RNA Splicing as a Target for a New Generation of Precision Medicines

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The drug-discovery pipeline: nusinersen

Pre-clinical development

Target discovery → Hit ID & MoA → Lead optimization → GMP manufacture → Safety & tolerability

2001 - 2011

Clinical testing

IND filing → Phase I → Phase II → Phase III → Approval → To patients


Cold Spring Harbor Laboratory
Ionis Pharmaceuticals
Biogen
FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

NUSINERSEN is indicated for the treatment of spinal muscular atrophy (SMA) in pediatric and adult patients.

2 DOSAGE AND ADMINISTRATION

2.1 Dosing Information

NUSINERSEN is administered intrathecally by, or under the direction of, healthcare professionals experienced in performing lumbar punctures.

Recommended Dosage

The recommended dosage is 12 mg (5 mL) per administration.

Initiate NUSINERSEN treatment with 4 loading doses. The first three loading doses should be administered at 14-day intervals. The 4th loading dose should be administered 30 days after the 3rd dose. A maintenance dose should be administered once every 4 months thereafter.

Missed Dose

If a loading dose is delayed or missed, administer NUSINERSEN as soon as possible, with at least 14-days between doses and continue dosing as prescribed. If a maintenance dose is delayed or missed, administer NUSINERSEN as soon as possible and continue dosing every 4 months thereafter.
contains nusinersen, which is a modified antisense oligonucleotide, where the 2'-hydroxy groups of the ribofuranosyl rings are replaced with 2'-O-2-methoxyethyl groups and the phosphate linkages are replaced with phosphorothioate linkages. Nusinersen binds to a specific sequence in the intron downstream of exon 7 of the SMN2 transcript. The structural formula is:

\[ \text{Diagram of nusinersen structural formula} \]
NUSINERSEN is supplied as a sterile, preservative-free, colorless solution for intrathecal use in a single-dose glass vial. Each 1 mL solution contains 2.4 mg of nusinersen (equivalent to 2.53 mg of nusinersen sodium salt). Each 1 mL also contains calcium chloride dihydrate (0.21 mg) USP, magnesium chloride hexahydrate (0.16 mg) USP, potassium chloride (0.22 mg) USP, sodium chloride (8.77 mg) USP, sodium phosphate dibasic anhydrous (0.10 mg) USP, sodium phosphate monobasic dihydrate (0.05 mg) USP, and Water for Injection USP. The product may contain hydrochloric acid or sodium hydroxide to adjust pH. The pH is ~7.2.

The molecular formula of NUSINERSEN is $C_{234}H_{323}N_{61}O_{128}P_{17}S_{17}Na_{17}$ and the molecular weight is 7501.0 daltons.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

NUSINERSEN is an antisense oligonucleotide (ASO) designed to treat SMA caused by mutations in chromosome 5q that lead to SMN protein deficiency. 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.

12.2 Pharmacodynamics

Autopsy samples from patients (n=3) had higher levels of SMN2 messenger ribonucleic acid (mRNA) containing exon 7 in the thoracic spinal cord compared to untreated SMA infants.

Cardiac Electrophysiology
Eukaryotic gene expression

- **Transcription**: DNA is transcribed into mRNA in the Nucleus.
- **Translation**: mRNA is translated into a Protein by ribosomes in the Cytoplasm.
- **Splicing**: The pre-mRNA is processed by a spliceosome to remove Introns and include Exons, forming the mature mRNA.

Key terms:
- **DNA**: Double-stranded nucleic acid that contains the genetic information.
- **mRNA**: Messenger RNA that carries the genetic code from the DNA to the ribosomes.
- **Exons**: Functional parts of genes that are translated into proteins.
- **Introns**: Non-functional parts of genes that are removed during splicing.
- **Spliceosome**: A large complex of proteins and RNA that mediates the splicing reaction.

Cellular compartments:
- **Cytoplasm**: The cytoplasm is the material contained in the cytoplasmic membrane of a cell, excluding the nucleus and other organelles.
- **Nucleus**: The nucleus is the control center of a eukaryotic cell, containing the cell's DNA.
Alternative splicing

- **Cassette exon**
- **Mutually exclusive exons**
- **Alternative last exons**

**Alternative first exons**

**Alternative last exons**
Genome browser: human TP53 gene
Antisense oligonucleotides

DNA (gene)

A - T
G - C
Pediatric neuromuscular disorder, autosomal recessive

Degeneration of α-motor neurons in the spinal cord and lower brainstem

1 in ~10,000 newborns

Inactivating mutations in \( SMN1 \), which codes for SMN protein
  - SMN functions in snRNP assembly and axonal mRNA transport

\( SMN2 \) paralog (unique to humans) expresses a small amount of functional protein

Variable severity (type 1-4) inversely proportional to \( SMN2 \) copy number

Spinal Muscular Atrophy
### Type 1 infantile-onset
- Age of symptom onset ≤6 months
- Very short life expectancy
- Median event-free survival is 10.5 months
- Never able to sit
- Most have 2 copies of SMN2

### Type 2 later-onset
- Age of symptom onset >6 months
- Shortened life expectancy
- Able to sit or stand, but not walk
- Muscle weakness/skeletal deformities
- Most have 3 copies of SMN2

### Type 3 later-onset
- Age of symptom onset >6 months
- Close to normal life expectancy
- Ability to walk declines over time
- Muscle weakness/skeletal deformities
- Most have 3-4 copies of SMN2

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*Finkel et al (2014) Neurology 83: 810*
Survival of motor neuron 2 (SMN2) gene makes both normal SMN and defective SMNΔ7 proteins

Functional SMN protein (10%)

Defective SMNΔ7 protein (90%)
Nusinersen

SMN2 pre-mRNA

Exon 7

18-nt target sequence present only in SMN1/2 intron 7

2'-O-(2-methoxyethyl) ribose
phosphorothioate
5-methyl cytosine

18mer ASO; MW 7127

Targeting spinal-cord motor neurons via lumbar puncture

Long half-life of nusinersen in the CNS
Loading doses: 12 mg @ 2 weeks x 4
Maintenance doses: 12 mg @ 4 months
Type 1 SMA patient, phase-2 clinical trial
Type 1 SMA patient, phase-2 clinical trial
Type 1 SMA patient, phase-2 clinical trial
a baby, Cameron Harding, nearly 3, receives a drug that keeps his motor neur...
Nusinersen firsts

- First and currently only approved drug for SMA
- First approved drug that corrects defective RNA splicing
- First approved nucleic-acid therapeutic for a neurological disease
- First disease-modifying drug for neurodegeneration
- First drug to demonstrate that pre-symptomatic treatment can markedly delay or prevent the onset of a neurodegenerative disease; SMA is being added to newborn-screening panel
- Currently >6,600 SMA patients on nusinersen worldwide
Anticipated developments in the next decade

- Genome-wide annotation of mRNA/protein isoforms
  - Structure, function, where and when they are made, relative amounts

- Improved understanding and prediction of how specific mutations affect splicing

- Continued improvements in antisense-drug delivery
  - Efficient and specific targeting to brain, spinal cord, liver, muscles, lung, blood cells, tumor cells, etc.
  - Delivery to amniotic fluid or umbilical cord for prenatal treatment of developmental disorders

- Small molecules
  - Insights into mechanism of action
  - Improved specificity
  - Structure-based design
  - Splicing inhibitors
Diseases targetable by splicing modulators

- **Spinal muscular atrophy**: Change alternative splicing of a backup gene (SMN2) to restore levels of functional protein (*nusinersen*, *risdiplam*, *branaplam*)

- **Duchenne’s muscular dystrophy**: Alter normal splicing of a mutant gene (DMD) to turn a severe disease into a milder one (*eteplirsen*, *drisapersen*)

- **Familial dysautonomia**: Restore correct splicing due to a mutation in the *IKBKAP* gene

- **Alzheimer’s disease**: Alter splicing of *APP* gene to reduce production of amyloid peptides that accumulate in senile plaques

- **Dravet syndrome**: Change alternative splicing of *SCN1A* gene to restore levels of functional protein (STK-001)

- **Cancer**: Change alternative splicing of relevant genes to harm tumor cells but not normal cells; some tumor cells are more sensitive to general splicing inhibition (*H3B-8800*, *E7107*)

- Many other disease targets (β-thalassemia, Usher syndrome, Hutchinson-Gilford progeria, frontotemporal dementia, ISCU myopathy, ataxia telangiectasia, cystic fibrosis, erythropoietic protoporphyria, Leber congenital amaurosis, multiple sclerosis, etc.)
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