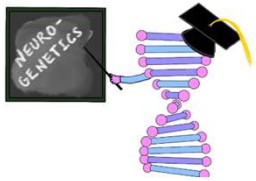
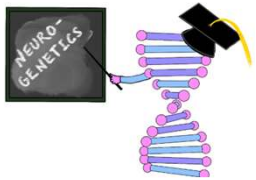
Therapies for Neurogenetic Diseases, 3 (ASOs and Virally-Delivered Therapies)

MODULE 14



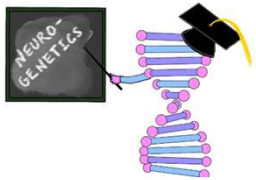
Learning Objectives

- List 3 criteria for inclusion on newborn screening testing
- Describe the genetic mechanisms of SMA and the mechanism of nusinersen
- Compare and contrast different therapeutic modalities:
  - ASO-based
  - CRISPR-based
  - gene replacement
  - protein replacement
- Identify barriers toward equitable delivery of gene targeted therapies

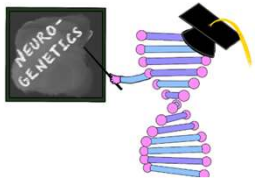


Chief Complaint

- 4-week-old girl who had a positive newborn screening for spinal muscular atrophy

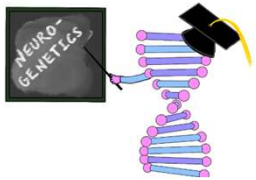


What are Your Next Steps in Management?



Next Steps in Management

- Family history (negative)
- Physical examination (mild neck weakness when prone)
- Confirmatory genetic testing
  - Confirmation of biallelic SMN1 pathogenic variants
  - SMN2 gene copy number
- Discussion about treatment options
- Baseline blood tests

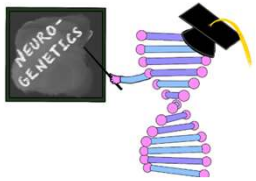


Genetic Testing Results

One homozygous Pathogenic variant identified in SMN1. SMN1 is associated with autosomal recessive spinal muscular atrophy.

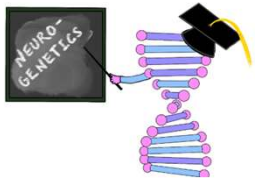
SMN2 copy number = 2.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
SMN1	Deletion (Entire coding sequence)	homozygous	PATHOGENIC



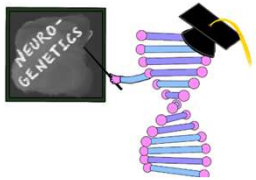
Interactive Breakout Groups

- Group A: What does the SMN1 gene encode? Where is this gene expressed?
- Group B: What is most common genetic cause of SMA? What is the mode of inheritance?
- Group C: What are the clinical subtypes of SMA? How do these correlate with SMN2 copy number?
- Group D: What's the difference between SMN1 and SMN2 genes?



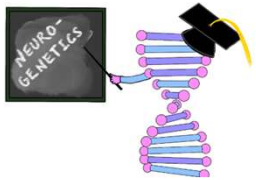
Spinal Muscular Atrophy, Type 1

- Hypotonia
- Areflexia/hyporeflexia
- Tongue fasciculations
- Hand tremor
- Proximal > distal muscle weakness
- History of motor difficulties, especially with loss of skills
- Recurrent respiratory infections
- Motor unit disease on EMG

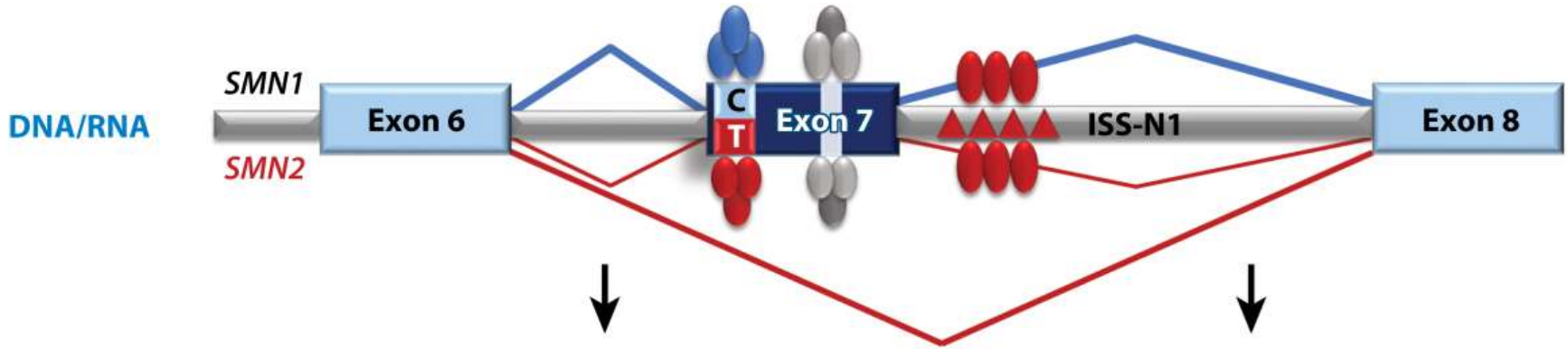


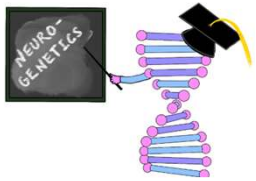
Survival Motor Neuron 1

- SMN1 and SMN2 genes are on chromosome 5q13
- The survival motor neuron (SMN) protein is expressed mostly in lower motor neurons
- Biallelic loss of function in SMN1 (deletion in 95%) results in SMA
- Autosomal recessive inheritance in 98%
  - 2% have de novo variant on 1 allele and inherited variant on other allele
- SMN2 is a like a “backup” copy of SMN1 but differs in 1 base at the start of exon 7, resulting in alternative splicing
- SMN2 copy number can modify phenotype



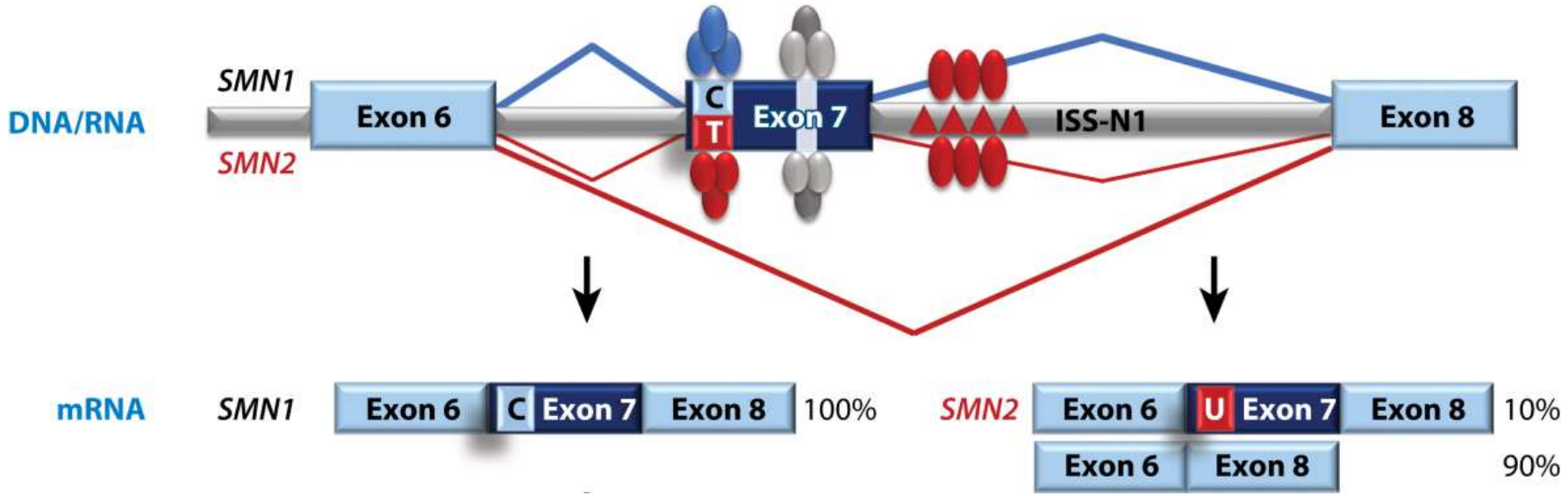
SMN1 and SMN2 Genes Differ by One Base

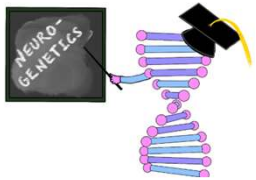




Wirth et al., 2020, Annu. Rev. Genom. Hum. Genet. 21:231-61

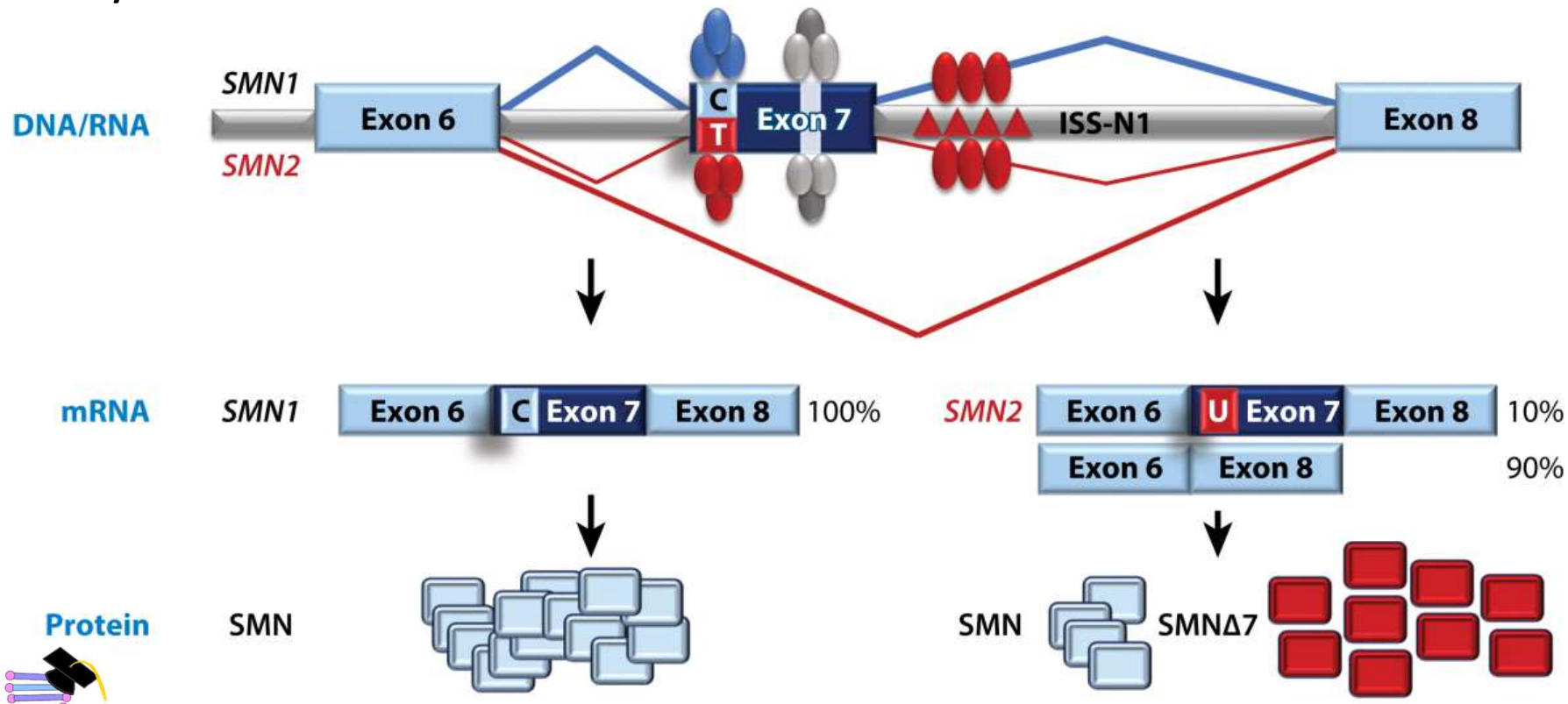
SMN2 Mostly Makes a Transcript Without Exon 7

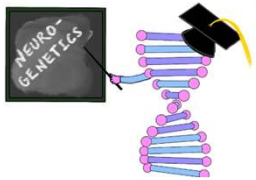




Wirth et al., 2020, Annu. Rev. Genom. Hum. Genet. 21:231-61

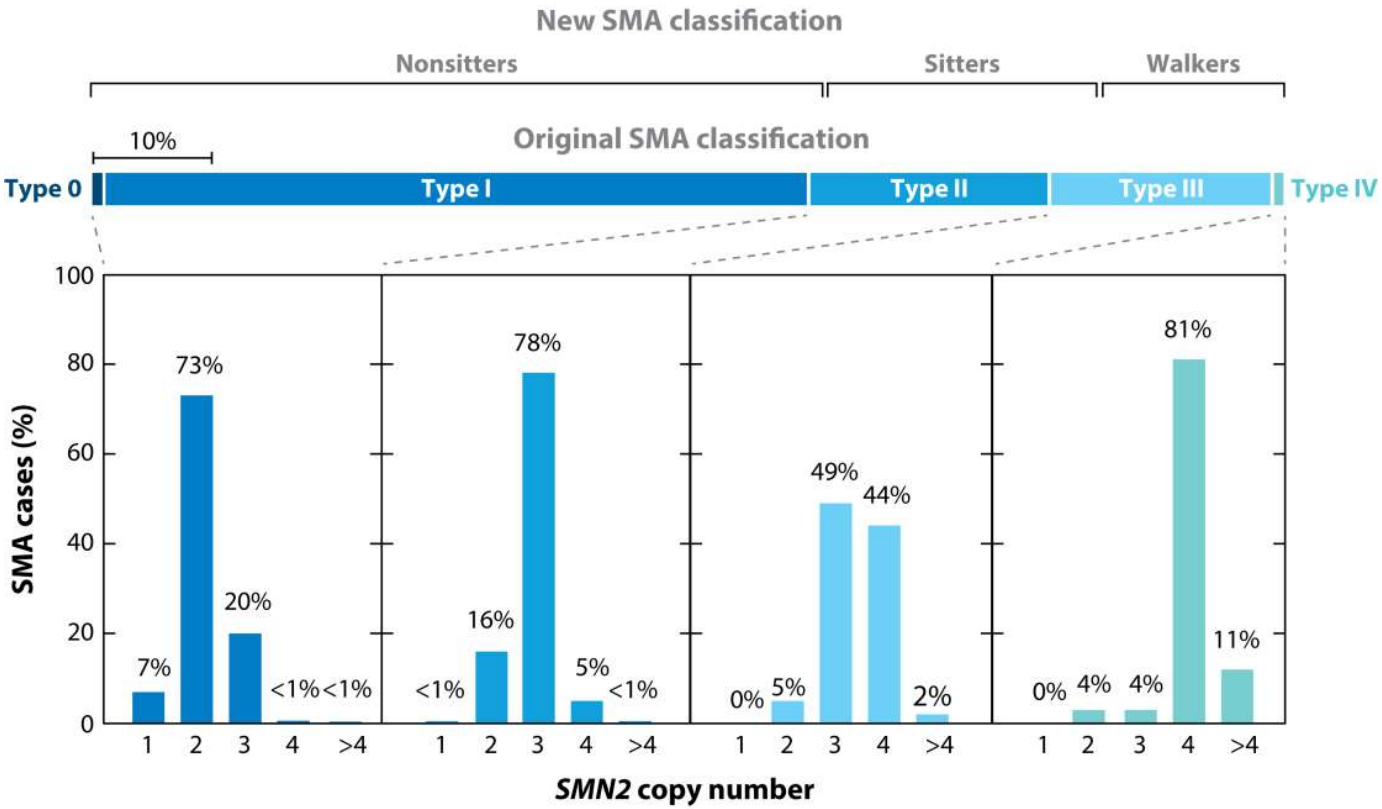
SMN Protein Lacking Exon 7 is Not Fully Functional

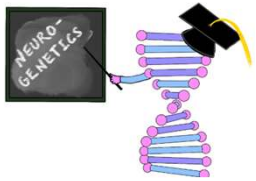




Wirth et al., 2020, Annu. Rev. Genom. Hum. Genet. 21:231-61

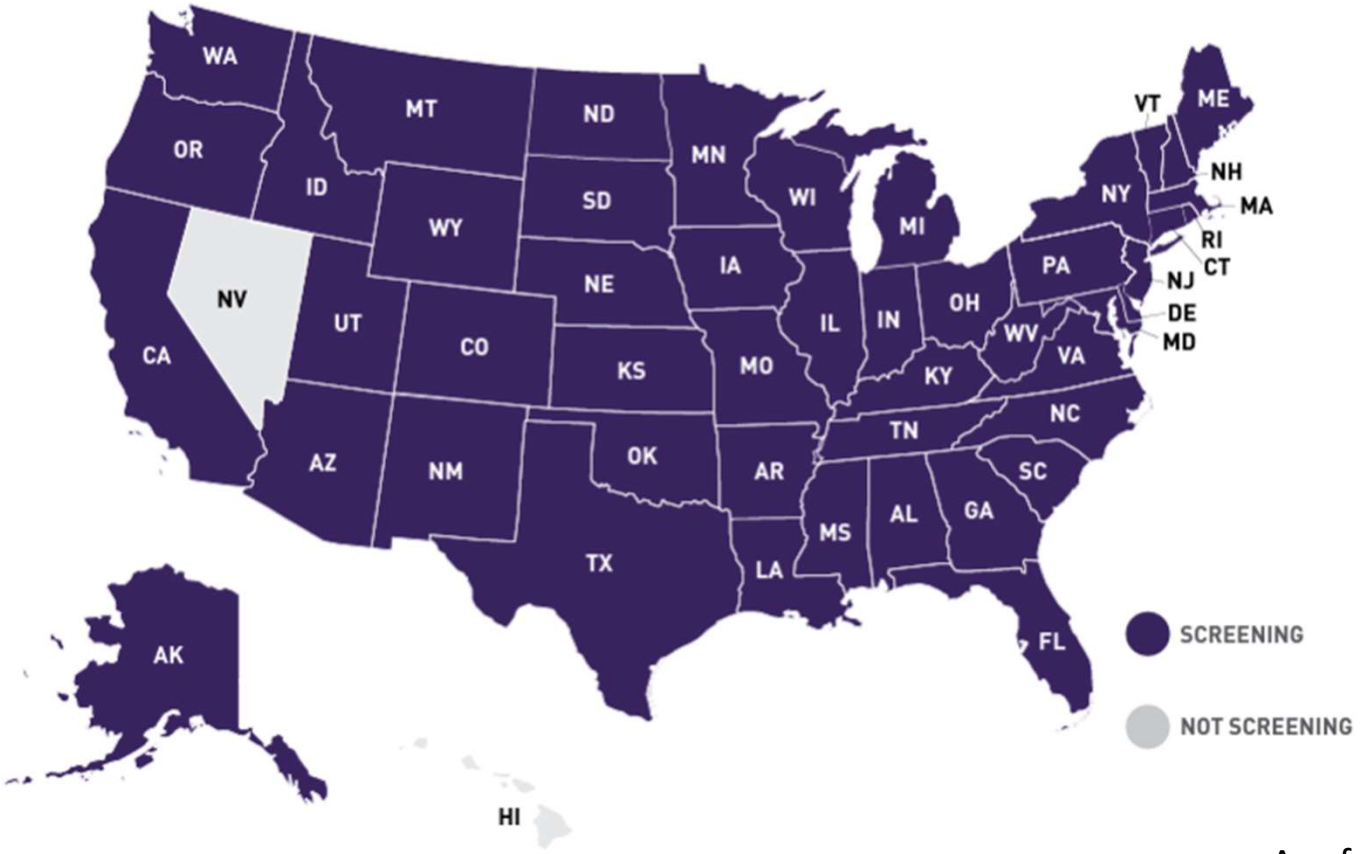
SMN2 Copy Number Correlates with SMA Subtype

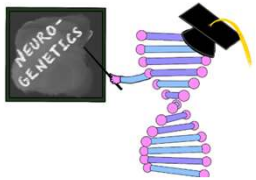




Wirth et al., 2020, Annu. Rev. Genom. Hum. Genet. 21:231-61

Newborn Screening for SMA in 48/50 states

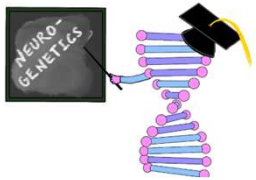




As of 2/22/23, curesma.org

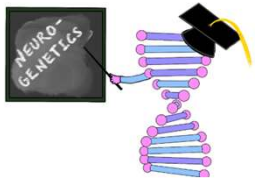
Genetic Testing for SMA

- First tier NBS for SMA is qPCR to look for absence of SMN1 exon 7 (most states)
- 5% of SMA cases are from point mutations in SMN1 and would be missed by NBS.
  - These cases would be clinically detected and then sequencing of SMN1 gene could be diagnostic
- Confirmatory testing is typically by multiplex ligation-dependent probe amplification (MLPA), which can reveal SMN1 and SMN2 copy number



Classic Newborn Screening Criteria

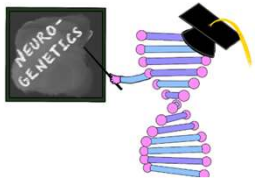
1. Important health problem.
2. Accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. Recognizable latent or early symptomatic stage.
5. Suitable test or examination.
6. Defined natural history
7. Cost effectiveness



Wilson JMG, Jungner G. *Principles and practice of screening for disease* Geneva: WHO; 1968.

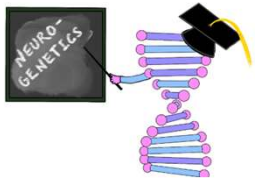
Therapies for SMA

- Onasemnogene abeparvovec
  - AAV9-based gene replacement therapy
- Nusinersen
  - Anti-sense oligonucleotide to increase exon 7 inclusion in SMN2 mRNA
- Risdiplam
  - Small molecule to increase exon 7 inclusion in SMN2 mRNA



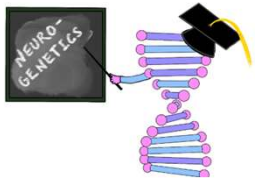
Onasemnogene Abeparvovec (OA)

- Adeno-associated virus vector-based gene therapy that expresses SMN1 gene
- Single intravenous infusion
- Warnings/precautions: Liver failure, systemic immune response, thrombocytopenia, thrombotic microangiopathy, elevated troponin-I
- Monitoring: Baseline anti-AAV9 antibody testing, liver function, platelet count, troponin-I

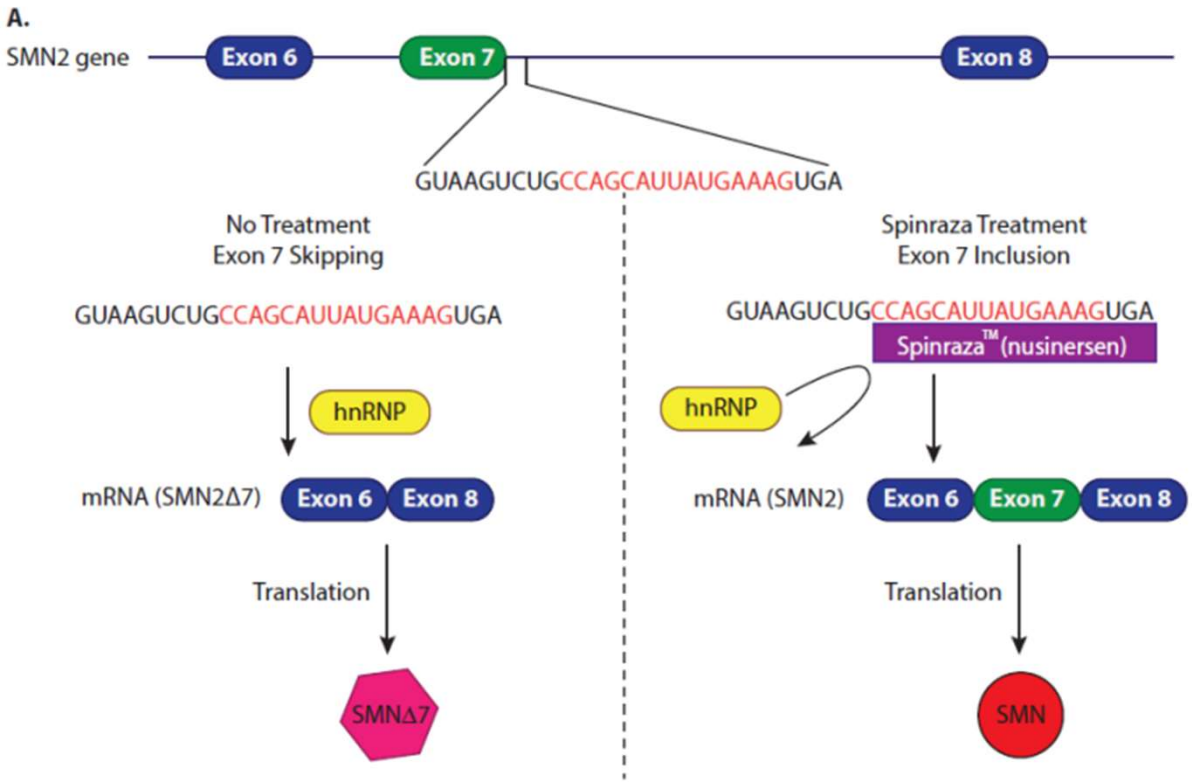


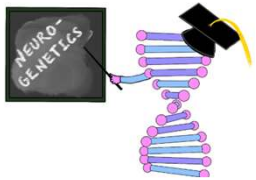
Nusinersen

- Intrathecal ASO, with 4 loading doses, spaced by 14 days for first 3 doses then 30 days between 3rd and 4th doses
- Then q4 month dosing
- Warnings/precautions: thrombocytopenia, renal toxicity
- Monitoring: platelets, coags, quantitative spot urine protein



Nusinersen Regulates Splicing of SMN2 to Increase Inclusion of Exon 7

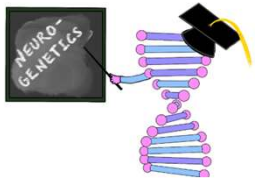




Bajan and Hutvagner, Cells 2020, 9, 137

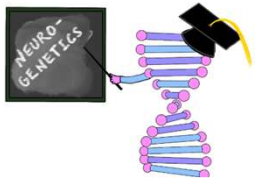
Risdiplam

- Daily oral medication that modifies SMN2 splicing (similar to nusinersen)



Efficacy of SMA treatments

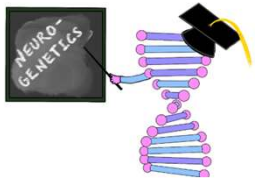
- No studies compared effectiveness of 3 treatments
- Each study used natural history data as control
- For presymptomatic infants that were treated:
    - With nusinersen, 22/25 (88%) were walking independently, median f/u of 2.9 years[1]
    - With OA, 10/14 (71%) were walking independently between 8.2-17.6 months[2]
    - With risdiplam, 3/6 (50%) were walking independently after 12 months of treatment[3]
- Separate trials for early-onset, late-onset, and previously-treated SMA



(1) NURTURE study (2) SPR1NT study (3) RAINBOWFISH study

Viral Vectors and Gene Replacement Therapy

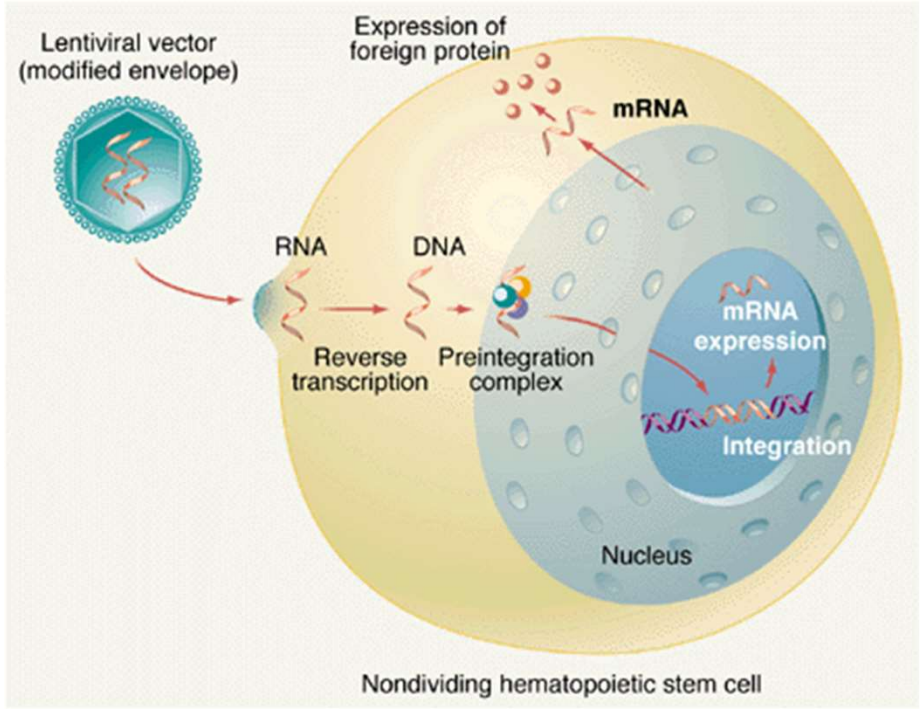
- Viruses can be packaged with genetic material to deliver genes into cells
- Viral vectors were first used in 1990 for SCID-X1
- Adenovirus vector-mediated therapy of OTC deficiency (2003)
  - Vector-associated toxicity, multi-organ failure, and death in an 18yo man
- Large number of adeno-associated viruses (AAV) discovered ~ 2005
- AAV ≠ Adenovirus

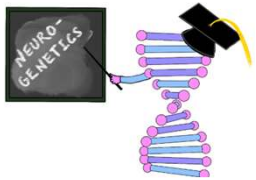


Jensen TL, et al., Front. Mol. Neurosci. 2021

Lentivirus

- Genomic integration – transduces postmitotic cells
- Low immunogenic potential
- Can carry relatively large transgenes
- Preferred tool for *ex vivo* gene therapy strategies
- Self-inactivating and replication-incompetent
- Lenti-viral based gene (elivaldogene autotemcel) therapy is now approved for X-ALD





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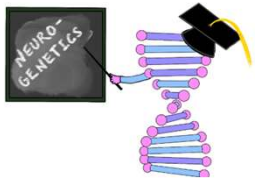


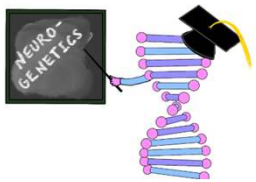
AAV

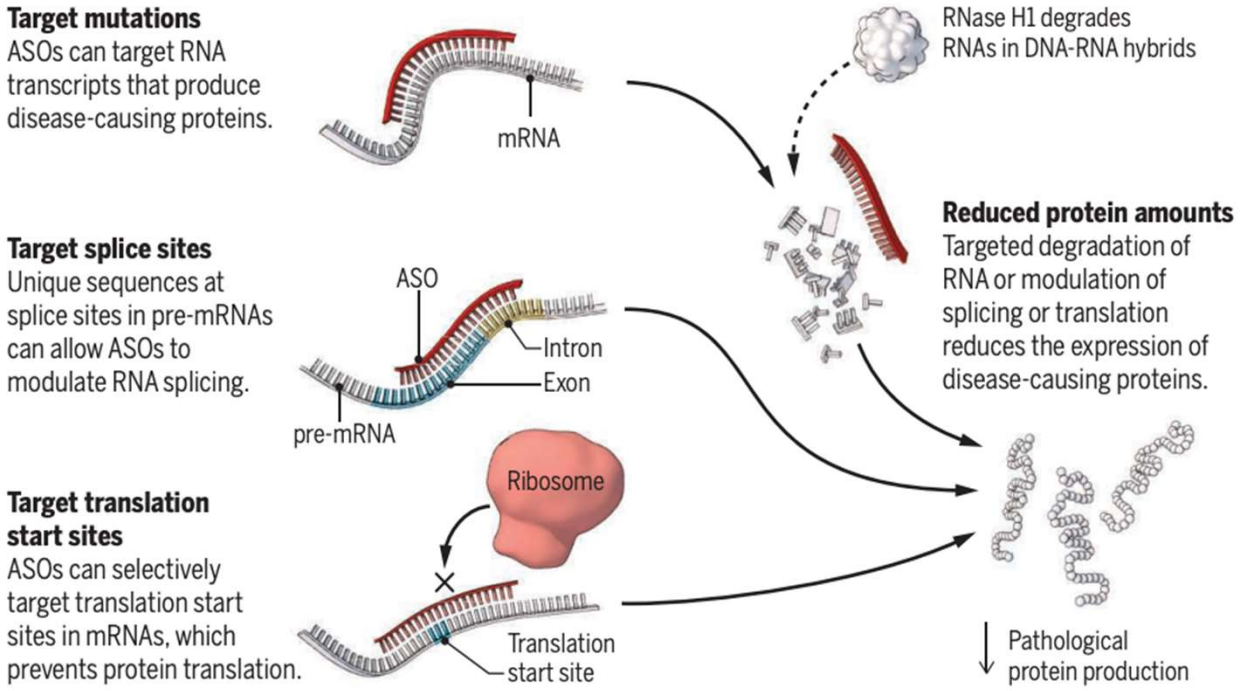
- Used in ~70% of gene therapies to date
- Pros:
    - Long term gene transduction (10+ years in human, 15+ in non-human primates)
    - Target broad distribution or specific cells
    - Some can cross the BBB
    - Lots of serotypes
- Cons:
    - Small cargo capacity
    - Liver/DRG toxicity at high dose
    - Immune response – though least immunogenic of viral vectors
    - Overexpression?

Jensen TL, et al., Front. Mol. Neurosci. 2021

Antisense Oligonucleotides - RNA Modulation

- Short (15-25 letter) snippets of synthetic DNA/RNA
- Chemically modified for stability and tight binding
- Designed to bind to specific genetic targets to **alter patterns of gene splicing or target RNA for destruction**

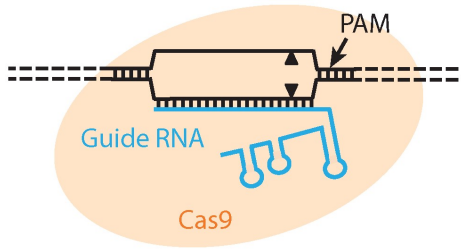


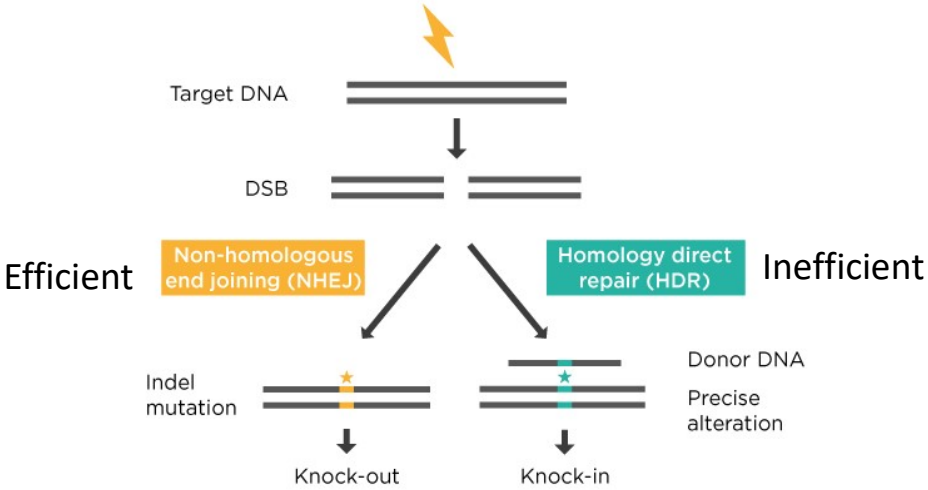


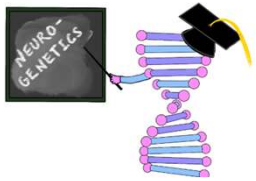
Leavitt and Tabrizi, Science, 2020

Gene Editing (e.g. CRISPR/Cas9)

- Cutting DNA is easy
  - Limited by recognition sequence
- Precisely fixing DSBs is hard
  - Base and prime editing may be a solution
- Delivering CRISPR/Cas9 is challenging
- Technology is rapidly advancing to overcome challenges



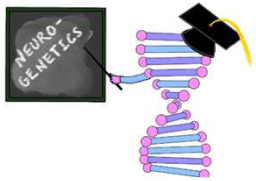




Carrol, Annu. Rev. Biochem. 2014, 83:409-39

Comparison of Therapeutic Approaches

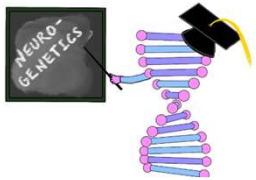
Therapeutic approach	Gene replacement	Gene editing	ASOs	Protein replacement
Variant-specific		✓	✓	
Gene-specific	✓	✓	✓	✓
Advantages				
One-time dose	✓	✓		
Effective for truncated/deleted genes		✓	✓	
Uses natural cell regulation	✓		✓	
Disadvantages				
Recurrent dosing			✓	✓
Artificial regulation		✓	✓	✓
Vector-based limitations	✓	✓		
Off-target effects	✓		✓	
High manufacturing cost	✓	✓		✓



Carvill GL, et al. NeuroTherapeutics 2021

ASO vs. Gene Therapy

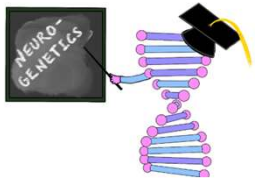
- ASO
  - Transient
  - Can't cross BBB
  - May be more rapidly developed for N-of-1 trials
  - Good safety profile
  - $$$$



- Gene therapy
  - Transgene expression 10+ years
  - Many vectors cross BBB
    - Local delivery may lower toxicity
  - Immunogenicity concerns
  - In vivo and ex vivo modification potential
  - $$$$$$$$$$$$$$$$$$$$$$$

Challenges to the Equitable Delivery of Gene-Targeted Therapies

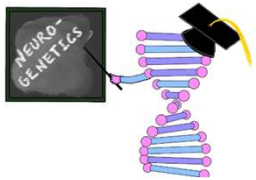
- Limited access to diagnosis
  - May be addressed with newborn screening
- Underrepresented communities in biomedical research, specifically genomic science
- High therapy cost may exacerbate existing inequities in health care system
- Not all rare diseases are proportionally represented
- Need for representation of diverse set of stakeholders in research and development (public, private entities, disease advocates, community groups)



Gaviglio et al., American Journal of Human Genetics, 2023, 193C:56-63

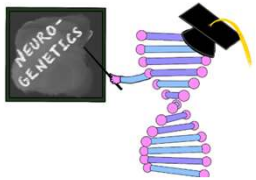
Back to Our Case (SMA with 2 copies SMN2)…

- AAV9 titers negative. Baseline labs normal.
- Patient got onasemnogene abeparvovec at 6 weeks of age
- At 2 years of age:
  - Full head control, sits independently, tall kneeling, and knee walking.
  - Uses straw cup, utensils, and has no problems with chewing/swallowing.
  - Tells her sister to “take deep breaths” when her sister is upset.



Suggested Reading

- GeneReviews: Spinal Muscular Atrophy
- Erdos, J., Wild, C., 2022. Mid- and long-term (at least 12 months) follow-up of patients with spinal muscular atrophy (SMA) treated with nusinersen, onasemnogene abeparvovec, risdiplam or combination therapies: A systematic review of real-world study data. European *Journal of Paediatric Neurology* 39, 1–10.
- Jensen, T.L., Gøtzsche, C.R., Woldbye, D.P.D., 2021. Current and Future Prospects for Gene Therapy for Rare Genetic Diseases Affecting the Brain and Spinal Cord. *Frontiers in Molecular Neuroscience* 14.
- Carvill, G.L., Matheny, T., Hesselberth, J., Demarest, S., 2021. Haploinsufficiency, Dominant Negative, and Gain-of-Function Mechanisms in Epilepsy: Matching Therapeutic Approach to the Pathophysiology. *Neurotherapeutics*.
- Shellhaas et al., 2021. Gene-Targeted Therapies in Pediatric Neurology: Challenges and Opportunities in Diagnosis and Delivery. *Pediatric Neurology* 125, 53–57.



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