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Bridging the Gap: Gene Therapy in a Spinal Muscular Atrophy Type 1 Patient

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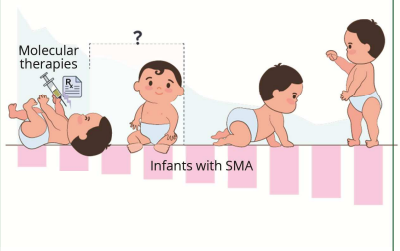
Abstract

Molecular therapies exploit understanding of pathogenic mechanisms to reconstitute impaired gene function or manipulate flawed RNA expression. These therapies include 1) RNA interference by antisense oligonucleotides, 2) mRNA modification using small molecules, and 3) gene replacement therapy, the viral-mediated intracellular delivery of exogenous nucleic acids to reverse a genetic defect. Several molecular therapies are approved for treating spinal muscular atrophy (SMA), a recessive genetic disorder caused Survival Motor Neuron (SMN)1 gene mutations. SMA involves degeneration of lower motor neurons, which leads to progressive muscle weakness, hypotonia, and hypotrophy. Onasemnogene abeparvovec is a gene replacement therapy for SMA that uses Adeno Associated Virus delivery of functional SMN1 cDNA to motor neurons. Two other molecular therapies modulate SMN2 transcription: nusinersen, an antisense oligonucleotide, and risdiplam, a small molecule designed to modify faulty mRNA expression. The most suitable individualized treatment for SMA is not established. Here, we describe remarkable clinical improvement in a 4-month-old patient with SMA type 1 who received onasemnogene abeparvovec therapy. This case represents an explanatory bridge from bench to bedside with regard to therapeutic approaches for genetic disorders in neurology. Knowledge of the detailed mechanisms underlying genetic neurological disorders, particularly monogenic conditions, is paramount for developing tailored therapies. When multiple disease-modifying therapies are available, early genetic diagnosis is crucial for appropriate therapy selection, highlighting the importance of early identification and intervention. A combination of drugs, each targeting unique genetic pathomechanisms, may provide additional clinical benefits.

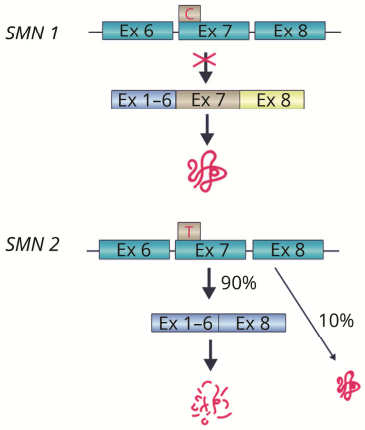
Neurology® Gene Therapy in a Patient with Spinal Muscular Atrophy Type 1 American Academy of Neurology



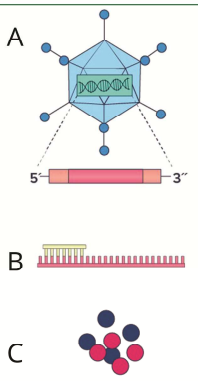
Case: A 4-month-old child with persistent hypotonia, motor delay, and failure to thrive

Diagnosis: Genetic diagnosis of spinal muscular atrophy (SMA) type I

Intervention and outcome: Gene therapy with onasemnogene abeparvovec with clinical improvement



Genetic disease mechanisms: Reduced production of survival motor neuron protein (SMN) following *SMN1* gene mutation with only partial rescue by *SMN2* gene



Gene-specific therapies in SMA:
A) Viral vector-based gene replacement therapy
B) RNA interference by antisense oligonucleotides
C) mRNA splicing modification by small molecule

Introduction

Genetic molecular therapies are developed to restore gene function or modulate RNA expression related to diseases arising from genetic defects. Three currently available therapeutic approaches in clinical neurology are classified based on mechanism of action: 1) RNA interference by antisense oligonucleotides (ASOs) or small-interfering RNAs (siRNAs), 2) mRNA splicing modification by small molecules, and 3) gene replacement therapy, which is the delivery of exogenous DNA encoding a functional version of the target gene[1]. Therapies under investigation or currently available include RNA interference drugs (inotersen and patisiran) for hereditary transthyretin amyloidosis and four ASOs (casimersen, viltolarsen, golodirsen, eteplirsen) for Duchenne muscular dystrophy (DMD) related to specific exon mutations[1]. Three spinal muscular atrophy (SMA) therapies based on these technologies (onasemnogene abeparvovec, nusinersen, risdiplam) and targeting the respective genetic mechanism have shown remarkable clinical benefits.

Case presentation

A 4-month-old female child presented with diffuse muscle hypotonia and no head control during a routine pediatric consultation. She was referred at age 4.5 months to our tertiary care center for persistent hypotonia, motor delay, failure to thrive, and meteorism. The patient's parents reported a reduction in spontaneous movements beginning as early as age one month. The family history was negative for known genetic neurological disorders, and she was born at term (weight 3440 g) following a normal pregnancy and delivery.

Neurological examination revealed poor head control, diffuse appendicular and truncal hypotonia, and severe lower limb weakness (Medical Research Council 1/5), weak hand prehension, and areflexia. Tongue fasciculations were absent. She presented no signs of chest deformity or respiratory distress. Electromyography showed a neurogenic pattern with active denervation in lower and upper limb muscles. Considering the motor deficits, neurological examination, and electrophysiological findings, our diagnosis was SMA type 1. Genetic testing confirmed a homozygous Survival Motor Neuron (SMN)1 deletion and two copies of *SMN2*. The child scored a 22 on the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), a reliable scale for assessing motor development in the first 2 years of life[2]. This scale consists of 16 items, each rated 0 (no response) to 4 (complete response), based on spontaneous upper and lower limb, truncal and pelvic movements, as well as head control and strength. The total score range is 0 to 64, with better motor function reflected in higher scores.

Testing for Adeno Associated Virus serotype (AAV)9 antibodies was negative. The parents were counseled regarding therapeutic alternatives, and the patient received an intravenous dose of onasemnogene abeparvovec (1.1×10^{14} vector genomes/kg) at age 5 months. Per therapeutic protocol, she also received prednisolone (1 mg/kg) starting the day before gene therapy began and continuing for 8 weeks. She was monitored weekly for laboratory abnormalities, including liver enzymes and platelets. Because of a slight transient increase in aspartate aminotransferase (51 U/L), the corticosteroid dose was increased temporarily to 2 mg/kg for one week and subsequently reduced progressively until cessation. No side effects were noted. The CHOP-INTEND score at 4 weeks post-infusion was 32, indicating clinical improvement. During the hospitalization, prophylactic bilevel positive airway pressure (BiPAP) was prescribed for overnight use.

Two months after therapy administration, the patient was hospitalized with viral pneumonia, requiring escalation of respiratory support with continuous BiPAP. She recovered after 4 weeks

with ongoing respiratory improvement noted up to 2 months after discharge. At 8 weeks post-discharge, her BiPAP requirement was downgraded to overnight only. Six months post-gene therapy treatment, the patient showed moderate head control and improved hypotonia, lower limb strength and hand prehension. She had preserved spontaneous breathing and swallowing and could sit without assistance for 5 seconds, with a CHOP-INTEND score of 42.

Discussion

This child is in a recent cohort of patients with SMA type 1 receiving onasemnogene abeparvovec gene therapy in a real-world setting. She showed motor improvement based on parent-reported functional skills and serial CHOP-INTEND assessment. Apart from a transient aspartate aminotransferase increase controlled by corticosteroids, we detected no other laboratory abnormalities or major side effects. Comprehensive follow-up data collection is ongoing to assess long-term efficacy and any delayed-onset side effects of onasemnogene abeparvovec.

SMA as a neurological genetic disease

SMA, a leading genetic cause of infant mortality[3], is archetypal in that knowledge of the underlying genetic mechanisms has allowed for use of gene-specific approaches to alter the disease course. Most SMA cases (95-98%) are caused by homozygous deletions in *SMN1,* which lies in the telomeric region of chromosome 5q13(Figure 1A)[4]. The remaining cases involve small intragenic *SMN1* mutations[5]. These mutations lead to progressive limb muscle weakness, hypotonia, and atrophy with motor function decline in and the development of bulbar difficulties and respiratory insufficiency[6]. A centromeric SMN2 variant[7] may be present in variable copy numbers on 5q13 and is an almost identical homolog of *SMN1* except for a few nucleotides[3]. A crucial C-to-T substitution in exon 7 at position 6 alters *SMN2* splicing, so that~90% of transcripts lack exon 7, and the resulting truncated SMN protein is rapidly degraded. The remaining 10% of protein produced is insufficient for motor neuron survival[3], and *SMN2* copy number is inversely correlated with phenotypic severity. Current advanced molecular treatments for SMA target repletion of SMN in motor neurons by replacing *SMN1* or acting on *SMN2* genes transcription.

Molecular therapies in Neurology

Molecular therapies target the underlying disease mechanism with the aim of reconstituting gene function or manipulating RNA expression. Three techniques are currently available in clinical neurology[1] (additional data listed in eTable 1 in the Supplement). Their mechanism of action depends on the underlying gene mutations, which may cause partial or complete loss of function, gain of toxic function, or both.

When loss of function is the prevalent mechanism, the aim of treatments is restoring expression of the affected protein. If the prevailing mechanism is gain of toxic function, the therapy goal is to suppress expression of the disease-related protein. Gene replacement with viral vectors (Figure 1B) or ASOs/miRNAs can be tailored to downregulate or upregulate target gene/mRNA expression. For instance, when protein expression is reduced or absent, as in SMA or DMD, gene replacement therapy and ASOs increase the expression of the missing protein. The viral vector delivers cDNA encoding a functional SMN protein or dystrophin, and ASOs modulate splicing of *SMN2* or the mutant dystrophin gene to increase overall levels of functional protein. Conversely, with hereditary transthyretin amyloidosis or SOD1-related amyotrophic lateral sclerosis[8], the underlying pathomechanism is associated with a toxic gain of function mutation, and viral vectors, siRNAs, or ASOs are used to reduce levels of the mutated protein.

Molecular therapies in SMA

EU and US regulatory bodies have approved three therapies for SMA: onasemnogene abeparvovec (Zolgensma), nusinersen (Spinraza) and risdiplam (Evrysdi) (Figure 1C and eTable 1). All three increase levels of functional SMN by reexpressing a healthy copy of *SMN1* cDNA or by modifying *SMN2* splicing. Onasemnogene relies on non-replicating, recombinant AAV9 that can cross the blood-brain barrier, at least in infants, to deliver a functional SMN cDNA transgene to motor neurons[9] (eTable 1, mechanism 1). It is given once intravenously and indicated for children under age 2 years with SMA type 1[9]**.** Nusinersen is a short single-stranded oligonucleotide that is delivered intrathecally and alters SMN2 pre-mRNA splicing to ensure exon 7 inclusion, boosting production of the functional protein[10] (eTable 1, mechanism 2).The small molecule risdiplam, an oral therapy for SMA, binds SMN2 pre-mRNA in two regions, increasing the quantity of functional SMN[9] (eTable 1, mechanism 3). The most suitable treatment for each patient, best initial approach and possible advantages of combination therapies remain unclear[9,11].

Early diagnosis is paramount for access to and benefit from molecular therapies in neurology, yet diagnostic delays persist[12]. Implementation of prenatal genetic testing or neonatal screening for SMA could lead to earlier diagnosis and improve access to personalized therapies[13]. Our patient showed a remarkable clinical amelioration despite being symptomatic with a low CHOP-INTEND score at treatment initiation. Although the diagnosis was made at a tertiary care center when she was age 4.5 months, symptoms may have manifested 3 months earlier. Our case highlights the need for neonatal screening to treat patients as soon as possible to yield maximum benefit from molecular treatments.

Other gene replacement therapies approaches are under investigation for a wide range of neurological diseases. In most cases, these experimental therapies target loss-of-function mutation diseases such as DMD**,** lysosomal disorders (e.g. Pompe, Gaucher, Fabry)[14], idiopathic Parkinson disease, Leber hereditary optic neuropathy, and Charcot-Marie-Tooth 1A[1]. Less frequently, viral-mediated delivery of microRNAs or short hairpin RNAs to silence disease genes is used for disorders involving gain-of-function mechanisms, such as amyotrophic lateral sclerosis (SOD1, C9orf72, FUS mutations) and Huntington disease[8]. In addition, genome editing with mRNA CRISPR-Cas9 is being explored for Huntington disease[13] and hereditary transthyretin amyloidosis[15].

The efficacy:safety ratio must be carefully evaluated for classical gene transfer approaches with AAV or other viruses as well as with genome editing. Overall, genetic molecular strategies offer new perspectives and hope for patients affected by genetic and acquired neurological diseases with known molecular mechanisms.

WNL-2022-201193_etab1 -- http://links.lww.com/WNL/C379

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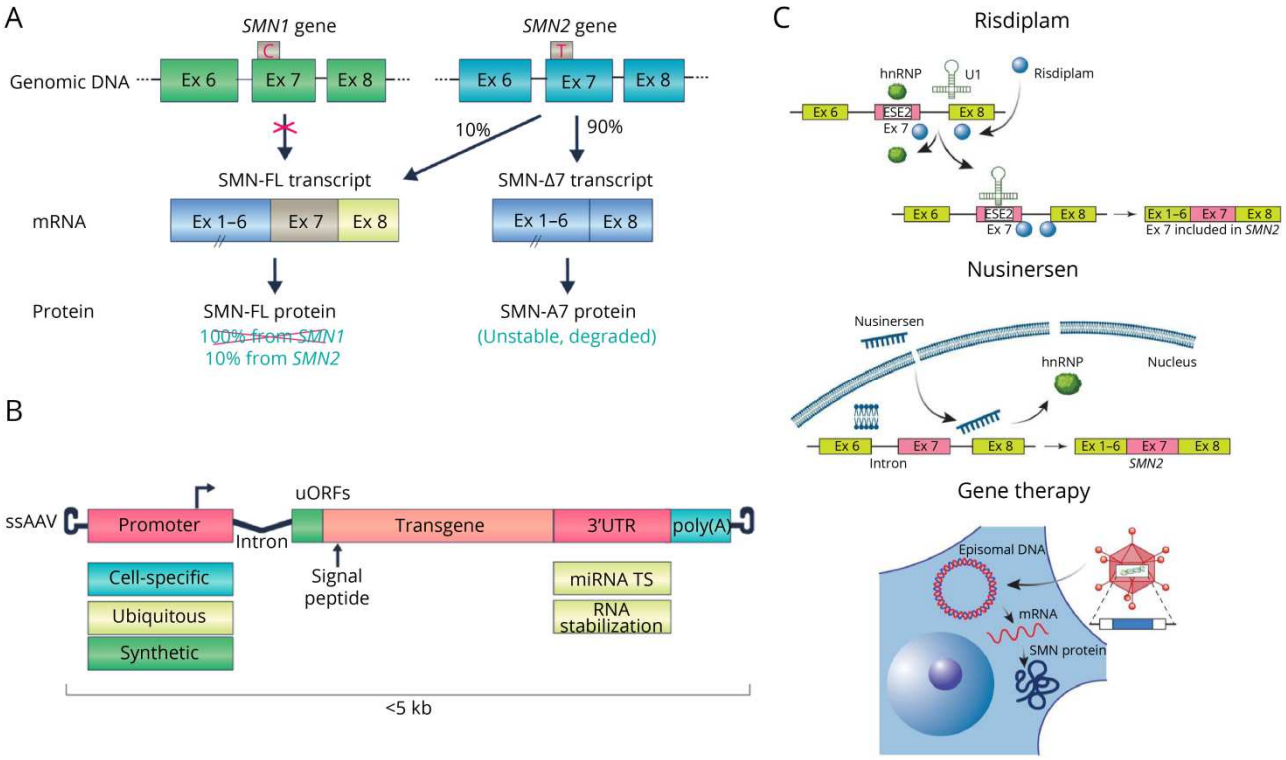
Figure 1: Molecular mechanisms of spinal muscular atrophy (SMA), basic design of Adeno Associated Virus (AAV)-based gene therapy and molecular therapies for SMA.

A) Mutations in the telomeric *SMN1* gene (red crosses) lead to reduced production of the functional survival motor neuron protein (SMN-FL). In SMN2, a transition switch from C to T alters the splicing of exon 7, and the resulting transcript (SMNΔ7) lacks the exon in 90% of cases, leading to an unstable, rapidly degraded product. The remaining 10% of SMN-FL from *SMN2* cannot sustain motor neuron survival, with consequent motor neuron death and muscle atrophy.

B) The general design of AAV-based gene therapy. A modular structure consisting of specific promoter (red), intron, open reading frames (ORFs, green), the 3' untranslated (UTR) region, and polyadenylation (poly(A), light blue) region enable targeted and cell-specific transgene (orange) expression. AAV vectors can carry genomes up to 5 kb in length.

C) Mechanisms of action of the three approved molecular therapies for SMA. The precise mechanism of action of risdiplam is unknown, but it supposedly displaces the heterogeneous nuclear ribonucleoproteins (hnRNP G) from exon 7 and enhances the interaction of U1 small nucleolar RNA (snRNA) with exon 7 splicing enhancer (ESE2). These molecular changes increase translation of *SMN2* into functional SMN. Nusinersen is a single-stranded antisense oligonucleotide taken up into cells by endocytosis. It binds nuclear SMN2 pre-mRNA and displaces the hnRNP protein that usually prevents retention of exon 7 in the final transcript, enabling production of complete SMN. Gene replacement with onasemnogene abeparvovec consists of *SMN1* cDNA carried by a non-replicating, non-integrating Adeno Associated Virus 9 (AAV9). When the cDNA is released in episomal form in the motor neuron cytoplasm, it begins to produce copies of SMN1 transcripts encoding functional SMN.



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