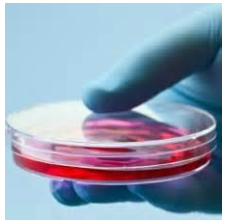
FROM MOLECULE TO PATIENT

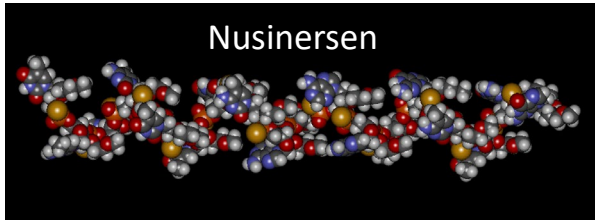
RNA Splicing as a Target for a New Generation of Precision Medicines

Adrian R. Krainer, Ph.D.
Cold Spring Harbor Laboratory
krainer@cshl.edu

Disclosures: Collaborator, royalties (Ionis Pharmaceuticals); consultant (Biogen); founder, board of directors, SAB, consultant, stock (Stoke Therapeutics); SAB (Skyhawk Therapeutics); SAB (Envisagenics); SAB (Autoimmunity Biologic Solutions)

The drug-discovery pipeline: nusinersen

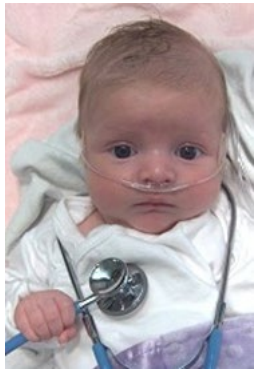




Pre-clinical development

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

2011 - 2017 2016

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.

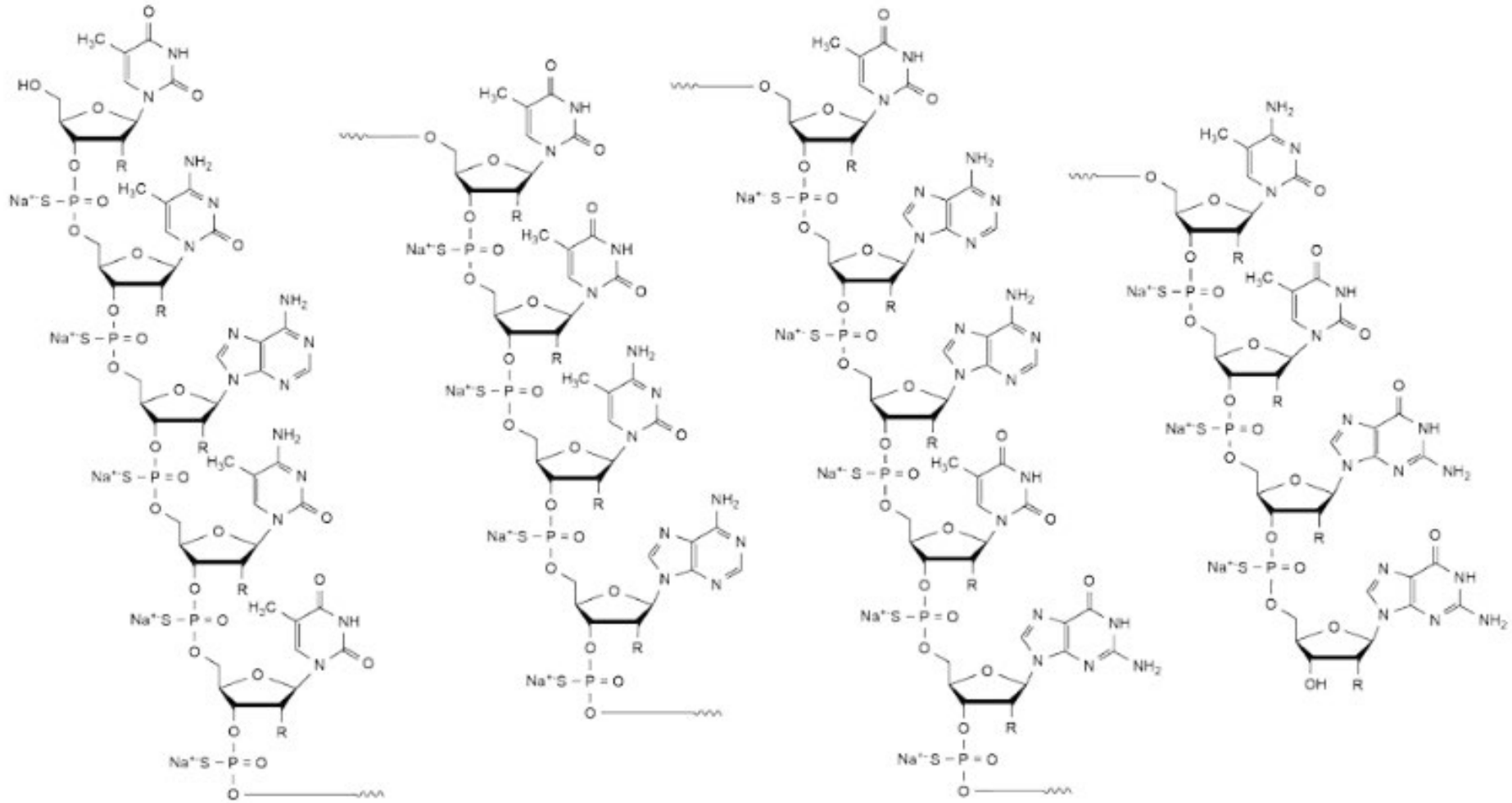
Initiate **NUSINERSEN** treatment with 4 loading doses. The first three loading doses should be administered at 14-day intervals. The 4th loading dose should be administered 30 days after the 3rd dose. A maintenance dose should be administered once every 4 months thereafter.

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.

11 DESCRIPTION

[redacted] 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:



R = $OCH_2CH_2OCH_3$

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

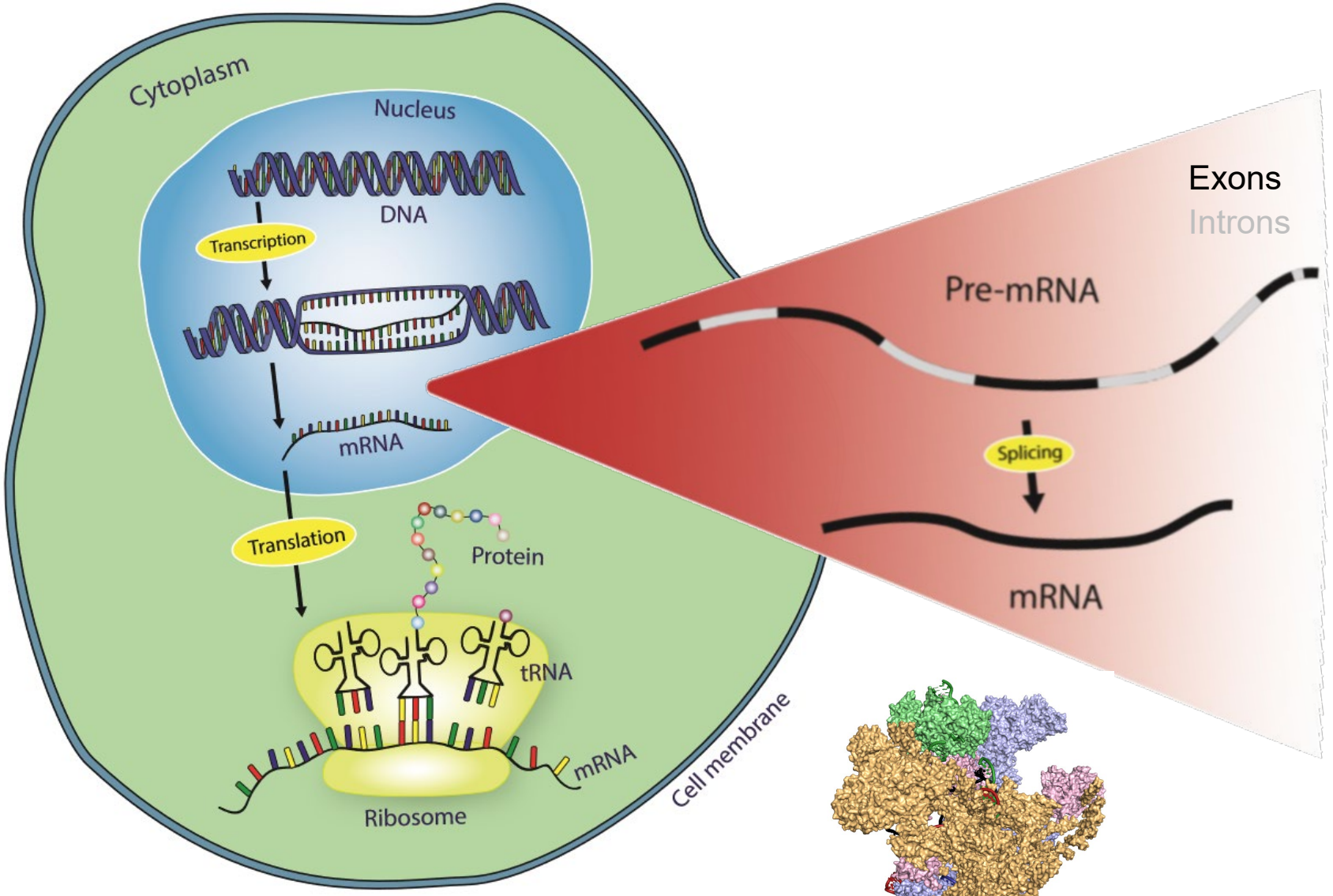
NUSINERSEN is an antisense oligonucleotide (ASO) designed to treat SMA caused by mutations in chromosome 5q that lead to SMN protein deficiency. Using in vitro assays and studies in transgenic animal models of SMA, **NUSINERSEN** was shown to increase exon 7 inclusion in *SMN2* messenger ribonucleic 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.

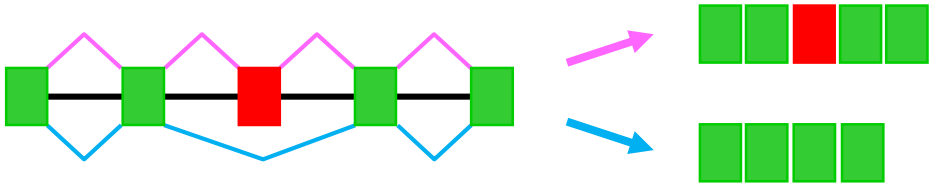
Cardiac Electrophysiology

Eukaryotic gene expression

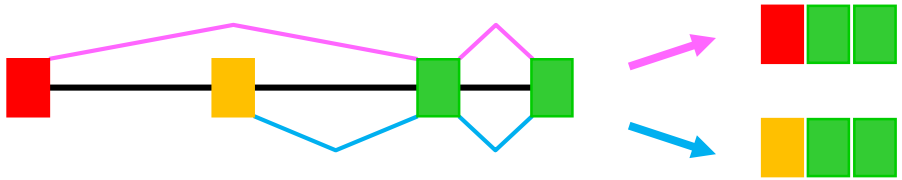


Spliceosome

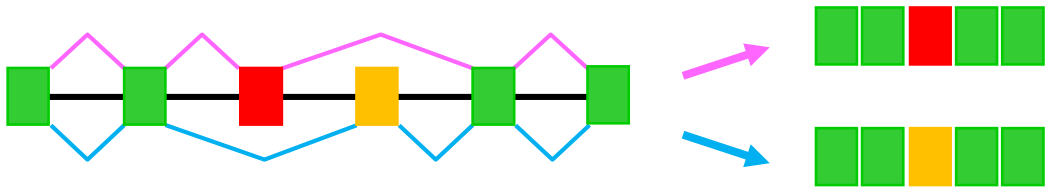
Alternative splicing



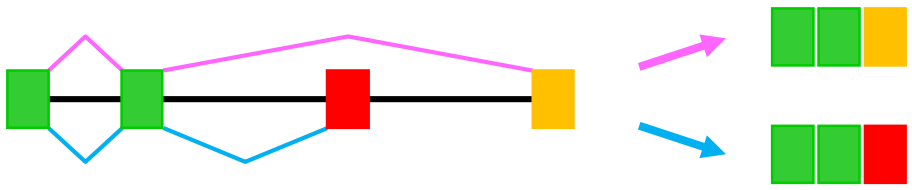
Cassette exon



Alternative
first exons

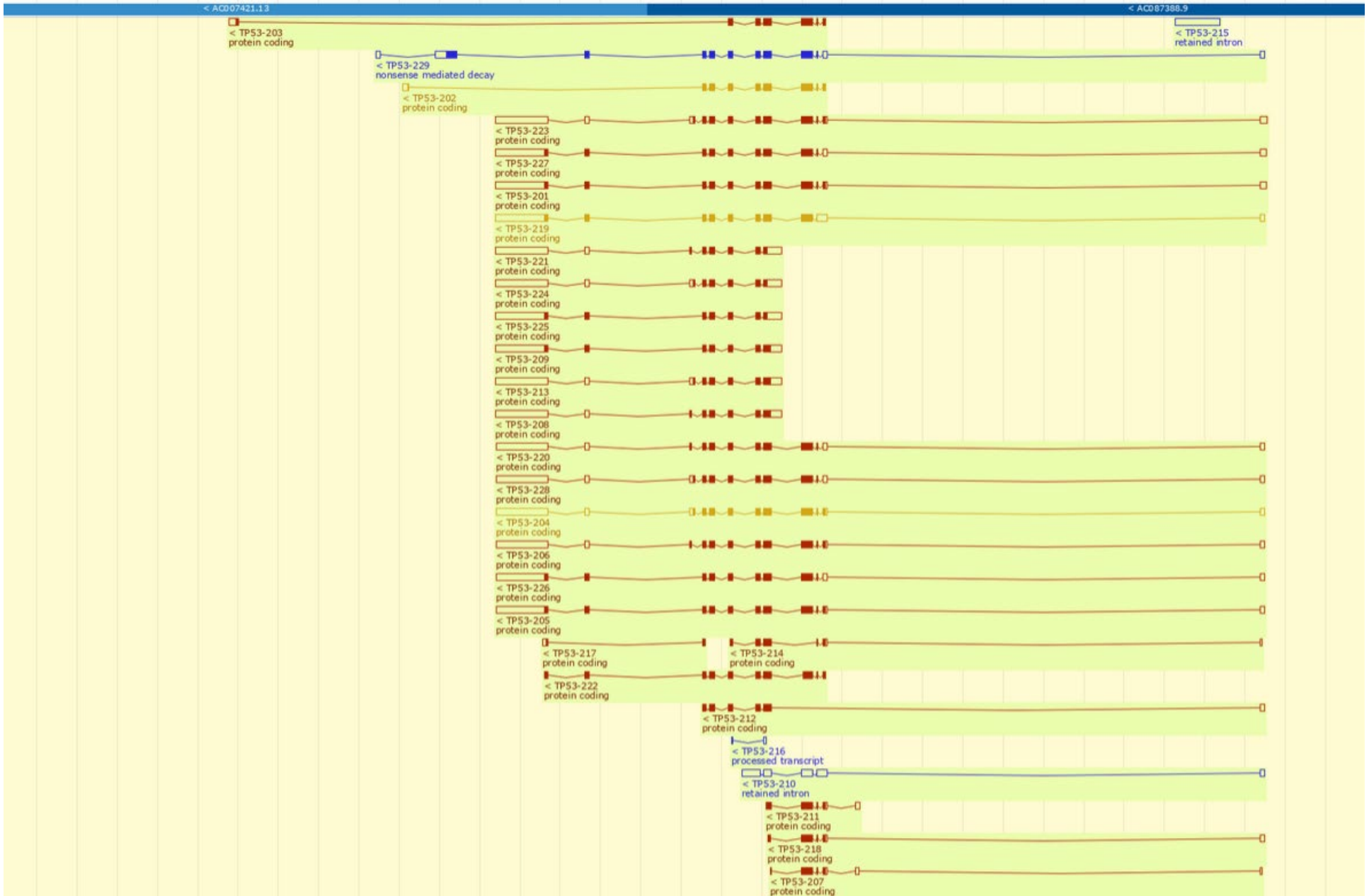


Mutually
exclusive exons



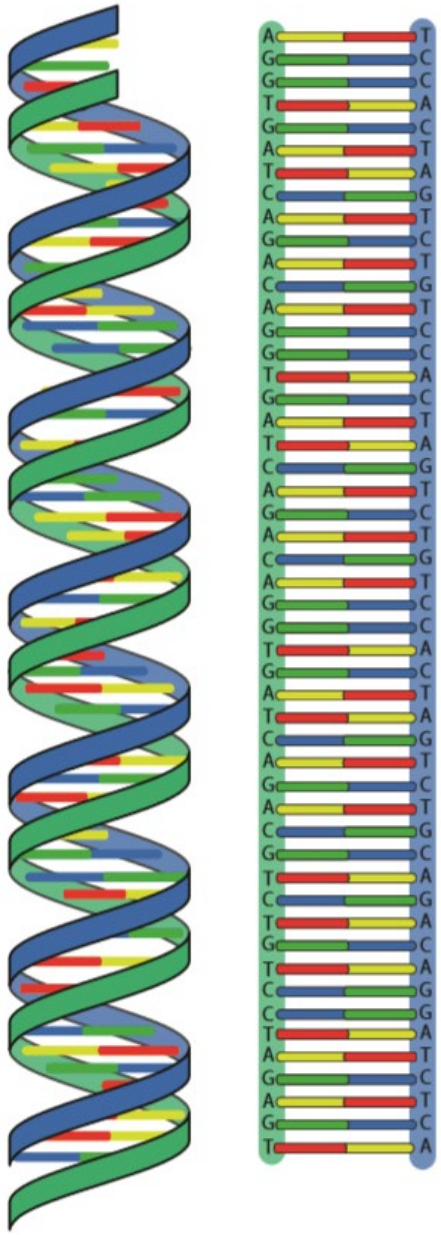
Alternative last
exons

Genome browser: human *TP53* gene



Antisense oligonucleotides

DNA (gene)

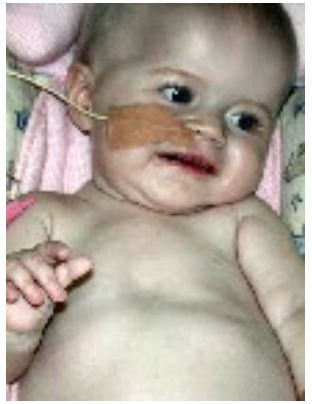


A – T

G – C

Spinal Muscular Atrophy

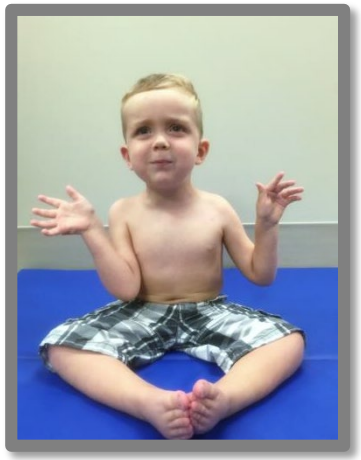
- **Pediatric neuromuscular disorder, autosomal recessive**
- **Degeneration of α-motor neurons in the spinal cord and lower brainstem**
- **1 in ~10,000 newborns**
- **Inactivating mutations in *SMN1*, which codes for SMN protein**
  - SMN functions in snRNP assembly and axonal mRNA transport
- ***SMN2* paralog (unique to humans) expresses a small amount of functional protein**
- **Variable severity (type 1-4) inversely proportional to *SMN2* copy number**



Broad spectrum of SMA disease severity correlates with *SMN2* gene copy number

Type 1 infantile-onset

- Age of symptom onset ≤6 months
- Very short life expectancy
- Median event-free survival is 10.5 months[a]
- Never able to sit
- Most have 2 copies of *SMN2*



Type 2 later-onset

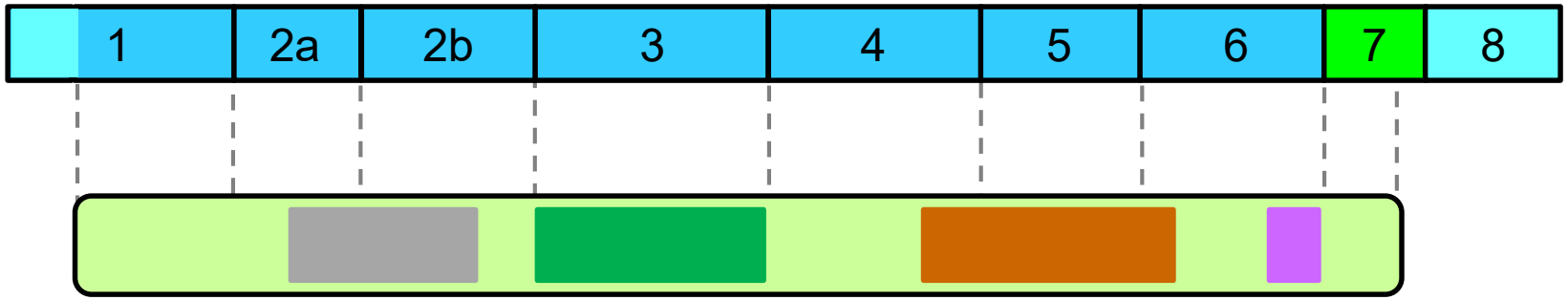
- Age of symptom onset >6 months
- Shortened life expectancy
- Able to sit or stand, but not walk
- Muscle weakness/skeletal deformities
- Most have 3 copies of *SMN2*

Type 3 later-onset

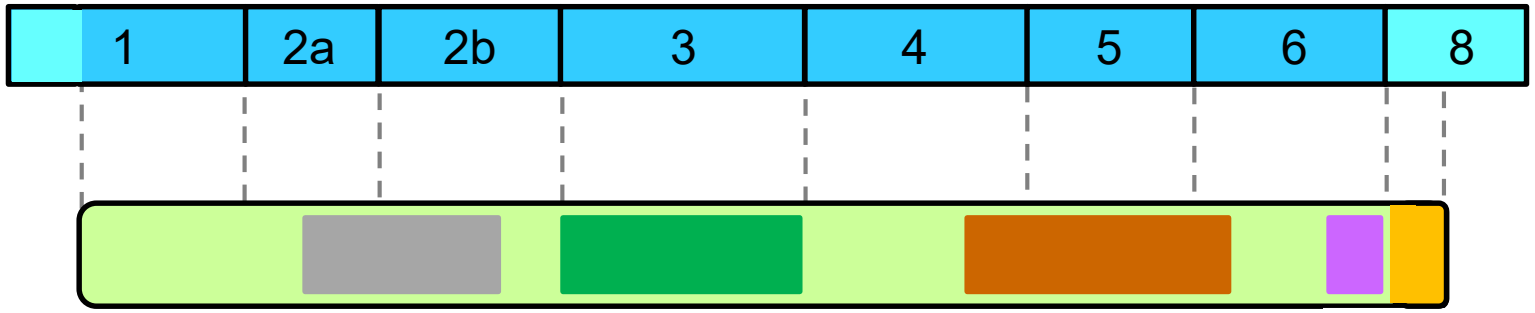
- Age of symptom onset >6 months
- Close to normal life expectancy
- Ability to walk declines over time
- Muscle weakness/skeletal deformities
- Most have 3-4 copies of *SMN2*

[a]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%)



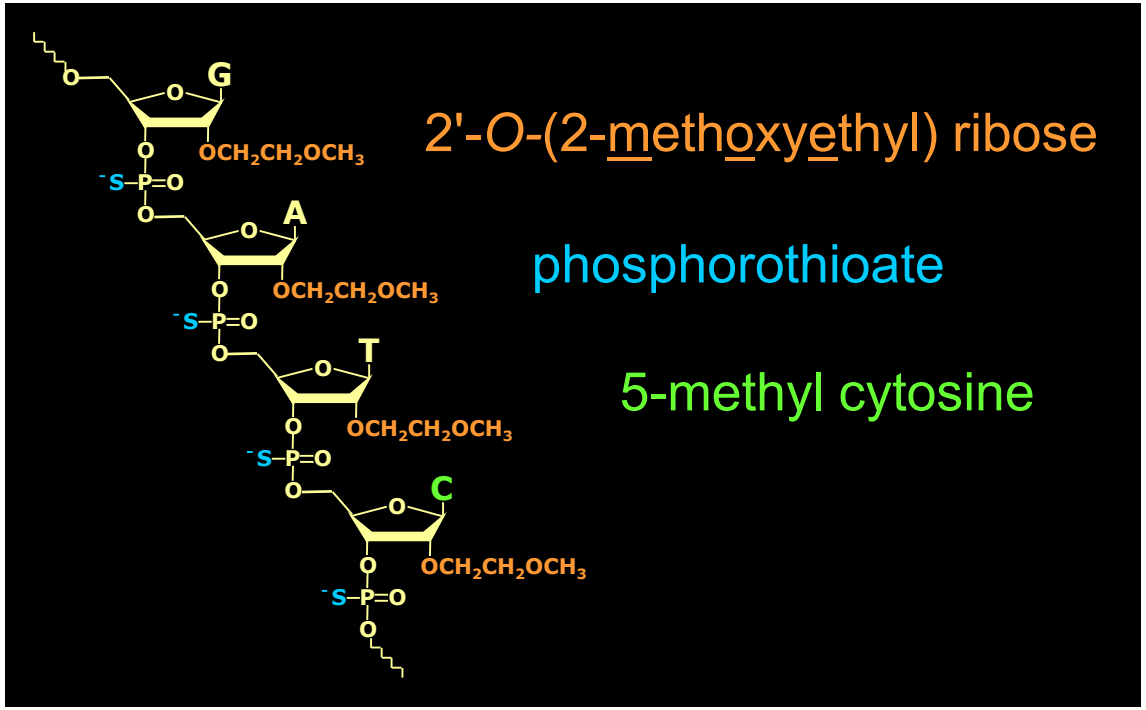
Defective SMNΔ7 protein (90%)

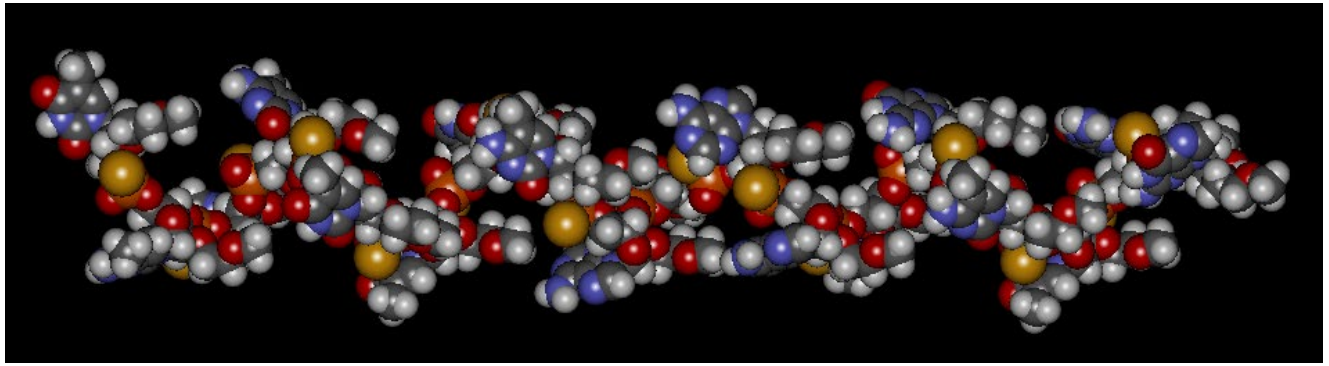
Nusinersen

SMN2 pre-mRNA

Nusinersen

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

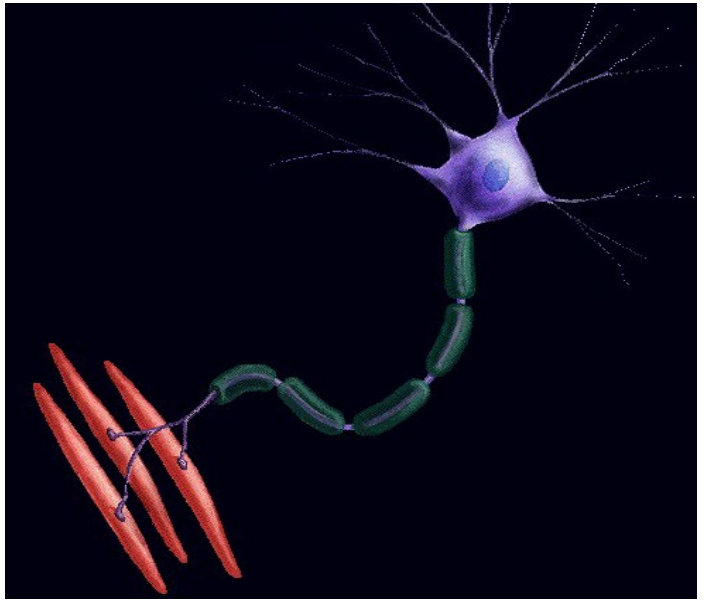




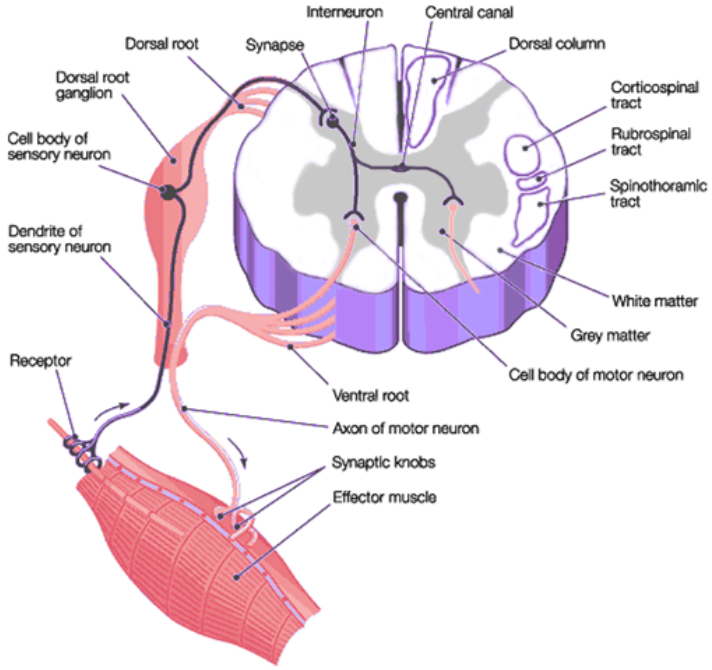
18mer ASO; MW 7127

Hua, Vickers, Okunola, Bennett & Krainer (2008) *American Journal of Human Genetics* 82: 834

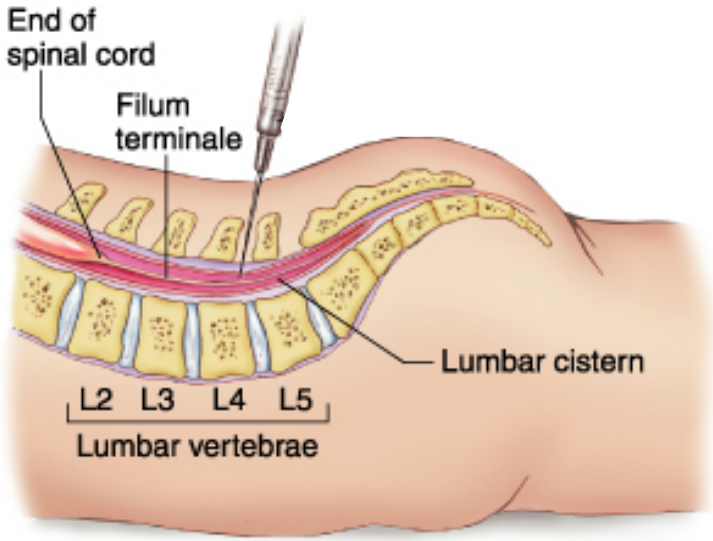
Targeting spinal-cord motor neurons via lumbar puncture



www.uofaweb.ualberta.ca



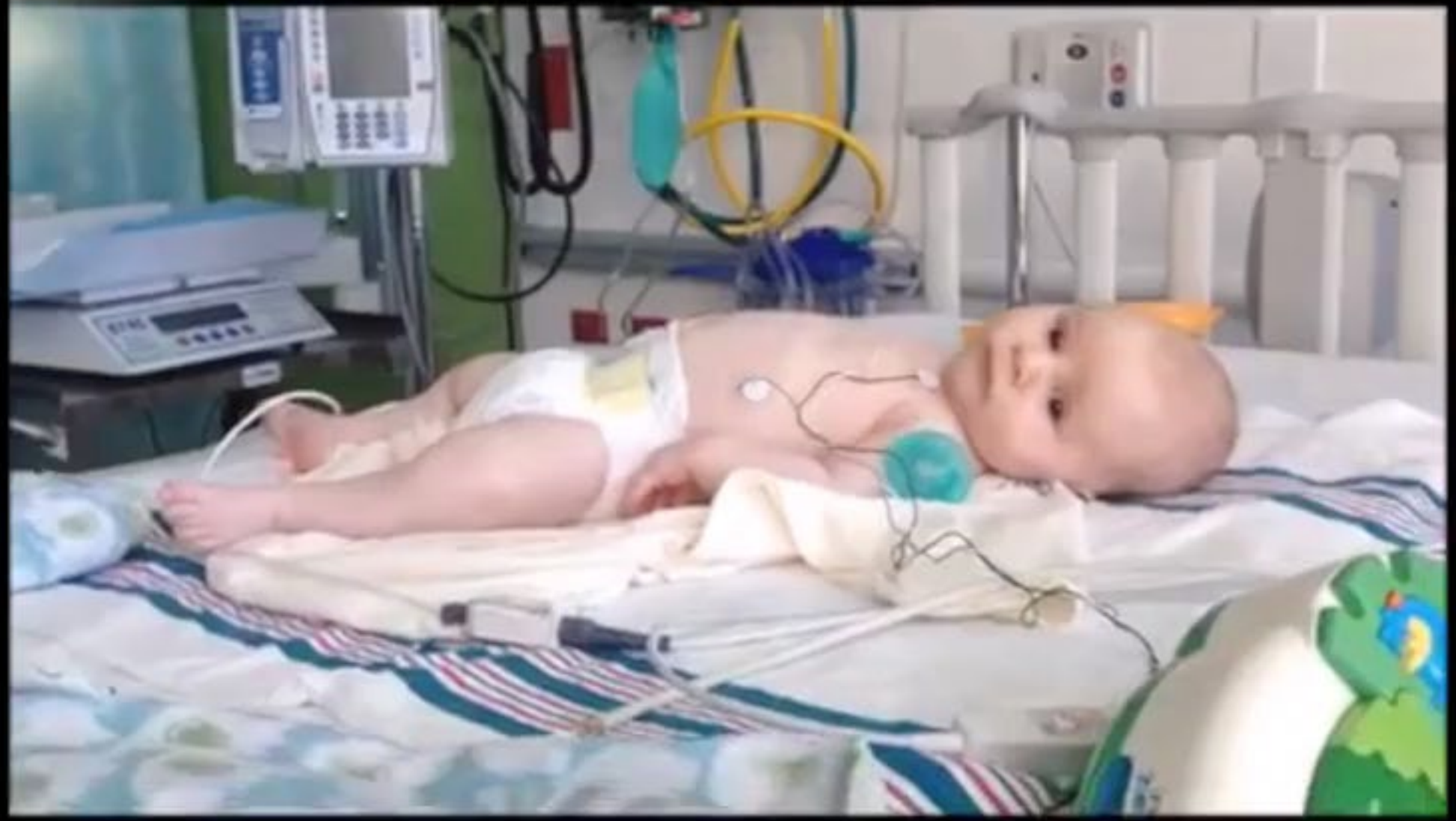
www.glittra.com/yvonne/neuropics.html



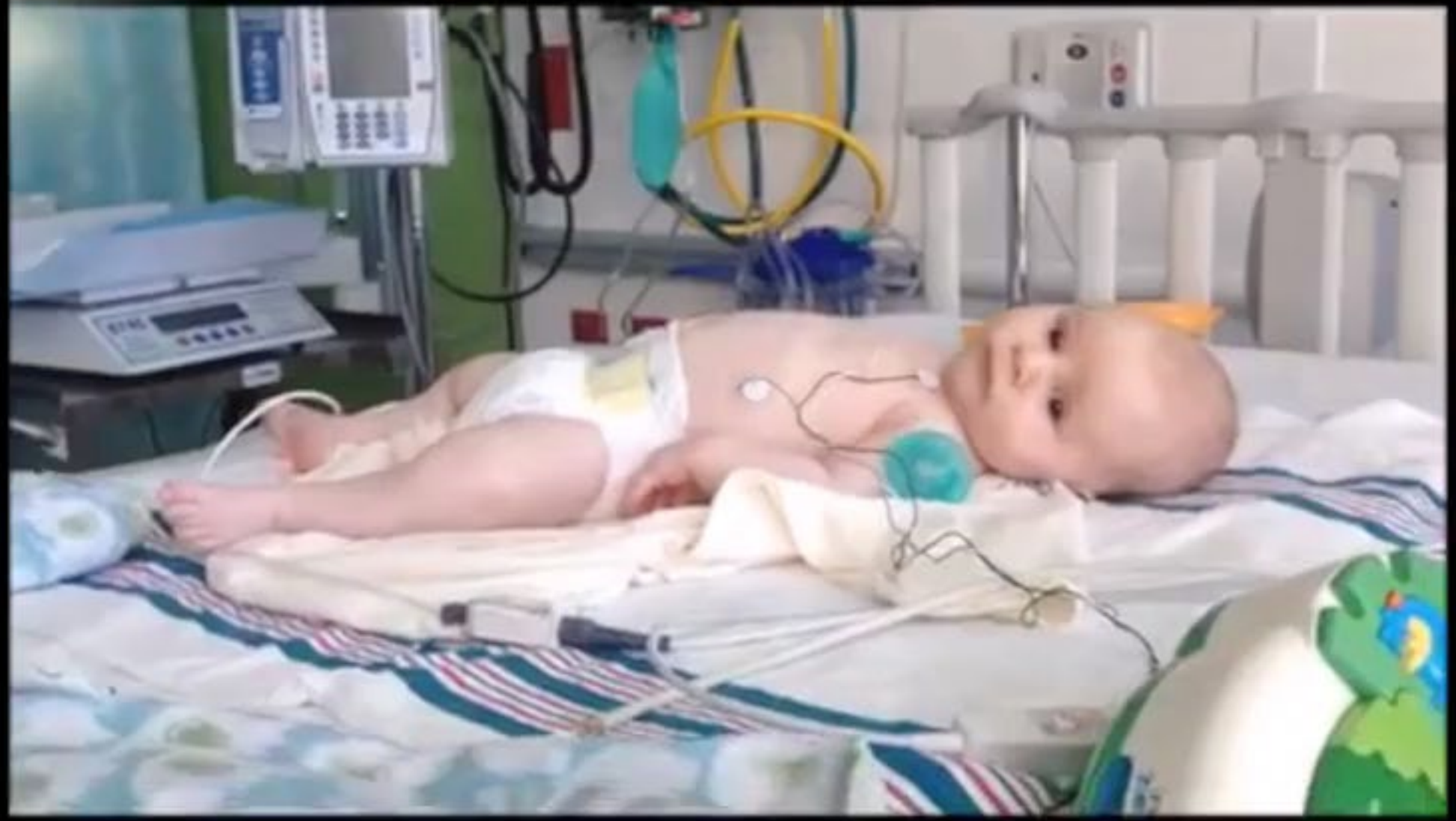
www.mdguidelines.com/lumbar-puncture

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



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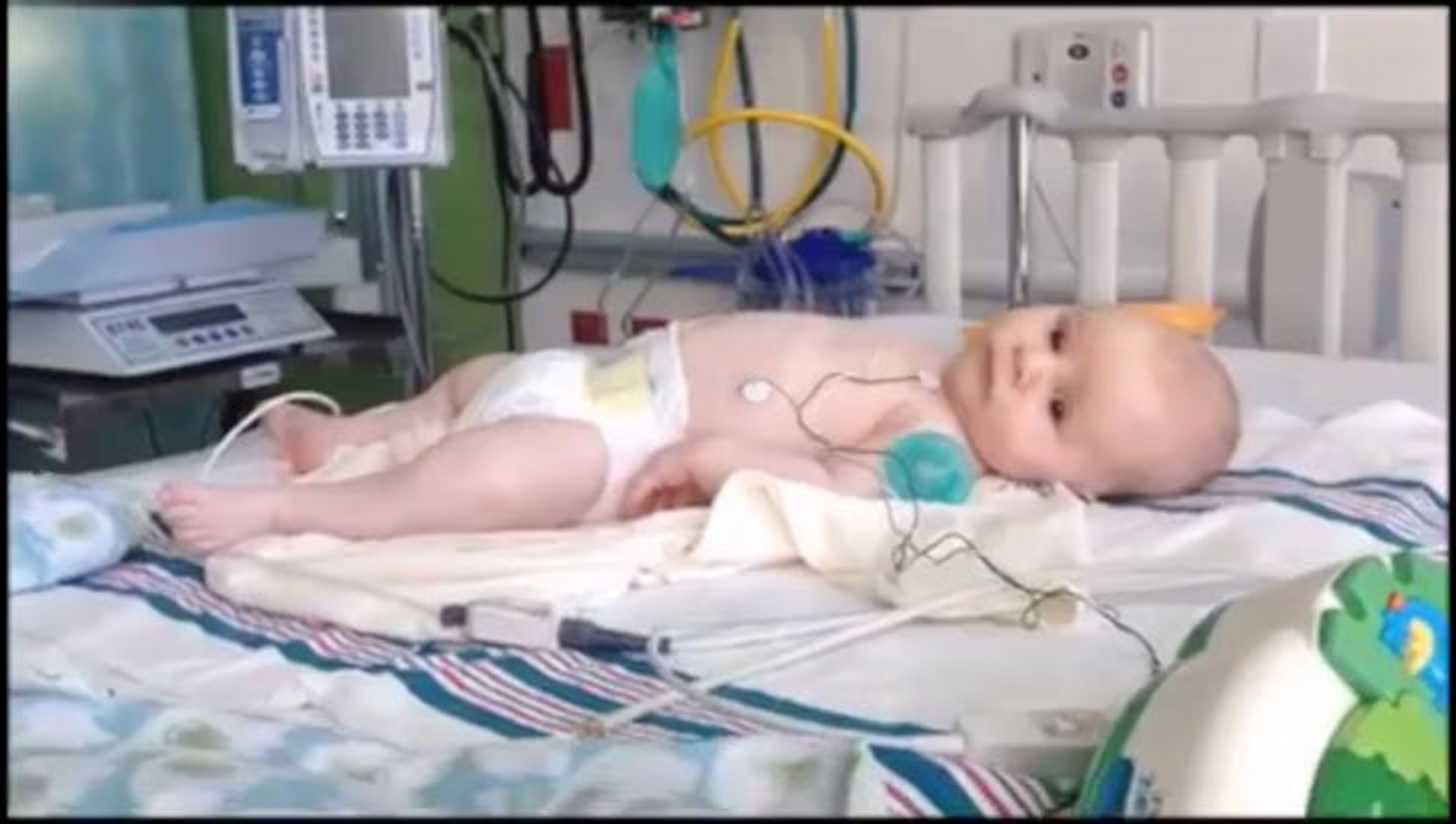


a baby, Cameron Harding, nearly 3, receives a drug that keeps his motor neur

December 2016

December 2018

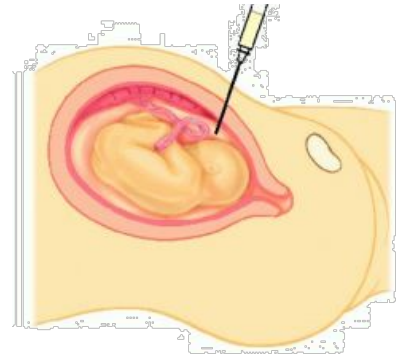
Nusinersen firsts

- 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



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.)

Acknowledgements

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CSH

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Tim Vickers
Brenda Baker

Biogen

Patients and families who participated in the nusinersen clinical trials

Clinical teams that conducted the clinical trials

The RNA and SMA scientific communities

MDA Fighting Muscle Disease

cure SMA

ST. GILES FOUNDATION

National Institute of General Medical Sciences

SMA FOUNDATION

fightsma

National Institute of Neurological Disorders and Stroke