Review on Gene Replacement Therapy for Spinal Muscular Atrophy

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Gene Replacement Therapy for Spinal Muscular Atrophy” submitted by Nurani Jannat (18346010**)** of Spring, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on March 2023.

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Ethics Statement

This study did not involve any human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues and field research.

Abstract

Spinal muscular atrophy (SMA) is initiated by the deletion or malfunction of the gene that encodes survival motor neuron 1 (SMN1) and this leads to muscular atrophy. It is one of the most prevalent hereditary that causes infant mortality. Eventually, various genetic interventions and approaches, including gene replacement, non-gene replacement, and gene editing have been explored up to this point as potential treatment options for spinal muscular atrophy and other neuromuscular illnesses. Whereas gene replacement therapy is most often claimed to treat infants and toddlers with SMA type I. When treating the underlying cause of this disease, researchers have discovered the greatest benefit to motor neurons early on. Here, I have reviewed the enormous advances made in genetically mediated therapeutics for SMA, with an emphasis on gene replacement therapy and the outcome of this therapy was found to be more efficacious than other treatments in terms of motor millstone result.

Keywords: Spinal Muscular Atrophy Type I; Gene Therapy; Motor Milestone.

Dedication

Undoubtedly, I want to dedicate my effort for this project to my family who has been trying so hard to give me a better life.

Acknowledgement

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List of Acronyms

AAV- Adeno-Associated Viral Vector

ALT- Alanine Aminotransferase

AST- Aspartate Aminotransferase

CMV- Cytomegalovirus

EBV- Epstein-Barr Virus

GGT- Gamma Glutamyl Transferase

HSV- Herpes Simplex Virus

INR- International Normalized Ratio

SMA- Spinal Muscular Atrophy

SMN- Survival Motor Neuron

TLR9 - Toll-like Receptor 9

U snRNP - U-rich Small Nuclear Ribonucleoprotein

HFMSE- Hammersmith Functional Motor Scale Expanded

ITRs - Inverted Terminal Repeats

BBB - Blood Brain Barrier

CHOP INTEND - Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders

Chapter 1

Introduction

1.1 Background

Symmetric, progressive, proximal more than distal muscle weakness and atrophy characterize spinal muscular atrophy (SMA), which is caused by deterioration of the anterior horn cells of the spinal cord and motor nuclei in the lower brainstem. SMA is one of the most common genetic causes of infant mortality that have been mentioned earlier. Approximately 6 million Americans, 10,000 to 25,000 children and adults in the United States are affected with SMA. In Bangladesh, the Neurosciences hospital gets some 30 patients with SMA every year. Researchers have found gene therapy as a new hope for treating this fatal disease. This disorder causes muscle to become frail and waste away. SMA affected patients lose a specific nerve cell in spinal cord which is motor neuron that command muscle movement. Through these neurons signals are being transmitted from the brain and spinal cord that instruct skeletal muscles to contract. In the absence of these neurons, muscles don't require signals that make muscle activity. Correspondingly, there is a specific protein called survival motor neuron (also known as SMN) which helps motor neurons to survive and work properly. This protein is produced using instructions from the SMN1 and SMN2 genes that play a pivotal role in motor neuron development. That is why, SMN protein shortage leads to motor neuron death, resulting to the signs and symptoms of spinal muscular atrophy. Biallelic SMN1 loss-of-function mutations cause the most common kind of SMA. Type 1, 2, and 3 of spinal muscular atrophy combined account for around 8% of all cases of infant mortality due to genetic disorders. SMN1 maps to the 5q13.2 region of chromosome 5, hence SMA that is linked to this gene is sometimes referred to as 5q SMA. Lesser deletions, nonsense variations,

frameshift variants, missense variants and splice site mutations containing alleles are also present in around 6% of people with SMN1-related SMA. Non-5q spinal muscular atrophies are a category of illnesses that have clinical symptoms with SMN1-related disease but have different underlying genetic causes and can be distinguished from classic SMA by careful examination. All future references to spinal muscular atrophy (SMA) shall be made in terms of 5q SMA only. This gene is highly similar to SMN1, with the exception of a single C to T transition in exon 7. This change causes exon 7 to be left out of the final SMN protein after transcription. Some 10-15% of SMN2 mRNAs, however, keep exon 7 and so generate full-length SMN protein. There are typically two copies of the SMN1 gene and one or two copies of the SMN2 gene in every cell in the human body. Around eight copies of the SMN2 gene have been found in some people, though this number varies. More than one copy of the SMN2 gene is associated largely with milder forms of this muscle illness that manifest later 41in life. Mutations in the SMN1 gene lead to protein shortages, which can be mitigated to some extent by the SMN protein encoded by the SMN2 genes (Abreu & Waldrop, 2021).

1.1.1 Classification

Based on the age of beginning and milestones reached, this disorder is categorized into four subgroups (Al-Zaidy et al., 2019).

- **Type 1 (Severe):** Type 1 commonly known as Werdnig-Hoffman disease, accounts for roughly 60% of SMA cases. The first signs typically show up shortly after birth or in between the first six months of existence. Type 1 spinal muscular atrophy affects the ability of infants to swallow and sucking. They fail to achieve developmental landmarks including head-holding and sitting. Children's susceptibility to respiratory infections and collapsed lungs increases as they age because their muscles weaken

(pneumothorax). Type 1 spinal muscular atrophy is fatal for nearly all children under the age of 2.

- **Type 2 (Intermediate):** Type 2 SMA (also known as Dubowitz illness) usually presents itself between the ages of 6 and 18 months in infants and toddlers. The lower extremities are frequently affected by this kind. Even if a child with type 2 SMA is able to sit, they likely won't be able to walk. The vast majority of kids with type 2 SMA reach adolescence.

- **Type 3 (Mild):** Type 3 SMA (also known as Kugelbert-Welander or juvenile-onset SMA) is characterized by the appearance of symptoms after the first 18 months of life. Symptoms of type 3 diabetes may not appear until a person is well into their 20s for some. Frequent respiratory infections, mild muscle weakness, trouble walking are also signs of type 3. Walking and standing skills may be impaired by symptoms if they continue for an extended period of time. Life expectancy is unaffected by SMA type 3.

- **Type 4 (Adult):** Rarely seen after age 30, adult-onset SMA is a devastating disease. Most persons with type 4 are able to maintain mobility and lead normal lives because the progression of muscle weakening symptoms is slow.

1.1.2 Pathogenesis of SMA

The q13 region of chromosome 5 is the location of the SMA-causing gene. The survival SMN1 mutation or deletion that brings about the failure to encrypt the survival motor neuron protein is the cause of SMA. Widely distributed housekeeping protein that aids in the transport of axonal mRNAs and contributes to the construction of the U-rich small nuclear ribonucleoprotein (U snRNP). A recent review provided a thorough breakdown of the SMN

protein's actions. SMN1 is situated on a side of the telomere in the almost symmetrical 5q13 region, which was created by the inversion duplication of a significant stretch. SMN2 being the parallel homolog of SMN1, acts as a disease moodier, is located at the centriole side of this region. However, SMN2 only produces a little quantity of the functional SMN protein, which is insufficient to make up for the reduction in SMN caused by the deletion of SMN1 and SMN2 are remarkably similar, with only 16 known base changes. The sixth base of exon 7—SMN1 has C whereas SMN2 has T—is the primary point of difference. Although the coding of amino acids is unaffected by this single-base change. As a result, the majority of the products of the SMN1 gene are full-length mRNAs that contain exon 7, whereas the products of the SMN2 gene are mRNAs that have exon 7 skipped. The termination codon of the mRNA 7 is shifted, resulting in a shortened and very unstable protein known as SMN. That is why, one of the options for treating SMA is to increase the level of exon 7 inclusion in SMN2. The amount of SMN protein is what determines how severe SMA is. Since a larger SMN2 copy number corresponds to higher levels of SMN protein. Additionally, by adjusting the inclusion of exon 7, additional regulations, including point mutations, can modify the severity of SMA patients. The precursor mRNA of SMN2 contains several cis-acting splicing regulatory elements, including intronic splice silencers (ISS), exonic splice silencers (ESS), exonic splice enhancers (ESE), and intronic splice enhancers (ISE), which control the inclusion of exon 7 by binding to trans-acting proteins. Patients with SMA who carried the mutation c.859G>C (p.Gly287Arg) displayed a less severe phenotype. The initial hnRNP-dependent ESS was disrupted by this location, which increases the inclusion rate of the SMN2 exon 7. The A-G transition at position -44 on SMN2 intron 6 was identified as a novel protective locus for SMA patients (Qiu et al., 2022).

1.2 Treatment

In the beginning, researchers who wanted to enhance SMN protein levels, concentrated on making SMN2 more efficient. Nusinersen is an antisense oligonucleotide used to promote SMN2 transcription and consequently, translation of functional SMN protein by binding the splicing silencer region on SMN2 pre-mRNA. The administration of this treatment necessitates repeated intrathecal treatments administered by injections. Risdiplam, an orally bioavailable small molecule, is an option in contrast to Nusinersen but Risdiplam can be administered orally in liquid form daily rather than intrathecally. Hence, an once-in-a-lifetime intravenous gene transfer therapy called Onasemnogene Abeparvovec-xioi was created as a potential alternative treatment (Feldman, Parsons, Dutmer, Veerapandiyan, Hafberg, Maloney, & Cara, 2020).

Table 1: Compare and Contrast among Most Renowned Approved Approaches

Treatment	**Mechanism**	**Administration Method**	**Date of FDA Approval**	**Restriction**
Spinal Muscular Atrophy				
Nusinersen (Spinraza®)	Antisense oligonucleotide altering splicing of survival motor neuron-2 (SMN2)	Intrathecal injections, 4 loading doses then every 4 months	December 23, 2016	None

Onasemnogene abeparvovec-cioi (Zolgensma®)	Viral-mediated gene One-time replacement	IV injection	May 24, 2019	Age should be <tow. Awareness for those with advanced disease (tetraplegia, permanent ventilator dependence)
Risdiplam (EvrysdiTM)	Splicing modifier of SMN2	enteral Once daily	August 7, 2020	2 months or more than that

1.2.1 Gene Replacement Therapy for Children with Type I SMA

Onasemnogene abeparvovec-xioi (formerly AVXS-101, Zolgensma®, Novartis Gene Therapies EU limited, and Dublin, Ireland) was the first in vivo systemically delivered gene replacement therapy to receive FDA Aapproval in May 2019. It is approved to treat SMA patients in more than 40 countries globally. Adenoassociated virus (AAV) vector-based gene therapy called AVXS-101 attempts to deliver a functional copy of the human SMN gene across the BBB by intravenous infusion. In the European Union and the United States, it is permitted to assist SMA patients with 5q SMA who have a biallelic mutation in the SMN1

gene and a clinical diagnosis of SMA type 1 or up to three copies of the SMN2 gene, as well as pediatric SMA patients with biallelic mutations in the SMN1 gene who are under the age of two (Abreu & Waldrop, 2021).

1.2.2 AAVs and Their Significance in Gene Therapy

Gene replacement therapy uses targeted delivery of operative genetic material to treat disorders. Years of research had gone into finding a virus that could transport genetic material beyond the impenetrable blood-brain barrier. Except one, the majority of the earliest studies on gene therapy for neurological illnesses bank on the direct injection of vectors through burr holes created into the skull. “To get something that you can dose at really high levels peripherally, and then get it into the CNS was what was really missing.” —Christian Lorson. Then the adeno-associated virus serotype 9 was discovered for the first time by Kaspar's team and an independent team in France (Dolgin, 2019). AAVs exhibit a number of properties that make them a good choice for this purpose right away. As the vector interacts with distinct cell-entry receptors from other similar viruses, Ohio State University researchers demonstrated in 2009 that AAV9 may pass through the anatomical gateway of mice brains to infiltrate tissues across the central nervous system (Dolgin, 2019).

It has been shown to penetrate the BBB in mouse, non-human primate, and human endeavor, raising the probability of systemic delivery to the nervous system. Another promising benefit of AAVs is that they instinctively exhibit a large tissue tropism, a characteristic mostly governed by their capsid, and are non-pathogenic also immunogenic in humans. Researchers have mixed genomes and capsids from several serotypes to alter tropism depending on their demand, then minimum 11 natural serotype capsids was found. Early research aimed at the

1.2.3 Motor Milestone Assessments

Although Zolgensma works in a somewhat different way, it has nonetheless proven quite successful. The medicine works by reintroducing a copy of SMN1 into the motor neurons which is functional, using a genetically modified virus. To the end, rather than focusing on optimizing a less-than-ideal alternative route, the therapy seeks to correct the underlying flaw that causes SMA. There is great hope for gene therapy, as seen by the increasing number of children who with the help of Zolgensma, are now reaching their motor milestones (Dolgin, 2019). Early-onset neuromuscular disease patients, especially those with SMA type 1, can have their motor skills accurately assessed using the CHOP-INTEND scale, a validated evaluation tool. This scale has 16 questions with a range of 0 to 64 potential answers. Rankings of the best performers are stable. It takes into account the limited patient ability to acquire and maintain specific postures, the level of exhaustion brought on by respiratory impairment, and the condition's most clinically important motor goals. This milestone is seen as therapeutically significant because patients with SMA1 hardly ever achieve and maintain scores above 40. When given the therapeutic dose of AVXS-101, the vast majority of SMA1 patients were able to attain independent sitting, regardless of age at dosing; however, those dosed early accomplished this milestone far more quickly, regardless of baseline motor capability. Children who had poor motor function to begin with showed rapid and clinically significant gains, including the capacity to sit independently which have been shown in figure: 2 and 3. These data sets underline the gravity of newborn screening for SMA1 and the potential impact of early therapy with AVXS-101 for optimal outcomes (Lowes et al., 2019).

1.2.4 Zolgensma over Nusinersen Millstone Attainment

Between Sept 21, 2019, and April 20, 2021,Onasemnogene Abeparvovec was administered to seventy six children affected by spinal muscular atrophy (58 had been early treated with Nusinersen and 18 were not), with 16 months mean age (range 0-59 months; interquartile range [IQR] 9-23 months) and a mean weight of 9 kilograms (ranging 4- 15 kilograms) (Weiß et al., 2022). Standard operating procedures for administering Onasemnogene Abeparvovec were developed using information from the US Food and Drug Administration and afterwards adapted using a German consensus guideline. The German consensus protocol states that at least four weeks must pass between the last dose of Nusinersen and the start of gene replacement therapy; however, we were unable to gather accurate information from all of the children in our cohort regarding the timing of these events. Clinical evaluation, genetic testing for SMN1 variants and SMN2 copy number, highest AAV9 titre within 30 days of medicaments, and a battery of laboratory tests, including a complete blood count, AST, ALT, creatine kinase, total bilirubin, troponin T or troponin I, and gamma-glutamyl-transferase, were all performed prior to gene replacement therapy. If necessary, polysomnography was also performed. To lessen the possibility of immune-mediated hepatopathy, all patients were hospitalized the day before treatment and given their first oral prednisolone dosage (1 mg/kg). The rectal administration of prednisolone was an alternative for those who could not take the oral form. Treatment with nervous system used AAV2, AAV5, and AAV8 capsids; however, AAV9 has gained popularity in recent years due to its greater bio distribution (Marrone et al., 2021). Gene replacement drugs were put on hold in situations of severe sickness or abnormally high liver enzyme levels. All other immunizations were put on hold until immunosuppressive therapy was no longer being administered, although respiratory syncytial virus prophylaxis was maintained until age 20. Under strict observation, all patients were given a 1:1.11014 vector genome intravenous infusion over 1 hour, with

their body weight used as a guide. Patients were cared for in accordance with EU directive 2000/54/EG's biosafety level 1 beginning at the time of infusion. Clinical evaluations and blood analysis were part of the standardized aftercare protocol at weekly intervals for the initial month, every two weeks for the second and third months, monthly till the sixth month, and once each three months until the twelfth month. Children experiencing adverse effects were monitored more frequently. Every patient was on a 30-day course of prednisolone (1 mg/kg daily, either orally or rectal dosage equivalent). Patients whose liver enzyme concentrations were normal 30 days following therapy (maximum 2-fold rise) were switched to hydrocortisone and their prednisolone doses were reduced by a quarter every week. Repeated elevations in liver enzyme levels would result in the maximum steroid dose being administered. If the liver enzyme levels were more than 400 U/L (6.67 kat/L), the dosage of steroids was risen up to 2 mg/kg per day, as per the German consensus procedure. Patients those have liver enzymes above 1000 U/L (16 67 kat/L) or those whose concentrations persisted to rise despite therapy with 2 mg/kg per day oral prednisone, high-dose intravenous methylprednisolone was investigated (20 mg/kg per day for 3-5 days).Patients with elevated troponin T or troponin I concentrations who showed no evidence of heart injury on echocardiography and electrocardiogram (ECG) were monitored every three to six months by a pediatric cardiologist. The most common reasons for protocol deviations are technical difficulties (such as a problematic venous condition) and patient noncompliance with scheduled checkups (lost to follow-up). Sixty patients had data available, and 49 of them showed huge improvement on the CHOP-INTEND and HFMSE. Children less than 8 months (n=16; mean change 138 [SD 85]; p00001), children between 8 and 24 months (n=34; 77 [SD 52]; p00001), and children > 24 months (n=6; 25 [SD 52]; p=100) all showed substantial improvements in CHOP INTEND scores 6 months following therapy. Results showed that 6

months after gene replacement therapy, the CHOP INTEND score raised by 8 points (p=0.0003) in the 45 children who had been pretreated with Nusinersen (Weiß et al., 2022).

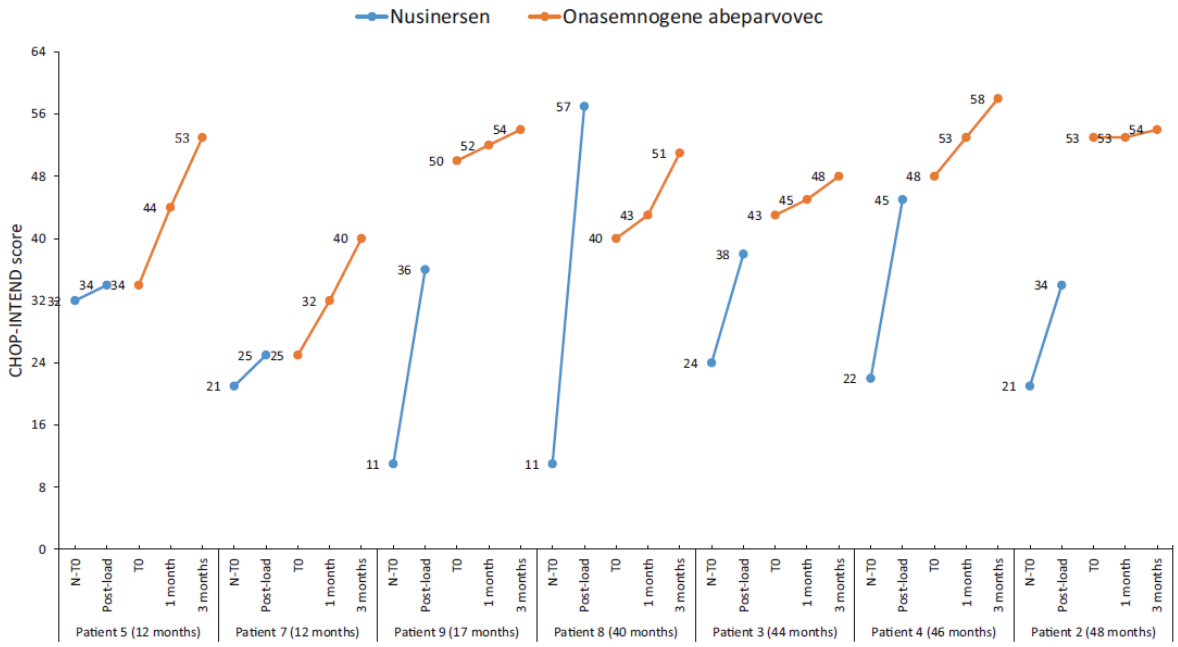

Figure 1: CHOP-INTEND Score for 7 Patients Who Were Switched to Zolgensma from Stable Nusinersen(Bitetti et al., 2022).

Figure 1 comprises the score of seven patients on stable dosing of Nusinersen were diverted into Onasemnogene Abeparvovec consumers. Treatment with Nusinersen is represented by blue scores; Analyses performed before (T0) and 1, 3, and 6 months after (T3, 6, and 12 months) Onasemnogene Abeparvovec treatment via intravenous administration are shown in orange. The number of patients and their ages are displayed along the horizontal axis, with the youngest patients listed first. However, it is worth mentioning that the two patients who are patient 5 and 7 in our trial, had the poorest response to Nusinersen as well as they had the biggest increase in baseline scores at the first and third month after changing the drug. This event may imply that Onasemnogene Abeparvovec may be more effective for those who did not respond well to Nusinersen. At the same time, the age and the initiation of the treatment is proportional to each other in this circumstance. However, in order to evaluate the

likelihood of these theories, testing must be conducted in larger patient populations. Newborn screening for spinal muscular atrophy (SMA) and presymptomatic therapy improves outcomes in children with genetically confirmed SMA. Implementing this task is somewhat challenging as everyone's mutation is not the same as well as the progressive degeneration of SMA changes rapidly inside the body and it varies from person to person specially for type-one. As suggested by multiple published preclinical and clinical evidence. It is now widely accepted that the irreversible loss of motor neurons in people with SMA type 1 occurs during pregnancy and the first few months of life. Over 90% of motor units are lost by 6 months of age, and there is significant evidence that irreversible motor neuron loss occurs in the perinatal period in individuals with SMA type 1. Onasemnogene Abeparvovec treatment before symptoms appear has the potential to alter the natural history of SMA by facilitating neuromotor development that is comparable to that of a typically developing kid (Bitetti et al., 2022).

1.3 Goal of the Study

SMA is claimed as a rare disease, 1 in every 6000-10000 is born with SMA but yet a fatal one, most of the babies with SMA type I gets the shortest lifespan, mostly die in early stage. Gene replacement therapy aims to slow down or stabilize the progression of disease by utilizing proteins of our body that are the building blocks of how we function, which is generated by specific genes. As per neurodegenerative disease genes play prime role as the root cause. Early diagnosis and treatment can reduce the severity of the disease. Considering that new born screening and gene replacement therapy ensures individual motor milestone achievement rather than other conventional methods. The goal of this study is to determine the effectiveness of gene replacement therapy in the treatment of SMA that are already in use and also those that are under ongoing researches.

Chapter 2

Methodology

Initially, several internet sources including Research Gate, Jstor, PubMed, Elsevier and Science direct were used thoroughly to search relevant papers for my topic. I have collected 30 papers and then chosen 13 out of those based on most recent years of publications along with the older ones to compare and contrast all the information. After that, comprehensive review was done blending and re-organizing all the information from those articles. Lastly, all the selected information was re-written and references were added with the help of Mendeley library to state the sources of those information. Thus, this review on 'Gene Replacement Therapy for Spinal Muscular Atrophy' was accomplished.

Chapter 3

Result & Discussion

3.1 Result

Zolgensma's approval process had flaws with outraged rising prescription prices and other side effects. Because of the high rate of this drug, it puts a question mark on its affordability and also it can be the biggest drawback of this therapy. It is highly expensive compared to Nusinersen drug. But on the other hand, using this medicine once in a lifetime is enough for a human being to be recovered for this reason it can be recommended (Bitetti et al., 2022). However; the research underlying these medicines gives hope that they may both pave new therapeutic ground. Also, the community of drug development department considers Zolgensma being the more lasting tale —its footprint not only on both patients along with the entire technology of gene therapeutics—may have been lost in the media firestorm. The acceptance of Zolgensma in May marked a watershed moment for the biotechnology sector. Today, receiving a diagnosis with SMA is not the same as being given a death sentence. Researchers are looking for guidance as they explore the new era of medicines to address the underlying causes of other debilitating neuromuscular illnesses. Former Biogen head of R&D Michael Ehlers predicts a "coming revolution" in the treatment of severe neurological illnesses (Abreu & Waldrop, 2021).

3.1.2 Side Effect

Recombinant AAVs have become the preferred delivery method for gene therapeutics. Their poor packaging capability, however, nonetheless represents a significant obstacle for the treatment of diseases brought on by mutations in big genes. Regrettably, the use of rAAVs has been constrained to the development of therapy for only a small subset of ailments due to their restricted packaging capacity of 4.7kb, excluding conditions brought on by mutations in genes larger than this threshold. (Marrone et al., 2021). AAV toxicity is primarily associated with the use of high vector dosages, which can have negative effects on the liver. Vomiting and increased aminotransferases are the most frequent adverse effects. A person with Type 1 SMA and premorbid elevated aminotransferases experienced acute liver injury with jaundice, AST 80X ULN, and ALT 45X ULN, as well as massive inflammation of the liver biopsy, seven weeks after receiving treatment through a managed access program. As a result, the medication Onasemnogene Abeparvovec-xioi carries a black box warning of acute liver injury. There are other descriptions of brief increases in cardiac troponin-I levels and brief declines in platelet counts, both of which have unknown clinical importance. (Feldman, Parsons, Dutmer, Veerapandiyan, Hafberg, Maloney, & Mack, 2020).

3.1.3 Remedy

Here, we analyzed the various tactics that have been tried over the past ten years to try to get around this restriction. Certain approaches have been shown to be more effective than others depending on the target tissue and cell type, and case-specific optimization. Including single vector techniques and dual/triple vector tactics (concatamerization, overlapping, hybrid, protein trans-splicing and mini-genes). To solve the AAV packaging issue, creative techniques targeted at increasing the AAV capsid size are being investigated in addition to cargo manipulation. This is a vastly unexplored area that might offer a more universally

accessible method of delivering large transgenes. The results of more investigation will indicate whether this is a practical course of action. Importantly, each of these solutions needs to be carefully designed, indicating the need to advance the state of the art. There are still many unmet medical needs that are awaiting effective gene therapy solutions. In the case of systemic neurological conditions, this is especially true (Marrone et al., 2021). After using corticosteroids, the youngster reached baseline level and had already finished the necessary 30-day course of 1 mg/kg of prednisolone and a 2-week taper. There are other descriptions of brief increases in cardiac troponin-I levels and brief declines in platelet counts, both of which have unknown clinical importance. All in all Onasemnogene Abeparvovec is a relatively risk-free and very successful medication when administered to kids who don't already have liver damage, and prednisolone doses are adjusted in accordance with close monitoring during the first few days after treatment (Abreu & Waldrop, 2021). Preclinical trials of Onasemnogene showed that patients with increased aminotransferases responded favorably to steroid Treatment. Therefore, prednisolone (1 mg/kg/day) has to be administered in the 24 hours prior to infusion and continued for 30 days after infusion, as stated on the Onasemnogene package insert. After two months of steroid treatment, the dosage can be gradually reduced over the next 28 days if liver function tests remain normal. However, despite taking steroids before to and during Onasemnogene therapy, both children presented in this article had liver failure between 3 and 8 weeks after infusion. The first child was tapered off steroids more quickly than is currently suggested, and both children were treated through a managed access program before current steroid recommendations were in place. Therefore, in the first two months following Onasemnogene medication, it is important to check liver tests frequently (every one to two weeks) and to increase therapy as needed for severe hepatitis or acute liver failure. Corticosteroids, often given intravenously at rates of 10–20 mg/kg daily, may need to be increased in dosage as part of the treatment plan. Since the liver histology reveals a

predominant CD8+ T-cell-mediated pathology, a T-cell targeted immunosuppressant (tacrolimus, sirolimus, everolimus, or antithymocyte globulin) may be worth trying if high-dose corticosteroids fail to do so. On the other hand, treatment with anti-IL-6, anti-tumor necrosis factor-a, or janus kinase inhibition may prove advantageous, as proinflammatory cytokines and type I interferon production caused by TLR9 activation may be implicated in the pathophysiology of liver injury in AAV9-facilitated gene therapy. On either extreme, harmful innate and humeral immune responses may be averted with pre-Onasemnogene treatment with a TLR9 inhibitor and/or B-cell depletion. As promising and transformative as these medicines are, we must never lose sight of the need of putting patient safety first and thoroughly investigating. There is a need for more research into the immune response and optimal prevention of liver harm (Feldman, Parsons, Dutmer, Veerapandiyan, Hafberg, Maloney, & Mack, 2020).

3.1.4 Cautions

Onasemnogene Abeparvovec-viral xioi's vector is not pathogenic for us, rather the guards and daycare providers of child’s those receive gene transfer, advised to cover hands with protective gloves when in close proximity to patient bodily fluids and waste. In terms of better hygiene it is suggested to obey for 4-6 weeks after administration to vulnerability brought on by viral sloughing. Manifestation of this incident, caretakers might grow dispensation to AAV9, barring them from potential future AAV9 treatments. Secondary maternal shifting of anti-AAV9 antibodies is potential danger of, breastfeeding was prohibited before, during, and 30 days after gene transfer in the phase 1 trial. However, the risks and benefits should be discussed individually with the treating physician. At the treating physician's option, vaccinations may be changed and administered at least one week before conveying gene. Till four weeks live atrophied vaccines shouldn't be administered after the

prednisolone treatment is through, however inactivated vaccinations is allowed at any time after gene transfer (Abreu & Waldrop, 2021). Newly diagnosed with SMA patients > twenty four months, one-time intravenous gene replacement therapy is a fruitful option; however, children pretreated with Nusinersen a wash-out period should maintained. In elderly patients, it is especially important to evaluate their safety in light of their long-term steroid dependency and the associated risks; to achieve therapeutic consultations necessitates standardized longitudinal estimation to a greater extent in the time ahead (Weiß et al., 2022).

3.2 Discussion

After reviewing all cumulative findings of the researchers, I would like state that, in terms of early age treatment especially for SMA type I, gene replacement therapy should be a prior choice as it aims to reduce the burden of severity at early stage and provides better milestone achievement. However, early age treatment brings about some limitations for instance individual body response by the age increases which differ from person to person. Moreover, elderly people will might not be benefited with this therapy. As researchers are devoting their efforts towards finding remedies against all possible drawbacks, we can hope that these limitations can also be concurred at a greater extend in future. As I am reviewing on gene replacement therapy, keeping aside the expenses Zolgensma should be drug of choice as a one-time intravenous application. In terms of age limitation there are other novel drug choices that have been discussed already such as Nusinersen, Risdiplam. Some indivudauls can also respond to these alternatives and treatment may vary with different sub types. Finally, it can be claimed that For SMA type I gene replacement therapy is a better option to choose.

Chapter 4

Conclusion

To sum up, Gene replacement therapy for spinal muscular atrophy is speculatively convenient for having revolutionized precision medicine for neuromuscular illnesses during the past decade. In addition to Risdiplam, several small molecule and gene therapies are being researched and developed for this and other neuromuscular disorders. Research on the safe and successful treatment of those with preexisting AAV antibodies, as well as efforts to broaden SMA gene therapy to those of older ages assessing multidisciplinary care and half-life of these drugs committing durable safety issue. Whether or not gene replacement plus other genetically mediated therapy yields optimal results is still uncertain. Because of the significant expenses associated with these therapies, more research into the topic is necessary. For this joint effort of advocacy groups, researchers, industry, government, physicians and patient is a must (Abreu & Waldrop, 2021).

4.1 Future Aspects

A proof-of-concept study claims base editing-mediated splicing repair as chemoprotective method that is also predicted to be an effective method in future (Lin et al., 2020). Correspondently, CRISPR/Cas9 based gene editing techniques have shown promise in treating SMA in vast animal models and are making strides toward potential human applications. In additament, indoprofen and aminoglycosides are two examples of a type of treatment known as protein stabilization therapy that works to keep SMN's protein level constant. However, current methods are not advanced enough to treat SMA. The goal of this approach is to provide fuel and shield neurons from damage with neuroprotective measures.

Riluxolone is the standard drug for this treatment. These methodologies are being honed too (Qiu et al., 2022).

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