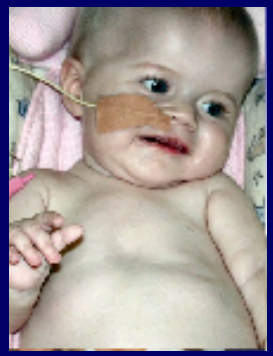
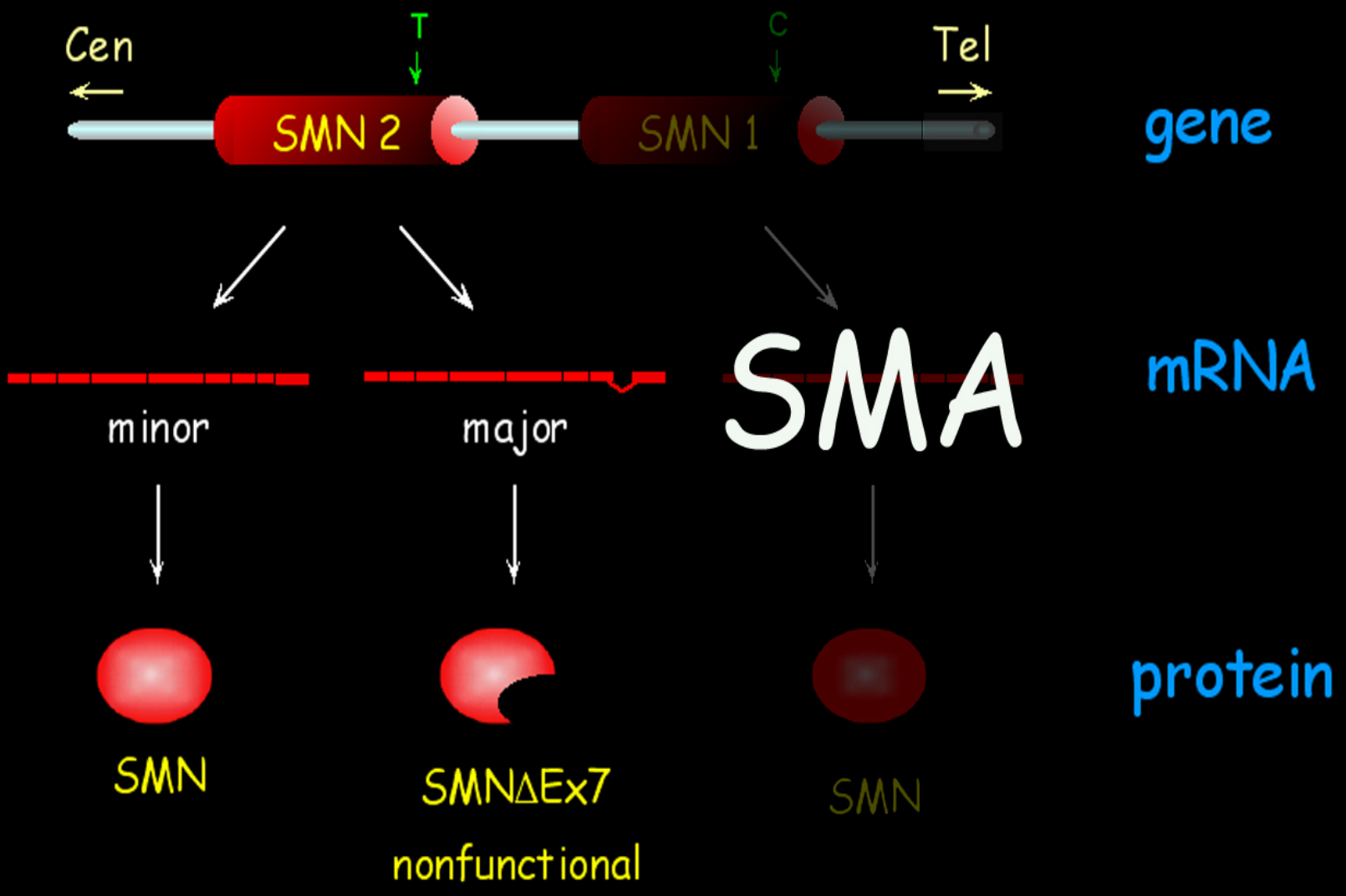
Spinal Muscular Atrophy

- **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**
  - SMN functions in snRNP assembly and axonal mRNA transport
- ***SMN2* paralog (unique to humans) provides partial function**
- **Variable severity (type I-IV) inversely proportional to *SMN2* copy number**

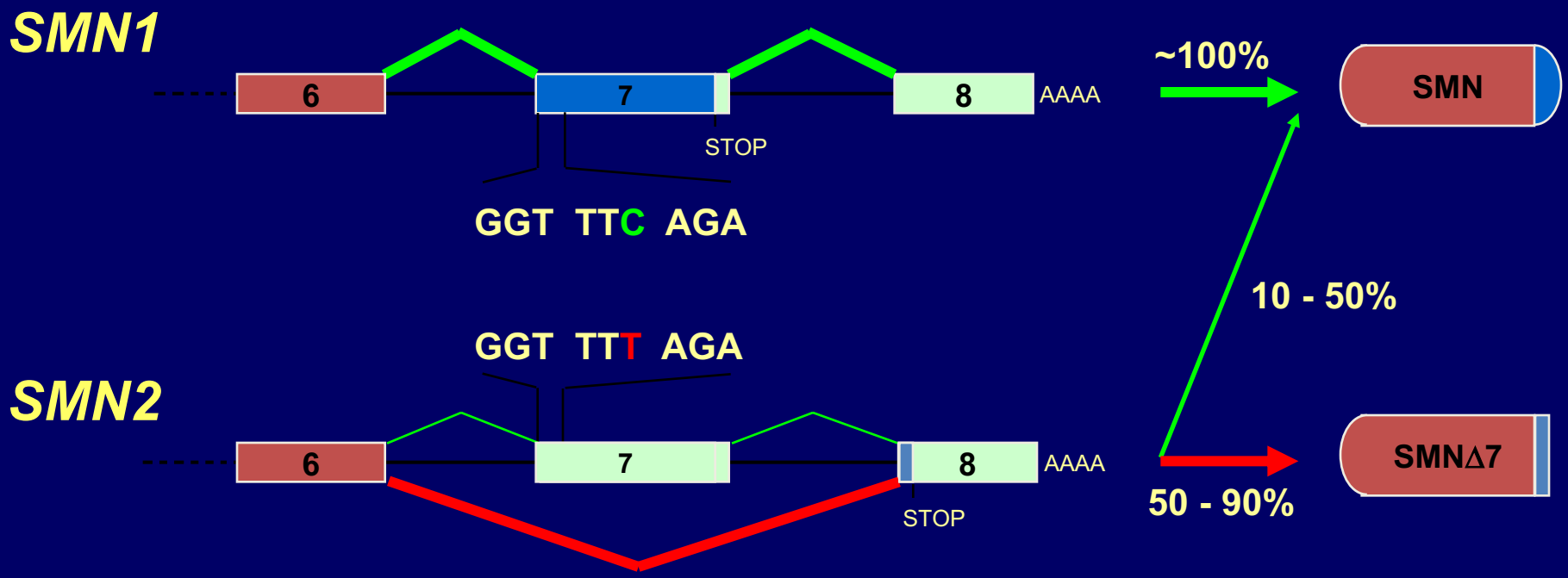


Courtesy of A. Krainer

SMN genes on human chromosome 5



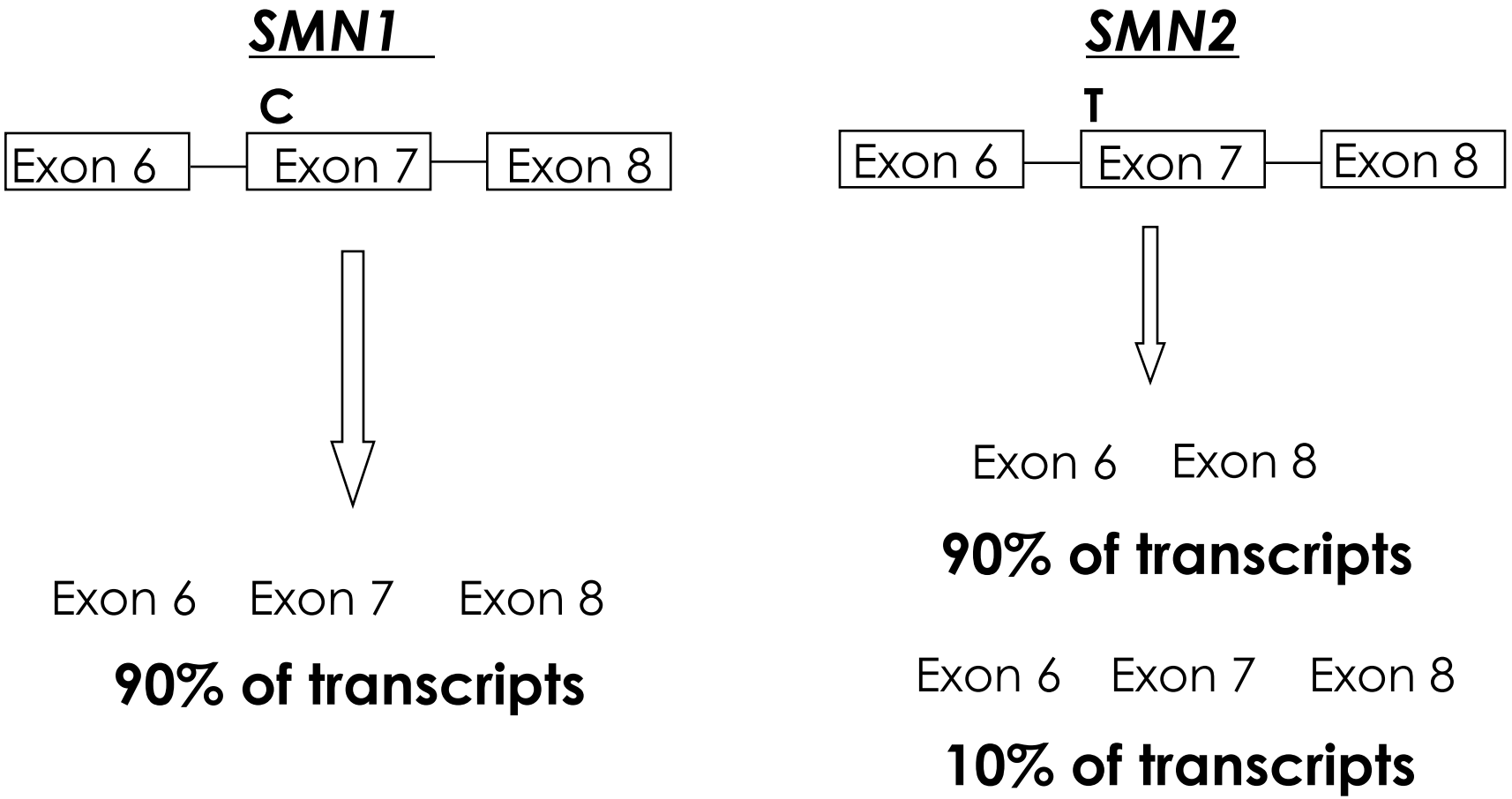
A single nucleotide difference causes exon skipping in *SMN2*



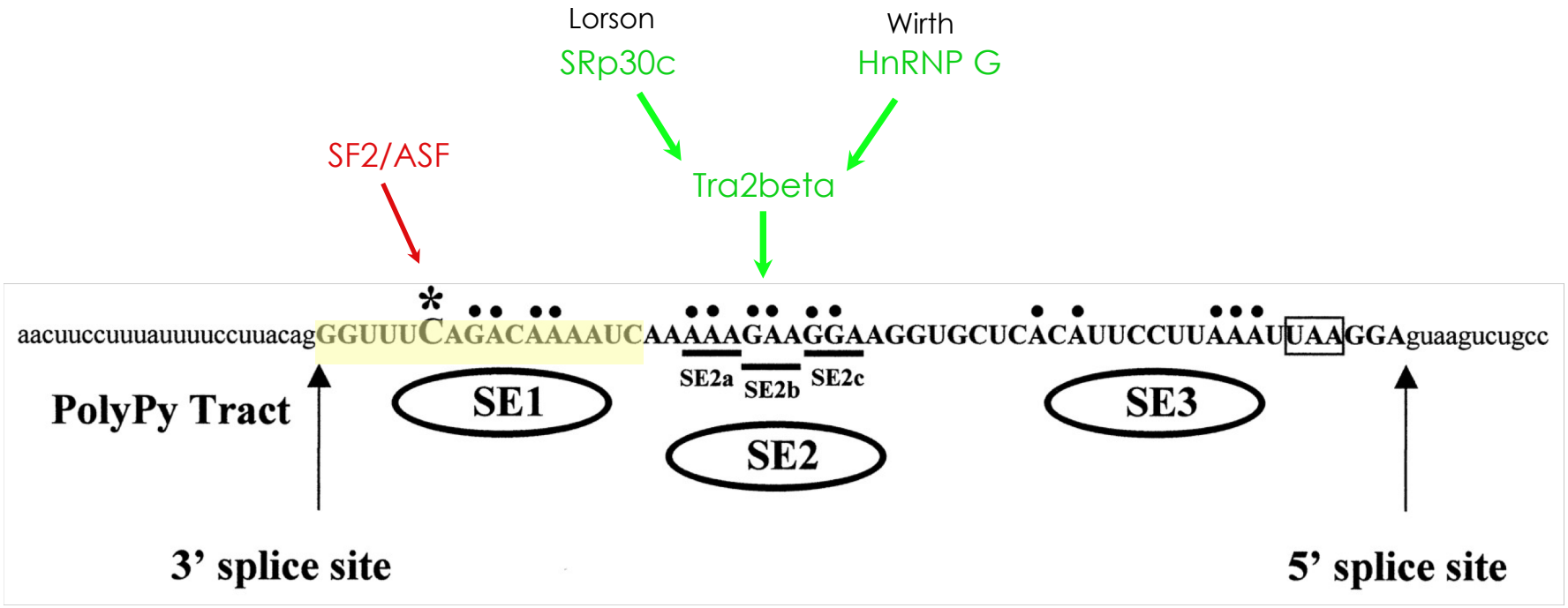
Courtesy of A. Krainer

Lorson et al (1999) *Proc Natl Acad Sci USA* 96: 6307
Monani et al (1999) *Hum Mol Genet* 8: 1177

- **Alternative Splicing of the *SMN* Genes**



SMN1 exon 7

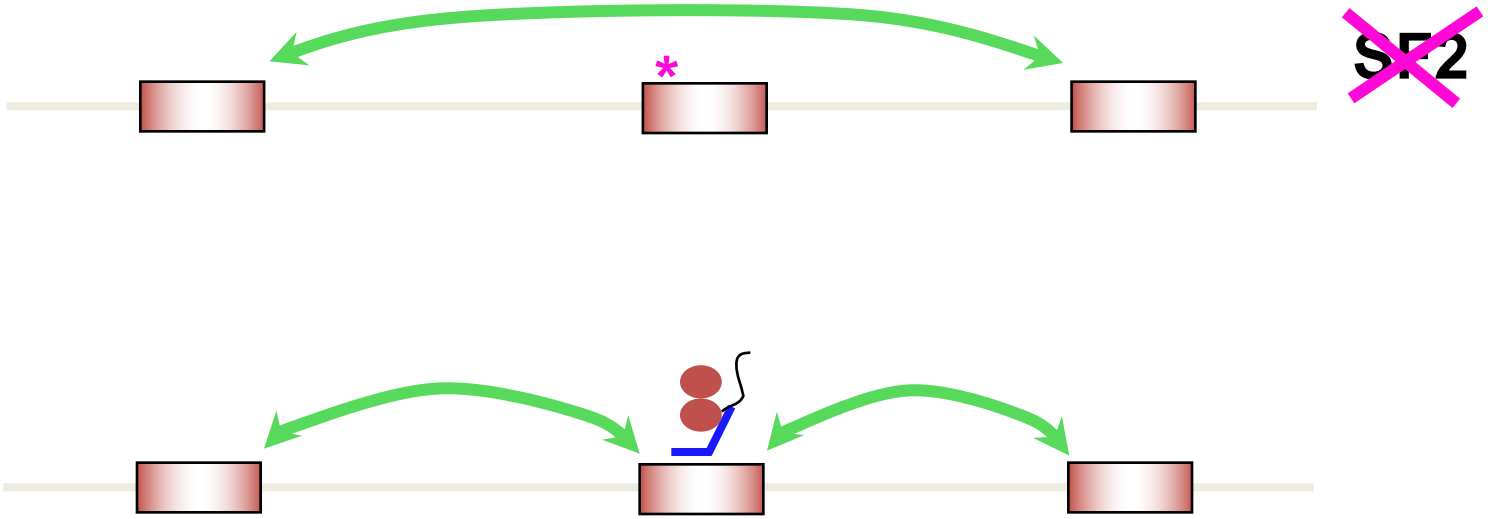


General inhibitory context

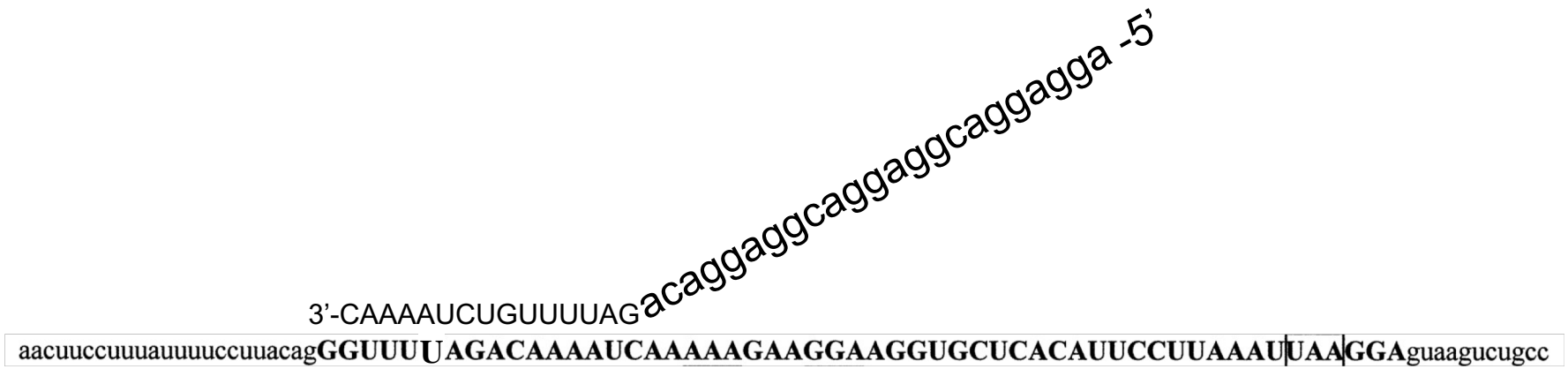
C-T A1

From: Hofmann et al. (2000). PNAS 97, 9618-9623

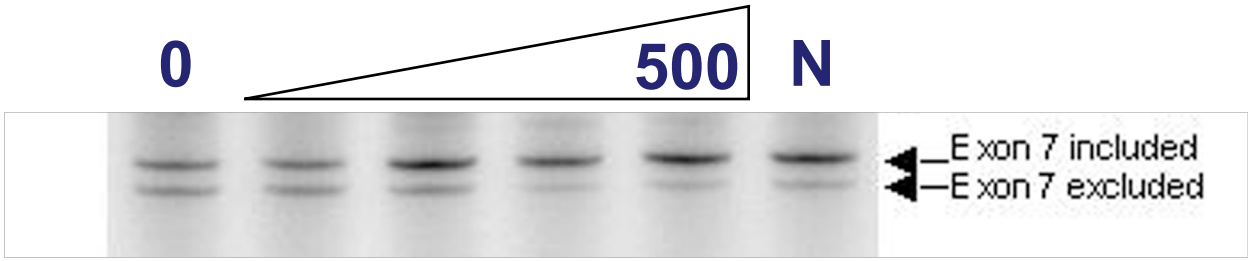
- Can they be rescued?



- **SMN2 exon 7**


3'-CAAAAUCUGUUUUAG
acaggaggcaggaggcaggagga -5'
aacuuccuuuauuuuccuuacagGGUUUUAGACAAAAUCAAAAAGAAGGAAGGUGCUCACAUUCCUUAAAUUAAGGAguaagucugcc

- **With endogenous *SMN2***



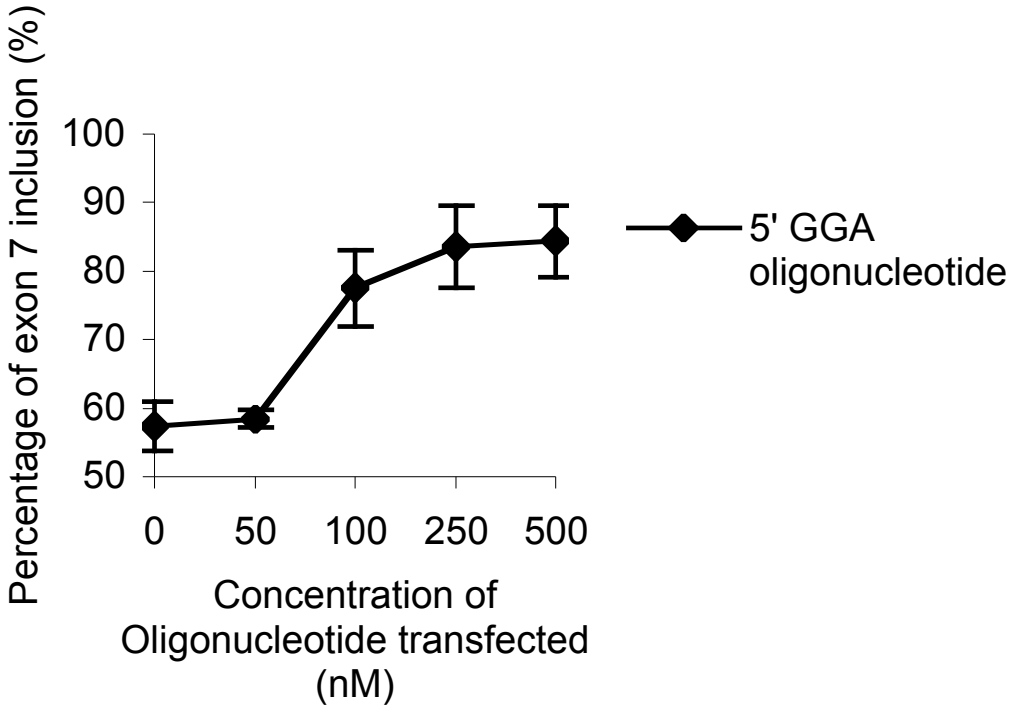
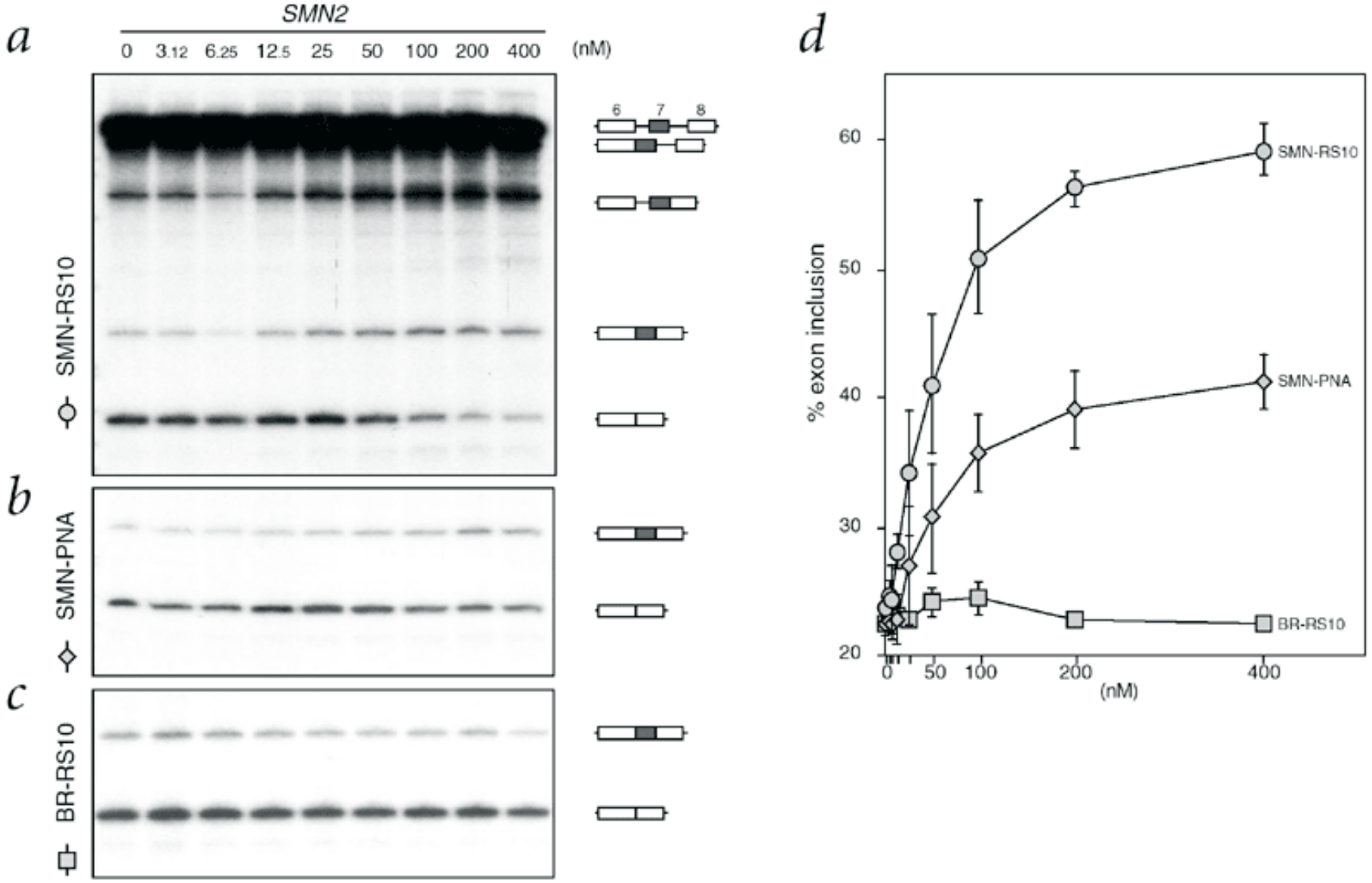


Figure 1: Design of synthetic compounds that specifically promote exon inclusion.

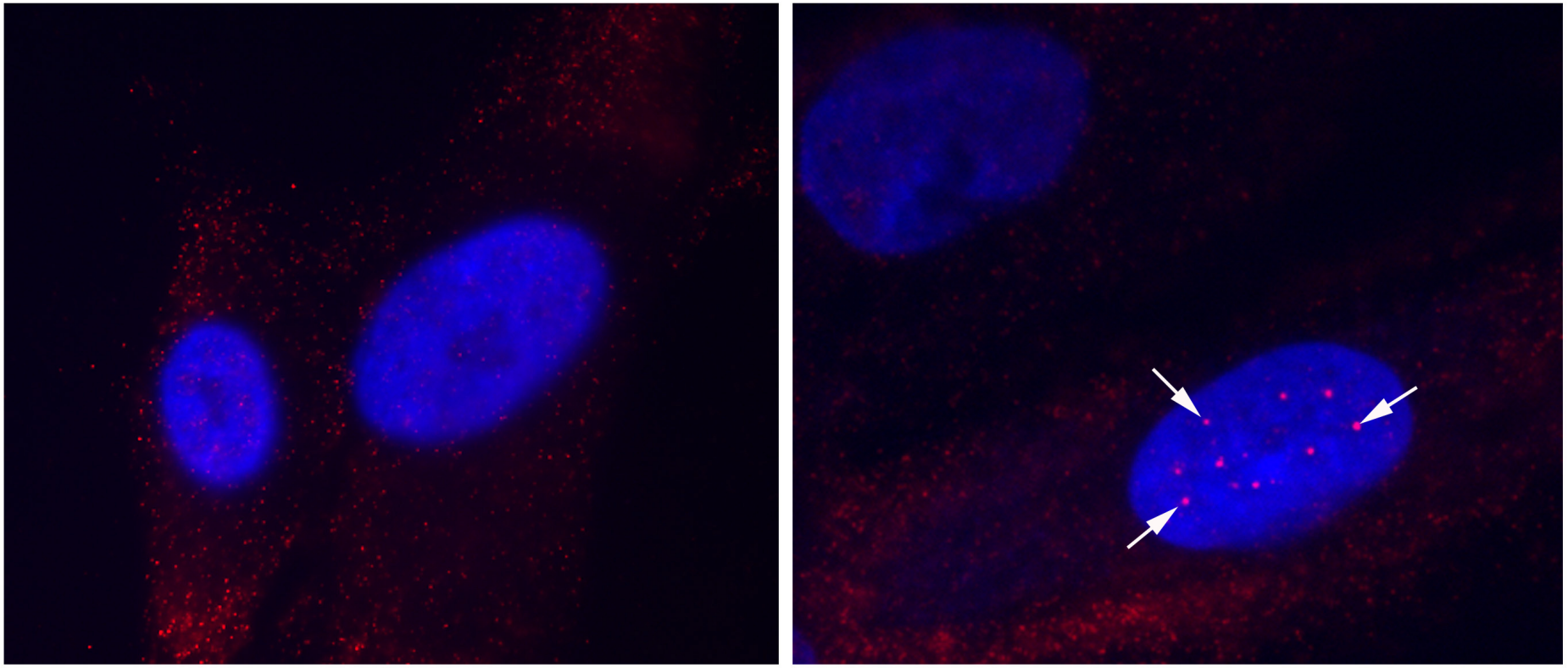
a, ESSENCE emulates the ESE-dependent function of SR proteins. SR proteins (top) bind to an ESE through their RNA-binding domain (RBD) and promote exon inclusion by recruiting the splicing machinery through their RS domain (RS). In the absence o f an ESE (middle), SR proteins cannot bind and the exon is skipped. An ESSENCE co mpound (bottom) tethers a minimal RS domain to the exon by Watson-Crick base pai ring and rescues splicing. Any sequence along the exon can be chosen as the target si te. ***b***, Chemical structure of the PNA–peptide chimeric molecule. All compounds were HPLC-purified after direct synthesis with the PNA moiety at the N terminus and th e recruiting peptide moiety at the C terminus to mimic the standard domain organiza tion of SR proteins. A two-glycine linker separates the two minidomains to provide fl exibility. Upper and lower case letters indicate amino acids and nucleotides, respecti vely. ***c***, Sequences and targeting strategy of the chimeric compounds directed to *BR CA1* E1694X exon 18 and *SMN2* exon 7. The underlined nucleotide at position +6 of b oth exons is substituted by g or c in wild type *BRCA1* and in *SMN1*, respectively. The compounds are represented from C to N terminus to emphasize the base pairing to t he target site (bold) on the exon (white box). Only partial sequences at the intron/e xon boundaries are shown. BR and SMN compounds are directed to *BRCA1* E1694X e xon 18 and *SMN2* exon 7, respectively. CON-PNA is a control PNA of unrelated sequ ence.



Rescue of *SMN2* exon 7 splicing to *SMN1* levels by ESSENCE.

SMN2-derived pre-mRNAs (10 fmol per 12.5 l reaction) were spliced in HeLa cell nuclear extract for 4 h at 30 ° C as described26. Increasing amounts of ***a***, SMN-RS10, ***b***, SMN-PNA, ***c***, BR-RS10 wer e added to the pre-assembled reactions. The identity of each band is schematically represented on the right. ***d***, Quantification, as above, of the experiments shown in (*a–c*), using mean values from at least three independent experiments.

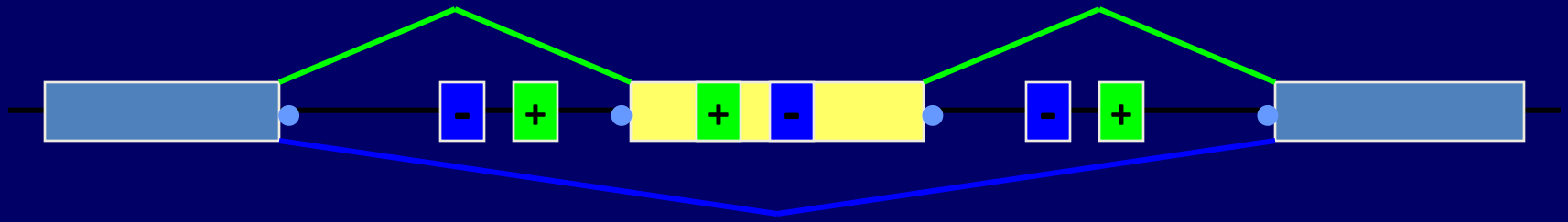
- **Recovery of gems**



A) B)

Rescue of *SMN2* exon 7 splicing to *SMN1* levels by ESSENCE restores gems localization of the SMN protein.
A) mutant cells, B) cells treated with ESSENCE

Exon/intron identity determinants



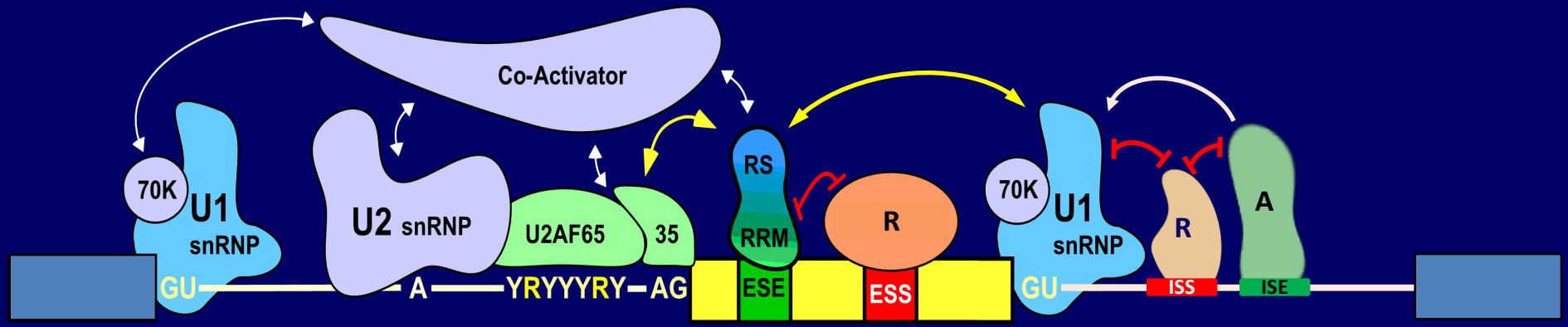
– Strength of splice sites

– Splicing enhancers: exonic (ESE) or intronic (ISE); bound by activators

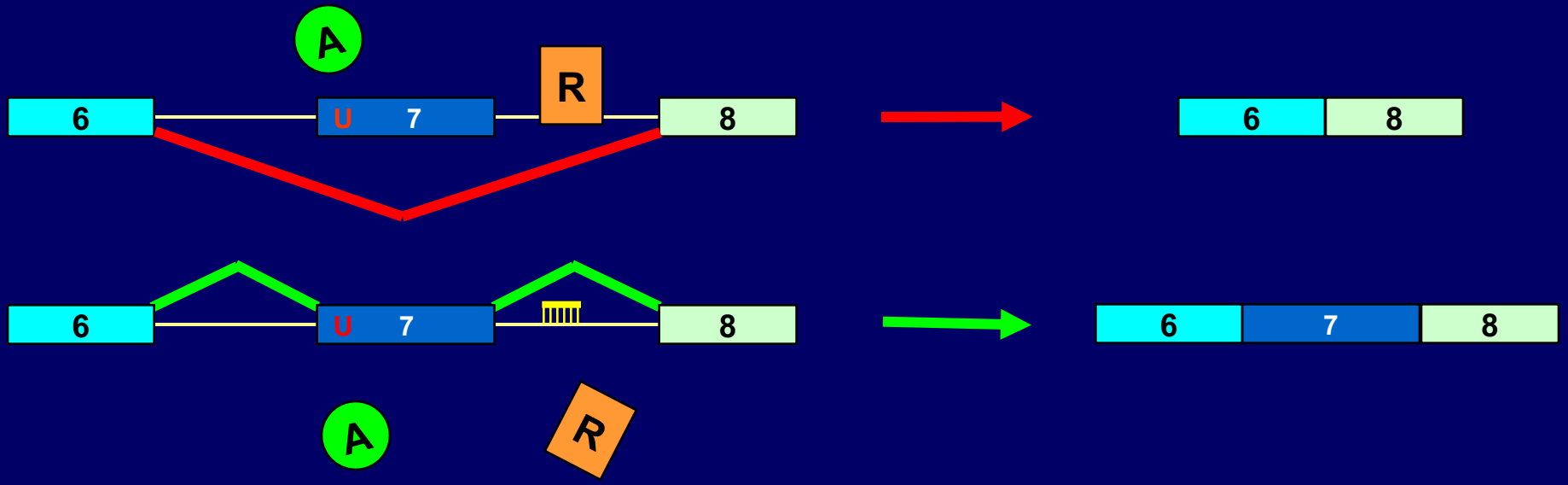
– Splicing silencers: exonic (ESS) or intronic (ISS); bound by repressors

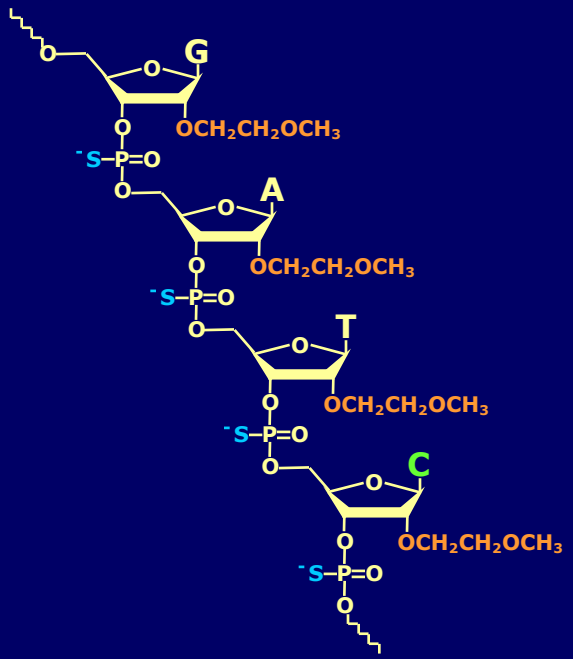
– RNA 2^{ary} structure

–Chromatin features



Antisense strategy to promote *SMN2* exon 7 inclusion





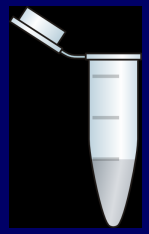
2'-*O*-(2-methoxyethyl) ribose

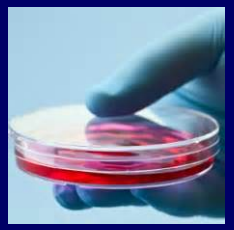
phosphorothioate

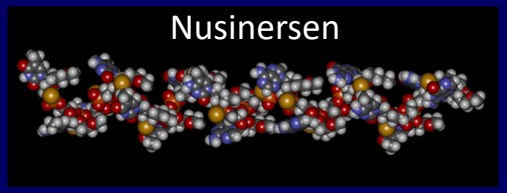
5-methyl cytosine

IONIS PHARMACEUTICALS

The drug-discovery pipeline







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

2011 - 2017

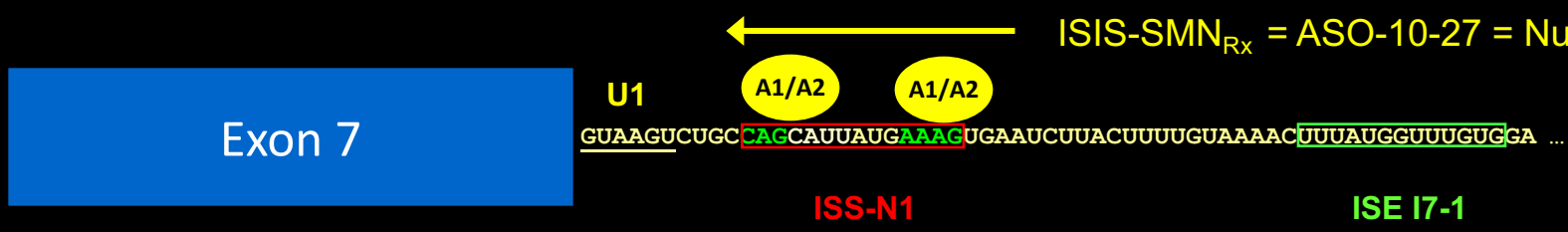
2016

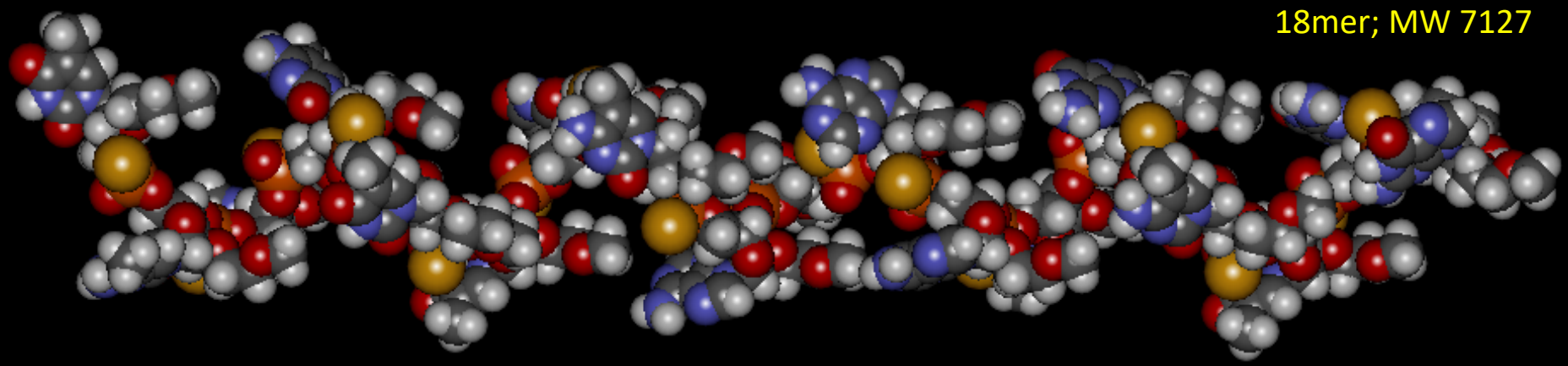
IONIS PHARMACEUTICALS

Biogen

Courtesy of A. Krainer

Nusinersen (Spinraza®)

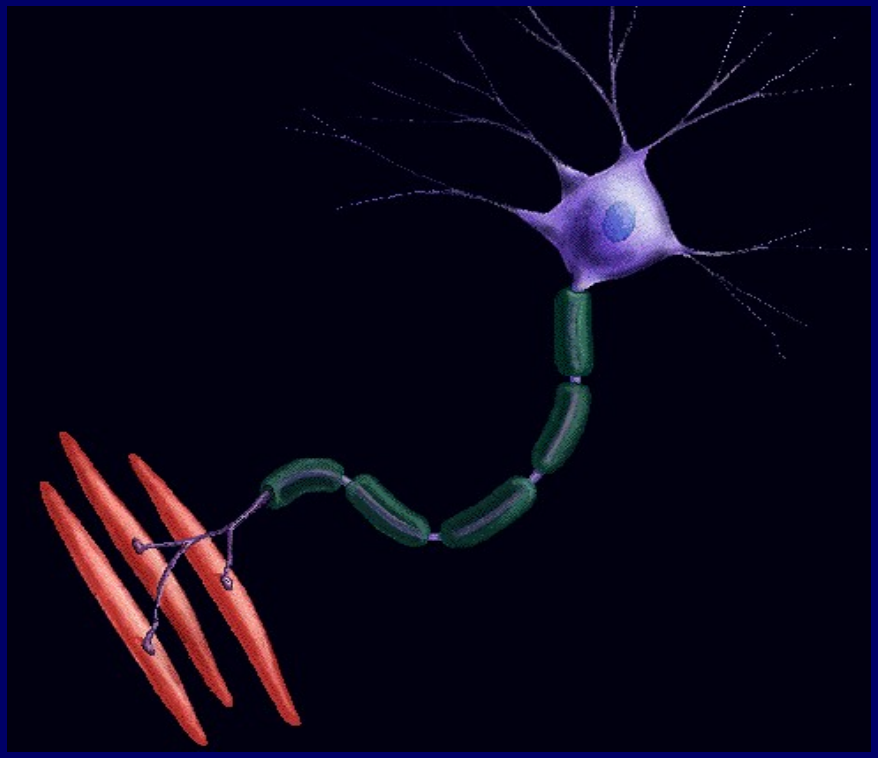




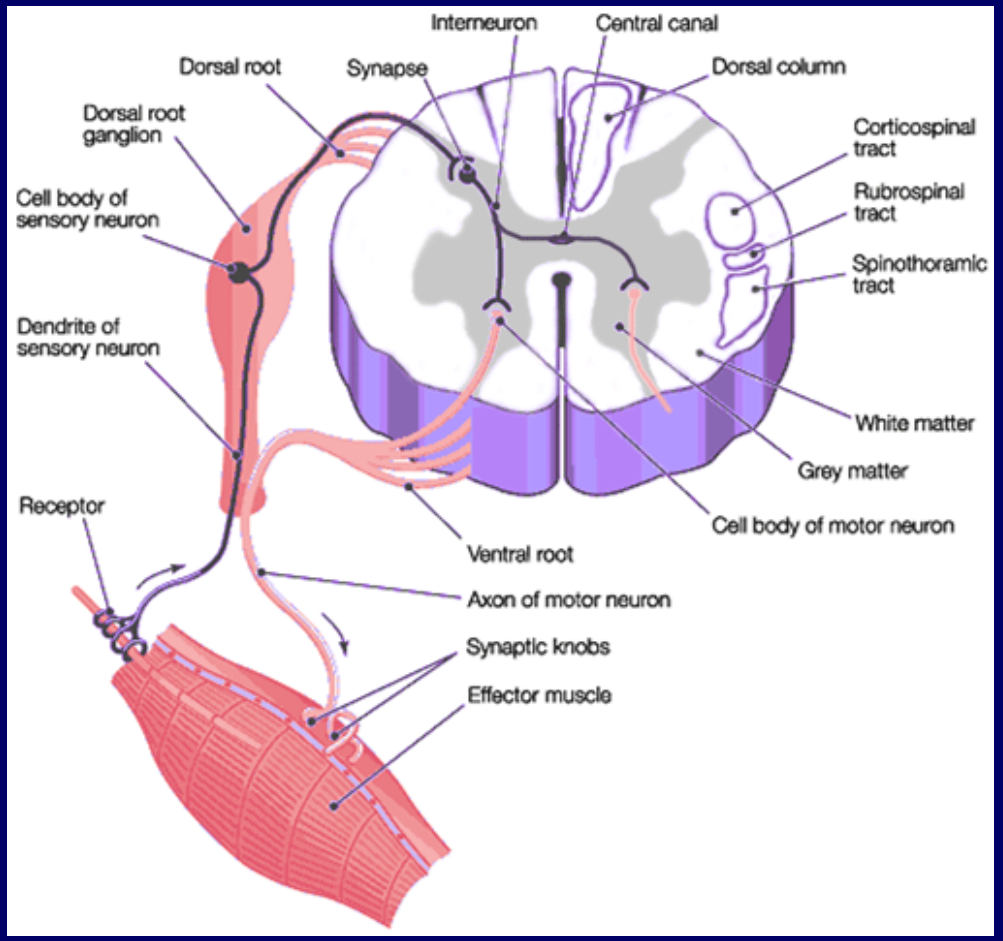
Hua et al (2008) *Am J Hum Genet* 82: 834
Hua et al (2010) *Genes Dev* 24: 1634
Passini et al (2011) *Science Transl Med* 3: 72
Hua et al (2011) *Nature* 478: 123
Rigo et al (2012) *Nature Chem Biol* 8: 555
Rigo et al (2014) *J Pharmacol Exper Ther* 350: 46

Singh et al (2006) *Mol Cell Biol* 26: 1333
Gladman & Chandler (2009) *Hum Genet* 126: 833

Spinal-cord α-motor neurons



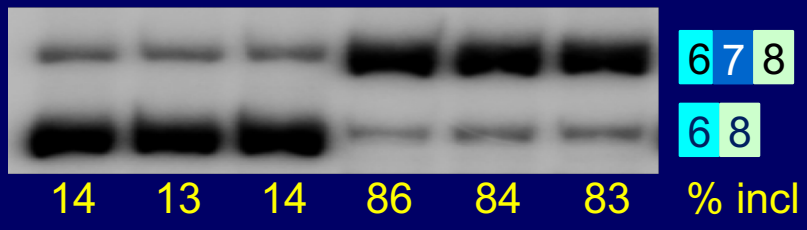
www.uofaweb.ualberta.ca



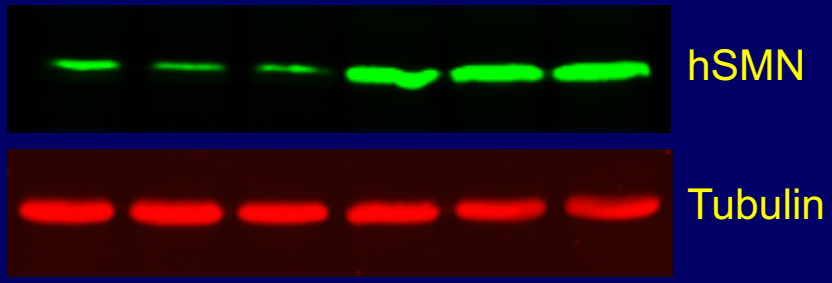
http://www.glittra.com/yvonne/neuropics.html

Intracerebroventricular injection of nusinersen in neonate transgenic mice with severe SMA

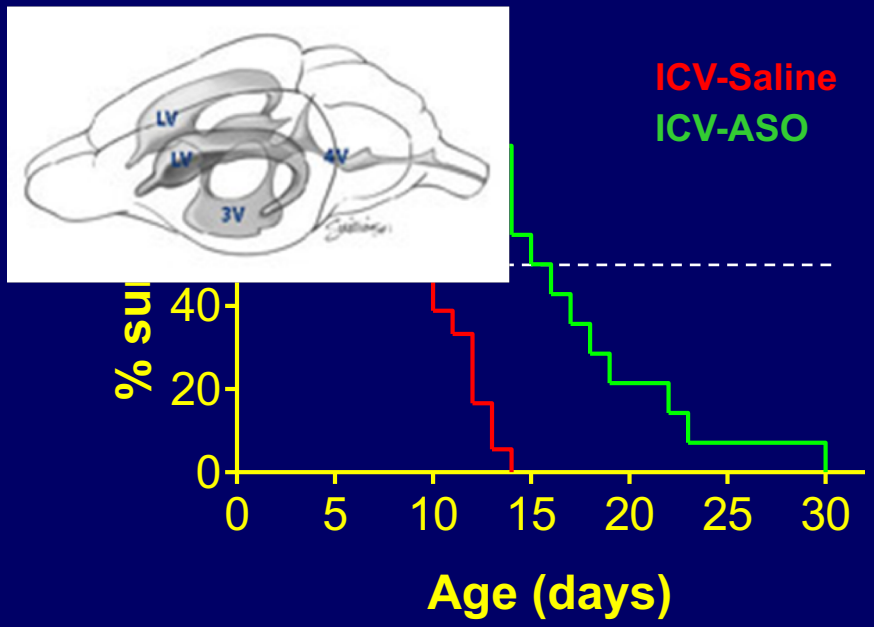
RT-PCR



Western



Dose: 20 µg ICV at P1
Tissue: whole spinal cord at P7
Smn$^{-/-}$; *SMN2*$^{2Tg/0}$

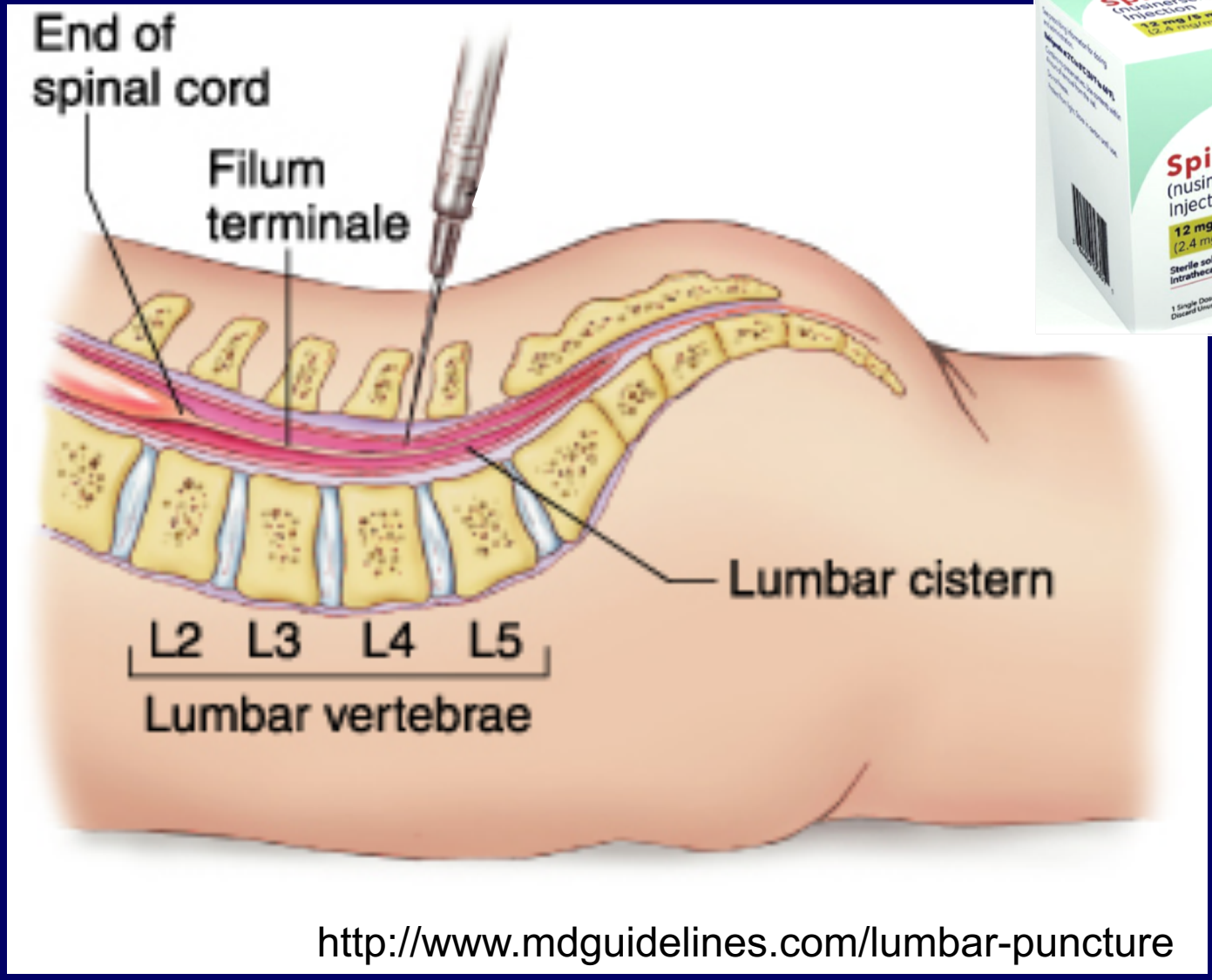


Mean life span

Saline: 10 d
ASO: 17 d
P<0.001 (Mantel-Cox)

Hua et al (2011) *Nature* 478: 123
Passini et al (2011) *Science Transl Med* 3: 72

Intrathecal drug delivery

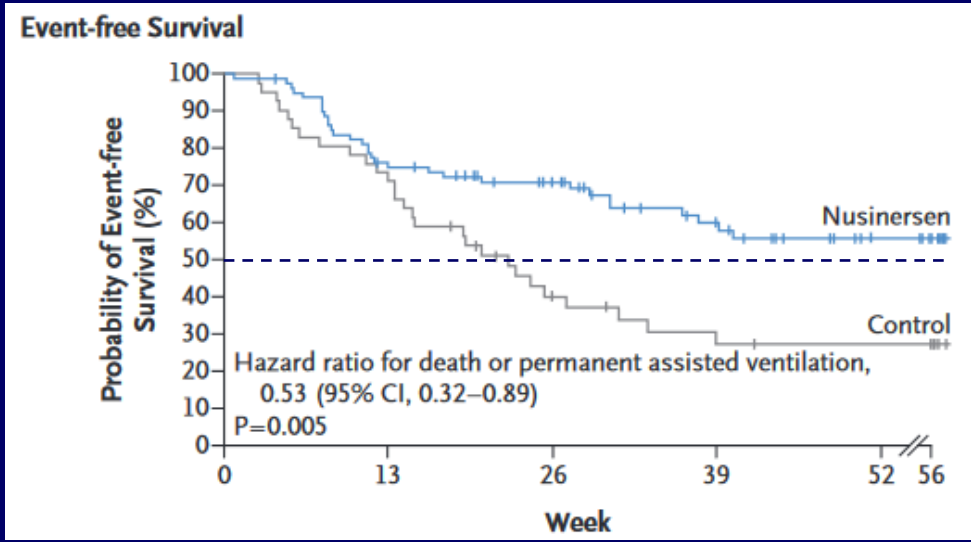


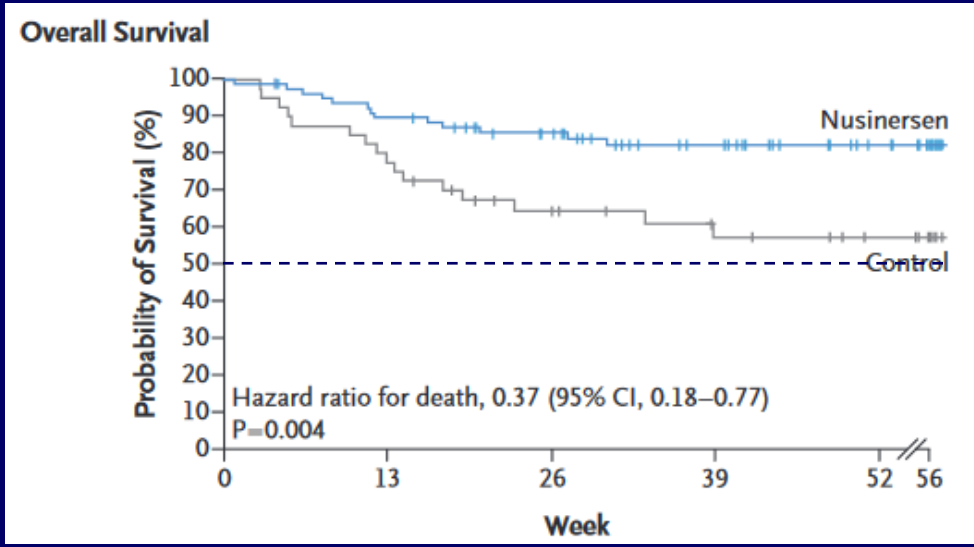
http://www.mdguidelines.com/lumbar-puncture

Spinraza clinical trials

- Began in November 2011
- Sponsored by Ionis and Biogen
- More than 300 SMA patients enrolled (infants, children, teens)
- Some children/teens have been on drug for 7 years, and infants for 5 years
- Statistically significant and clinically meaningful improvements in survival and motor-function endpoints
- Continued motor improvements over time; not just preventing further decline
- Pivotal phase-3 trials (ENDEAR and CHERISH) terminated early, based on interim analyses
- Pre-symptomatic treatment (NURTURE trial) results in even more remarkable outcomes

Increased event-free and overall survival in Endear phase-3 study (type I SMA infants)





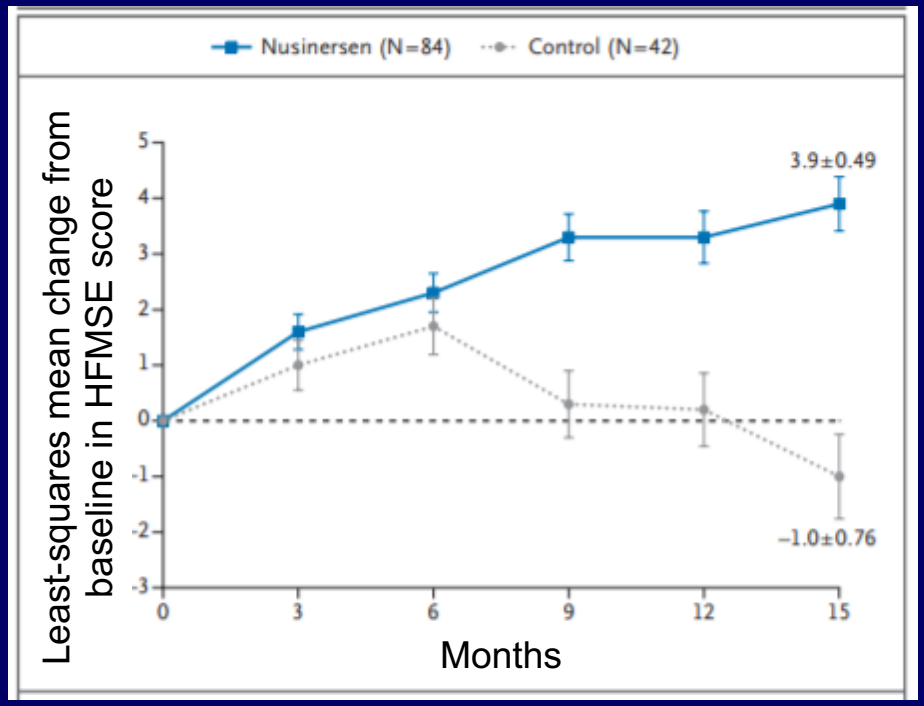
Finkel et al (2017) *New Engl J Med* 377: 1723

Type I SMA natural history

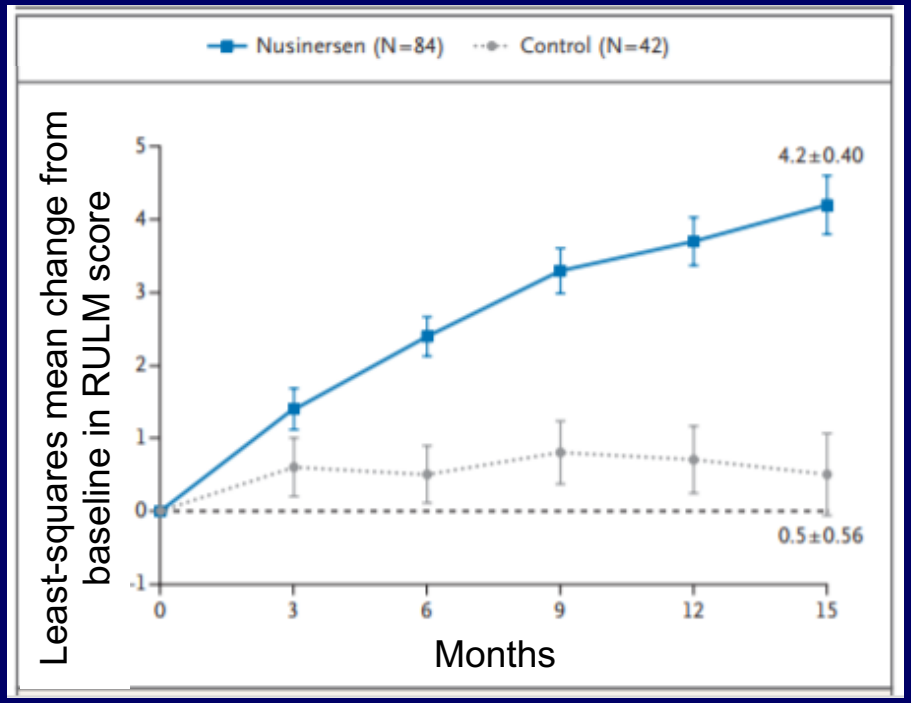
- Infant onset
- Can never sit independently
- Median time to death or permanent ventilation: 10.5 months (2 copies of *SMN2*)
- By 18 months, 85% have met the endpoint
- Steady decline in muscle function over time
  - Mean rate of decline in CHOP INTEND scale: 1.27 points/year

Finkel et al (2014) *Neurology* 83: 810

Increased muscle-function scores in nusinersen-treated type 2 SMA children



HFMSE

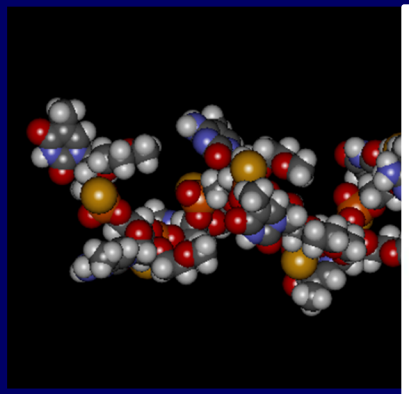


RULM

CHERISH phase-3 trial; 126 children ages 2 to 9

Mercuri et al (2018) *N Engl J Med* 378: 625

Nusinersen: a drug of many firsts



GALIEN FOUNDATION

PRIX GALIEN USA

Best Biotechnology Product 2017
for extraordinary achievement
in scientific innovation that improves t
he state of human health

- First (and so far
- First approved d
- First approved nucleic-acid therapeutic administered by lumbar puncture
- First approved nucleic-acid therapeutic for a neurological disease
- First drug to demonstrate that pre-symptomatic treatment can markedly delay or prevent disease onset of a neurodegenerative disease
- First disease-modifying drug for neurodegeneration

Updates

- November 2018: ~6,000 patients currently on Spinraza worldwide; >10% of adults with SMA in the US
- >200 sites are already dosing Spinraza across the U.S.
- Missouri, Utah, Minnesota, Indiana, and New York recently added SMA to newborn screening panel; ongoing efforts in other states; already adopted in Taiwan
- July 3, 2018: US Health & Human Services Secretary Alex Azar approves the recommendation that newborn screening for SMA be implemented nationwide; 4 million newborns born annually in the US will be screened for 35 conditions, including SMA
- Spinraza approved in 35 countries; reimbursed in 28

Biogen