Spinal muscular atrophy (SMA) is a complex congenital myoneural disease, distinguished by gradually increasing muscular weakness, resulting in significant disability and atrophy of muscles. The disease is caused by homozygous or heterozygous omission of survival motor neuron (SMN1) gene or mutation in SMN1 gene which causes loss of its encoding function to produce SMN protein. This SMN1 gene is positioned on chromosome 5q13.

In healthy individuals, SMN1 is primarily responsible for the production of survival motor neuron protein. Along with SMN1 gene, humans bear a homologue SMN2 gene replica which varies from SMN1 by single nucleotide. While SMN2 gene go through a splicing process, it produces an mRNA lacking exon 7 that thereby results in a very little, insufficient amount of intact SMN protein. Majority of the protein encoded from SMN2 is unstable, only about 10% of protein manufactured from SMN2 is stable and functional. In simple terms, SMA patients experience the loss of motor neurons in the spinal cord that control muscle movement, due to insufficient amounts of SMN protein. In the absence of these motor neurons, muscles don’t receive nerve signals to initiate muscle movement, causing muscle weakness and wasting.

More than 95% of cases of SMA are of the autosomal recessive variety, which is brought about by homozygous deletions of or mutations in SMN1 gene positioned in the long arm of chromosome 5. Werdnig and Hoffmann defined this disease for the first time in the 1890s and the genetic defect was localized to chromosome 5q with the identification of SMN gene in 1995. With an incidence of 1:11,000 live births, it is mostly diagnosed in infants and children but adults can also manifest the signs of SMA.

For treatment, considerable endeavors have been dedicated to formulating potential therapeutic solutions along the lines of substituting SMN1 or reducing SMN2 exon omission to boost the level of functional protein. Gene replacement and disease-modifying therapies provide hope to the sufferers and their families globally. In order to create a widely agreed statement
Classification and Clinical Description

The SMA manifests a wide range of clinical severity and its clinical traits can be graded into four primary categories-SMA Type I, II, III and IV mainly founded on the age of commencement of symptoms and highest motor milestones attained. Symptoms as well as prognosis of the disease vary depending on SMA phenotype. The variance observed among the clinical cases of SMA is basically attributed to changing SMN2 gene copy numbers. SMN2 gene lacks exon 7 owing to alternative splicing, thus producing an unstable SMN protein. Even after the lack of exon 7 in SMN2, it also translates small amounts of workable SMN protein. The severeness of clinical phenotypes is inversely associated to the number of SMN2 copies in genetic material. Type I, II and III SMA embodies around 60, 27 and 12% of all SMA cases respectively. Figure 1 illustrates the SMN2 gene copies present, milestones achieved and clinical manifestations associated with each type of SMA.

Type 0 SMA

An added category of SMA is Type 0 SMA includes neonatal patients that exhibit significant hypotonia, weakness and a history of reduced fetal movements. Such patients are referred to as type 0 neonates. Most likely, the onset of the weakness is at a prenatal stage. Atrial septal abnormalities, facial diplegia, areflexia, and joint contractures can be seen in type 0 infants. Respiratory failure is a significant issue and life expectancy decreases. The majority of infants with Type 0 SMA are not able to live beyond 6 months of age.

Type 1 SMA

About 60% of patients diagnosed with SMA can be categorized as type 1 SMA, which is also referred as Werdnig-Hoffman illness. Type 1 SMA is the most serious and prevalent kind. It is differentiated by the beginning of illness prior to 6 months of age and fatality before second birthday of the child. It is also typified by a speedy depletion of respiratory and motor function within a year after birth. Babies with the SMA type I usually develop clinical signs such as decreased resting tension in muscles, poor head control, lower or missing tendon reflexes and symmetrical flaccid paralysis preliminary to the 6 month mark. The inability to sit unassisted is a critical indication of this type of SMA.

Weakened intercostal muscles with the spared diaphragm, cause paradoxical chest movements while breathing and a bell-shaped chest, thus referred to as “belly breathing”. Tongue weakness and fasciculation with poor suck and swallow are often present. Such infants are at the risk of aspiration, increasing the danger of aspiration pneumonia, a significant contributor to health and fatality.

Type 2 SMA

This phenotype of SMA is of moderate severity and its symptoms begin to manifest after 6 months of age upto 18 months. Children are able to sit unaied, whereas they will not be able to walk unassisted at any point of life. The gradual proximal leg weakness in this intermediate variant of SMA is more pronounced than the arm weakness. On examination areflexia and hypotonia both exist. The comorbidities largely revolve around joint contractures, mandibular ankylosis, and problems in bones and joint formation may appear due to the presence of increasing scoliosis and muscle weakness. Scoliosis combined with weakness of intercostal muscles may result in severe restrictive pulmonary illness. Cognition is normal in these children. When weak swallowing is present, it might inhibit weight gain. Respiratory insufficiency is observed to be the common cause of death during adolescence.

Type 3 SMA

Adults along with children could suffer from Type 3 SMA, but they normally attain important motor milestones including autonomous ambulation. Some patients might demand a walking aid in the early years of life, however, others might persist in independent walking and witness an active adult life with slight muscle weaknesses indicating profound symptom variability existing in Type 3 SMA. This SMA phenotype, also referred as Kugelberg-Welander disease, typically manifests symptoms of muscular fatigue, gradual weakness and wasting of lower limb muscles after they have already achieved unassisted walking. There are patients with clinically diverse conditions who have SMA type III. Their proximal muscle weakness, however, develops during infancy. Patients who lose their capacity to walk may get scoliosis and other health conditions caused due to reduced mobility including osteoporosis and obesity. Life expectancy and cognitive ability are not reduced in this group. These individuals normally have little or no respiratory muscle weakness.

Type 4 SMA

SMA type 4 patients fall on the milder end of the spectrum. This type includes patients who underwent adult onset and experienced mild course of illness. They make up less than 5% of SMA cases. Similar to type 3, these people can walk around, however, unlike type 3, their symptoms first appear in maturity, sometimes at age 30 or even later but sometimes onset can be in younger once also. Patients categorized under this type are spared of respiratory as well as nutritional problems and motor impairment is mild.
Before the revelation of the genetic etiology of SMA, this disease baffled the medical field with an unanswered question that in what way a single gene defect is being able to create such a broad spectrum of clinical manifestations and severity. An explanation to this query was initiated by the discovery made in Melki laboratory in 1995. This discovery revealed that a homozygous removal of the SMN1 gene is the reason for causing SMA in a majority of cases, regardless of its severity. The human genome carries numerous copies of SMN gene but has only one copy of telomeric SMN i.e. SMN1 and several copies of centromeric SMN i.e. SMN2. SMN1 cause to produce a 38 kDa protein having chain of 294 amino acids. This protein is ubiquitous as it is coded in all somatic tissues and safeguarded in wide variety of fauna. Out of these two forms of SMN genes, each patient suffering from SMA is deficient in an efficient SMN1 gene and rely upon the available SMN2 gene for providing SMN protein required for function. A major portion of SMN protein translated from the SMN2 gene is unstable and ineffective, while this gene also produces small quantities of functional SMN protein. Thus, SMN2 gene is incompetent for generating functional SMN protein in sufficient quantities. Consequently, the amount of functional and integral SMN protein expressed by SMN2 decides the severity of the disease and the variation in SMN2 gene copy number in SMA patients solves the riddle. Several investigations on genotype/phenotype too have validated an association between a greater number of SMN1 gene copy number and less severe SMA phenotypes and vice-versa. The homozygous deletion of SMN1 or mutation in SMN1, along with the clinical manifestations is considered as diagnostic for SMA. Thus, the diagnosis of SMA is essentially based on SMN1 molecular testing and it also help to recognize the carriers. The sensitivity of diagnostic testing can be enhanced by screening for intragenic mutations in SMN1. SMN2 copy number has decisively proved to improve the phenotype and provide valuable insights for prognosis. SMN2 copy number is not the singular modifier of SMA phenotypes, even though it is considered to be the essential explanation of illness severity. Other phenotypic moderators have been explained and information on additional modifiers is anticipated as the comprehension about the pathogenesis of SMA at the molecular level, is deepened.

**Treatment**

Over the past few years, numerous feasible interventions founded on the common precept of augmenting the coding of the SMN protein have been tried. Such interventions encompass therapeutically active molecule therapies as well as gene therapies to augment SMN2 translation, antisense oligonucleotide-founded interventions to increase fusion of exon 7 into mRNA transcribed from SMN2 gene, and virus-vector-based interventions for substituting the complete SMN1 gene. Latest treatment alternatives include splicing modification of SMN2 and replacement of SMN1 gene using gene therapy. It has been observed that early initiation of these treatments can substantially alter the natural course and future outcome of the disease. However, collected proofs for such latest therapies are limited, based on the small size of a group of patients considering age, disease phenotype and disease progression.

In 2007, the International Standard of Care Committee for Spinal Muscular Atrophy (ISCCSMA) presented the general guidance on treatment in first-ever general agreement on standards of the care provided in the treatment of SMA. There has been a consensus that multidisciplinary medical care is required with interdisciplinarity handling of respiratory, gastric and intestinal, orthopedic, psychosocial and nutritional issues along with rehabilitation and palliative care.

Treatment approaches can be broadly divided into SMN-dependent gene therapies and treatments targeting SMN autonomous factors. SMN contingent gene therapies incorporate splicing modulation of SMN2 (onasemnogene abeparvovec) and substituting SMN1 gene (onasemnogene abeparvovec), whereas therapies focusing on SMN independent aspects involve muscle strengthening interventions, treatment of spinal deformities and neuroprotection. Figure 2 shows the authorized treatments and complementary therapies in development for SMA.

Hereupon, the review broadly discusses SMN-dependant gene therapies such as therapies targeting to correct the splicing of SMN2 and therapy substituting the SMN1 gene. Treatments targeting SMN SMN-independent factors have also been discussed.

**SMN dependant gene therapy**

Gene therapy is a therapeutic approach with the aim to correct underlying genetic problems via rectification of mutated genes or site-specific alterations or replacement of an abnormal gene causing an abnormality or disorder with a normal gene. Out of different challenges associated in the method of gene therapy,
the most critical is the complication involved in delivering the gene into the stem cell. Thus, a molecular carrier termed as “vector” is employed to deliver the gene. Such vector needs to possess various characteristics including high specificity, efficiency in the release of single or multiple genes, and at the same time be unrecognizable to the immune system. Finally, the vector should have the capability to express the gene for the patient’s entire life.  

The first authorized medicine to treat SMA specifically, is aimed at restoring SMN production by modifying SMN2 splicing.  

Splicing modulation of SMN2  
Nusinersen was the first approved drug for SMA and is the noted turning point in SMA treatment. United States Food and Drug Administration (USFDA) approved this therapy in December 2016, while European Medicines Agency (EMA) provided approval in July 2017 for all 5q chromosome-associated SMA types. Nusinersen is an antisense oligonucleotide (ASO) intended to tether to the SMN2 pre-mRNA and support the insertion of exon 7. It causes alteration of SMN2 pre-RNA splicing process by inducing inhibition of splicing factors. This improves the incorporation of SMN coding section, referred as exon 7, into mRNA transcript and consequently augments level of functional protein. This molecule’s inability to penetrate the blood-brain barrier necessitates its’ administration into the cerebrospinal fluid through the intrathecal route. On the basis of current experience, drug shows no severe side effects, but some side effects associated with other ASOs such as nephrotoxicity, blood clotting disorders and thrombocytopenia need to be acknowledged. Another drawback is its invasive intrathecal delivery, multiple times in a year and its CNS-specific distribution which led to issue of systemic symptoms not fully focused on.

Risdiplam is an orally deliverable small molecule and third approved therapy for SMA. It specifically binds at two sites in SMN2 pre-mRNA and modulates splicing of SMN2. The ability to bind at two sites with precision, enhances amounts of integral SMN mRNA transcript and functional protein, lowering the influence on splicing of other untargeted locations and thus decreasing the potential of side effects. It reaches the central nervous system as well as peripheral organs and increases SMN protein levels in those areas, compared to intrathecally administered Nusinersen whose effect is limited to motor neurons of the central nervous system. Its’ other advantage is that it is an orally administered molecule. One of the limitations of risdiplam is its’ targeting of the cell division regulator FOXM1 at high concentrations of risdiplam, increasing the risk of oncogenic side effects. This necessitated the strict monitoring of dosage in clinical trials.

Branaplam is a potential drug to modulate SMN2 splicing for improving the expression of functional SMN. It was recognized for SMN2 exon 7 insertions. Additionally, it has been demonstrated to stabilize SMN2 pre-mRNA. When administered daily, it exhibited an increment in exon 7 insertion and a subsequent increment in the production of SMN protein in animal model. This led to an increase in body mass and increase in life expectancy. The first clinical trial of branaplam started in 2015 in which the subjects were infants below 6 months of age suffering from SMA and possessing two copies of SMN2. This is Phase 1 and 2 study, estimated to complete in 2023.

Another recently identified drug, TEC-1, having a molecular configuration comparable to risdiplam, has led to enhanced production of SMN protein, extended survival, and improved symptoms of severe SMA in pre-clinical studies. It has an added advantage over risdiplam of having lesser off-target splicing alterations.

In another finding, a calcium channel blocker molecule, flunarizine was found to modulate splicing incidences in HeLa cells and promote intron preservation in SMN. Such activity of flunarizine was confirmed in an animal model where it improved the muscular functionality and viability of spinal motor nerve cells in mice with SMA. When tested in SMA patients, it enhanced the accumulation of SMN in Cajal bodies in fibroblasts.

Gene Replacement for SMN1  
Onasemnogene abeparvovec (Zolgensma) is a gene replacement therapy for SMN1, in which wild-type SMN1 gene is delivered to motor neuron cells to replace mutated SMN1 gene. Onasemnogene abeparvovec is the second approved therapy for SMA, before which Nusinersen was the only treatment available for SMA. This therapy employs nonreplicating adeno-associated virus capsid (scAAV9) as a vector to carry and transfer wild-type SMN1 gene. This construct is composed of a vector harboring SMN1 complementary recombinant DNA that have been able to cross the brain–blood barrier. It generates the expression of SMN protein for a prolonged duration. Administered through the intravenous route, it is a one-time-administered gene therapy to replace mutated gene.

Data generated from clinical trials demonstrates noteworthy enhancement in achieving motor milestones and survival without the assistance of ventilation, in the treated subjects, but this data is limited. Another limitation linked with this therapy is its hefty price which is deterring widespread access to drug and its use. However, it can be concluded that this therapy could be more economical in the long term when compared to multiple intrathecal injections required with nusinersen. Another issue is the lack of long-term clinical trials data without which safety profile of this therapy is incomplete.

The FDA approved the drug for conducting clinical trials in May 2019. The therapy bears the designations of FDA breakthrough therapy and European priority medicines (PRIME). It was evaluated by EMA under a fast-track evaluation process and was granted Conditional Marketing Authorisation in 2020.

STR1VE study was a phase 3 trial conducted on symptomatic participants having type ISMA and who carried two copies of SMN2 gene. The purpose was to assess the safety and efficacy of the therapy. This study was completed in November 2019. In STR1VE-US, 91% participants achieved the
conclusive terminal point of event-free survival at 14 months and 59% achieved the terminal point of getting the capability to sit for 30 seconds or more at the age of 18 months. 68.2% of patients did not require non-invasive ventilatory support while in study, whereas 81.8% did not need ventilatory support at the age of 18 months.

In August 2022, Novartis declared its plans to modify the labeling of the Onasemnogene abeparvovec (Zolgensma) drug due to reported events of fatal acute liver failure on use of the drug. Such an announcement was made after reports of fatal acute liver failure in two patients treated with Zolgensma.

The fact that only one injection is required and it will lead to SMN protein expression in the whole body provides the decisive edge to this therapy. Preclinical trials in primates and piglets using large intravenous doses, were reported to show acute liver damage and toxicity to sensory neurons. Another problem associated with the therapy would be the SMA patients who are previously carrying anti-AAV9 antibodies.

Muscle Boosting Therapies
Reldesemtiv (formerly recognized as CK-2127107 and Tirasemtiv) is a small-molecule and second-generation fast–skeletal muscle troponin activator. It was originally introduced to boost skeletal muscle function for disorders showing weakness of muscle and fatigue. It preferentially adheres to the fast skeletal muscle troponin complex and enhances its sensitivity towards calcium. It also has the potential usefulness for the treatment of people suffering from debilitating diseases and conditions linked with muscular weakness and fatigue. Its use in SMA is justified by numerous evidences.

Treatment for Spinal Deformities
Since non-sitters have a low chance of life, spinal management has been considered as a potential treatment for them if their respiratory and nutritional systems are stable. Under the condition that pulmonary function will not be hampered, specific rigid braces that permit a steady sitting position may be utilized. They may be followed up with a supine Cobb angle or one obtained while sitting with a trunk brace. The situation of spinal care in these patients is constantly evolving as a result of the introduction of new medicines that increase survival and improve overall functional outcomes.

Smn Independent Therapeutic Targets: Future Prospects
Before the gene therapies for SMA were discovered, multiple approaches were tried to improve the SMN amounts utilizing drugs which were not specifically aiming at SMN genes. Such therapies can definitely be reviewed as adjunct therapies to further improve SMN expression.

Knowledge of histone acetylation controlling SMN expression led to the investigation of Histone deacetylase (HDAC) inhibitors in SMA models. Research on HDAC inhibitors in neural cells of SMA clinical cases demonstrated that HDACs inhibitors class I could improve SMN expression. An example of class I HDAC inhibitor, valproic acid has showed a largely favorable impact on motor function, but provided slight proof of improvement in survival. Other molecules having HDAC suppressive activity encompass trichostatin A, resveratrol, etc. Such drug entities found to be successful in SMA pre-clinical studies, but are yet to be explored fully for its clinical application.

R-loop resolution is a possible therapeutic goal in SMA treatment. Senataxin, a DNA repair factor shows a depressed production in animals with SMA. But when it is expressed excessively in spinal cord motor neurons of SMA animal model, it has been shown to decrease R-loop creation and DNA damage. This suggests potential of senataxin as a therapeutic target in SMA treatment. Zinc-finger protein also designated as ZPR1 is another nuclear factor identified to have depressed production in SMA models. Animal models was found to show enhanced righting reflexes, augmented motor neuron viability, increased diameter of muscle fibers and improved survival of mouse model by twice on increasing its expression. An augmented expression of SMN itself was observed with ZPR1 overexpression but could have more protective mechanisms too.

Stabilization of the SMN protein by inhibiting the decomposition of SMNΔ7 which is the product of SMN2 gene copies could be considered as another therapeutic strategy. This will allow even lower magnitude expression of SMN2 to show a more prominent influence on intracellular pathways. Indoprofen, which is a non-steroidal anti-inflammatory drug (NSAID) was found to selectively enhance SMN2-luciferase reporter protein and thus increased SMN protein quantities in fibroblasts of clinical cases. Other compounds namely azithromycin, bortezomib also have demonstrated stabilization of SMN Δ7. However, these mentioned approaches have not reached stage of clinical trials for SMA yet. These therapies can be used as an adjunct along with other SMN-targeting treatments for improved clinical outcomes.

Numerous autonomous factors unrelated to SMN found to have participated in SMA pathogenesis in recent years based on both in vivo and in vitro research, and they might serve as potential targets for new treatments.

One of the targets that need to be explored for the management of SMA is autophagy involving autophagosomes. A rise in autophagosomes was observed both in vivo and in-vitro, SMA motoneurons’ cytoplasm investigations. Further, it has been demonstrated that intracerebroventricular administration of 3-methyladenine inhibits autophagy and along with alleviating autophagic features, it increases lifespan and improved control on movements in SMA animal models. Another entity with therapeutic potential is agrin which is a synaptic organizer necessary to effectively transmit signals between neuron and muscles. Muscles in SMA animal model have shown a reduction in agrin expression levels by half. Moreover, the application of C-terminal section of agrin to SMA animal model is able to bring back the interaction between muscles and motor neurons and positively affected the development of the neuromuscular junction. Replacement of agrin gives results akin to alleviation of SMA symptoms and
leads to prolonged survival in SMA model mice. Even though the outcomes of pre-clinical studies on discussed therapeutic targets are promising, additional evidence are necessary before the clinical translation of new compounds is initiated. 

CONCLUSION

A noted milestone in the history of SMA was the approval of Nusinersen in 2016. It was followed by approval in 2019 and in 2020 to Onasemnogene abeparvovec and Risdiplam respectively. Even though these therapies not proved to be a panacea for the patients of SMA, they had given them hope with improved clinical outcomes. These three therapies work by offsetting the shortage in SMN protein via genetic manipulation or gene replacement. These therapies have been able to entirely enhance the phenotypic category of the patients, who will no longer show the natural course of SMA. After achieving the prolongation of lifespan and improvement in neuromuscular functions, the lingering concern is the non-CNS symptoms in clinical cases receiving treatments targeting CNS. Another thing to note down for these licensed therapies is that, for none of the three therapies, the long-term effects are available, except in the case of nusinersen. For nusinersen, we have 5 years of experience of use. Long-term effects will manifest only after several years from the initiation of therapy. All the information and insight we have, have been made available through clinical trials with specific inclusion criteria, conducted and funded by pharmaceutical companies. We are gradually getting Phase 4 data from a broad range of clinical cases over a prolonged duration of treatment, which is accentuating the weaknesses of current SMN replacement therapies. Furthermore, only patients who receive therapy early in life have much better prospects and we can also see the section of patients who are unresponsive to these therapies. In addition, high price tag of these therapies acts as an added deterrent to access to treatment. One injection of onasemnogene abeparvovec costs around $2.1 million, turning it the costliest therapy globally. High cost is a major inhibition to access to therapy particularly patients living in low and middle countries and those who are not covered under any health insurance. Crowdfunding had provided a ray of hope to access gene replacement therapy, but not covered under any health insurance. Crowdfunding had provided a ray of hope to access gene replacement therapy, but protracted. Such high expenditure can become a serious economic concern for patients and their families. Moreover, treated patients remain disabled and require a high level of care. Presently, there is no availability of any clinical guidelines to assist clinicians and families in the selection of a treatment over others for a particular clinical case. Numerous clinical trials over the therapies with varied inclusion criteria, assessment parameters and endpoint measures had made comparison between these therapies a difficult task. Such issue is anticipated to be solved through the trials of patient volunteers precisely aimed at safety and efficacy comparison between therapies.

It is now well accepted that for better outcomes of SMN replacement therapies, intervention should be started at the earliest possible manner. The golden rule to treat SMA is therefore to include congenital screening of neonates for SMA. There has been awareness about the limitations of these therapies, notably for patients with late diagnosis, because early treatment is the key for better outcomes. This necessitates the inclusion of approaches that encompass therapies autonomous to SMN, like those mentioned in the discussion. Such a multidisciplinary approach, involving use of drugs with established safety parameters, could possibly make medical interventions reasonably safer and accessible to the patient. Clinical trials also underscore the call for adjunct therapies to provide better quality of life to patients with SMA. Initiation of intervention at early age along with the utilization of adjunct therapy seems to be a sound approach to treat SMA patients.

REFERENCES


