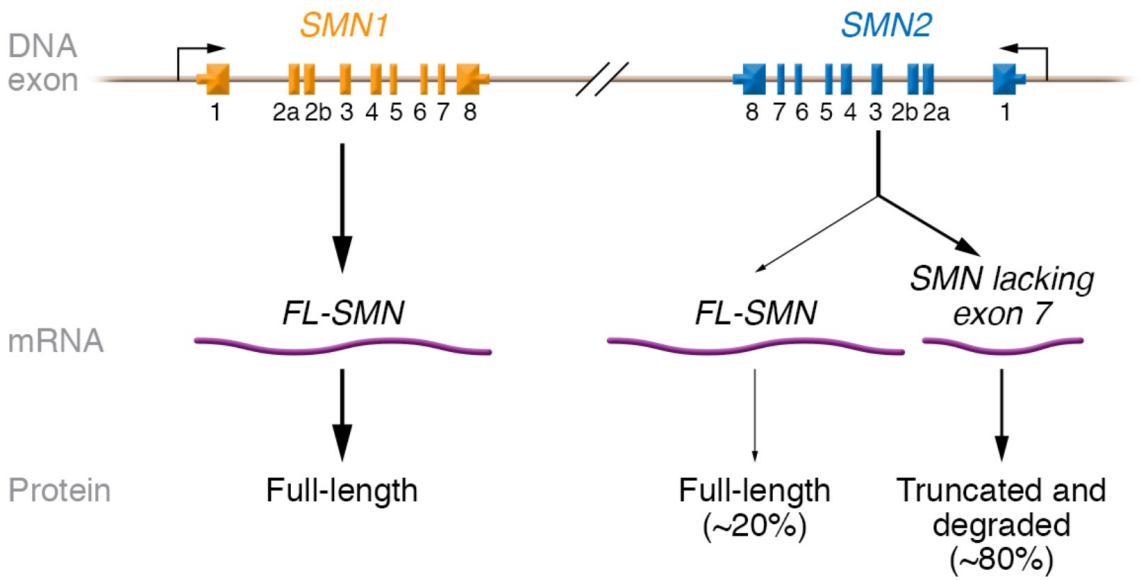
Treatments for Spinal Muscular Atrophy

Survival of Motor Neuron protein

SMN gene(s): key for motoneuron functional development



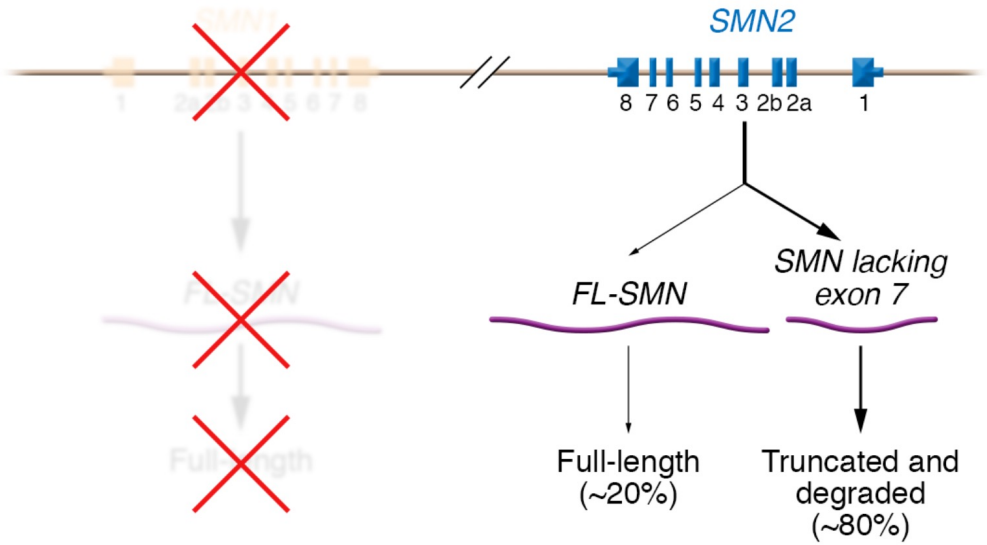
SMN complex:

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

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

Spinal Muscular Atrophy

Cause: loss of SMN1 activity



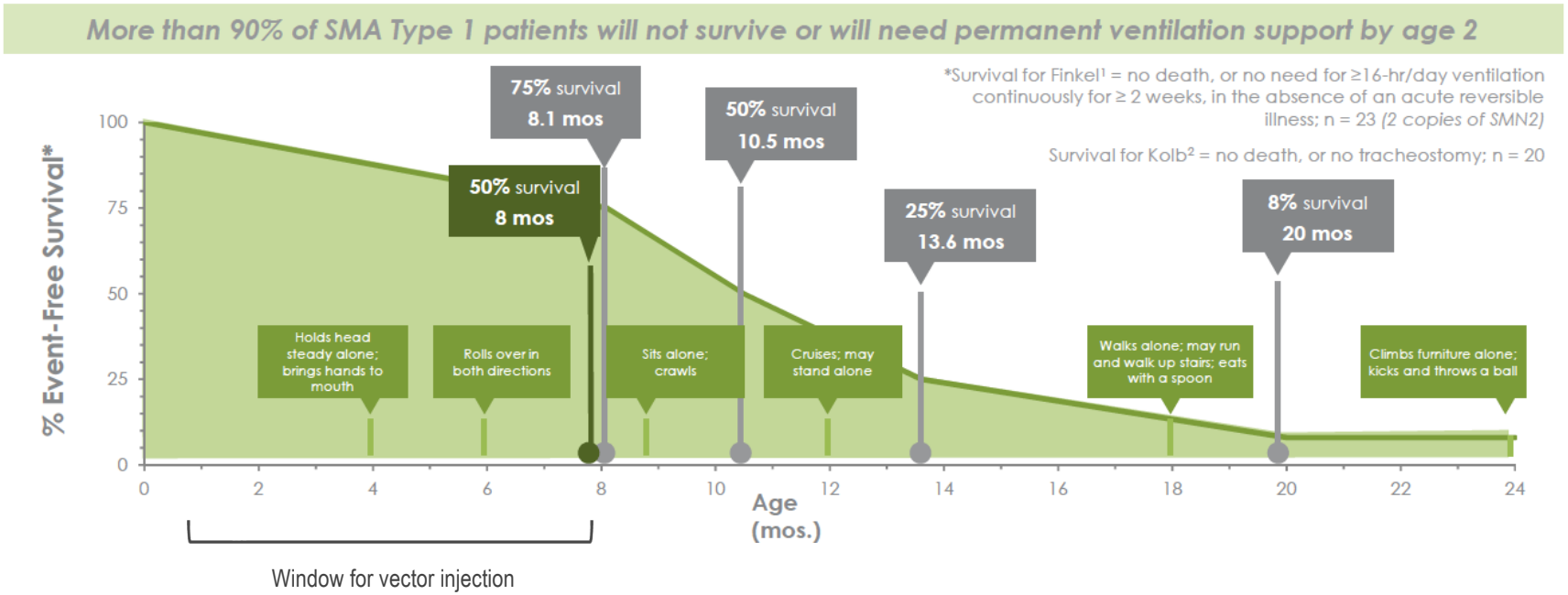
SMA type I

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

Motoneuron loss, muscle atrophy, weakness

Spinal Muscular Atrophy: disease natural history

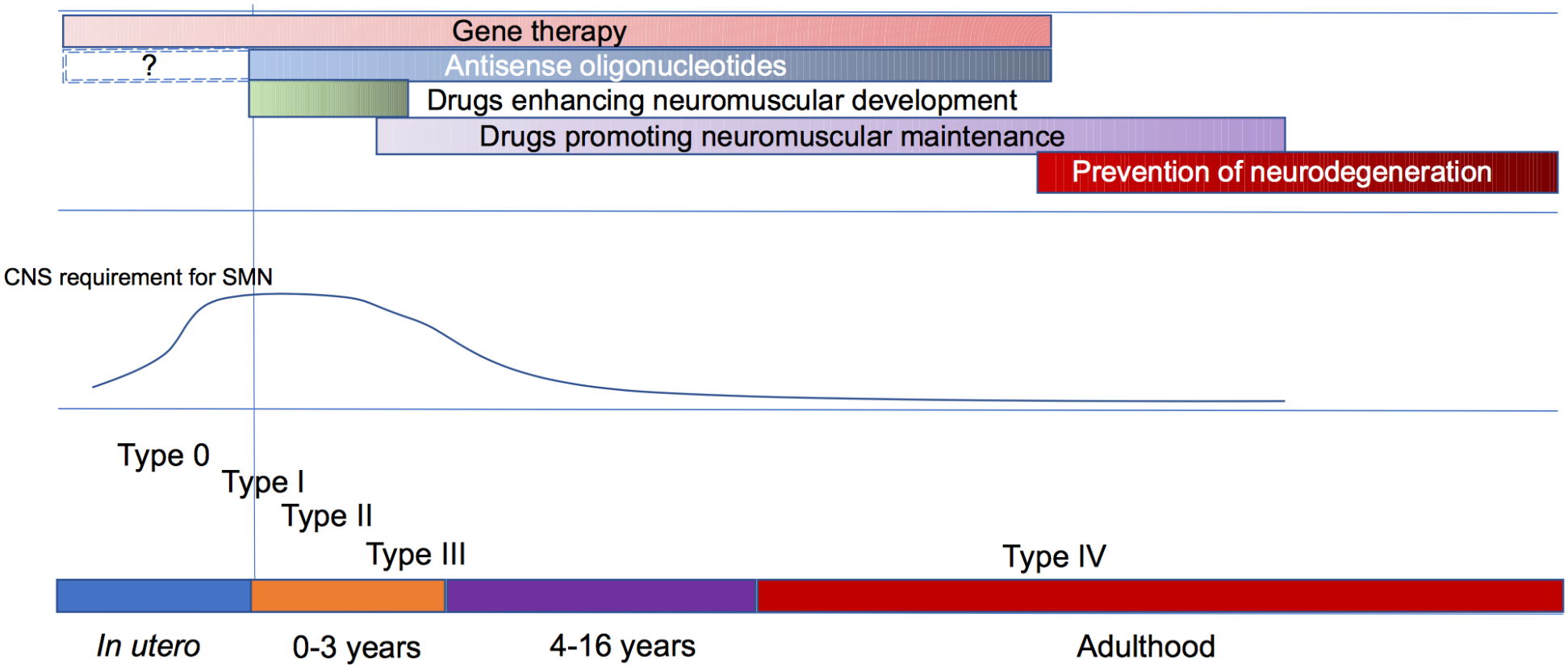
SMA type I: natural course of the disease



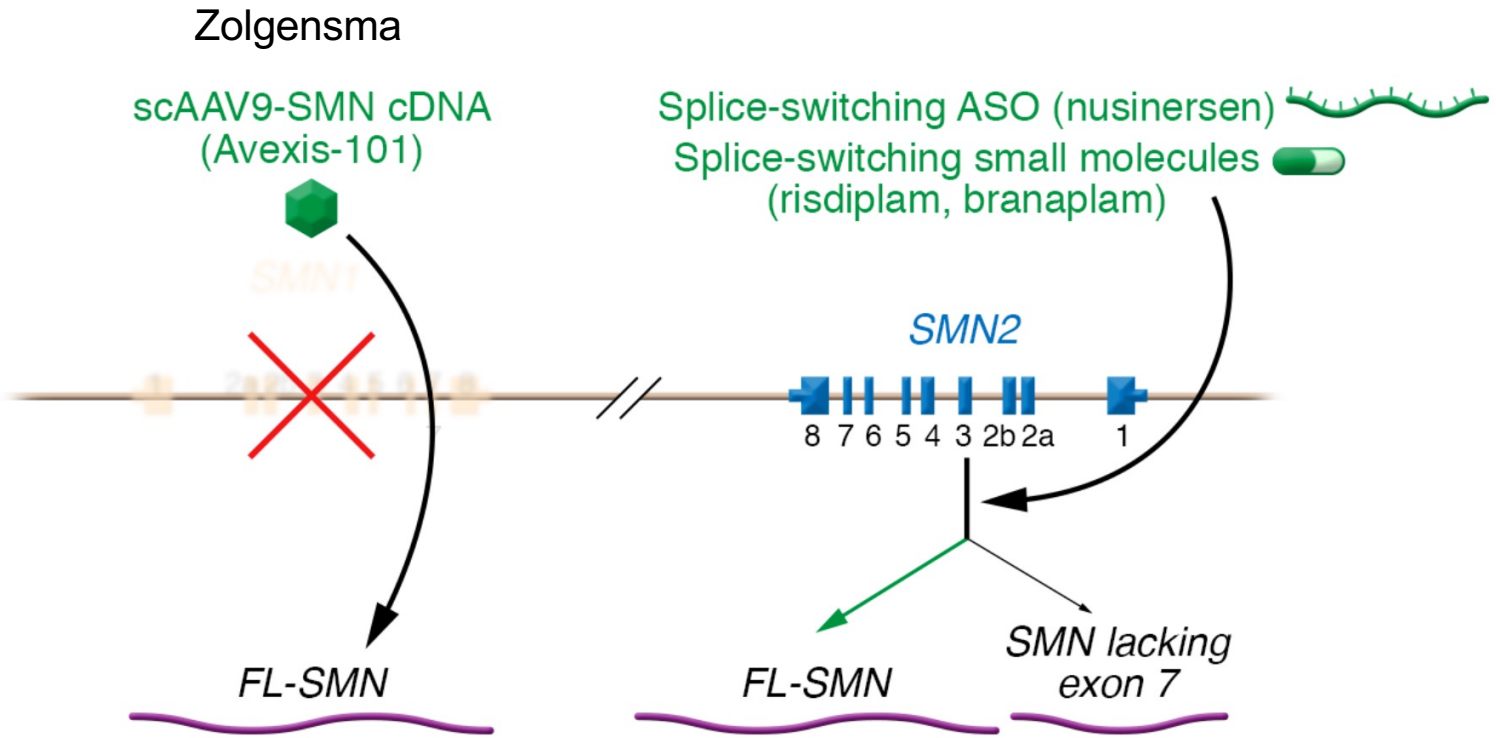
At 13.6 months of age:
only 25% are expected to survive w/o treatment

Gene therapy for SMA: treatment

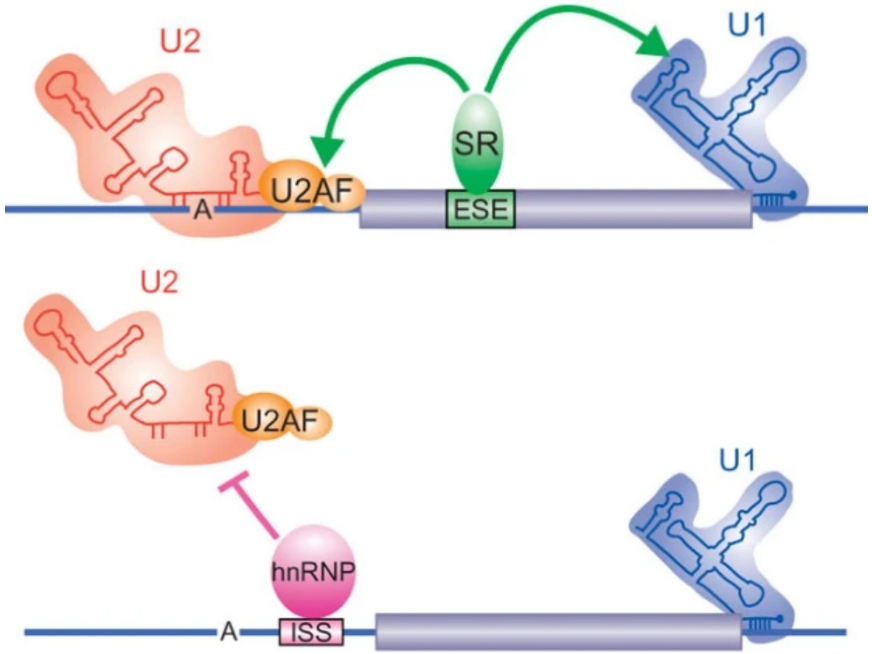
CNS requirement for SMN activity



SMA: rationale treatments



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.

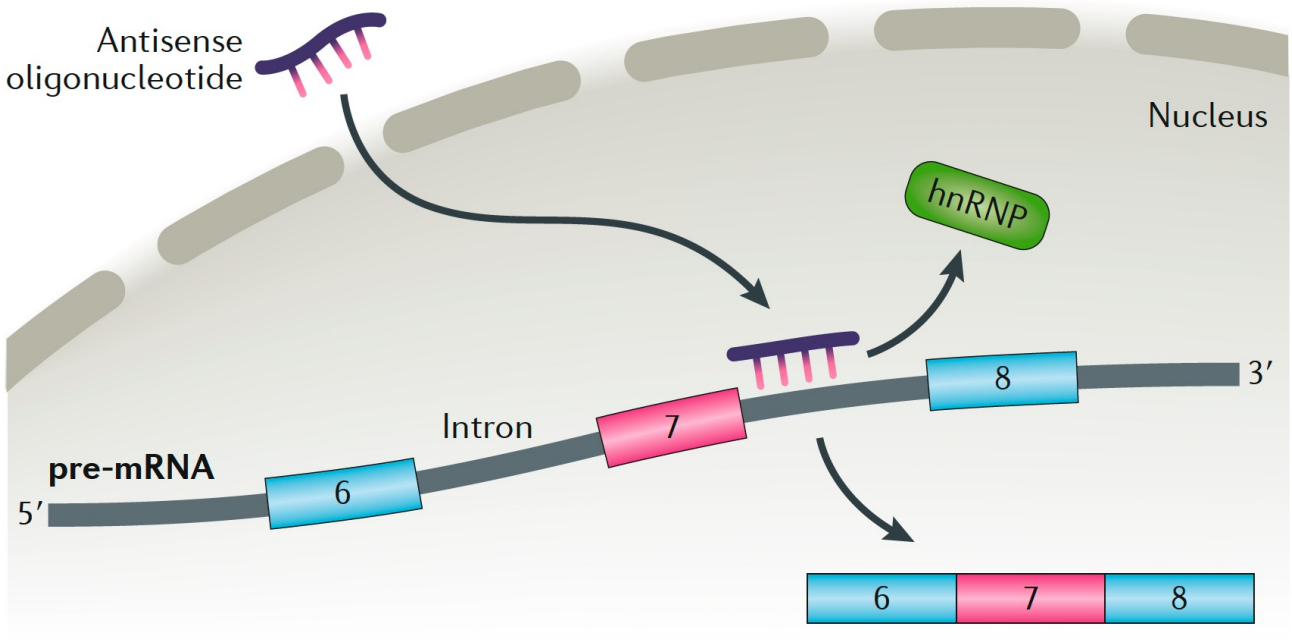
Splicing silencer sequence (ISS) in SMN2 exon 7 is strengthened as a result of the C-to-T transition.

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

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.



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.

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

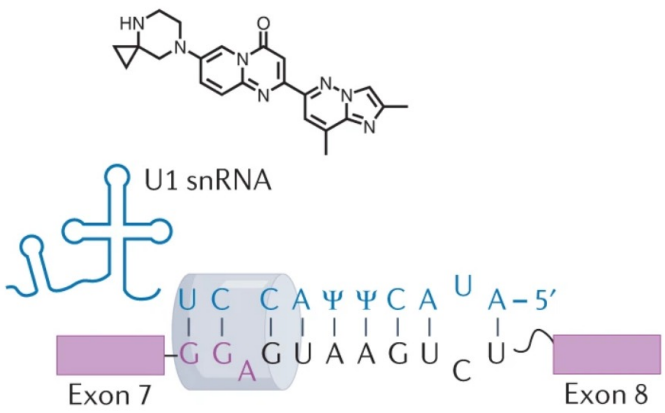
- Extensive high-throughput screening to identify a small molecule acting as a 'molecular glue' to increase exon 7 splicing. The molecule binds on two sites within the exon 7 of the SMN2 transcript, namely exonic splicing enhancer 2 (ESE2) and 5′ splice site. The mechanism of action is based on the recruitment 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.

Modification of mRNA splicing as therapeutic approach

e Molecular glue that directs *SMN2* pre-mRNA exon inclusion

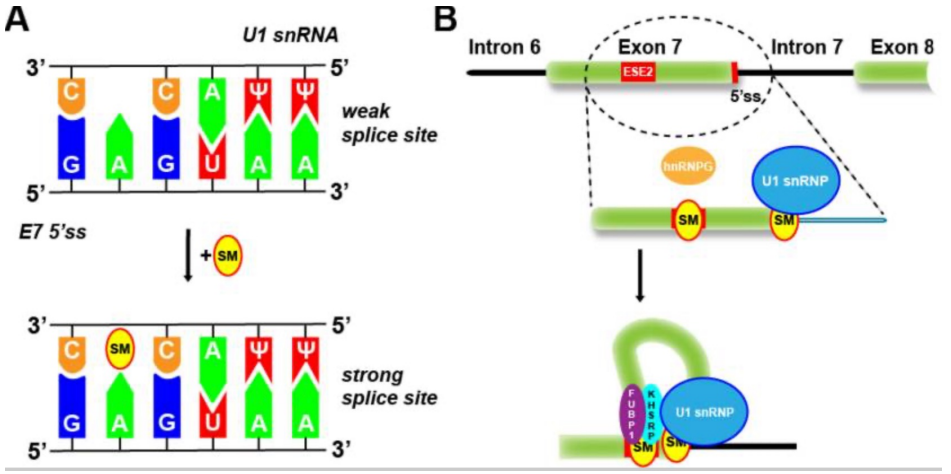


MOA: promotes exon inclusion by stabilizing the binding of the splicing machinery at the exon 7–intron junction

Selective molecular glue
K_d of ternary complex = 15 nM; $EC_{1.5x}$ (SMA patient-derived fibroblasts) = 7 nM

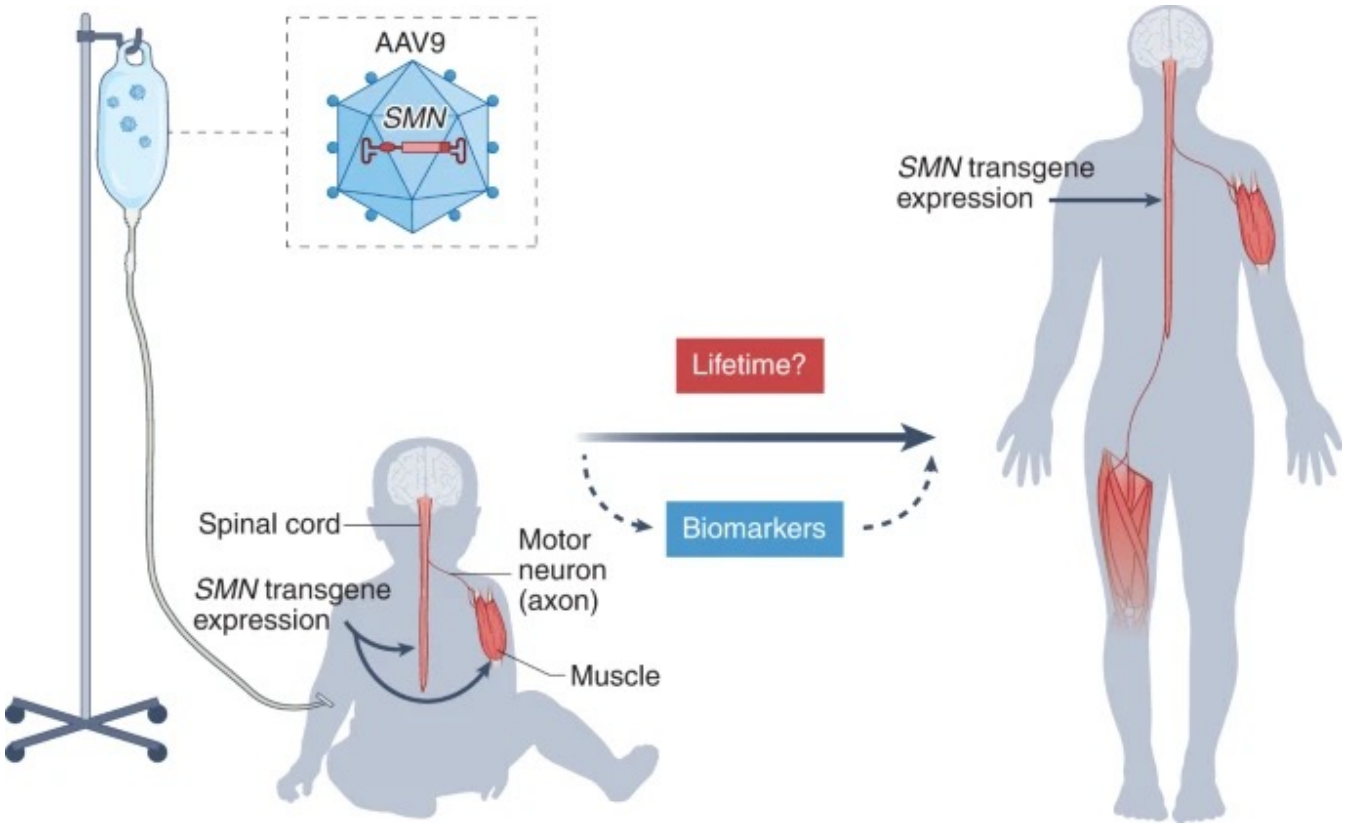
Small molecule:

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



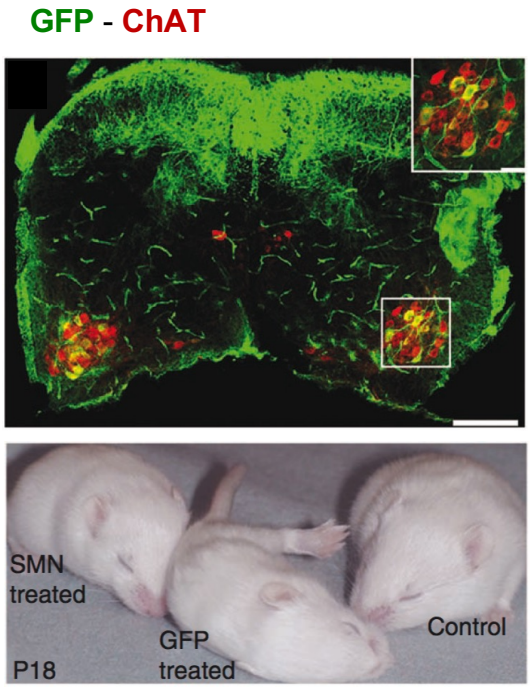
Nature Reviews Drug Discovery (Nat Rev Drug Discov) ISSN 1474-1784 (online)

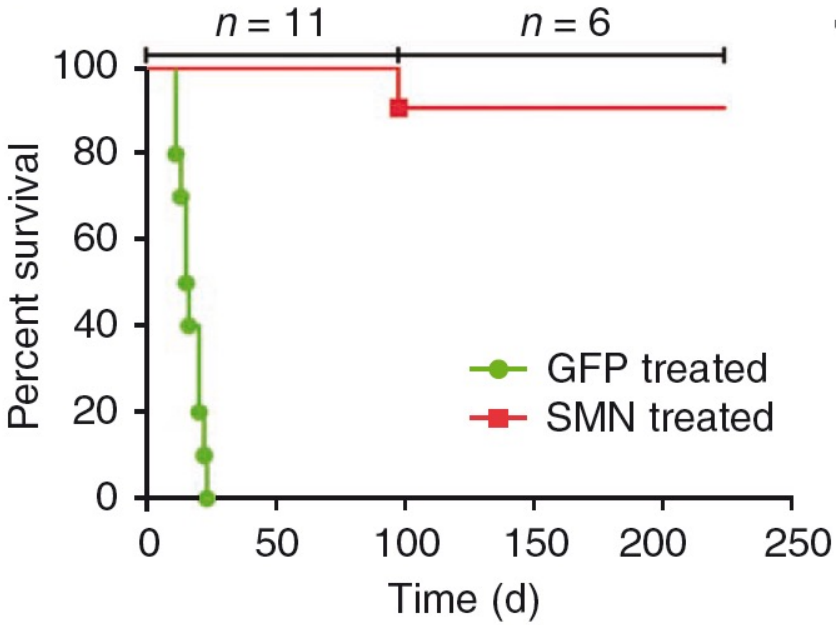
Gene therapy for SMA



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

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.

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

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

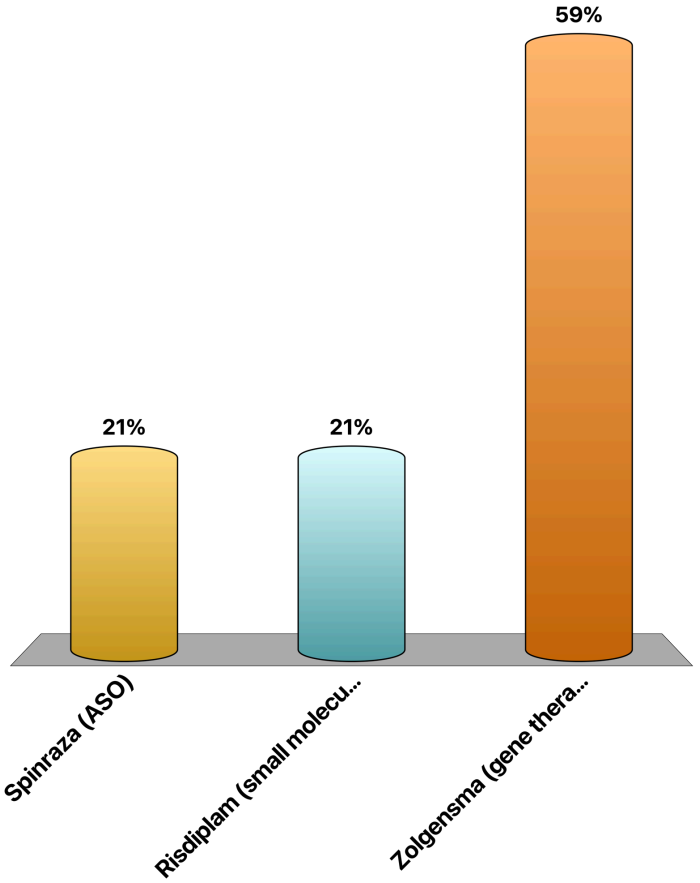
B. Zolgensma: gene therapy (scAAV9-cba-SMN)

C. Risdiplam (RO7034067): small molecule

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

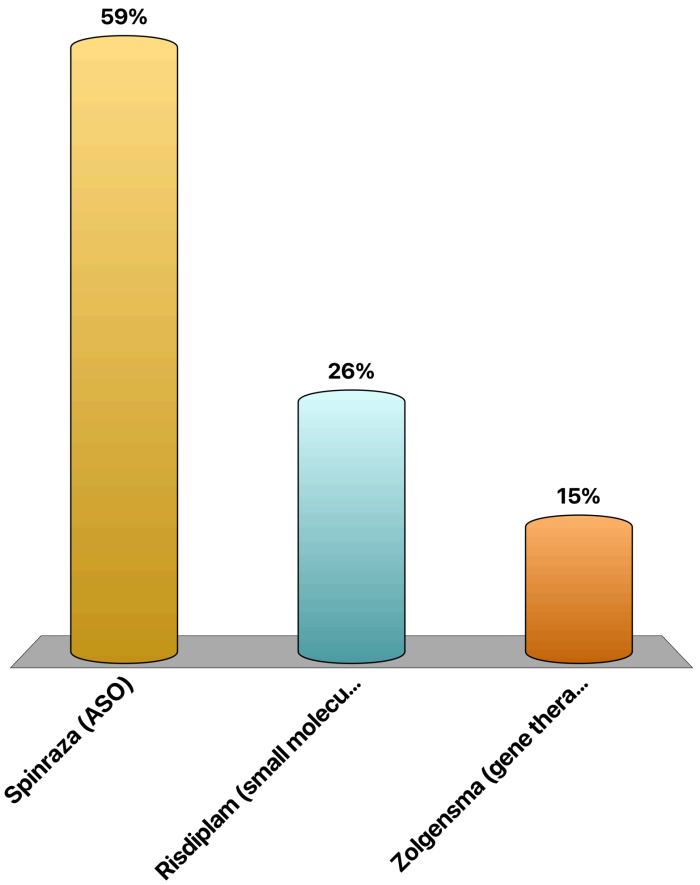
Which treatment do you think is more appropriate for SMA type I ? (by order of priority)

A. Spinraza (ASO) - 3/2
B. Risdiplam (small molecule) - 2/3
C. Zolgensma (gene therapy) - 1



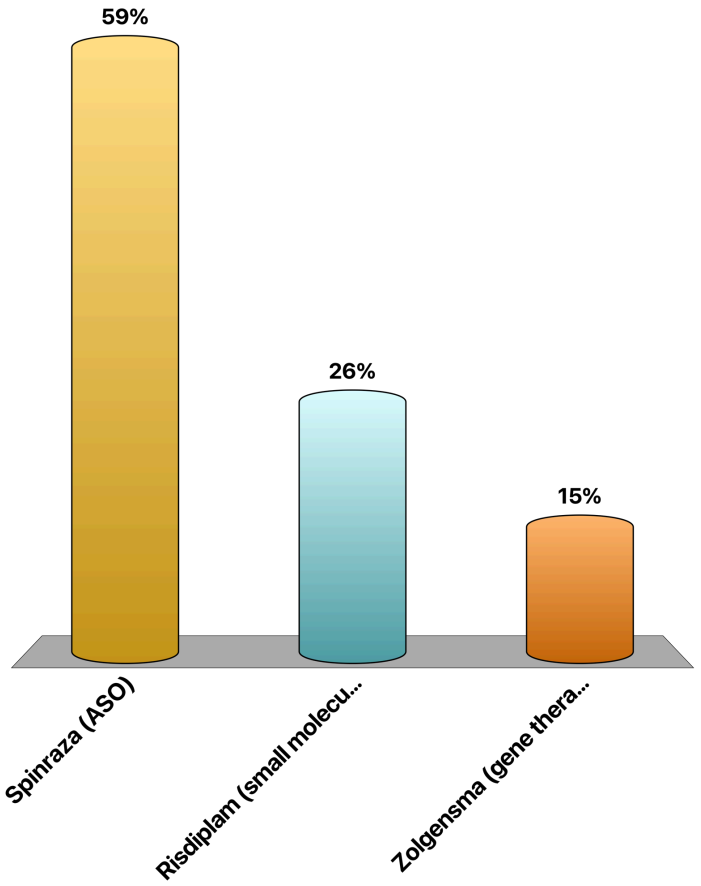
Which treatment do you think is more appropriate for SMA type II and III ? (by order of priority)

A. Spinraza (ASO) - 1/2
B. Risdiplam (small molecule) - 2/1
C. Zolgensma (gene therapy) - 3



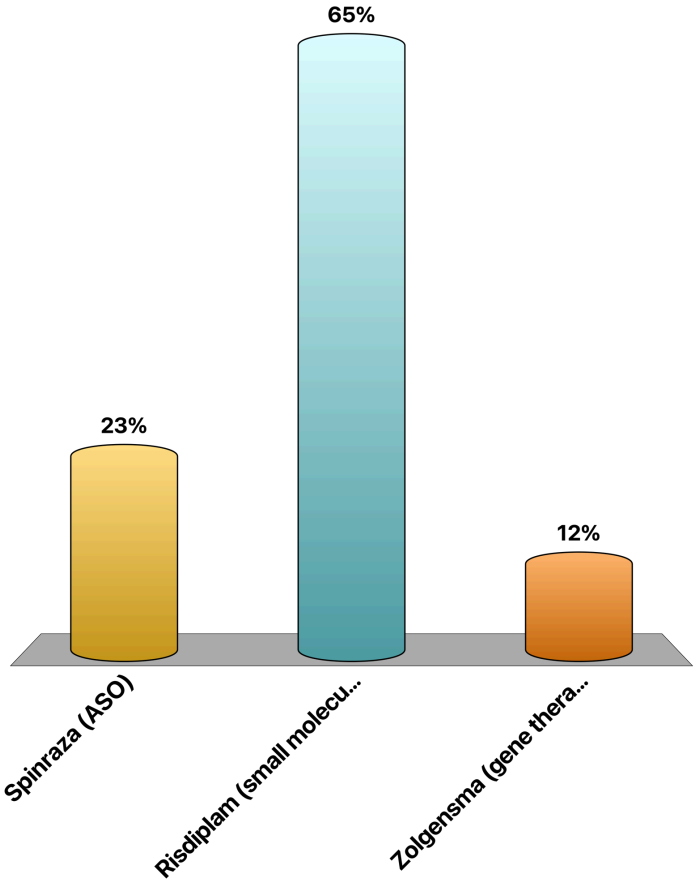
Based on the mechanisms of action, which are the possible combinations of treatments?

A. Spinraza + Risdiplam
B. Risdiplam + Zolgensma
C. Spinraza + Zolgensma



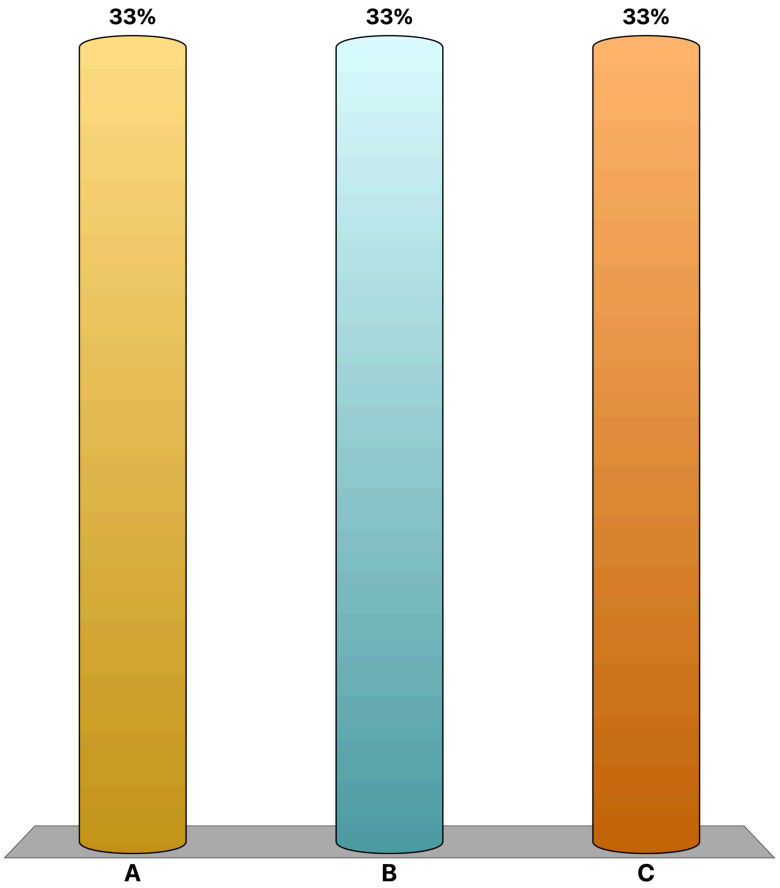
For which treatment is the risk of side effects the most important ? (by order of priority)

A. Spinraza (ASO) - 3
B. Risdiplam (small molecule) - 2/1
C. Zolgensma (gene therapy) - 1/2



Which treatment is the most effective in SMA type I ?

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



Which treatment is the most accessible to patients ?

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