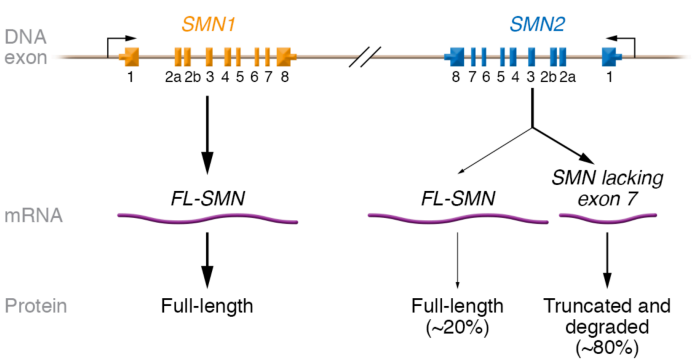
Exercise 1

Treatments for Spinal Muscular Atrophy

1

Survival of Motor Neuron protein

SMN gene(s): key for motoneuron functional development



SMN complex:

- Role in RNA metabolism, actin dynamics
- Key for axonal outgrowth and stabilization of neuromuscular junctions

J Clin Invest. 2018;128(8):3219-3227

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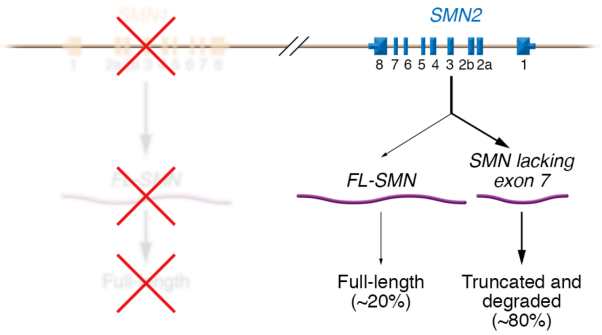
Spinal Muscular Atrophy

Cause: loss of SMN1 activity



SMA type I

Most frequent genetic cause of mortality in children
Incidence: 1:10'000 births / year

Motoneuron loss, muscle atrophy, weakness

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Spinal Muscular Atrophy

- SMA is due to the loss of the SMN1 gene.
- A second gene present in humans, SMN2, can partly rescue SMN1 function.
- The number of SMN2 copies varies between 1 and 4 or even more.

SMA type	Age of onset	Death	Motor abilities / defects	SMN1	SMN2 copies
I	0-6 months	< 2 yrs	Never sit	Deleted/ mutated	
II	7-18 months	> 2 yrs	Sit, never walk		
III	> 18 months	Adult	Stand and walk Scoliosis Weakness		
IV	10-30 yrs	Adult	Walk during adulthood Weakness		

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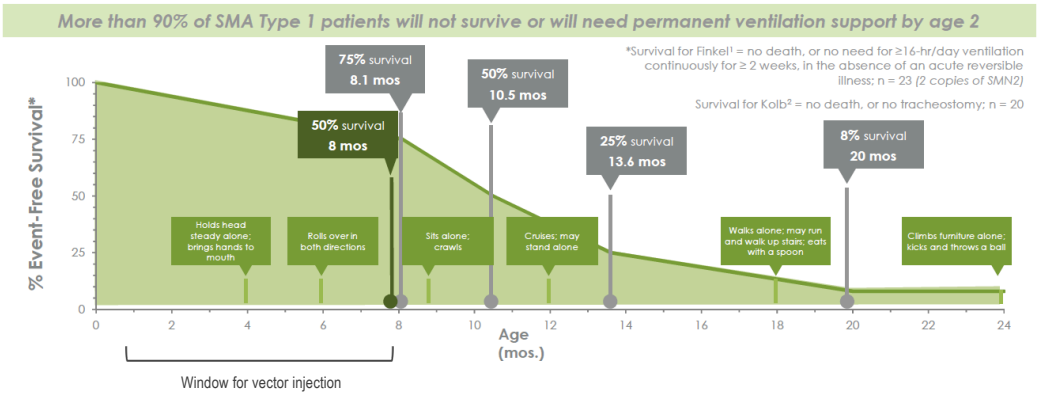
Spinal Muscular Atrophy: disease natural history

SMA type I: natural course of the disease



At 13.6 months of age:
only 25% are expected to survive w/o treatment

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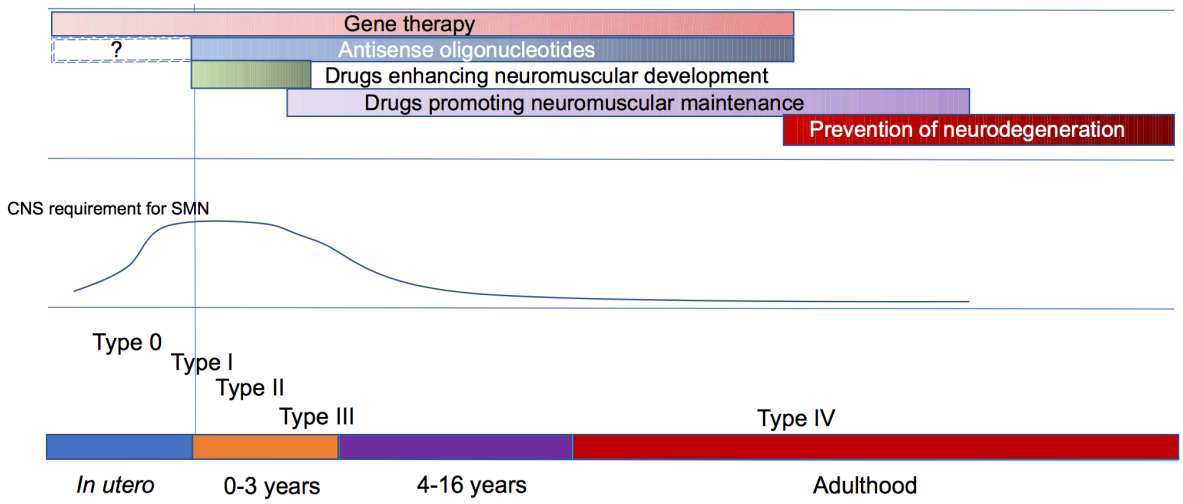
Gene therapy for SMA: treatment

CNS requirement for SMN activity



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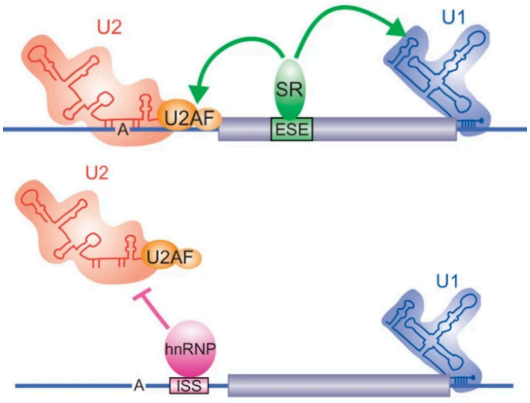
Modification of mRNA splicing as therapeutic approach



Splicing activation

Splicing repression

It is the SRSF1 (splicing enhancer) binding site that is weakened by the C-to-T substitution at nucleotide 6 in SMN2 exon 7,
⇒ predominant skipping of this exon.

Splicing silencer sequence (ISS) in SMN2 exon 7 is strengthened as a result of the C-to-T transition.

Nature Structural & Molecular Biology volume 16, pages 13–15 (2009)
J. Cell Biol. Vol. 199 No. 1 21–25; doi/10.1083/jcb.201207087

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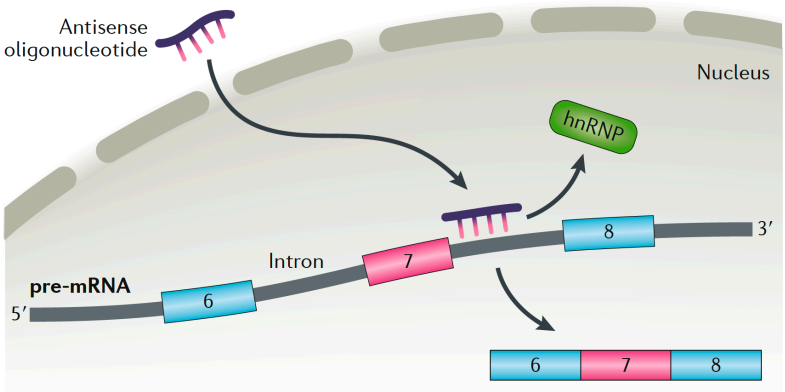
Modification of mRNA splicing as therapeutic approach

Nusinersen:

ASOs targeting a site near the 5' splice site in SMN2 intron 7 could efficiently promote exon 7 inclusion. They acted by preventing binding of the splicing repressors HNRNPA1 and HNRNPA2. In addition, chemical modifications in the backbone (phosphorothioate) and nucleotides (2'-O-methoxyethyl, or 2'-MOE) of the ASOs improved their pharmacological properties.



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Nusinersen: what are the molecular feature(s) in the mechanism of action of compound which are key for therapeutic efficacy?

- Specific binding to the intronic splicing silencing site (ISS-N1) to prevent the binding of hnRNP.
- Stabilization of the antisense oligonucleotide by chemical modification (2'-O-methoxyethyl-modified (MOE) nucleotides with phosphorothioate backbone). The good tolerance, wide distribution throughout the cerebrospinal fluid, and a half-life of >6 months allow for intrathecal injection of Nusinersen once every 4 months after the initial phase of treatment.

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Risdiplam: what are the molecular feature(s) in the mechanism of action of compound which are key for therapeutic efficacy?

- Extensive high-throughput screening to identify a small molecule acting as a 'molecular glue' to increase exon 7 splicing. The molecule binds on two sites within the exon 7 of the SMN2 transcript, namely exonic splicing enhancer 2 (ESE2) and 5′ splice site. The mechanism of action is based on the recruitment of the U1 snRNA.
- Second optimization to eliminate off-target effects by increasing the selectivity for SMN2. Decreasing off-target splicing events led to the discovery of risdiplam.
- Compound available via oral administration.

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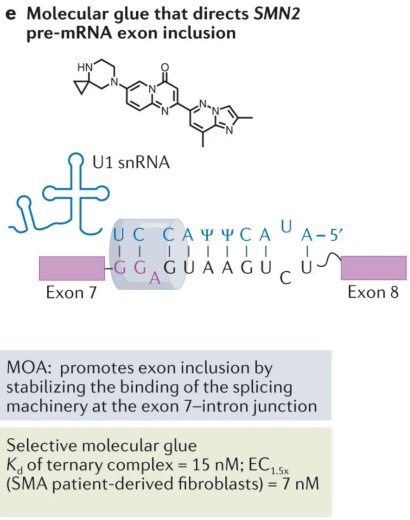
Modification of mRNA splicing as therapeutic approach



Nature Reviews Drug Discovery (Nat Rev Drug Discov) ISSN 1474-1784 (online)

Small molecule:

Risdiplam stabilizes the interaction between U1 snRNA and SMN2 intronic sequence.

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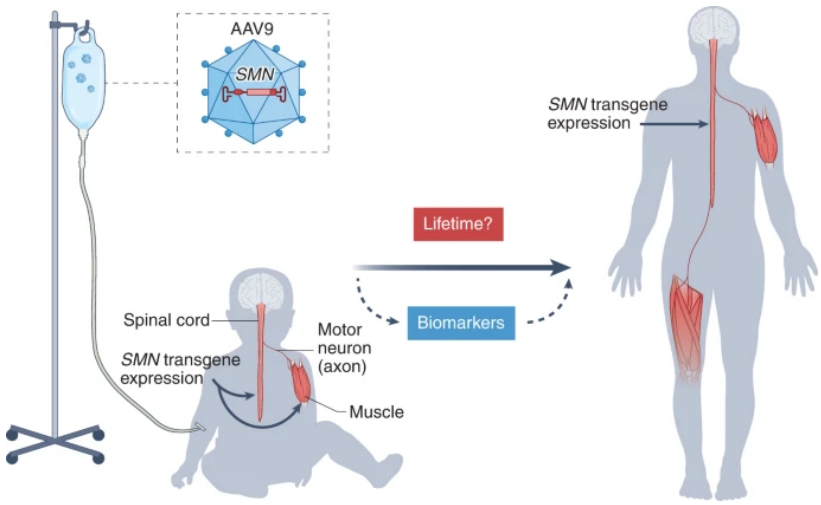
Gene therapy for SMA



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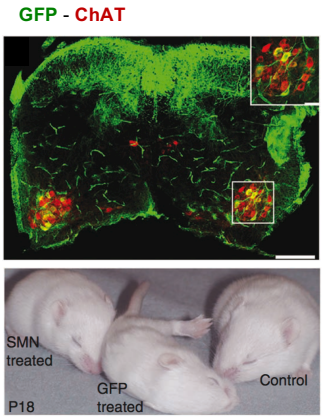
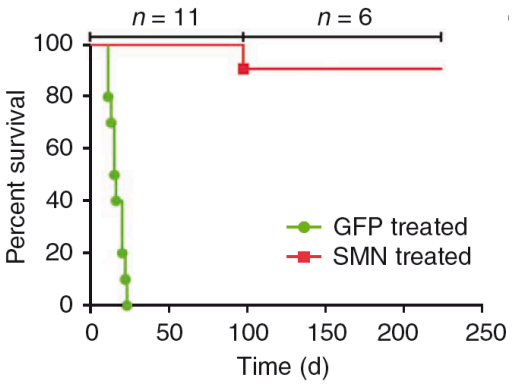
AAV-based gene therapy treatment for SMA

- **Intravenous injection of** scAAV9-cba-SMN to increase SMN1 activity in the Δ7-SMN SMA mice





Foust KD et al, Nature Biotechnology 2010

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Zolgensma: what are the molecular feature(s) in the mechanism of action of compound which are key for therapeutic efficacy?

- AAV9 can pass the blood-brain barrier following intravenous administration. This feature allows a broad targeting of the CNS with the SMN-encoding vector.
- High dosing ($1^{E}14$ VG/kg body weight) before 6 months of age is critical for therapeutic efficacy in SMA type I.

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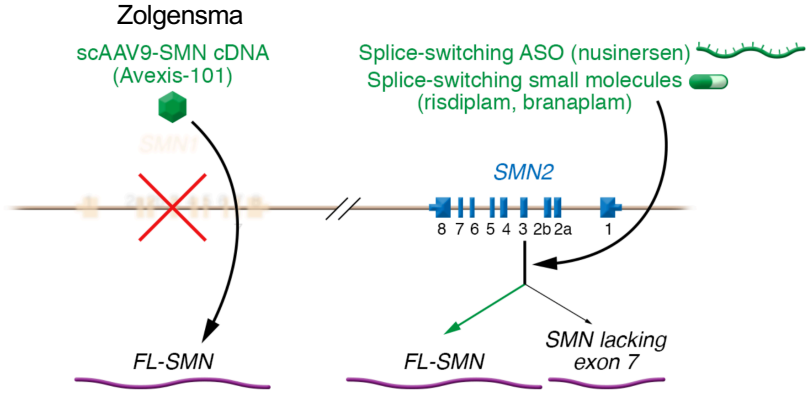
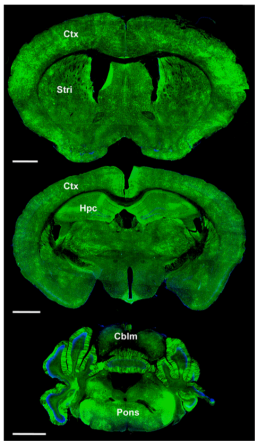
Gene therapy for Spinal Muscular Atrophy

SMA: rationale treatments

Transgene delivery to the CNS
following intravenous injection of AAV9





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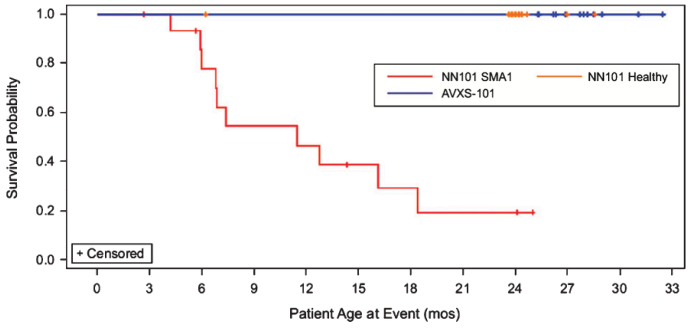
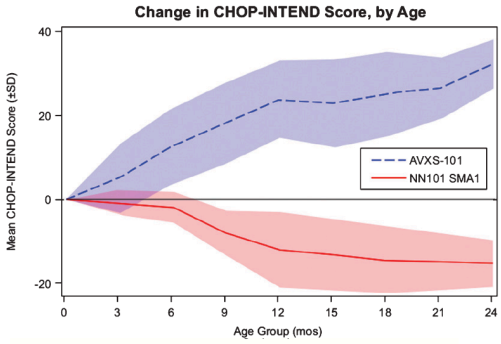
Gene therapy for SMA: clinical trial

Clinical trial for Zolgensma (**scAAV9-cba-fISMN**): follow up

Patients: SMA type I, 2 copies of SMN2 (1-8 months old)
Treatment: 1-8 months old, intravenous administration

Dosing: $1.1^{E}14$ VG/kg body weight





Journal of Neuromuscular Diseases 6 (2019) 307–317

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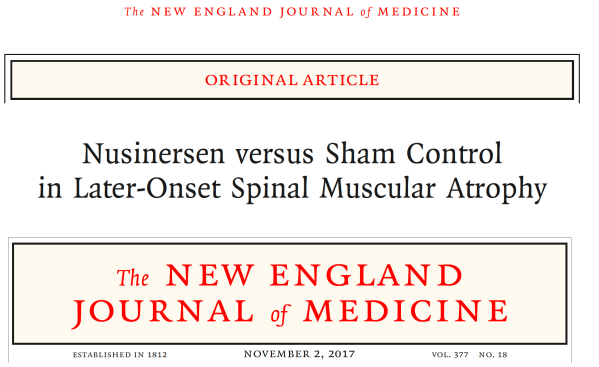
Material for exercise

Key publications on the different treatment strategies:

- ASO (Nusinersen):


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy

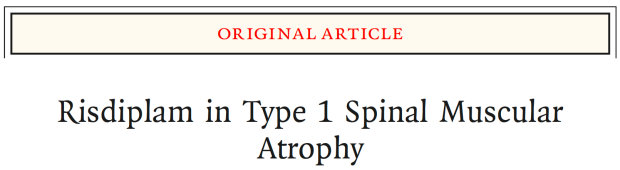
- Gene therapy (Zolgensma):

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 NOVEMBER 2, 2017 VOL. 377 NO. 18

Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

- Small molecule (Risdiplam):


ORIGINAL ARTICLE

Risdiplam in Type 1 Spinal Muscular Atrophy

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SMA pathology: current treatments

Several companies have developed therapeutic approaches against spinal muscular atrophy. The following compounds are currently applied or are considered as potential treatments:

A. SPINRAZA (Nusinersen): antisense oligonucleotide

B. Zolgensma: gene therapy (scAAV9-cba-SMN)

C. Risdiplam (RO7034067): small molecule

Based on the mechanism of action of each treatment, address te following questions:

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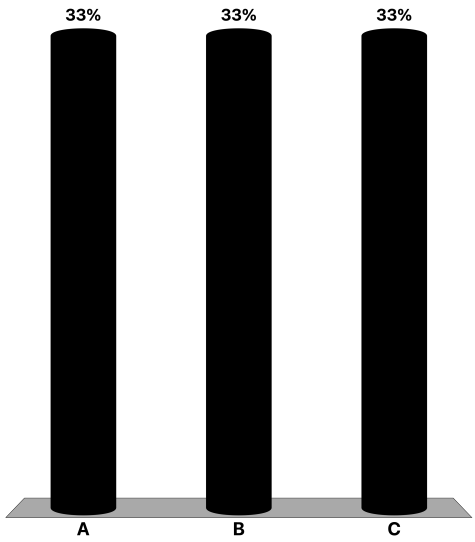
Which treatment do you think is more appropriate for SMA type I ? (by order of priority)

A. Spinraza (ASO)
B. Risdiplam (small molecule)
C. Zolgensma (gene therapy)



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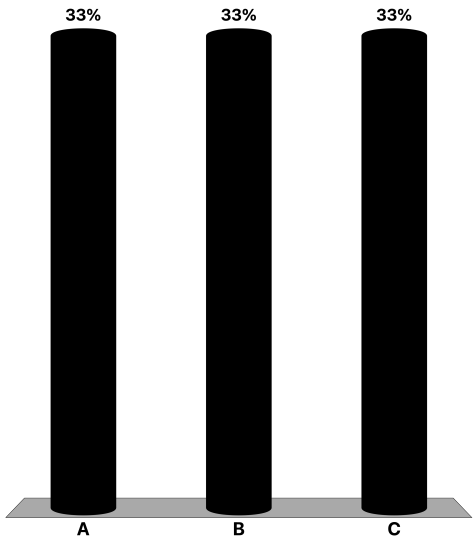
Which treatment do you think is more appropriate for SMA type II and III ? (by order of priority)

A. Spinraza (ASO)
B. Risdiplam (small molecule)
C. Zolgensma (gene therapy)



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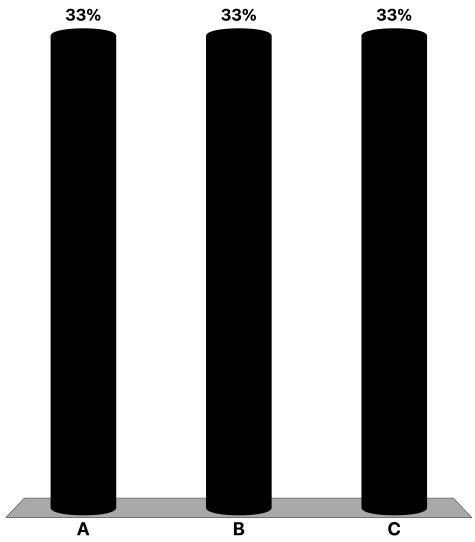
For which treatment is the risk of side effects the most important ? (by order of priority)

A. Spinraza (ASO)
B. Risdiplam (small molecule)
C. Zolgensma (gene therapy)



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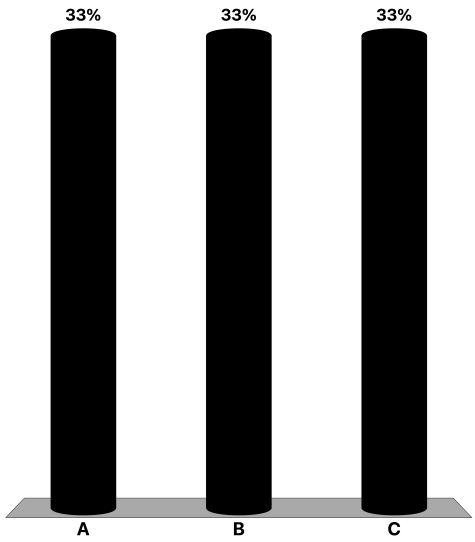
Which treatment is the most effective in SMA type I ?

A. Spinraza (ASO)
B. Risdiplam (small molecule)
C. Zolgensma (gene therapy)



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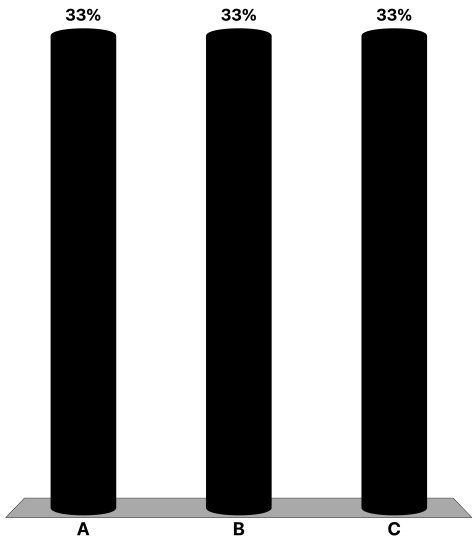
Which treatment is the most accessible to patients ?

A. Spinraza (ASO)
B. Risdiplam (small molecule)
C. Zolgensma (gene therapy)



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