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REVIEW ARTICLE

Spinal Muscular Atrophy: A Genetic Disease In Kerala.

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ABSTRACT

Spinal muscular atrophy (SMA) is a genetic disease affecting the central nervous system, peripheral nervous system, and voluntary muscle movement (skeletal muscle). It is characterized by weakness and wasting (atrophy) in muscles used for movement (skeletal muscles). It is caused by a loss of specialized nerve cells, called motor neurons that control muscle movement. The weakness tends to be more severe in the muscles that are close to the centre of the body (proximal) compared to muscles away from the body's centre (distal). The muscle weakness usually worsens with age. There are many types of spinal muscular atrophy that are caused by changes in the same genes. The types differ in age of onset and severity of muscle weakness; however, there is overlap between the types. It has been unfortunate to see the rising number of Spinal Muscular Atrophy (SMA) cases in the state of Kerala. The drug is of USA origin which nearly costs 18 crores in Indian market, which is available only when there is a requested demand. The patients are unable to afford such a great amount all the time. Zolgensma is a gene therapy medicine for treating spinal muscular atrophy, a serious condition of the nerves that causes muscle wasting and weakness. Nusinersen, an antisense oligonucleotide, is administered directly into cerebrospinal fluid. It alters SMN2 pre-RNA splicing so exon 7 is included, increasing expression of functional SMN protein.

Keywords: Spinal muscular atrophy (SMA), Gene therapy, Onasemnogene abeparvovec, Nusinersen, Antisense oligonucleotide.

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INTRODUCTION

Most of the nerve cells that control muscles are located in the spinal cord, which accounts for the word spinal in the name of the disease. SMA is muscular disease because its primary effect is on muscles, which don't receive signals from these nerve cells. Atrophy is the medical term for getting smaller, which is what generally happens to muscles when they're not stimulated by nerve cells. SMA involves the loss of nerve cells called motor neurons in the spinal cord and is classified as a motor neuron disease (figure 1). In the most common form of SMA (chromosome 5 SMA, or SMN-related SMA), there is wide variability in age of onset, symptoms, and rate of progression. In order to account for these differences, chromosome 5-related SMA, which often is autosomal recessive, is classified into types 1 through 4.The age at which SMA symptoms begin roughly correlates with the degree to which motor function is affected: The earlier the age of onset, the greater the impact on motor function. Children who display symptoms at birth or in infancy typically have the lowest level of functioning (type 1). Later-onset SMA with a less severe course (types 2 and 3, and in teens or adults, type 4) generally correlates with increasingly higher levels of motor function.

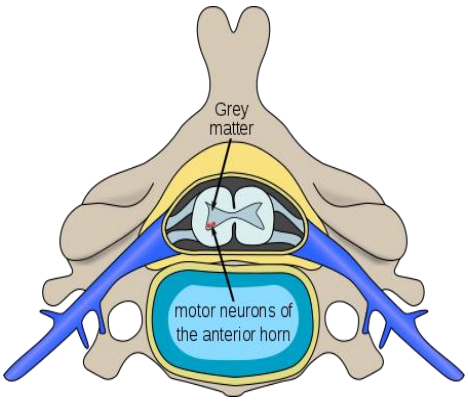

Figure 1: Location of neurons affected by spinal muscular atrophy in the spinal cord

Types of spinal muscular atrophy

There are four primary types of SMA:

Type 1 (severe): About 60% of people with SMA have type 1, also called Werdnig-Hoffman disease. Symptoms appear at birth or within an infant's first six months of life. Infants with type 1 SMA have difficulty swallowing and sucking. They don't meet typical milestones like holding up their heads or sitting. As muscles continue to weaken, children become more prone to respiratory infections and collapsed lungs (pneumothorax). Most children with type 1 SMA die before their second birthday.

Type 2 (intermediate): Symptoms of type 2 SMA (also called Dubowitz disease) appear when a child is between six months and 18 months old. This type tends to affect the lower limbs. Children with type 2 SMA may be able to sit up but can't walk. Most children with type 2 SMA live into adulthood.

Type 3 (mild): Symptoms of type 3 SMA (also called Kugelbert-Welander or juvenile-onset SMA) appear after a child's first 18 months of life. Some people with type 3 don't have signs of disease until early adulthood. Type 3 symptoms include mild muscle weakness, difficulty walking and frequent respiratory infections. Over time, symptoms can affect the ability to walk or stand. Type 3 SMA doesn't significantly shorten life expectancy.

Type 4 (adult): The rare adult form of SMA doesn't typically appear until the mid-30s. Muscle weakness symptoms progress slowly, so most people with type 4 remain mobile and live full lives [1].

Causes of SMA

Chromosome 5 SMA is caused by a deficiency of a motor neuron protein called SMN, for "survival of motor neuron." This protein, as its name implies, seems to be necessary for normal motor neuron function. SMN plays a pivotal role in gene expression in motor neurons. Its deficiency is caused by genetic flaws (mutations) on chromosome 5 in a gene called SMN1. The most common mutation in the SMN1

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gene within patients diagnosed with SMA is a deletion of a whole segment, called exon 7 [2]. Neighbouring SMN2 genes can in part compensate for nonfunctional SMN1 genes as there is 99% identity between these two genes. Other rare forms of SMA (non-chromosome 5) are caused by mutations in genes other than SMN1 [3]. Spinal muscular atrophy is inherited in an autosomal recessive pattern (figure 2), which means that the defective gene is located on an autosome. Two copies of the defective gene – one from each parent – are required to inherit the disorder: the parents may be carriers and not personally affected. SMA seems to appear de novo (i.e., without any hereditary causes) in around 2–4% of cases.

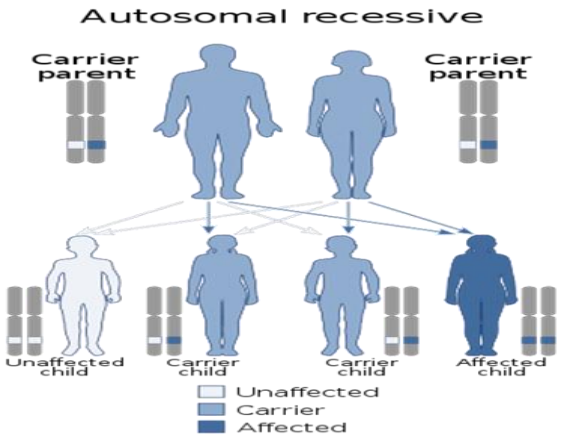

Figure 2: Spinal muscular atrophy has an autosomal recessive pattern of inheritance.

Symptoms of SMA

SMA symptoms cover a broad spectrum, ranging from mild to severe. The primary symptom of chromosome 5-related (SMN-related) SMA is weakness of the voluntary muscles. The muscles most affected are those closest to the center of the body, such as those of the shoulders, hips, thighs, and upper back. The lower limbs seem to be affected more than the upper limbs, and deep tendon reflexes are decreased.[4] Special complications occur if the muscles used for breathing and swallowing are affected, resulting in abnormalities in these functions. If the muscles of the back weaken, spinal curvatures can develop. There's a great deal of variation in the age of onset and level of motor function achieved in chromosome 5-related SMA. These are roughly correlated with how much functional SMN protein is present in the motor neurons, which in turn correlates with how many copies of SMN2 genes a person has. Sensory, mental, and emotional functioning are entirely normal in chromosome-5 SMA. Some forms of SMA are not linked to chromosome 5 or SMN deficiency. These forms vary greatly in severity and in the muscles most affected. While most forms, like the chromosome 5-related form, affect mostly the proximal muscles, other forms exist that affect mostly the distal muscles (those farther away from the body's center) at least in the beginning (figure 3).

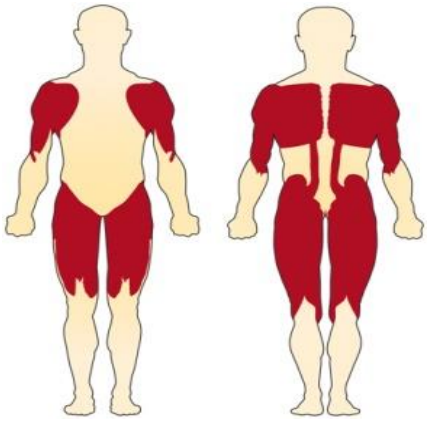

Figure 3: The muscles closer to the center of the body (proximal muscles) are usually more affected in SMA than are the muscles farther from the center (distal muscles).

Complications of SMA

Over time, people with SMA experience progressive muscle weakness and loss of muscle control. Potential complications include:

- Bone fractures, hip dislocation and scoliosis (curvature of the spine).
- Malnutrition and dehydration due to problems eating and swallowing that may require a feeding tube.
- Pneumonia and respiratory infections.
- Weak lungs and breathing problems that may require breathing support (ventilation).

Progression of SMA

In chromosome 5-related SMA, the later the symptoms begin and the more SMN protein there is, the milder the course of the disease is likely to be. While in the past, infants with SMA typically did not survive more than two years, today most doctors now consider SMN-related SMA to be a continuum and prefer not to make rigid predictions about life expectancy or weakness based strictly on age of onset. SMA is the most common genetic cause of mortality in infants [5].

Frequency

Spinal muscular atrophy affects 1 per 8,000 to 10,000 people worldwide. Spinal muscular atrophy type I is the most common type, accounting for about half of all cases. Types II and III are the next most common and types 0 and IV are rare. It is estimated to affect roughly 10,000 children and adults in the United States, and about 1 in every 50 Americans is a genetic carrier. The disease can affect infants and adults of any race or gender.

Causes

Mutations in the SMN1 gene cause all types of spinal muscular atrophy. The number of copies of the SMN2 gene modifies the severity of the condition and helps determine which type develops. The SMN1 and SMN2 genes both provide instructions for making a protein called the survival motor neuron (SMN) protein. Normally, most functional SMN protein is produced from the SMN1 gene, with a small amount produced from the SMN2 gene. Several different versions of the SMN protein are produced from the SMN2 gene, but only one version is functional; the other versions are smaller and quickly broken down. The SMN protein is one of a group of proteins called the SMN complex, which is important for the maintenance of motor neurons. Motor neurons transmit signals from the brain and spinal cord that tell skeletal muscles to tense (contract), which allows the body to move. Most people with spinal muscular atrophy are missing a piece of the SMN1 gene, which impairs SMN protein production. A shortage of SMN protein leads to motor neuron death, and as a result, signals are not transmitted between the brain and muscles. Muscles cannot contract without receiving signals from the brain, so many skeletal muscles become weak and waste away, leading to the signs and symptoms of spinal muscular atrophy [6].

Typically, people have two copies of the SMN1 gene and one to two copies of the SMN2 gene in each cell. However, the number of copies of the SMN2 gene varies, with some people having up to eight copies. In people with spinal muscular atrophy, having multiple copies of the SMN2 gene is usually associated with less severe features of the condition that develop later in life. The SMN protein produced by the SMN2 genes can help make up for the protein deficiency caused by SMN1 gene mutations. People with spinal muscular atrophy type 0 usually have one copy of the SMN2 gene in each cell, while those with type I generally have one or two copies, those with type II usually have three copies, those with type III have three or four copies, and those with type IV have four or more copies. Other factors, many unknown, also contribute to the variable severity of spinal muscular atrophy.

Learn more about the genes associated with Spinal muscular atrophy

- SMN1
- SMN2

Inheritance

Spinal muscular atrophy is inherited in an autosomal recessive pattern, which means both copies of the SMN1 gene in each cell have mutations. In most cases, the parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they typically do not show signs and symptoms of the condition. In rare cases, a person with spinal muscular atrophy inherits an SMN1 gene mutation from one parent and acquires a new mutation in the other copy of the gene that occurs during the formation of reproductive cells (eggs or sperm) or in early embryonic development. In these cases, only one parent is a carrier of the SMN1 gene mutation. Individuals who have more than the usual two copies of the SMN2 gene usually do not inherit the extra copies from a parent. They typically arise during a random error when making new copies of DNA (replication) in an egg or sperm cell or just after fertilization.

Diagnosis

Some SMA symptoms resemble those resulting from neuromuscular disorders like muscular dystrophy. To find the cause of symptoms, your healthcare provider will perform a physical exam and get a medical history. Your physician may also order one or more of these tests to diagnose SMA:

Blood test: An enzyme and protein blood test can check for high levels of creatine kinase. Deteriorating muscles release this enzyme into the bloodstream.

Genetic test: This blood test identifies problems with the SMN1 gene. As a diagnostic tool, a genetic test is 95% effective at finding the altered SMN1 gene. Some states test for SMA as part of routine newborn screenings.

Nerve conduction test: An electromyogram (EMG) measures the electrical activity of nerves muscles and nerves.

Muscle biopsy: Rarely, a physician may perform a muscle biopsy. This procedure involves removing a small amount of muscle tissue and sending it to a lab for examination. A biopsy can show atrophy, or loss of muscle.

Diagnosis of SMA during pregnancy

If you're pregnant and have a family history of SMA, prenatal tests can determine if your unborn baby has the disease. These tests slightly increase the risk of miscarriage or pregnancy loss. Prenatal tests for SMA include:

Amniocentesis: During amniocentesis, your obstetrician inserts a thin needle into your belly to draw out a small amount of fluid from the amniotic sac. A lab specialist (pathologist) checks the fluid for SMA. This test takes place after the 14th week of pregnancy.

Chorionic villus sampling (CVS): Your obstetrician removes a small tissue sample from the placenta through the mother's cervix or stomach. A pathologist checks the sample for SMA. CVS can take place as early as the 10th week of pregnancy.

Treatment

Although there's no cure for SMA, these treatments can help kids who have it:

Disease-modifying therapy: Nusinersen (or Spinraza™), a new treatment for SMA that was approved in 2016. This medicine increases the amount of protein the body needs from the missing SMN1 gene [7]. It works by making the "back-up" gene, the SMN2 gene, look more like the SMN1 gene and produce the needed protein. This is given through a spinal tap. Four doses are given over 2 months, and then every 4 months after that. Studies have shown significant improvement in breathing, motor function, and survival. A different medication, risdaplam (Evrysdi®), helps adults and children older than two months. People take risdaplam daily by mouth (orally) [8].

Gene replacement therapy: Children younger than two may benefit from a one-time intravenous (IV) infusion of a drug called onasemnogene abeparvovec (Zolgensma®). This therapy replaces a missing or faulty SMN1 gene with a functioning gene.

- Breathing support through a mask/mouthpiece or a breathing machine. If a breathing machine is needed, a tube may be placed into the windpipe (called a tracheostomy).
- Treatments to help kids cough and clear mucus, which can help prevent infections
- Proper nutrition. Sometimes a feeding tube is place through the nose into the stomach (an NG tube) or directly into the stomach (called a gastrostomy tube, or G-tube). This way, feedings can go right into the stomach.
- Medicine.
- A splint, brace, or sometimes surgery for scoliosis.
- Physical therapy and occupational therapy.
- Counseling and support groups [9].

Other Names for this Condition

- 5q SMA
- Proximal SMA
- SMA
- SMA-associated SMA
- Spinal amyotrophies
- Spinal amyotrophy
- Spinal muscle degeneration
- Spinal muscle wasting

Zolgensma

Zolgensma is a gene therapy medicine for treating spinal muscular atrophy, a serious condition of the nerves that causes muscle wasting and weakness. It is intended for patients with inherited mutations affecting genes known as SMN1, who have either been diagnosed with SMA type 1 (the most severe type) or have up to 3 copies of another gene known as SMN2 [10].

Mechanism of action

Zolgensma is an adeno-associated virus (AAV) 9 based gene therapy designed to deliver a copy of the SMN1 gene to encode for human SMN protein. It is a recombinant form of self-complementary AAV9, which contains human SMN protein-encoding transgene. The Zolgensma drug will be available in single-use vials, each containing a nominal concentration of 2.0 × 1013 vector genomes (vg) per millilitre for intravenous infusion.

Patients with spinal muscular atrophy have a defect in a gene known as SMN1, which the body needs to make a protein essential for the normal functioning of nerves that control muscle movements. The active substance in Zolgensma, onasemnogene abeparvovec, contains a functional copy of this gene. When injected, it passes into the nerves from where it provides the correct gene to make enough of the protein and thereby restore nerve function.

Zolgensma Administration

Zolgensma is given once as an infusion (drip) into a vein lasting about 1 hour. The infusion should take place in a clinic or hospital under the supervision of a doctor experienced in managing spinal muscular atrophy. Before and after receiving the infusion, the patient will have a number of tests, including liver and blood tests, and will be given corticosteroid medicines to reduce the risk of side effects. The medicine can only be obtained with a prescription. For more information about using Zolgensma, see the package leaflet or contact your doctor or pharmacist.

Side effects

Zolgensma can cause mild or serious side effects. Mild side effects of Zolgensma may include vomiting. Most mild side effects may go away within a few days or a couple of weeks. But if they become more severe or don't go away, talk with your child's doctor or pharmacist.

Serious side effects and their symptoms can include:

- High levels of troponin I (a protein that helps the heart muscles contract).
- Thrombotic microangiopathy (damage to small blood vessels).Symptoms can include:
- High blood pressure
- Bruising
- Seizures
- Low urine output
- Allergic reaction.
- Liver damage.
- Low platelet levels [11].

Benefits of Zolgensma have been shown in studies

A main study showed that Zolgensma reduces the need for artificial ventilation in babies with spinal muscular atrophy. In this study, 20 out of the 22 babies given Zolgensma were alive and breathing without a permanent ventilator after 14 months, when normally only a quarter of untreated patients would survive without needing a ventilator. The study also showed that Zolgensma can help babies sit unaided for at least 30 seconds. 14 out of the 22 babies given Zolgensma were able to do so after 18 months, a milestone that is never achieved in untreated babies with severe forms of the disease.

Zolgensma authorised in the EU

The main study of Zolgensma showed that a one-time infusion can improve survival in these patients and reduce the need for a permanent ventilator to breathe. It can also help them reach development milestones. As for its safety, the side effects of Zolgensma are considered manageable; the most common side effect in the study, raised liver enzymes, resolved after treatment with a steroid. The European Medicines Agency therefore decided that Zolgensma's benefits are greater than its risks and it can be authorised for use in the EU. Zolgensma has been given 'conditional authorisation'. This means that there is more evidence to come about the medicine, which the company is required to provide. Every year, the Agency will review any new information that becomes available and this overview will be updated as necessary.

Nusinersen

In December 2016, the US Food and Drug Administration (FDA) approved nusinersen (Spinraza, Biogen, Cambridge, MA) to treat SMA. Nusinersen, an antisense oligonucleotide, is administered directly into cerebrospinal fluid. It alters SMN2 pre-RNA splicing so exon 7 is included, increasing expression of functional SMN protein. Although nusinersen was FDA approved for treatment of all forms of SMA, the initial clinical trials were limited to patients up to age 14 years, diagnosed with SMA-1,-2, -3, not on mechanical ventilation support. Two subsequent phase 3 trials were completed for SMA-1 and SMA-2/-3 and demonstrated improved motor milestones and event-free survival, better than expected based on natural history studies. Efficacy assessments for patients receiving nusinersen are based on serial assessments of performance on age-appropriate standardized motor scales. Treatment requires complex financial and logistics because of the very high drug cost, intrathecal administration, and medical fragility of the patients. Treatment implementation also engenders ethical considerations related to cost, insurance coverage, limited clinical data on groups of patients not in clinical trials, and questions of duration of treatment. Nusinersen has been integrated into the treatment of many SMA patients [12].

Mechanism of action

Spinraza contains an antisense oligonucleotide (ASO), which controls the mutations caused in the chromosome 5q. This selectively binds and targets RNA and regulates gene expression. It has the

potential to enhance the amount of functional SMN protein in infants and children with SMA [13]. Using in vitro assays and studies in transgenic animal models of SMA, nusinersen was shown to increase exon 7 inclusion in SMN2 messenger ribonucleic acid (mRNA) transcripts and production of full-length SMN protein. Nusinersen acts to replace the SMN protein deficit which causes SMA, by increasing the splicing efficiency of the SMN2 pre- mRNA. More specifically, nusinersen in an 18-mer 2'-MOE phosphorothioate antisense oligonucleotide that acts as a splice-altering oligonucleotide. Nusinersen was designed to pair with a specific target sequence on the SMN2 pre-mRNA to displace heterogeneous ribonucleoproteins (hnRNPs) at the intronic splice silencing site-1 (ISS-1) between exons 7 and 8 to allow for more complete translation of SMN protein from the paralogous gene SMN2. Further reinforcing this concept, SMA phenotype is closely tied to SMN2 copy number. SMN2 serves to produce SMN protein, however at a greatly reduced rate because of differential splicing caused by the binding of the hnRNPs at the ISS-1 [14].

As an antisense oligonucleotide (ASO), SPINRAZA targets an underlying cause of motor neuron loss by binding to a specific sequence in the SMN2 gene to increase the production of functional SMN protein in the central nervous system (CNS).Delivery of SPINRAZA to the CNS helps infuse the motor neurons with nusinersen and maintain a high concentration and long half-life in the spinal cord, where it's needed most [15].

Side effects

People treated with Nusinersen had an increased risk of upper and lower respiratory infections and congestion, ear infections, constipation, pulmonary aspiration, teething, and scoliosis. There is a risk that growth of infants and children might be stunted. In older clinical trial subjects, the most common adverse events were headache, back pain, and other adverse effects from the spinal injection, such as post-dural-puncture headache. In 2018, several cases of communicating hydrocephalus in children and adults treated with nusinersen emerged; it remains unclear whether this was drug related [16].

Reported cases of SMA in Kerala

It has been really unfortunate to see the rising number of Spinal Muscular Atrophy (SMA) cases in the state of Kerala. It is said that there are more than 100 reported cases in the state suffering from SMA, of whom only a few have received the treatment under the compassionate use programme extended by pharmaceutical companies. Due to the high cost of the drug in the market, the access of drug to majority of the patients became a nightmare.

While considering the recent reported cases, all of children below 15 years of age, are in need of Zolengsma injection. A child 'X' of 2 months age is diagnosed with SMA type 1, which is the most severe early onset forms of Spinal Muscular Atrophy. This disorder attacks the baby's nerves and muscles, and as it progresses, makes it extremely difficult for them to carry out basic activities like sit up, lift their head, swallow milk and even breathe. The baby is currently (2021) four months old and along with many other problems such as legs paralyse and difficulty to breath etc. But it was not the case for another six month old. He was diagnosed with SMA when he was one month old, and he required ventilator support to breathe. For him, the Zolengsma injection was an emergency. After waiting six months for the drug in the ventilator, he took his last breath.

Another 15 year old girl from Kerala, was left paralysed when she was only two-and-a-half. She was diagnosed with Spinal Muscular Atrophy and years later her little brother of one and half years old also got diagnosed with SMA, the inherited disease that damages the nerve cells, called motor neurons, in the spinal cord. He was in a critical condition where he could only live if he get the Zolengsma injection before two years of age. The social media helped to raise funds for these kids.

Looking onto the context of last 10 years in Kerala, SMA were brought to light by media due to the protest head by the patients. There were patients from zero years to 28 years of age, all suffering from this disease. They collectively formed 'Kerala SMA forum' for the better consideration from the government and society. But, till now the government gave no green card to their needs. When some odd cases of SMA gathers huge attention, the government tries to reduce the tax that falls under the import section.

The drug is of USA origin which nearly costs 18 crore in Indian market, which is available only when there is a requested demand. The patients are unable to afford such a great amount all the time and hence, the treatment often occurs by the collective effort from the society.

We could also witness cases where children died of SMA disease which went unreported. It's been late for the government and researchers of the country either to develop a medication which makes the patients accessible or to find an alternative. They all are citizens who need proper care and attention equally from the government and the society.

CONCLUSION

SMA is the most common genetic disease of the spinal motor neuron. It can manifest any time from prior to birth to adulthood with varying severity and disease impact, and it is the most common genetic cause of death in infants. Though discovery of the causative gene occurred almost 20 years ago, no disease modifying treatments are yet available. Nevertheless, effectively designed supportive care can reduce disease burden and improve quality of life significantly, but continued refinement of supportive care is needed. Despite major progress in our understanding of the biological consequences of SMN reduction, the pathogenic mechanism of SMA by which low levels of SMN protein lead to selective loss of motor neurons remains undefined. Regardless, preclinical development has led to several SMN-restoring therapies that have shown dramatic success in animal models of the disease, and several of these candidates are currently being testing in early phase clinical trials. The US Food and Drug Administration (FDA) on May 24, 2019 approved onasemnogene abeparvovec, a drug for the treatment of spinal muscular atrophy (SMA). The drug, developed by the Swiss drug maker Novartis, has been marketed under the trade name Zolgensma. The drug has been approved to be used in children under 2 years of age, who are confirmed to be a case of SMA through genetic testing. The treatment is going to cost $2.125 million (approximately 14 crore rupees) and the drug is given as one-time infusion over 1 h. Nusinersen is the first treatment available for patients with SMA. In clinical trials, it was found to be safe and resulted in improvement in motor function. The SHINE trial is ongoing and will provide additional data on longer-term treatment with nusinersen. The introduction of nusinersen to the market represents new hope for patients diagnosed with SMA. Since, we have an increased number of SMA cases in Kerala; it's all up to the researchers in the country, to develop a safe, effective and affordable new medication for SMA.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] Michael Rubin, Spinal Muscular Atrophies (SMA), New york Presbyterian Hospital-Cornel Medical Center, 2020.
[2] Ogino S, Wilson RB. Human Genetics 2002; 111(6):477-500.
[3] Darras BT. Neurology 2011; 77 (4): 312-4. doi:10.1212/WNL.0b013e3182267bd8.
[4] David Arnold W, Darine Kassar, John T. Muscle and Nerve 2015; 51(2):157-167.
[5] Roberts DF, Chavez J, Court SD. Archives of Disease in Childhood 1970; 45(239):33–38.
[6] Suzie Lefebvre, Lydie Bürglen, Sophie Reboullet, Olivier Clermont, Philippe Burlet, Louis Viollet *et.al*. Cell 1995; 80(1): 155-165.
[7] Spinraza- nusinersen injection, solution. DailyMed, 2020.
[8] SMA News Today. Evrysdi (Risdiplam).
[9] Mena T. Scavina, KidsHealth.org. 2018.
[10] Zolgensma https://www.ema.europa.eu/en/medicines/human/EPAR/zolgensma
[11] Zolgensma https://www.medicalnewstoday.com/articles/drugs-zolgensma
[12] Erin E. Neil, Elizabeth K. Bisaccia. Journal of Pediatric Pharmacology and Therapeutics. 2019; 24(3): 194–203.
[13] Spinraza (nusinersen) for the Treatment of Spinal Muscular Atrophy (SMA) https://www.clinicaltrialsarena.com/projects/spinraza-nusinersen-for-the-treatment-of-spinal-muscular-atrophy-sma/
[14] Nusinersen https://go.drugbank.com/drugs/DB13161
[15] SPINRAZA. Prescribing Information. Cambridge, MA: Biogen; 2020.

[16] New warning of nusinersen-related communicating hydrocephalus. Reactions Weekly.2018; 1714 (1): 3.