Stem cell therapy approaches

As motor neurons are subject to degeneration in SMA, another potential therapeutic approach is the replacement of these lost motor neurons with stem cells and stem cell-derived cells. Stem cell transplantation can be performed with the goal of cell replacement, or to promote cell survival. Stem cell-mediated neuroprotection can occur through the secretion of neurotrophic factors and other mechanisms including the prevention of excitotoxicity. Corti *et al.* investigated the injection of both neuronal precursor cells derived from embryonic stem cells and primary neuronal stem cells in a model of SMA [57]. Neuronal stem cells injected into the CSF showed migration into the spinal cord of SMA mice. Some of these cells differentiated into motor neurons. Through both cell replacement and the secretion of trophic factors, these cells brought about improvement in motor function and survival (31.25%) in SMA mice, which is modest in comparison with some of the gene therapy approaches (**Table 1**). Direct

Table 1. Summary of important preclinical work for SMA.

	Treatment/vector	Animal model	Route	Outcome
Corti *et al.* [57]	Neuronal stem cell	Δ7	CSF-interthecial	↑survival 5 days (31.25%) ↑weight ↑motor function
Azzouz *et al*. [43].	EIAV RabG SMN	Δ7	Intramuscular	↑survival 6 days (38%) ↑SMN ↑MN
Foust *et al.* [44]	scAAV9 SMN	Δ7	Intravenous	↑survival 250 days (< 1000%)
Dominques *et al.* [45]	scAAV9 SMN (codon optimized)	Δ7	Intravenous	↑340 days ↑Weight ↑motor function
Valori *et al.* [46]	scAAV9 SMN (codon optimized)	Δ7	Intravenous	↑69 days ↑Weight ↑Motor function
Passini *et al.* [50]	AAV8 SMN	Δ7	ICV and direct spinal cord injection	↑157 days
Glascock *et al.* [52]	scAAV9 SMN	Δ7	Intravenous and ICV	↑200 days ICV mice gained more weight than IV mice
Osman *et al.* [53]	Bifunctional RNA, increasing SMN2	Δ7	ICV	↑SMN levels ↑weight gain & motor function ↑20 days

CSF: Cerebrospinal fluid; EIAV: Equine infectious anemia virus; ICV: Intracerebroventricular; scAAV9: Self-complementary AAV9; SMA: Spinal muscular atrophy; SMN: Survival of motor neuron.

injection of motor neurons derived from embryonic stem cells also survived in the anterior horns of the spinal cord in Δ7 SMA mice. The formation of new neuromuscular junctions was observed and also increased survival time. The Keirstead's group at University of California, Irvine, has invested significant effort into motor neuron transplantation. This group utilized human motor neurons progenitor cells in three models of neurodegenerative diseases: SMA, amyotrophic lateral sclerosis (ALS) and spinal cord injury. These motor neuron progenitor cells which have been produced from human embryonic stem cells are transplanted directly into the spinal cord models of all three disease states. In these models, human motor neuron progenitors showed engraftment, and produced increased levels of NGF and NT-3 neurotrophic factors. The secreted neurotrophic factors improved motor neuron survival in the animals demonstrating the potential for stem cell-mediated treatment of SMA.

Induced pluripotent stem (iPS) cells represent another interesting approach for both the treatment and understanding the mechanisms of SMA. iPS cells derived from SMA patients can be differentiated into motor neurons for further study of the disease pathology [58]. These cells can also be modified and transplanted back into the patients from whom they were obtained. This autografting approach might eliminate the hurdle of graft rejection and need for immune suppression currently used for stem cell transplantation. There is debate that cells obtained from a diseased patient, despite modification, may revert to a disease phenotype over time. Also, recent work has shown that iPS cells

may have immunogenic properties which need to be carefully evaluated before proceeding to clinical applications [59].

Further small animal work needs to be undertaken to optimize the delivery site, time of delivery and optimal cell type for stem cell approaches for SMA. The safety of large animal spinal cord stem cell transplantation has been extensively investigated by the researchers at Emory University [60-62]. They also developed a surgical platform for the delivery of stem cells to the spinal cord, which improves the accuracy and safety over previous methods, and is currently being utilized in a Phase I clinical trial for stem cell delivery for ALS [63]. This existing precedent for stem cell delivery in clinical and the promising preclinical data show the translational potential of stem cells for the treatment of SMA. Nonetheless, application of this technology to infants with SMA will present unique challenges.

6. Clinical trials

Though currently there are no active genes or stem cell-based clinical trials for SMA, the translational outlook is bright. California Stem Cells, Inc. has filed an investigational new drug (IND) application to the Food and Drug Administration (FDA) for a stem cell-based therapy for SMA. IND status is one of the major regulatory hurdles a therapeutic must pass in order to progress to clinical trial. The regulatory process that a therapy must go through in a rigorous one. The review by Aboody *et al.* gives an excellent overview of this process [64]. There are other hurdles which may occur when translating a new therapy to humans. In relation to viral

vectors, the choice of vector is critical. Many potential patients may have neutralizing antibodies to certain AAV serotypes. The prevalence of these neutralizing antibodies in healthy populations has been examined [65]. It may become necessary to screen patients prior to enrolment in a trail using AAV vector to ensure that the vector will not be subjected to an immune response, at least one clinical trial now has a screen for AAV neutralizing antibodies as part of the exclusion criteria (NCT00516477). Researchers are currently looking at way to circumvent these neutralizing antibodies by approaches such as directed evolution [66]. Another important caveat to keep in mind with vectors that give 'long-term' expression such as AAV and LV is will expression last the life-time of the patient or will 'booster' doses be required. If booster doses are required we are confronted again with the issue of neutralizing antibodies. We must also always be aware of possible species difference that may occur between rodent models to human patients. A therapy which corrects SMA in a murine model may not be as effective when used in a human and we should always keep this caveat in mind.

It is important to note that while viral vector direct delivery to the CNS is currently in clinical trials in adults and children, the invasive surgery approach may not be viable in infants, which would be the likely patient cohort for a SMA trial. Nonetheless, it is important to note that clinical trials for both Canavan (21 subjects) and Batten (10 subjects) disease involved direct delivery of viral vector to the CNS of children and, therefore, there is already a precedence for direct delivery of viral vectors to the CNS of young children [67,68].

7. Summary

SMA is a devastating childhood neurodegenerative disease for which there is no effective treatment. The genetic mechanism in SMA is defined and well understood and the target cells (motor neurons) have been well defined. SMA is an extremely attractive disease target for successful advances in gene and stem cell therapies. Advances in viral vector *SMN1* delivery have shown extremely encouraging results to date, rescuing the phenotype of SMA mice and greatly improving survival. Stem cell technology has also made great strides at advancing the SMA agenda with embryonic-derived motor neurons cells at a preclinical stage. Though both these therapeutic routes have made incredible process, there are many hurdles which remain. It is clear from the body of work summarized in **Table 1** that the main focus of SMA research is gene therapy. Currently, gene therapy offers the greatest potential success, due to the knowledge of the genetic defect in SMA and the development of suitable vectors such as AAV9. At present in animal models, gene therapy is offering the most dramatic recovery. Stem cells remain a promising alternative or augmentative treatment but further work will need to be done to strengthen the case of stem cell therapy in SMA. An animal model which can more accurately reproduce the human disease

state is required. Ideally, a model would have a disease progression similar to the human disease, have a reliable reproducibility and should have a suitable therapeutic window to allow potential therapies to have an effect. Once these therapies have proven effective in small animal models, large animal safety and preclinical work must be completed and all regulatory milestones met before any of these potential therapies can make it to clinical trail and potentially to FDA approval.

8. Expert opinion

When moving forward with any new therapeutic after proof-of-efficacy in an animal model, it must so go through a rigorous regulatory process in order to obtain IND status from the FDA. When considering SMA, there are issues specific to the disease that need to be carefully dealt with in order to choose a viable treatment.

Due to the invasiveness and risk involved with the surgery required to deliver the therapy, very careful consideration must be taken to justify a clinical trial with young children. As type I SMA is fatal by 2 years of age, should these children be the first to receive a prospective therapy? Due to the severe progression of the disease and lack of an effective alternative treatment, there is obvious justification for the risk of the surgery in these patient groups. Because there is no current prenatal test for SMA nor is there widespread neonatal screening, early recruitment into a trial becomes very difficult. As discussed above, in animal models of SMA early delivery is critical to therapeutic outcome, this early delivery time in the mouse model is equivalent to prenatal delivery in humans. In humans, diagnosis is usually not made until 6 months of age, which is most likely too late for a therapeutic to be able to reverse the severe phenotype of type I SMA and may only prolong the life of an infant with a fatal disease. Therefore, biopharmaceutical companies may not want to invest in a trial in type I SMA, due to the low probability of success and return on investment. With type II and III SMA, there is a larger therapeutic window with a milder phenotype and, therefore, a greater chance of a potential therapeutic to have a positive effect. Nonetheless, because the phenotype is milder and some of these patients have the potential to live into adulthood, it becomes harder to justify the risk of the surgery. This leaves us with the question: is it ethical to give the first chance of therapy to type II and III children who have a greater chance of a successful outcome, rather than first giving the treatment to type I infants who have a more urgent need of a therapy, but may not lead to a successful clinical trial?

There are three major delivery options for gene or cell therapies to SMA patients: direct injection into either the CNS or affected muscles, diffuse systemic delivery or delivery to the CSF. When direct and systemic delivery of *SMN* via scAAV9 were directly compared, though both routes had a therapeutic effect, direct delivery gave more significant results [52]. Also, intravenous delivery of either viral vector or cells may require larger doses and off-target effects cannot be dismissed.

Off-target effects may also be a concern with CSF delivery, but to a lesser extent, with therapeutic delivery confined to within the BBB. Until we have a greater ability to target both viral vector and stem cell to the area of interest following systemic delivery, direct delivery may be a most viable avenue to pursue. The cause of death in type I SMA is flail chest due to failure of the intercostal muscles. In order to directly target the motor neurons involved in controlling the intercostal muscles, delivery to the entire thoracic spinal cord would be necessary. Targeting the thoracic cord is a much more challenging surgery than targeting either the lumbar or cervical cord. The smaller grey to white matter ratio in the thoracic cord makes precise delivery into the cord more difficult. Also, a multilevel laminectomy in the thoracic region is a very invasive procedure that can lead to postoperative complications such as scoliosis and kyphosis in patients who survive the procedure. The translation of systemic delivery of AAV9 to humans also has its own challenges which need to be considered. Systemic delivery may require larger doses of vector, for which the biomanufacturing may prove prohibitively expensive. With the systemic delivery of AAV9 there is a risk of off-target effects in other tissues. Also as mentioned

earlier, the issue of neutralizing antibodies must also be kept in mind.

Both gene and stem cell therapies offer exciting potential therapeutic approaches for the treatment of SMA. There are still important questions which remain to be answered such as what the optimal therapeutic window for human delivery is, or whether we should focus on *SMN1* replacement or *SMN2* readthrough, or what the ideal site of delivery is. We also need to ensure that future trials are carefully designed due to the surgical risk of performing these invasive surgeries on infants and young children.

Declaration of interest

NMB is a consultant of Celgene, Medtronic, Neural Systems and Intellectual Property and has sponsored research from Genzyme/Sanofi. NMB is the inventor of devices to enable safe and accurate injection of the human spinal cord. Neuralstem Inc. has purchased an exclusive license to this technology. NMB received an inventor's share of this fee, and has the rights to royalty payments for distribution of this technology. EMD has no competing interests.

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