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ISS-N1 MAKES THE FIRST FDA-APPROVED DRUG FOR SPINAL MUSCULAR ATROPHY

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Abstract

Spinal muscular atrophy (SMA) is one of the leading genetic diseases of children and infants. SMA is caused by deletions or mutations of *Survival Motor Neuron 1* (*SMN1*) gene. *SMN2*, a nearly identical copy of *SMN1*, cannot compensate for the loss of *SMN1* due to predominant skipping of exon 7. While various regulatory elements that modulate *SMN2* exon 7 splicing have been proposed, intronic splicing silencer N1 (ISS-N1) has emerged as the most promising target thus far for antisense oligonucleotide-mediated splicing correction in SMA. Upon procuring exclusive license from the University of Massachussets Medical School in 2010, Ionis Pharmaceuticals (formerly ISIS Pharamaceuticals) began clinical development of Spinraza™ (synonyms: Nusinersen, IONIS-SMN$_{RX}$, ISIS-SMN$_{RX}$), an antisense drug based on ISS-N1 target. Spinraza™ showed very promising results at all steps of the clinical development and was approved by US Food and Drug Administration (FDA) on December 23, 2016. Spinraza™ is the first FDA-approved treatment for SMA and the first antisense drug to restore expression of a fully functional protein via splicing correction. The success of Spinraza™ underscores the potential of intronic sequences as promising therapeutic targets and sets the stage for further improvement of antisense drugs based on advanced oligonucleotide chemistries and delivery protocols.

Introduction

Spinal muscular atrophy (SMA) is the leading genetic cause of infant mortality [1]. SMA is characterized by the degeneration of α-motor neurons in the spinal cord, leading to progressive muscle weakness followed by respiratory insufficiency [1,2]. SMA is caused by low levels of Survival Motor Neuron (SMN) protein due to homozygous deletion or mutation of the *SMN1* gene [3,4]. SMN is involved in several critical functions including but not limited to snRNP assembly, snoRNP assembly, telomerase biogenesis, transcription, translation, DNA repair, RNA trafficking, selenoprotein synthesis, stress granule formation, and various signaling pathways [5]. SMA is unique among genetic disorders in that humans carry a second copy of the *SMN* gene, *SMN2* [3,6]. However, due to a translationally silent C-to-T mutation (C6U in RNA) at the 6th position of exon 7, *SMN2* exon 7 is inefficiently

spliced producing a truncated protein SMNΔ7, which is unstable and only partially functional [6,7,8]. While several additional splice isoforms are generated by alternative splicing of both *SMN1* and *SMN2* [9-12], transcripts lacking exon 7 appear to be the major isoform produced by *SMN2* in all tissues except in testis [10,13]. Therefore, mechanism of *SMN2* exon 7 splicing has been intensively studied [14-21]. Due to the potential for *SMN2* to produce full-length SMN protein, it remains the principal target for therapies designed to increase production of functional SMN protein in conditions of SMA [22,23,24].

Discovery of ISS-N1 as potential therapeutic target

Multiple approaches have been explored as potential methods to increase production of SMN protein from *SMN2*, including increasing transcription [22,24,25,26], modulating *SMN2*

exon 7 splicing [27-30], inducing translational read through of *SMNΔ7* transcript [31], and increasing stability of SMN protein [32,33]. One of the most promising methods is the redirection of *SMN2* splicing of exon 7 through antisense oligonucleotides (ASOs), short oligonucleotides designed to anneal to complementary sequences within a gene of interest [30,34]. ASOs can exert their influence on *SMN2* exon 7 splicing through multiple ways, including but not limited to blocking binding of *trans*-acting protein factors by steric hindrance [35,36], causing structural rearrangements within the target RNA molecule [37,38,39], or recruiting additional *trans*-acting protein factors to the target molecule, in the case of bifunctional ASOs [40,41,42].

As the most promising target for an ASO-based therapy of SMA, Intronic Splicing Silencer N1 (ISS-N1) was discovered in the Singh laboratory in 2004 at University of Massachusetts Medical School, Worcester, MA

(US patent 7838657) [43]. ISS-N1 confers a very strong inhibitory effect on inclusion of *SMN2* exon 7 and sequestration of ISS-N1 by an ASO leads to full splicing correction in SMA patient cells [44]. Because of the strong inhibitory effect, ISS-N1 is also referred to as the master checkpoint of splicing regulation of *SMN2* exon 7 [45]. Discovery of ISS-N1 was possible thanks to the in vivo selection that revealed that the 5' splice site of *SMN* exon 7 is very weak [17,19,46,47]. Subsequent studies revealed that ISS-N1 is a complex regulatory element being affected by the presence of other regulatory elements upstream and downstream of ISS-N1 [20,36,38,48]. ASOs targeting ISS-N1 are predicted to enhance *SMN2* exon 7 inclusion by at least two mechanisms; first, by blocking binding of hnRNP A1 to two target motifs in the region [35], second, by causing secondary structural rearrangements and preventing an inhibitory long-distance interaction with downstream sequences deep within intron 7 [37,38,39]. Numerous studies have demonstrated the efficacy of ASOs targeting ISS-N1 in both SMA patient cells and mouse models of SMA and using multiple ASO chemistries [28,34,35,44,49-59]. Based on the number of the independent studies performed, ISS-N1 would easily rank as the most studied antisense target for splicing correction for human disease. ISS-N1 targeting ASOs remain the most potent drugs for SMA therapy in independent pre-clinical studies [28,53].

Clinical development of Spinraza™ for the treatment of SMA

Ionis Pharmaceuticals (formerly ISIS Pharmaceuticals) obtained license for exclusive use of ISS-N1-targeting ASOs from University of Massachusetts Medical School, Worcester, MA in 2010. Spinraza™ (nusinersen), formerly known as IONIS-SMN$_{Rx}$ or ISIS-SMN$_{Rx}$, is a 2'-O-methoxyethyl (2'MOE) modified ASO targeting ISS-N1 (Figure 1A). Ionis Pharmaceuticals began phase 1 clinical trials of ISIS-SMN$_{Rx}$ in 2011 and results were very encouraging. Subsequently, Nusinersen/Spinraza™ has been the subject of multiple phase 2 and 3 clinical trials by Ionis

Pharmaceuticals/Biogen (Figure 1B) [60,61]. Shown to be both safe and effective in raising SMN protein levels and reducing the disease severity of SMA, Spinraza™ has recently been approved by the FDA for the treatment of both mild and severe SMA (Figure 1B). This represents the first FDA-approved drug for the treatment of SMA, as well as a proof-of-concept for the targeting of an ISS by an ASO for the treatment of a major genetic disease associated with the infant mortality.

Although a promising first step in the treatment of SMA, there is still much progress to be made and many other promising approaches to follow. Most clinical trials of Spinraza™ have focused on treatment of symptomatic infants and children already diagnosed with SMA,

by which time many changes have already occurred in motor neurons [62]. One promising approach which is the target of an ongoing clinical trial (NCT02386553) is to treat infants diagnosed with SMA-causing mutations but who have not yet experienced symptoms, thus preventing motor neuron degeneration before it can begin. Other approaches to increase expression of SMN, such as treatment with histone deacetylase (HDAC) inhibitors to increase transcription [25], have not shown sufficient efficacy for treatment of SMA by themselves, but may prove effective in combination with Spinraza™. SMA is not only a disease of motor neurons; low SMN levels can independently impact a number of somatic tissues [57,58,63-66] as well as the testis [13].
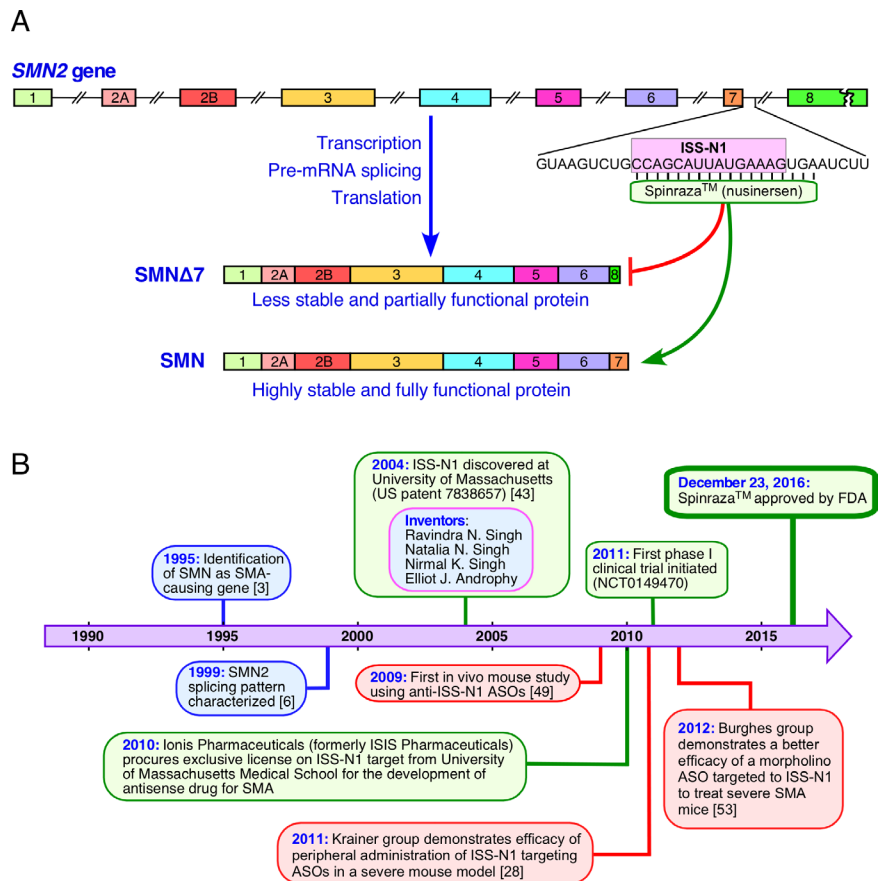

Figure 1. Spinraza™ represents the first FDA-approved drug for the treatment for SMA. (A) Overview of *SMN2* genomic sequence and mechanism of Spinraza™ action. Exons are represented by colored boxes. Introns are represented by broken lines. Region of ISS-N1 downstream of exon 7 is shown. ISS-N1 is represented by pink box, annealing location of Spinraza™ is indicated. Protein products of *SMN2* are shown below. Spinraza™ acts by redirecting splicing from the dysfunctional SMNΔ7 product to the full-length SMN. (B) Timeline of Spinraza™ target discovery, licensing, and therapeutic development. Purple arrow represents passage of time. Blue, red, and green ovals indicate critical developments in SMA research, landmark studies involving ISS-N1 ASOs, and critical stages in development of Spinraza™, respectively.

Currently, it is not known whether lumbar injections can fully ameliorate these peripheral defects. In addition, there are several other promising ASO targets within the *SMN2* pre-mRNA, such as ISS-N2 deep within intron 7 [38], Element 1 within intron 6 [27,67] and a GC-rich sequence that partially overlaps ISS-N1 [68,69]. A recent report showed utility of an ASO targeting an antisense sequence of *SMN2* [70]. In cell-based assays, a dual-masking ASO has shown a better efficacy than an ISS-N1 targeting ASO [71]. However, ISS-N1 still needs to be targeted to maintain the high efficacy of the dual-masking ASO [71]. Currently, it is not known how a variety of targets may be affected by different ASO chemistries or treatment with a combination of ASOs, and thus all ASO targets remain of interest for future research.

Conclusions and Future directions

Recent approval of Spinraza™ (Nusinersen) by US FDA as the first therapy for SMA is a major step forward for SMA patients worldwide. Spinraza™ also becomes the first antisense drug to restore the inclusion of an exon during pre-mRNA splicing. While invention of ISS-N1 was made in Singh laboratory more than a decade ago, credit of therapeutic development goes to several researchers who independently validated the therapeutic efficacy of ISS-N1 targeting ASOs. In particular, pioneering pre-clinical studies in the laboratory of Dr. Adrian Krainer at Cold Spring Harbor Laboratory in collaboration with Drs. Frank Bennet and Frank Rigo at Ionis Pharmaceuticals (formerly ISIS Pharmaceuticals) were critical for the Clinical development of Spinraza™. The exclusive licensing of ISS-N1-targeting ASOs from UMass Medical School allows IONIS Pharmaceuticals to develop additional drugs based on ISS-N1 target. Studies in the laboratories of Dr. Arthur Burghes at The Ohio State University and Dr. Francesco Muntoni at University College London independently validate the efficacy of ISS-N1-targeting morpholino ASOs [53-59]. SMA patients will tremendously benefit if additional antisense drugs based on morpholino and other chemistries are developed. This could be particularly important for patients who cannot tolerate the chemistry of Spinraza™. As we move forward with ASO-based therapy of SMA, there will be a need to develop non-invasive procedures for an effective delivery of drug into brain and spinal cord. With the FDA approval of Spinraza™, SMA disease transitions to the next phase in which long-term efficacy of Spinraza™ will be carefully monitored. We hope for a positive outcome that will have a transformative effect on the development of the next generation of the antisense drugs for SMA as well as for several other genetic diseases.

Acknowledgement

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