New Biologics for Neuromuscular Disorders

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Financial disclosures

- Basil T. Darras, MD,(BTD) has served as an ad hoc scientific advisory board member for AveXis, Biogen, Cytokinetics, Vertex, Genentech, Roche, and Sarepta; Steering Committee Chair for Roche and DSMB member for Amicus Inc.; he has no financial interests in these companies.
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- Dr. Darras has also received royalties for books and online publications from Elsevier and UpToDate, Inc.

Spinal muscular atrophy (SMA) is…

A generic term

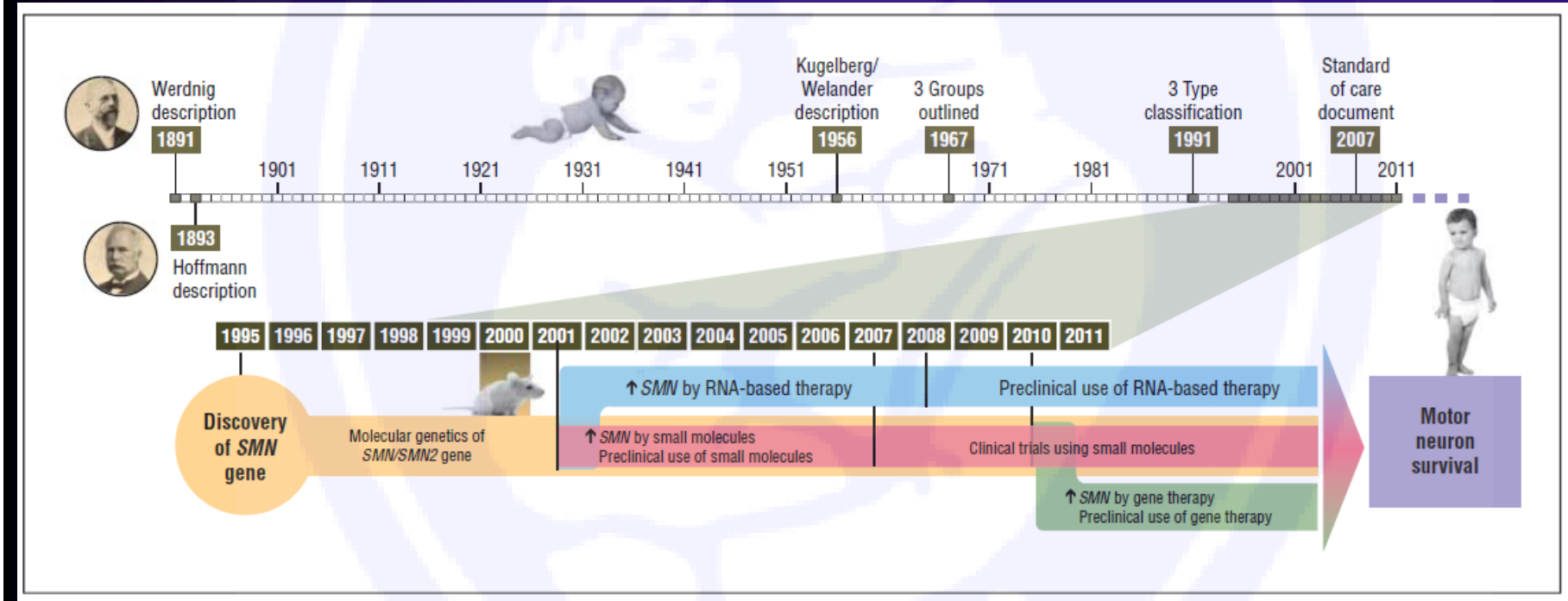
SMAs are genetic disorders, characterized by degeneration and loss of motor neurons in the anterior horns of the spinal cord and brain stem, leading to symmetrical muscular atrophy and weakness

Chromosome 5q SMA and "non-5q" SMAs

5q Proximal SMA is…

- An autosomal recessive disorder caused by loss or mutation of the *SMN1* gene and retention of the *SMN2* gene.
- *SMN1* and *SMN2* genes encode the "survival (of) motor neuron (SMN)" protein
- SMA is caused by *decreased levels rather than complete loss of the SMN protein*, leading to selective dysfunction of motor neurons in the spinal cord and brain stem

Spinal Muscular Atrophy Timeline: Description to Classification (1891-1991): 100 years



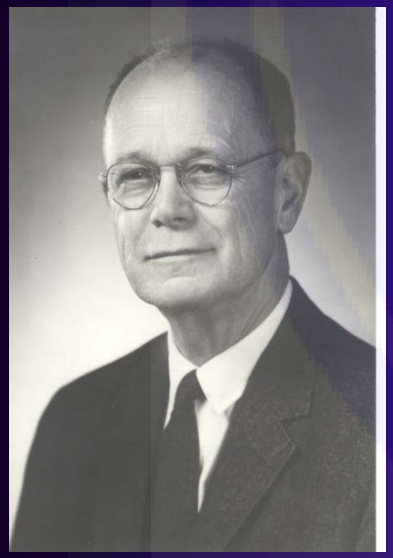
Kolb SJ, Kissel JT. Arch Neurol 2011 Aug;68:979.

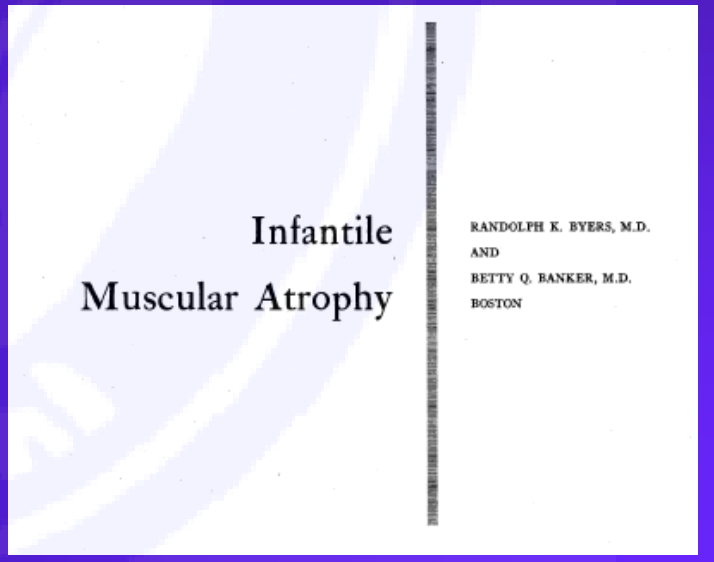
Classification of SMA (1961)

- *Arch Neurol. 1961 Aug: 5:140-164*

Byers RK, Banker BQ

Boston Children's Hospital





Classification of SMA (Munsat et al. ENMC, 1991)

Type	Age at Onset*	Life Span
Type 0	Prenatal	<6 months (1999)
Type I	0 – 6 months	<2 years
Type II	6-18 months	~70% alive at 25 years
Type III		Almost normal
IIIa	<3 years	
IIIb	>3 years	
Type IV	>21 years	Normal

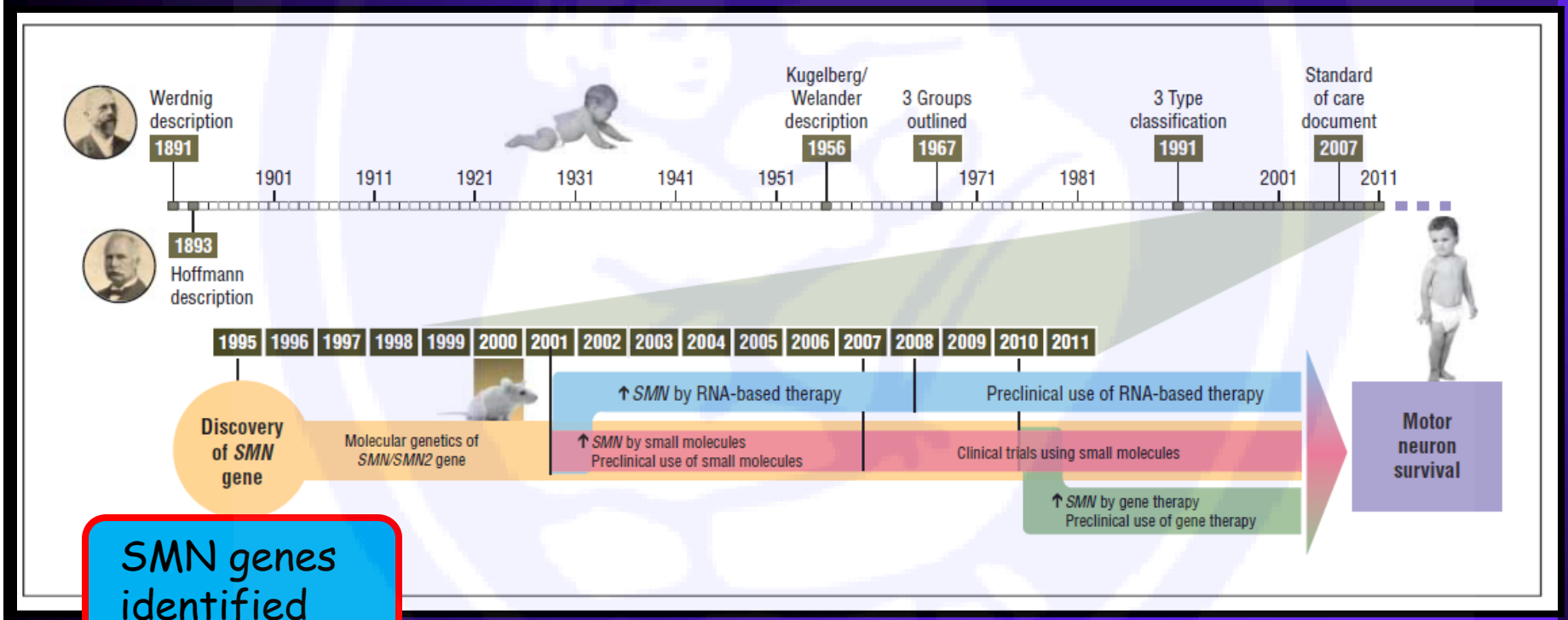
* 1/3 can not be classified accurately by age at onset

Classification of SMA: Continuum of severity- Phenotypic spectrum

FUNCTIONAL CLASSIFICATION:

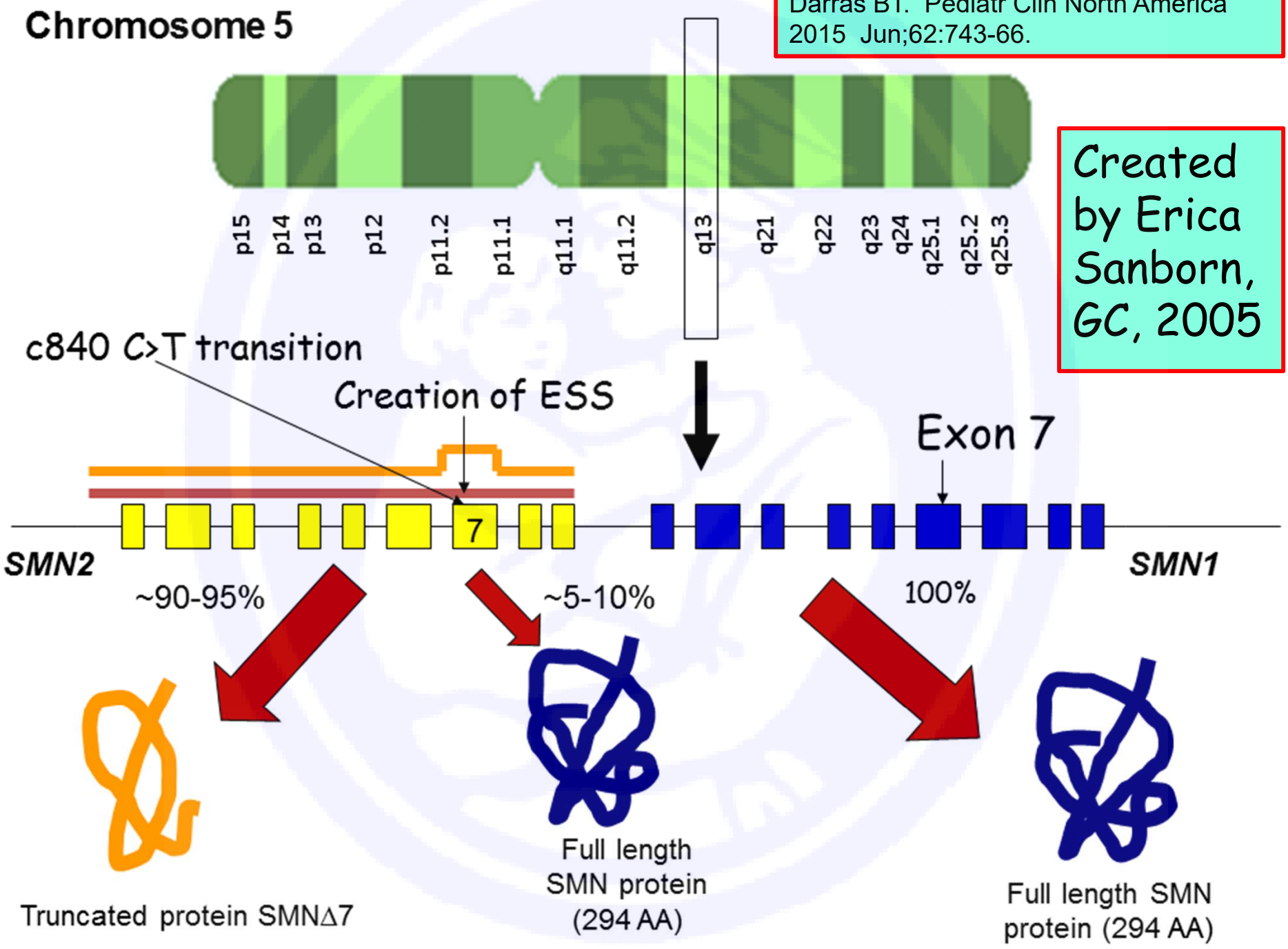
- **SMA, Type I (severe---"non-sitters")**
  - Onset: birth to 6 months
  - Course: never sit unsupported
  - Survival: < 2 years without aggressive treatment
- **SMA, Type II (intermediate---"sitters")**
  - Onset: < 18 months (7-18 months)
  - Course: never stand or walk but sit at some time
  - Survival: 98% to age 5 years, 70% to age 25 years
- **SMA, Type III (mild---"walkers")**
  - Onset: > 18 months (IIIA < 3 years, IIIB > 3 years)
  - Course: able to stand and walk at some time
  - Survival: Almost normal life span

Spinal Muscular Atrophy Timeline



Kolb SJ, Kissel JT. Arch Neurol 2011 Aug;68:979.

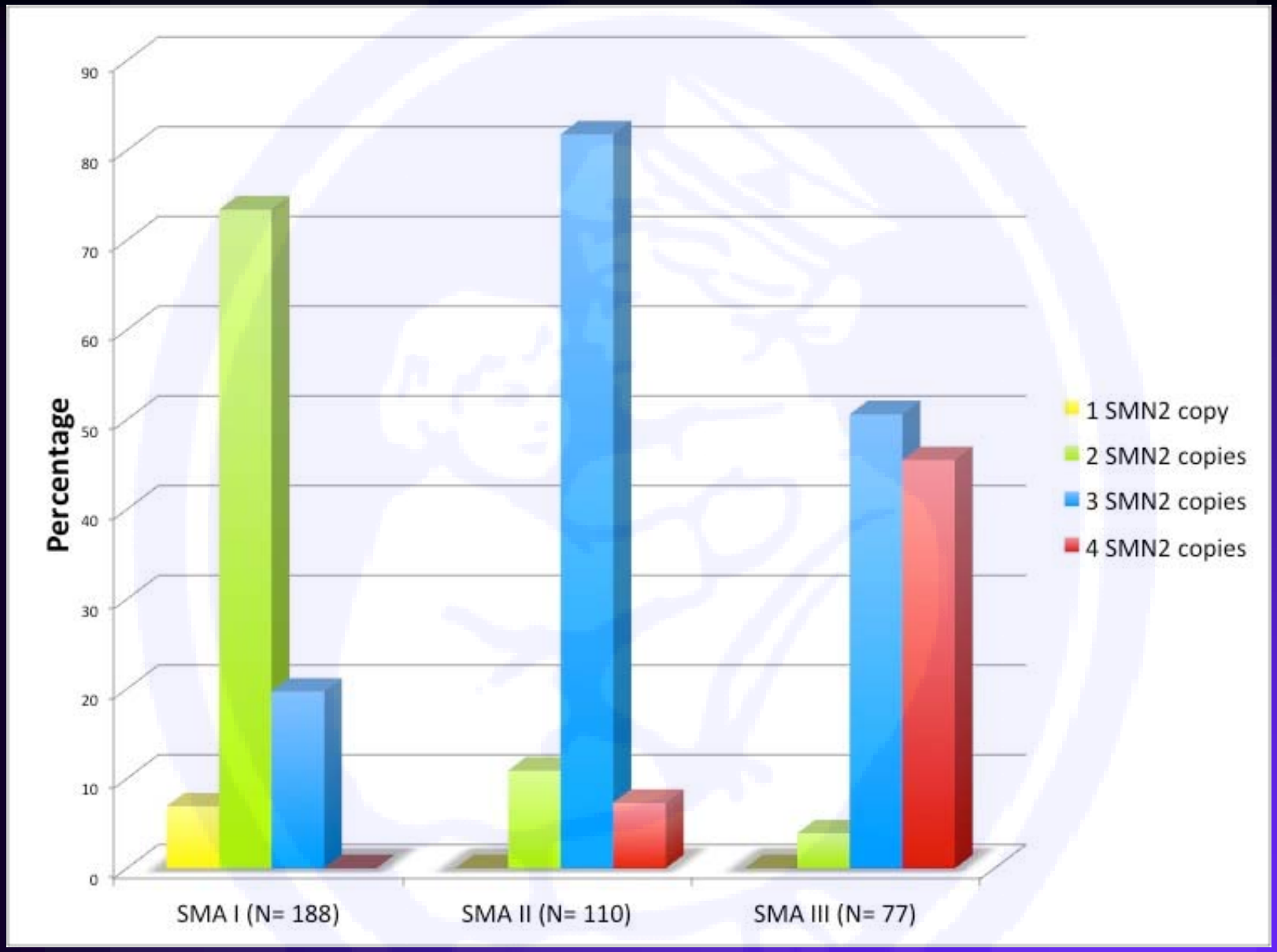
Darras BT. Pediatr Clin North America 2015 Jun;62:743-66.



Created by Erica Sanborn, GC, 2005

SMA Type & *SMN2* Copy Number

- **SMA I** — 80% have 1-2 copies *SMN2*
- **SMA II** — 82% have 3 copies *SMN2*
- **SMA III** — 96% have 3-4 (1% has 5) copies *SMN2*
- **Carrier** — 1 copy *SMN1* / 0-3 copies *SMN2*
- **Normal** — 2-3 copies *SMN1* / 0-3 copies *SMN2*
- **Normal?** — 0 copies *SMN1* / 5 copies *SMN2*



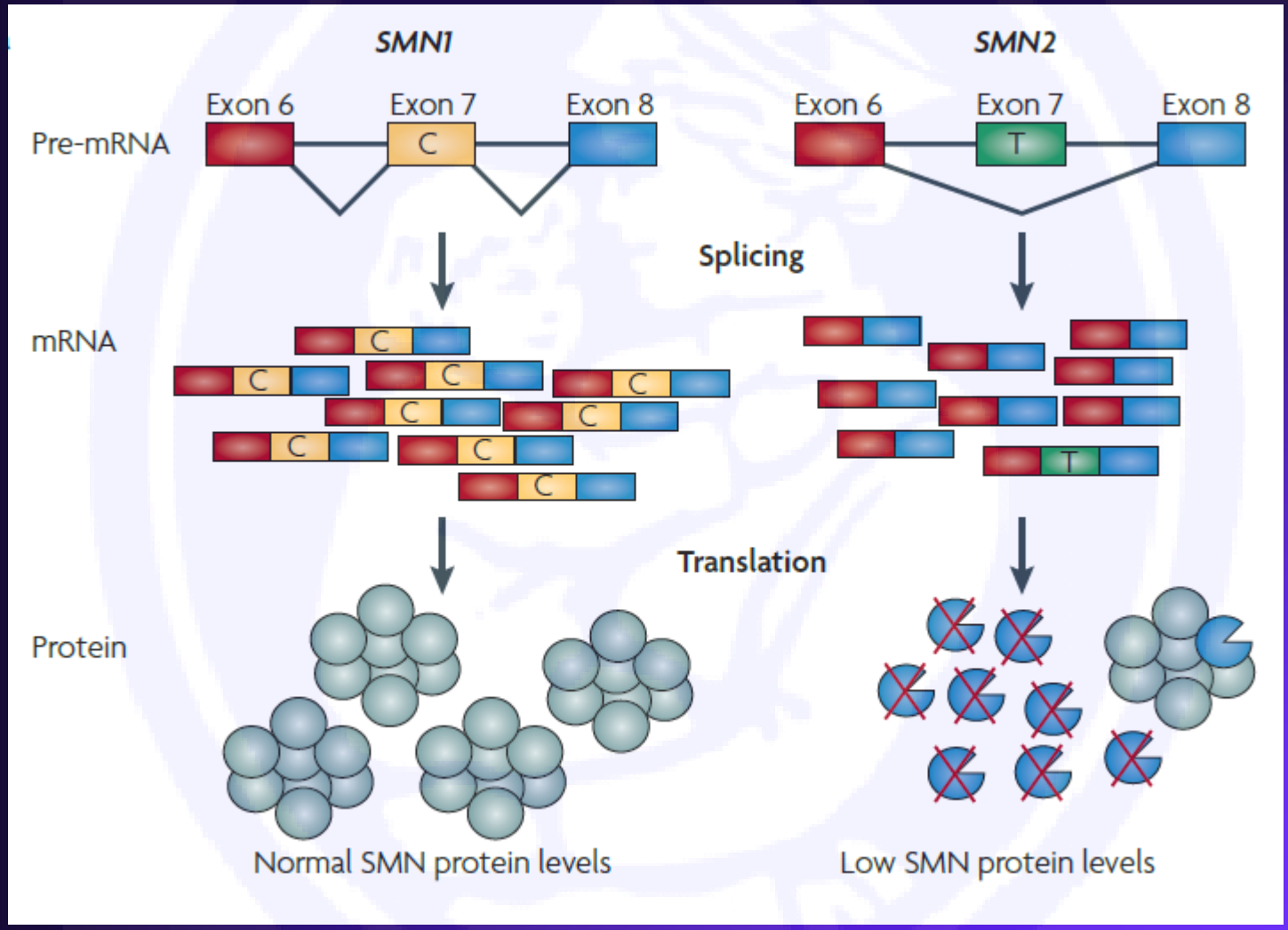
Feldkotter M et al. Am J Hum Genet 2002;70:358-368.

The *SMN2* Gene

An important gene

- A major phenotypic modifier
- It allowed the creation of animal models by introducing a number of *SMN2* copies into mouse SMN knockouts (SMN Δ7 mouse)
  - Severe phenotype (death at 14-17 days)
  - *Smn -/- Smn2 +/+ Smn delta7 +/+*
- Therapeutic target using *SMN2* splicing modulators and upregulators

Hsieh-Li HM et al. Nature Genet 2000



Butchbach MER. Human Gene Therapy 2011;22:121-125.

SMA Therapeutics

- Goal of most drug trials is to increase the full-length SMN protein production from *SMN2* gene
- Unique "translational" disease
  - Genetic defect same in all patients
  - Clear target

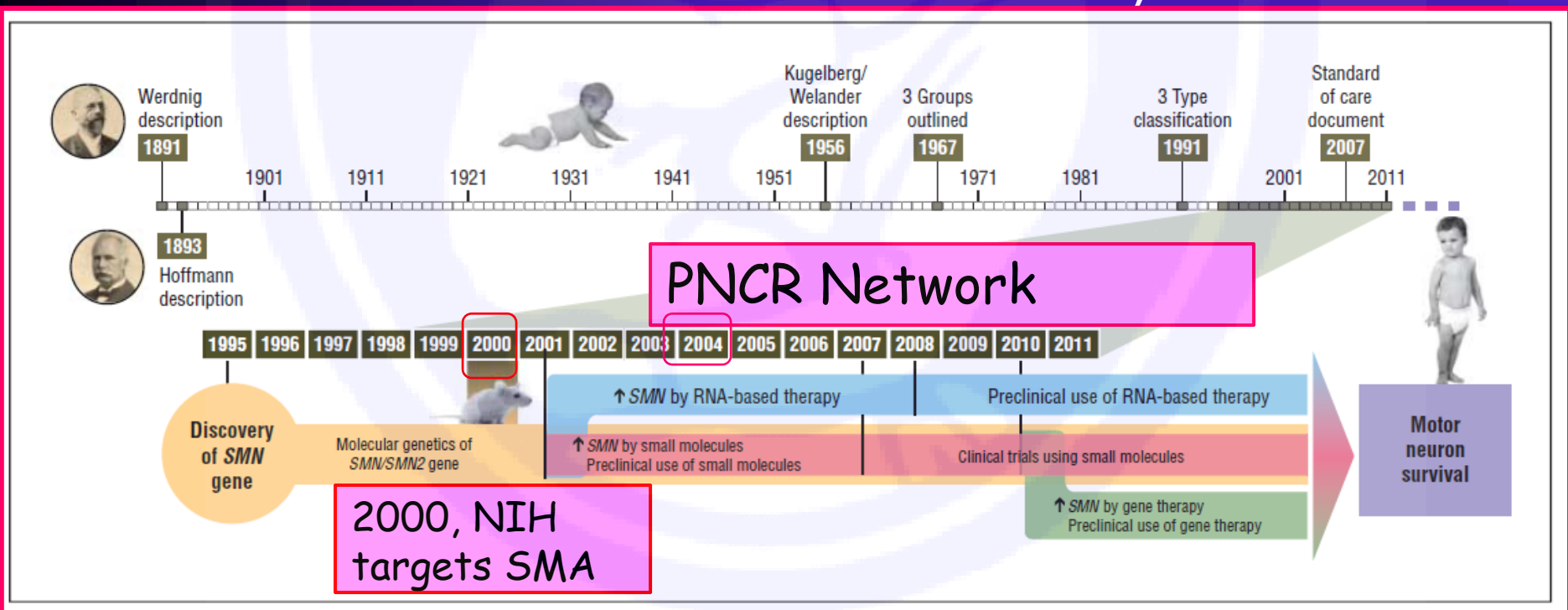
Therapeutic Strategies for SMA

Neuroprotection

- Riluzole
- Gabapentin
- Thyrotropin-Releasing Hormone

Amplification of SMN protein production

- Phenylbutyrate
- Hydroxyurea
- Valproic Acid
- Trichostatin A
- Quinazoline (Repligen RG3039)



Pediatric Neuromuscular Clinical Research (PNCR) Network for SMA: Participating sites

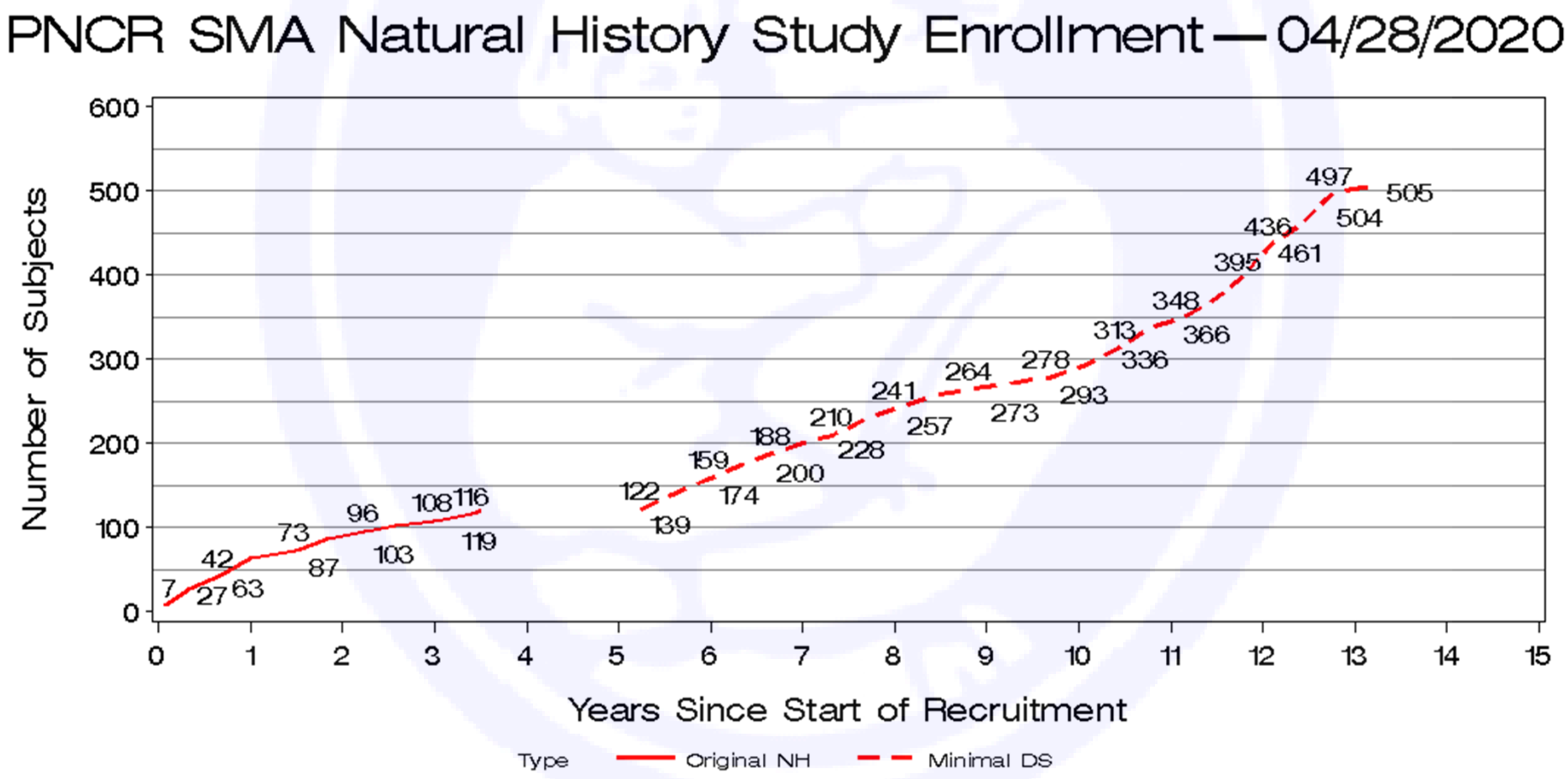
- Columbia U. New York
- Boston Children's
- University Rochester
- CHOP Philadelphia
- Nemours, Orlando
- Stanford, CA

Supported by SMA Foundation, NY

PNCR Network for SMA (2004): Objectives

- To study the natural history of SMA in a genetically and clinically well defined cohort of SMA patients
- To establish network infrastructure
- To collect data needed for trial planning
- To develop and validate outcome measures
- To conduct clinical trials for SMA
- To interact with patient population
- To provide comprehensive care integrated with research

Natural History Study: status 04/2020



SMA Natural History Study

PNCR Network

- 496 subjects enrolled over a 13-year period
  - NH study (4 years, 119 subjects): 3 publications
  - Minimal dataset study (7 years, 377 subjects)
- Developed, validated, tested 3 outcome measures
  - Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders CHOP INTEND (Type I)
  - Hammersmith Functional Motor Scale- Expanded HFMSE (Types 2 and 3)
  - 6- Minute Walk Test 6MWT (Type 3)
- Created an extensive biomaterials repository

HFMSE

ELSEVIER

Neuromuscular Disorders 17 (2007) 693–697

www.elsevier.com/locate/nmd

An expanded version of the Hammersmith Functional Motor Scale for SMA II and III patients

Jessica M. O'Hagen [a,*,1], Allan M. Glanzman [b,1], Michael P. McDermott [d], Patricia A. Ryan [a], Jean Flickinger [b], Janet Quigley [d], Susan Riley [d], Erica Sanborn [d], Carrie Irvine [f], William B. Martens [f], Christine Annis [f], Rabi Tawil [f], Maryam Oskoui [a], Basil T. Darras [d,e], Richard S. Finkel [b,c], Darryl C. De Vivo [a]

PNCR Network for SMA. Natural History Study: Results

- *Archives of Neurology 2011;68(6):779-786 (Types II and III)*
- *Neurology 2012;79:1989-1897 (Types II and III)*
- *Neurology 2014; 83:810-817 (Type I)*

Observational Study of Spinal Muscular Atrophy Type 2 and 3

Arch Neurol. 2011;68(6):779-786.

Functional Outcomes Over 1 Year

Petra Kaufmann, MD, MSc; Michael P. McDermott, PhD; Basil T. Darras, MD; Richard Finkel, MD; Peter Kang, MD; Maryam Oskoui, MD; Andrei Constantinescu, MD, PhD; Douglas Michael Sproule, MD; A. Reghan Foley, MD; Michele Yang, MD; Rabi Tawil, MD; Wendy Chung, MD, PhD; Bill Martens, BA; Jacqueline Montes, PT, MA; Jessica O'Hagen, PT, DPT; Sally Dunaway, PT, DPT; Jean M. Flickinger, PT; Janet Quigley, PT; Susan Riley, PT, MS, DPT; Allan M. Glanzman, PT, DPT; Maryjane Benton, RN; Patricia A. Ryan, OT, MA; Carrie Irvine, BS; Christine L. Annis, BS; Hailly Butler, BS; Jayson Caracciolo, MPH; Megan Montgomery, BS; Jonathan Marra, BS; Benjamin Koo, BS; Darryl C. De Vivo, MD; for the Muscle Study Group; The Pediatric Neuromuscular Clinical Research Network for Spinal Muscular Atrophy

Prospective cohort study of spinal muscular atrophy types 2 and 3

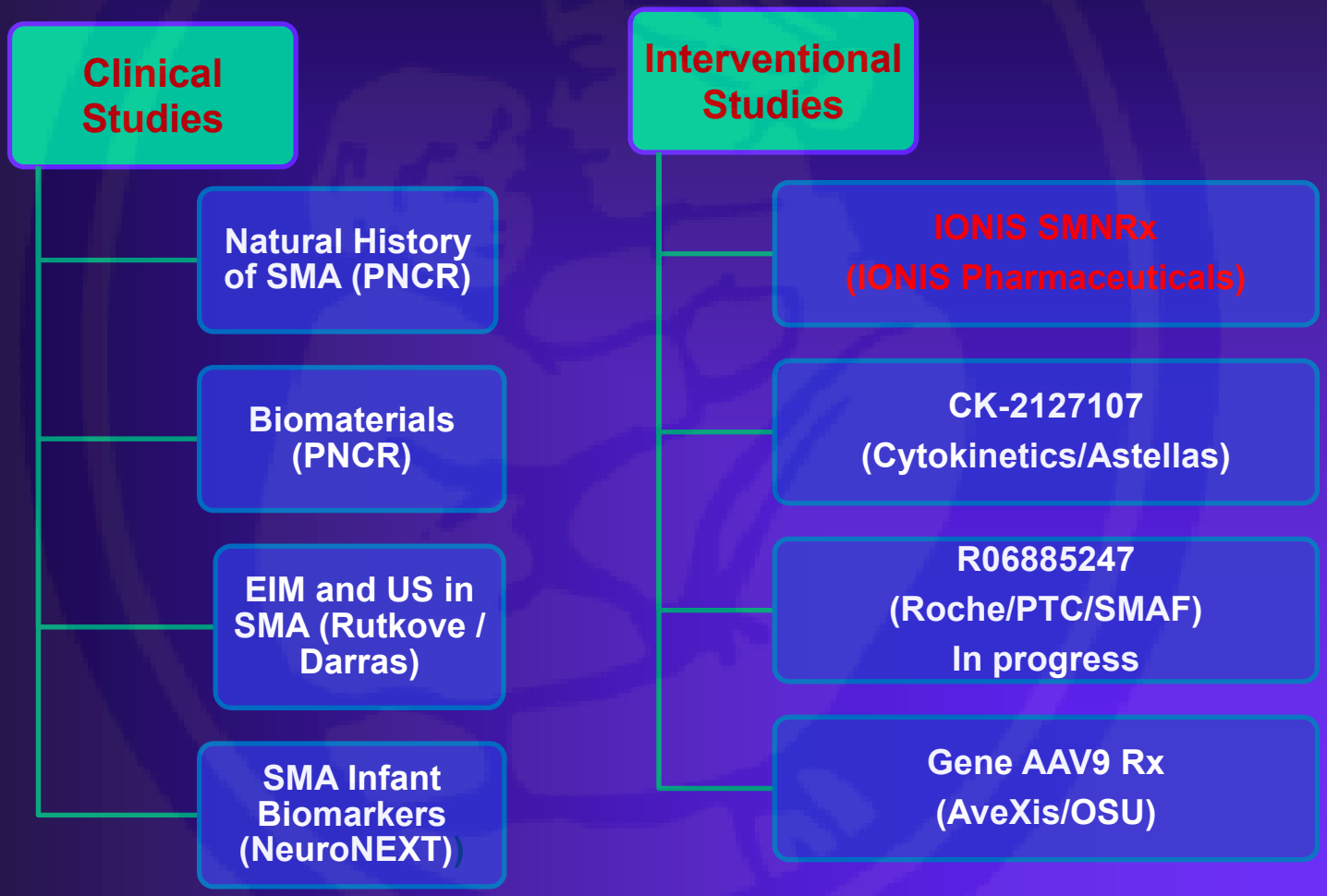
Petra Kaufmann, Michael P. McDermott, Basil T. Darras, et al.

Neurology 2012;79;1889; Published online before print October 17, 2012;

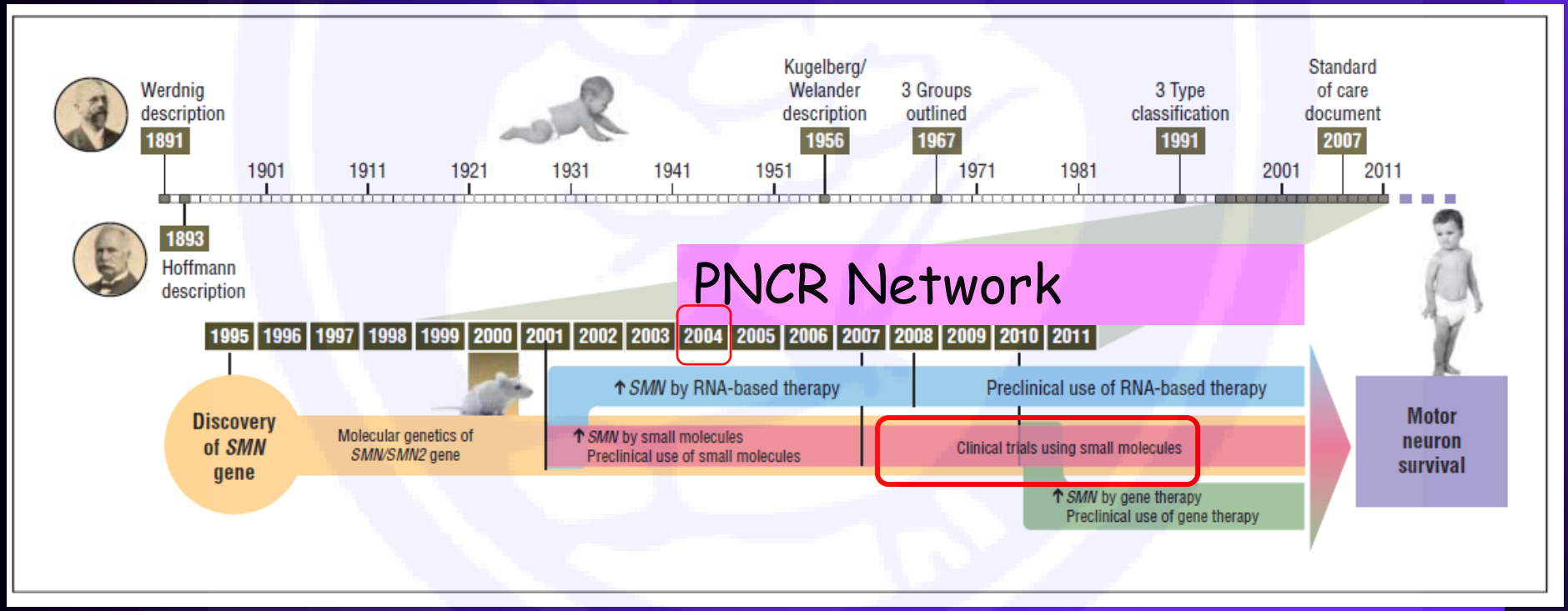
Summary of NHS of SMA Type II and 3 key findings

- Stable course over 12 months in terms of motor and pulmonary function
- Slow decline in motor and pulmonary function in SMA II and III over 48 months
- Patients under the age of 6 years may improve without intervention; hence the need for RDBCTs
- Implications for short clinical trials
  - Treatments need to cause improvement in function rather than slowing/arrest of decline

Spinal Muscular Atrophy (SMA) Studies at Boston Children's Hospital



Spinal Muscular Atrophy Timeline



Kolb SJ, Kissel JT. Arch Neurol 2011 Aug;68:979.

Therapeutic Strategies for SMA

1. Neuroprotection
   - Riluzole-F
   - Gabapentin-F
   - Thyrotropin-Releasing Hormone
   - Olesoxime (TRO19622) "Trophos" compound-F

2. Amplification of SMN protein production
   - Phenylbutyrate-F
   - Hydroxyurea-F
   - Valproic Acid-F
   - Trichostatin A
   - Quinazoline (Repligen RG3039)-T
   - RG7916 (PTC-Roche)

3. Muscle anabolism
   - Albuterol
   - Carnitine
   - Creatine
   - Anti-myostatin

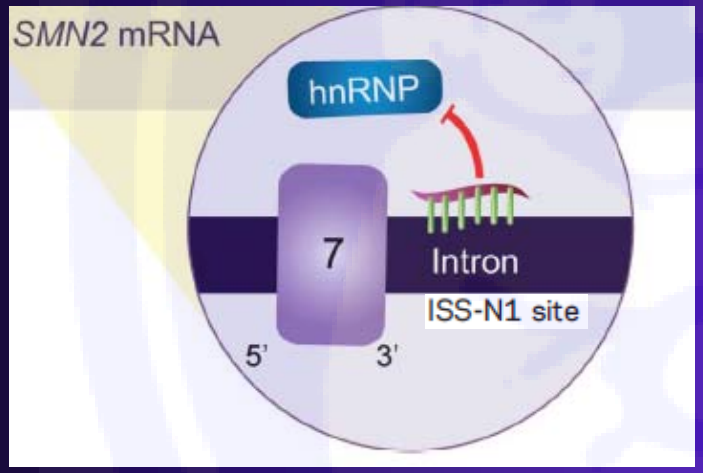
4. Cell therapy (stem cells)

5. Replacement of SMN1 (gene therapy)

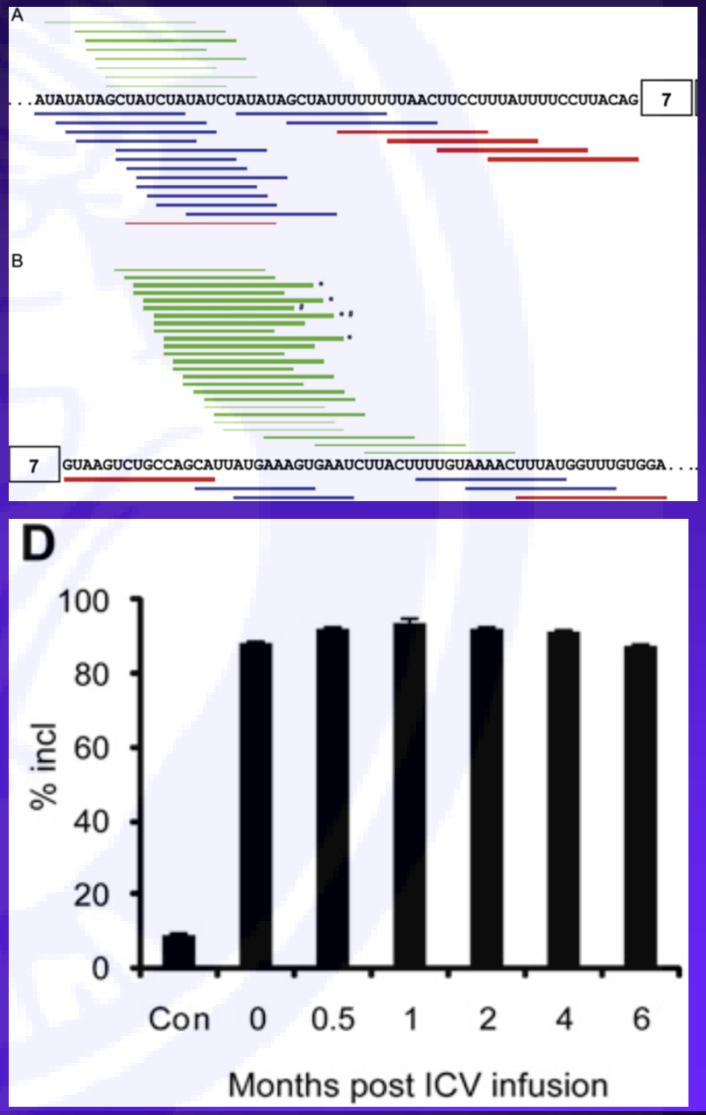
6. SMN2 exon 7 inclusion (antisense oligo-nucleotides, ASOs)

Oligo Rx: *SMN2* Exon 7 Inclusion

- Screening of oligos for exon 7 retention in *SMN2* mRNA



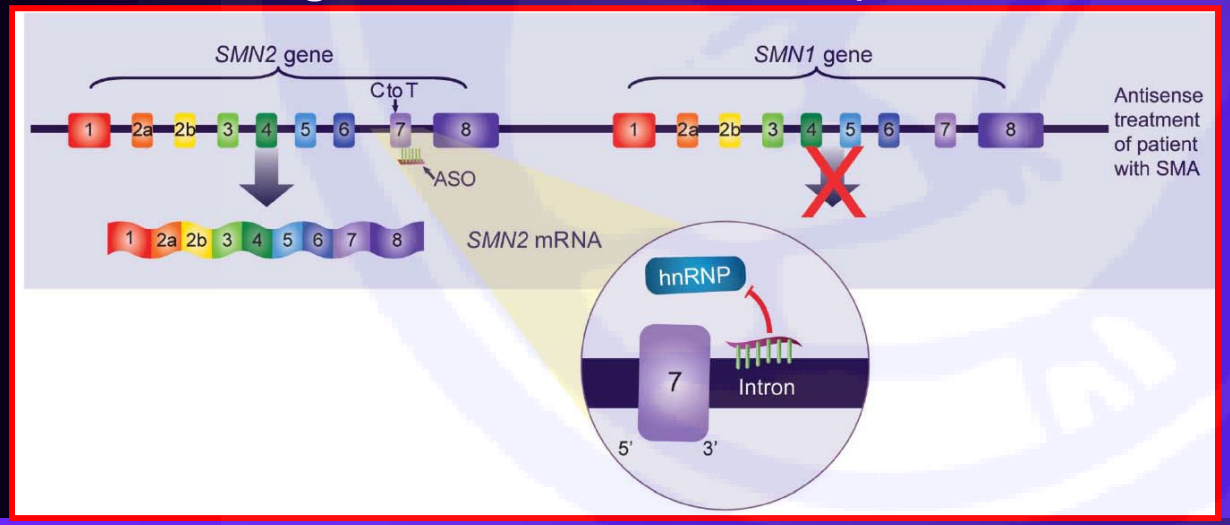
- Long-term retention of exon 7 after ICV infusion in SMA mice

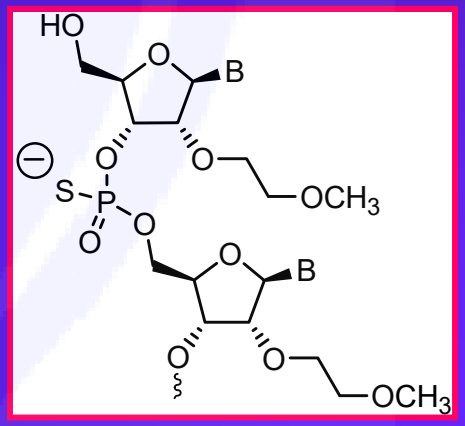


Hua et al 2008, 2010

IONIS-SMN_{Rx}: Modulating Splicing of *SMN2* to Increase Normal SMN Protein

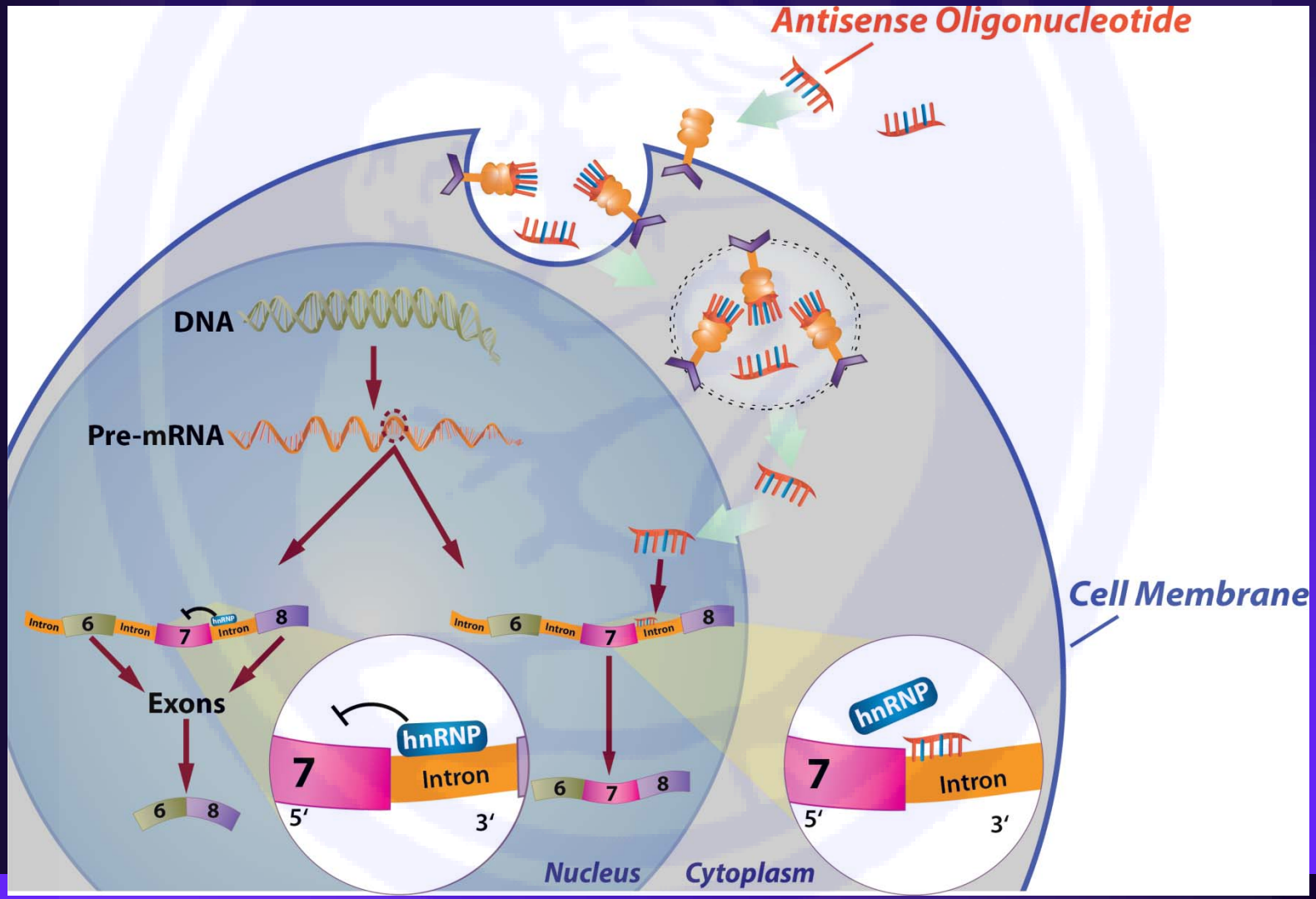
- Uniformly 2'-*O*-methoxyethyl modified (MOE) antisense drug
- Corrected the splicing disorder in *SMN2*, resulting in the production of fully functional SMN protein in model systems
- In mild and severe mouse models of SMA provided a phenotypic and pathological benefit when delivered centrally*
- Distributes broadly to spinal cord motor neurons after intrathecal delivery in monkeys*
- Has a long half life in CNS tissue (> 6 months in animal models)





*Hua et al., Genes Dev., 2010; Passini et al., Sci Transl Med, 2011; Hua et al., Nature, 2011

Mechanism of Action for SMN Antisense Drug



Courtesy Frank Bennett, PhD, IONIS Pharmaceuticals, Inc.

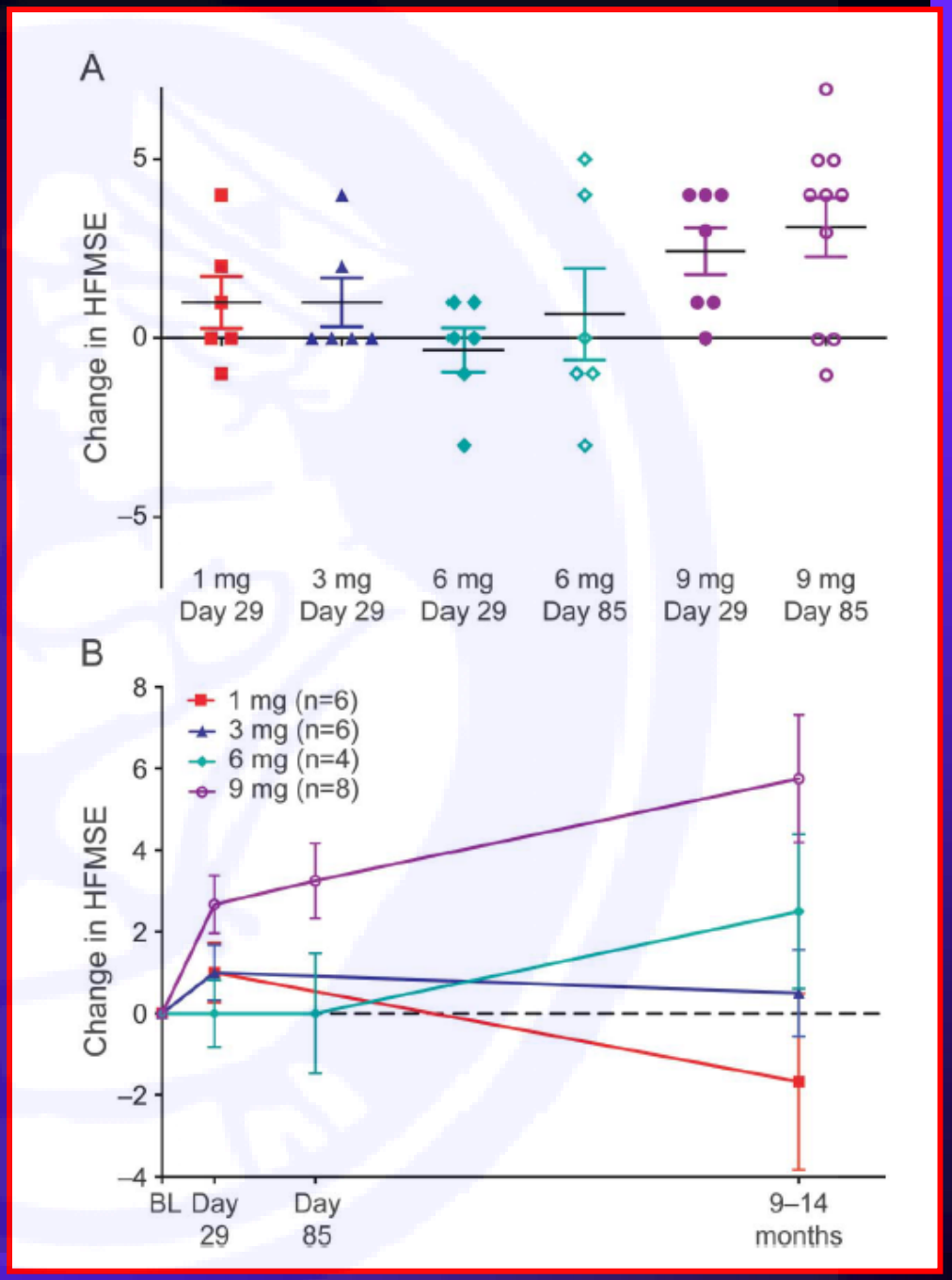
Results of an Open-Label, Escalating Dose Study to Assess the Safety, Tolerability, and Dose Range Finding of a Single Intrathecal Dose of ISIS-SMN_{Rx} in Patients with Spinal Muscular Atrophy (CS1)

1 – Boston Children's Hospital; 2 – Columbia University Medical Center; 3 – University of Utah; 4 – UT Southwestern Medical Center;
5 – Isis Pharmaceuticals, Inc.

No safety or tolerability issues (1,3,6,9 mg) n: 6-6-6-10

No serious adverse events or dose-limiting toxicities were reported in 28 patients with later onset SMA, Type II and III (2-14 years)

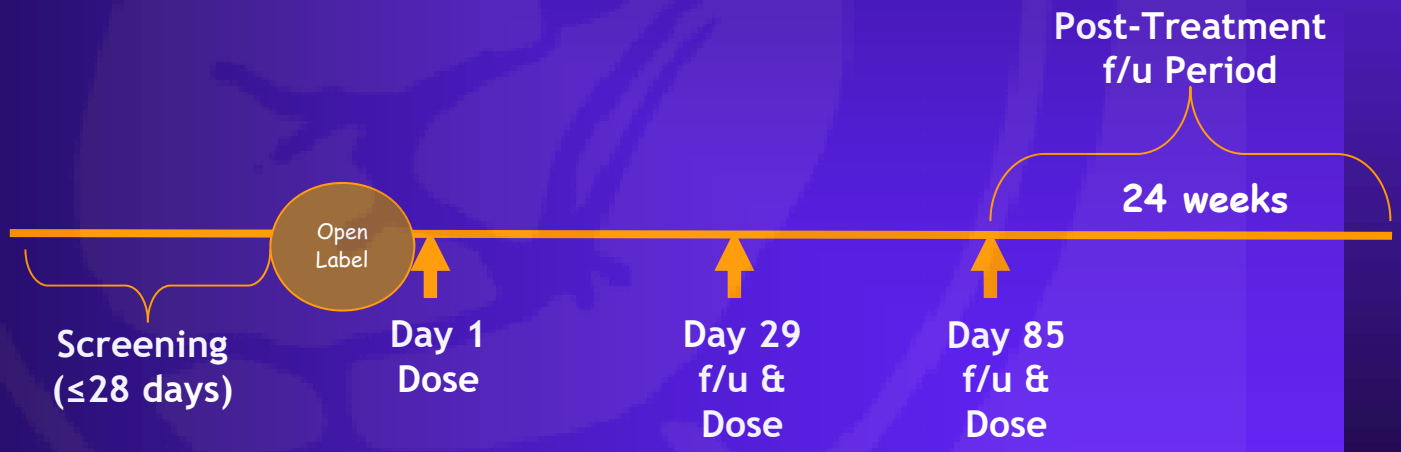
Mean change in HFMSE scores through 9-14 months post-dose



Phase 2 Multiple-Ascending Dose, Open-Label Study in Medically Stable SMA Patients 2-15 Years of Age

- **Objectives:**
  - ***Evaluate the safety and tolerability of multiple intrathecal doses of ISIS-SMN$_{Rx}$***
  - ***Evaluate CSF, plasma PK, and clinical outcomes related to SMA (including HMFSE)***
- **Status:**
  - **3 mg, 6 mg, and 9 mg cohorts completed; 12 mg cohort was added**

Cohort	Total Dose	n
3 mg	9 mg	8
6 mg	18 mg	8
9 mg	18 mg	9
12 mg	36 mg	9



SUBJECT DEMOGRAPHICS	N=25
SMA Type	Type 2 = 10; Type 3 = 15
Ambulatory/Non-ambulatory	9/16
Mean age (range)	7.5 years (2-15)
SMN2 Copy #	2 copies = 1; 3 copies = 20; 4 copies = 4

Interim Results of a Phase 1/2 Study of ISIS-SMN_{Rx} in Children with Spinal Muscular Atrophy

Darras B[1], Chiriboga C[2], Swoboda K[3], Iannaccone S[4], Montes J[2], Castro D[4], Holuba N[2], Rausch N[3], Ramos C[3], Visyak N[1], Dunaway S[2], Trussell D[3], Pasternak A[1], Neilson L[4], De Vivo D[2], Norris D[5], Bennett F[5], Bishop K[5]

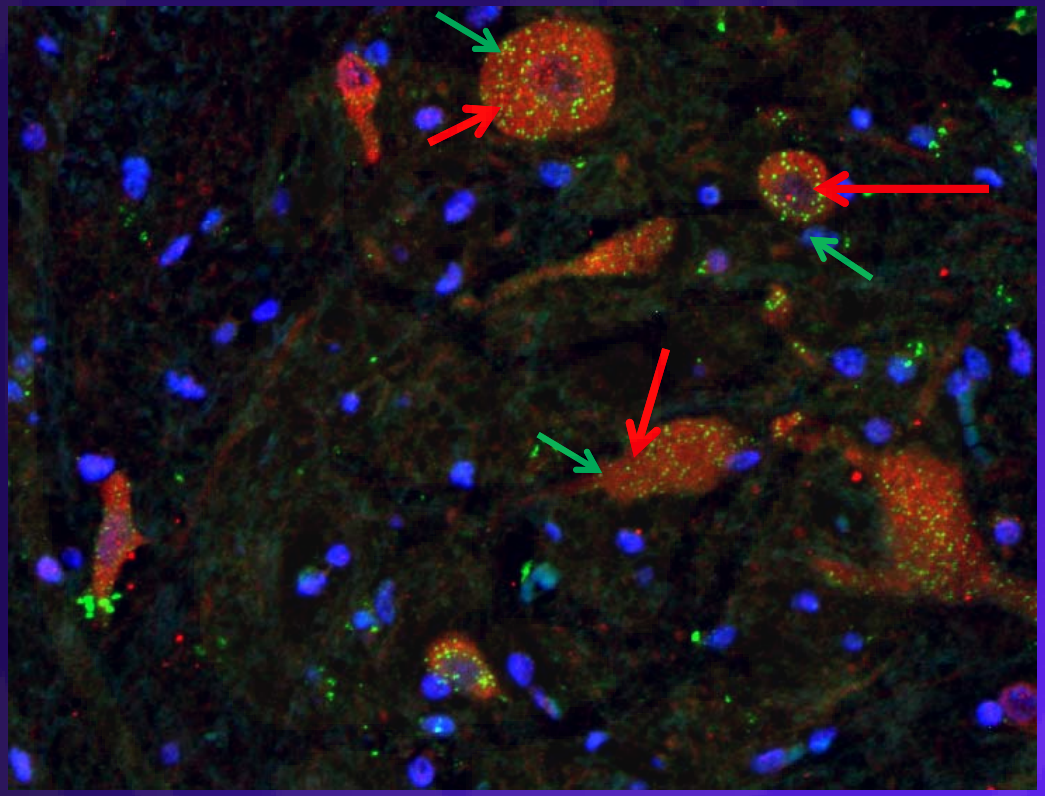
1 – Boston Children's Hospital; 2 – Columbia University Medical Center; 3 – University of Utah; 4 – UT Southwestern Medical Center; 5 – Ionis Pharmaceuticals, Inc.

World Muscle Society Meeting, Berlin, 2014

Summary of Results of Phase I/II Open-Label Studies in Children with SMA

- IONIS-SMN$_{Rx}$ had been well tolerated when given as multiple doses up to 12 mg - no safety or tolerability concerns have been identified
- CSF and drug concentrations were dose-dependent and consistent with predictions; CSF half-life was about 4-6 months
  - Observations supported infrequent administration
- Dose and time dependent SS increase in HFMSE scores observed (even 9-14 months after last dose)
- Additional secondary endpoints (6 MWT, ULM) supportive, although open-label study and small numbers limit interpretation
- These data informed the design of Phase 3 registration-enabling studies in infants and children with SMA

SMN Protein was Found in Neurons of ISIS-SMN$_{Rx}$-Treated SMA Infant in which ISIS-SMN$_{Rx}$ was Present



ISIS-SMN$_{Rx}$
Green

SMN Protein
Red

DAPI Stain (for nuclei)
Blue

Immunofluorescence Staining for SMN Protein and ISIS-SMN$_{Rx}$

ENDEAR (Nusinersen) Phase 3 Study in SMA Infants

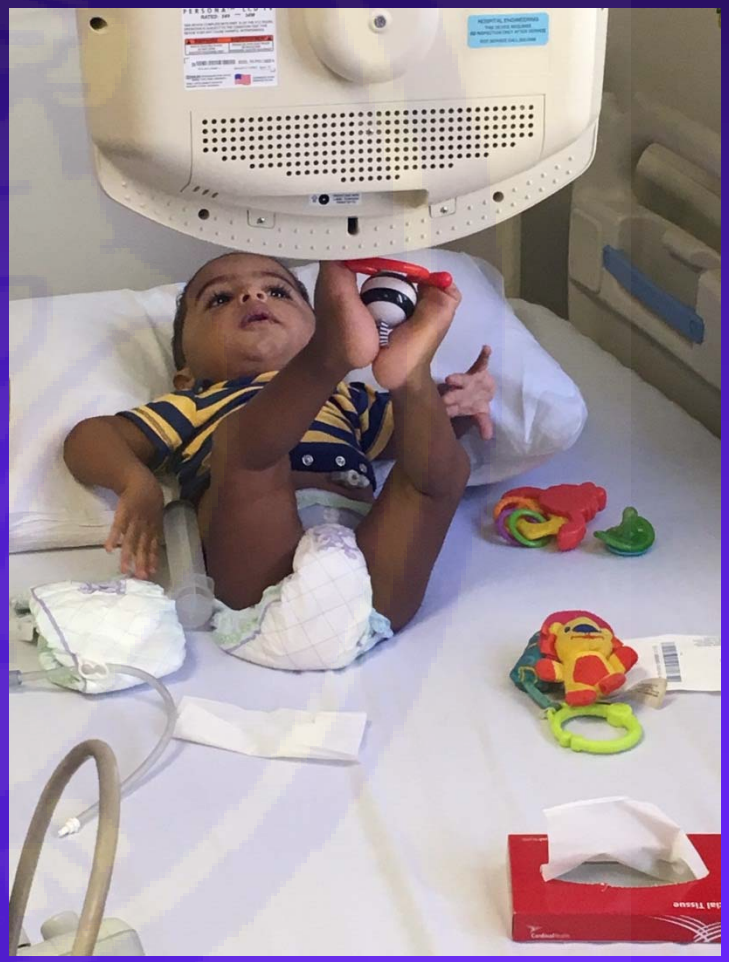
A Phase 3, randomized (2:1), double-blind, sham-procedure controlled study in infants with SMA Type I

- ***Global study in ~120 SMA infants ≤ 7 months old with 2 copies of SMN2***
- ***13-month study duration***
- **Evaluate the efficacy and safety of IONIS-SMN$_{Rx}$**
  - ***Primary efficacy endpoint is time to death/permanent ventilation***
  - ***Additional efficacy endpoints include CHOP INTEND and motor milestones***

Study initiated August 2014 Interim analysis: July 2016

Cohorts
Sham control
12 mg ISIS-SMN$_{Rx}$

ENDEAR (Nusinersen) Phase 3 Study in SMA Infants – Milestones



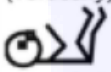
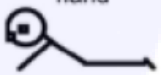
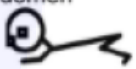
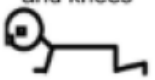
ISIS-SMN$_{Rx}$ (Nusinersen) Phase 3 Study in SMA Infants ENDEAR- Milestones

HINE

Hammersmith Infant Neurological Examination

Section II: Developmental milestones

THE JOURNAL OF PEDIATRICS
VOLUME 135, NUMBER 2, PART 1

	0	1	2	3	4
Head control	Unable to maintain head upright normal up to 3m	Wobbles normal up to 4m	Maintained upright all the time normal from 5m		
Sitting	Cannot sit	With support at hips  normal at 4m	Props  normal at 6m	Stable sit  normal at 7-8m	Pivots (rotates)  normal at 9m
Voluntary grasp – note side	No grasp	Uses whole hand	Index finger and thumb but immature grasp	Pincer grasp	Not included
Ability to kick in supine	No kicking	Kicks horizontally but legs do not lift	Upward (vertically)  normal at 3m	Touches leg  normal at 4-5m	Touches toes  normal at 5-6m
Rolling	No rolling	Rolling to side (normal at 4m)	Prone to supine (normal at 6 m)	Supine to prone (normal at 6 m)	
Crawling or bottom shuffling	Does not lift head	On elbow  (normal at 3 m)	On outstretched hand  (normal at 4m)	Crawling flat on abdomen  (normal at 8m)	Crawling on hands and knees  (normal at 10m)
Standing	Does not support weight	Supports weight (normal at 4m)	Stands with support (normal at 7m)	Stands unaided (normal at 12m)	
Walking		Bouncing (normal at 6m)	Cruising (walks holding on) (normal at 12m)	Walking independently (normal by 15m)	

ENDEAR
(Nusinersen) Phase 3 Study in SMA Infants

- **Motor milestones: Interim Analysis**
  - Percentage of patients achieving a motor milestone response

NUSINERSEN group: 41% (21/52)

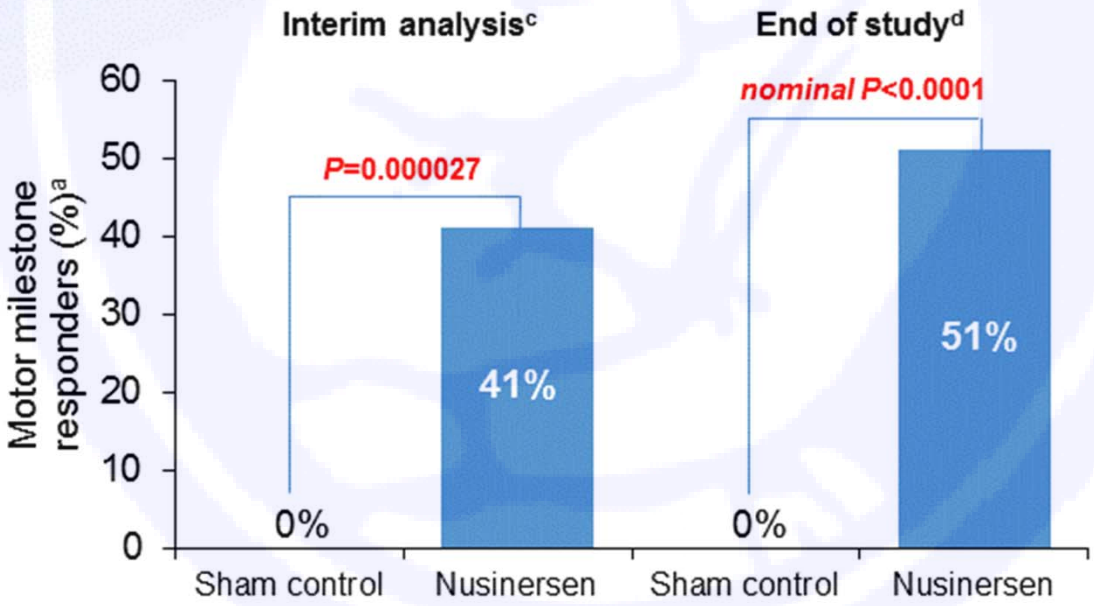
SHAM-CONTROL group: 0% (0/30)

- **p < 0.0001**

SPINRAZA (nusinersen) was approved by FDA on 12/26/16

Primary Endpoint: HINE Motor Milestone Responders

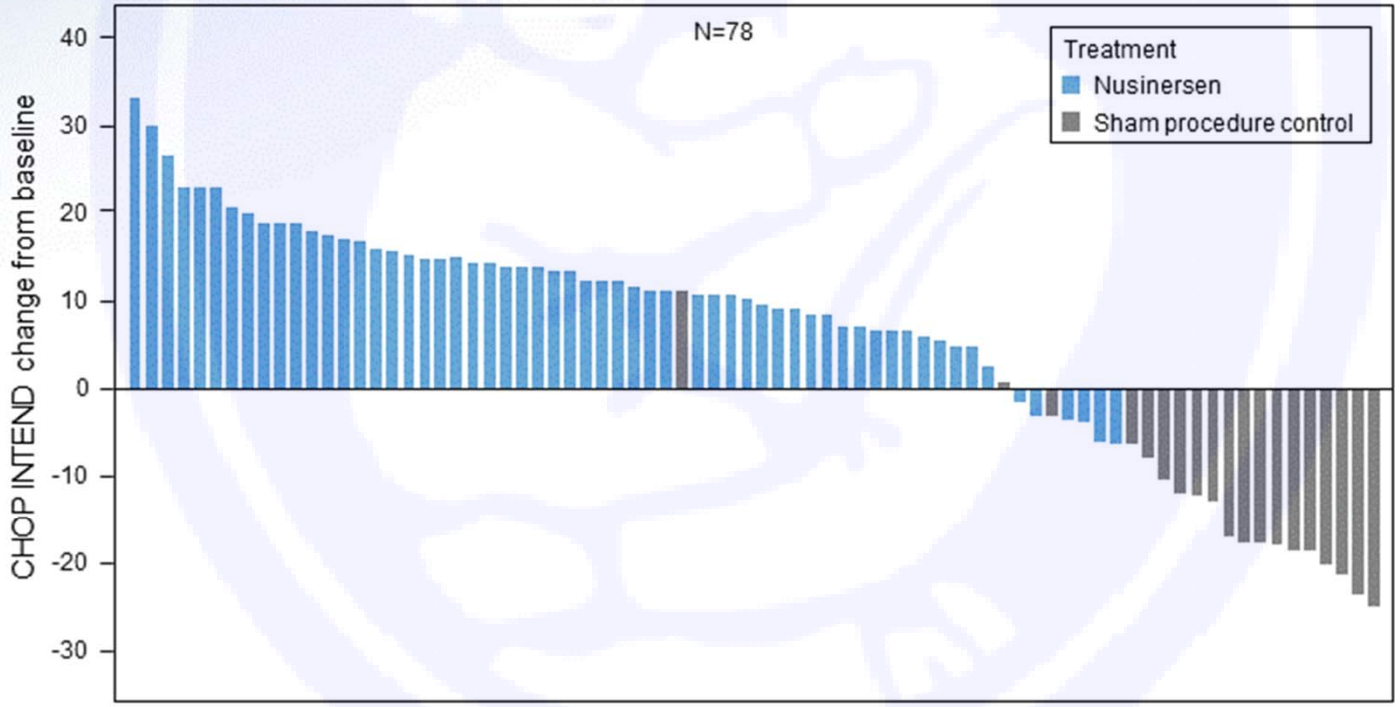
- **Motor milestone responder definition[a]:** more HINE[b] categories with improvement than worsening
  - **Improvement:** ≥2-point improvement in ability to kick (or maximal score), or ≥1-point improvement in any other milestone, excluding voluntary grasp
  - **Worsening:** ≥2-point worsening in ability to kick (or zero score), or ≥1-point worsening in any other milestone, excluding voluntary grasp



[a]Study participants on permanent ventilation were included. Participants who died or withdrew were counted as non-responders. [b]Modified section 2 of the HINE as described by Haataja L, *et al*. [*J Pediatr*. 1999;135(2 pt 1):153-161] excluding voluntary grasp. [c]The interim efficacy analysis was conducted on June 15, 2016, once ~80 participants had the opportunity to be assessed at the Day 183 visit; n=78. [d]The end of study analysis was conducted on November 21, 2016. Infants with opportunity for at least a Day 183 assessment were included; n=110. The interim endpoint was re-evaluated with final study data with no alpha spending.

CHOP INTEND Motor Function Scores at End of Study

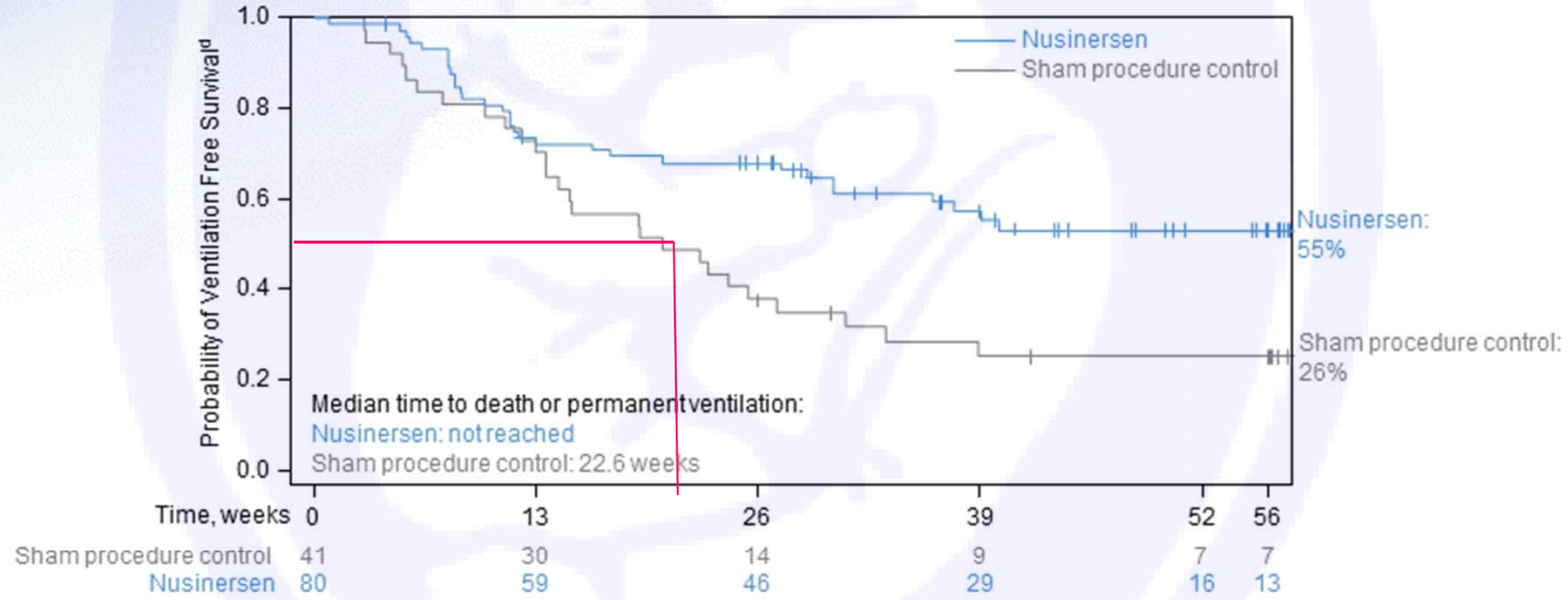
- More improvement and less worsening in motor function assessment (CHOP INTEND) in nusinersen-treated patients[a]



[a]Versus sham-control treated infants. Infants with opportunity for at least a 6-month (Day 183) assessment were included in the analysis; last available assessment of 6-month (Day 183), 10-month (Day 302), or 13-month (Day 394) was used. Shortest bars indicate zero value. Of the 110 infants in the efficacy set, 29 died (nusinersen, n=13; sham procedure control, n=16) and 3 withdrew for a reason other than death (nusinersen, n=2; sham procedure control, n=1) and were not included in this analysis.

Event-Free[a] and Overall Survival at End of Study

- The risk of death was 63% lower in nusinersen-treated infants[b] (*P*=0.0041[c])
- The risk of death or permanent ventilation was 47% lower in nusinersen-treated infants[b] (*P*=0.0046[c])



Outcome	Sham procedure control	Nusinersen
Death, n (%)	16 (39%)	13 (16%)
Alive without permanent ventilation, n (%)	13 (32%)	49 (61%)

All infants randomized who received at least one dose of nusinersen or sham procedure were included in the analysis. [a]Event-free survival = time to death or permanent ventilation (permanent ventilation was defined as tracheostomy or ≥16 hours ventilatory support per day for >21 days in the absence of acute reversible event in the determination of an independent endpoint adjudication committee). [b]Versus control infants. [c]Log-rank statistical test stratified by disease duration. [d]Estimated from the Kaplan-Meier method. HR = hazard ratio.

6

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy

R.S. Finkel, E. Mercuri, B.T. Darras, A.M. Connolly, N.L. Kuntz, J. Kirschner, C.A. Chiriboga, K. Saito, L. Servais, E. Tizzano, H. Topaloglu, M. Tulinius, J. Montes, A.M. Glanzman, K. Bishop, Z.J. Zhong, S. Gheuens, C.F. Bennett, E. Schneider, W. Farwell, and D.C. De Vivo, for the ENDEAR Study Group*

N Engl J Med 2017;377:1723-32.
DOI: 10.1056/NEJMoa1702752

N ENGL J MED 377;18 NEJM.ORG NOVEMBER 2, 2017

video

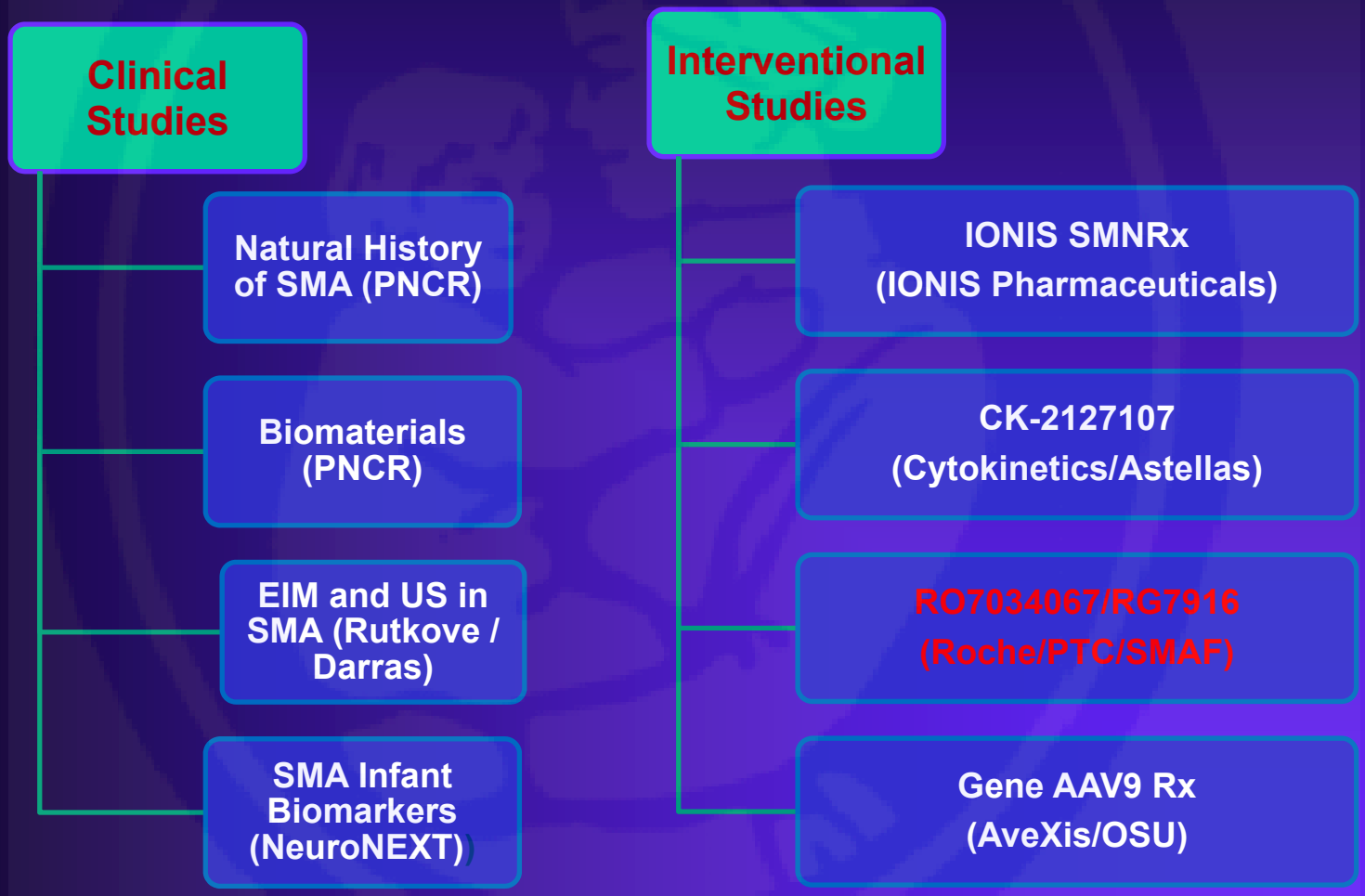
(Nusinersen) Phase 3 Study in SMA Type II - CHERISH

- A Phase 3, Randomized (2:1), Double-blind, Sham-Procedure Controlled Study in Children with SMA
  - *Global study in ~120 SMA children with SMA Type II*
  - *15-month study duration*
    - Determine the efficacy and safety of IONIS-SMN$_{Rx}$
      - *Primary endpoint is change in Hammersmith motor function score.*

It also met its primary endpoint.

Cohorts
Sham control
12 mg IONIS-SMN$_{Rx}$

Spinal Muscular Atrophy (SMA) Studies at Boston Children's Hospital



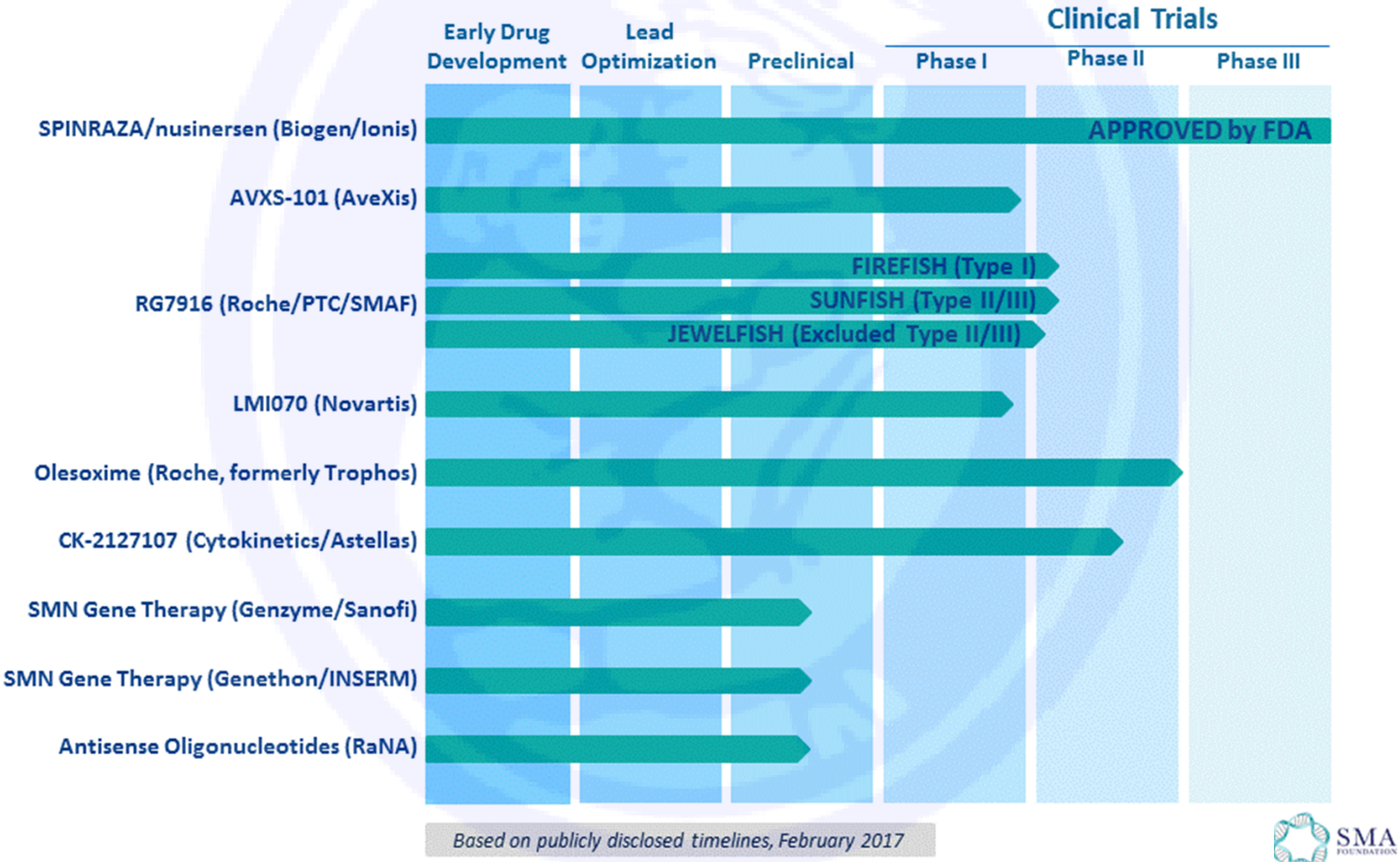
PTC-Roche Pharmaceuticals: Oral SMN2 splicing modifier (1)

- RO6885247: a small molecule to treat SMA by altering SMN splicing
  - Extended life of SMNΔ7 mice from 14-17 days to more than 150 days.
  - Single dose study in Europe on adult healthy volunteers was completed, and SMA trial (MOONFISH) was initiated.
  - Phase 1 (MOONFISH) study was discontinued (retinal toxicity in non-human primates)

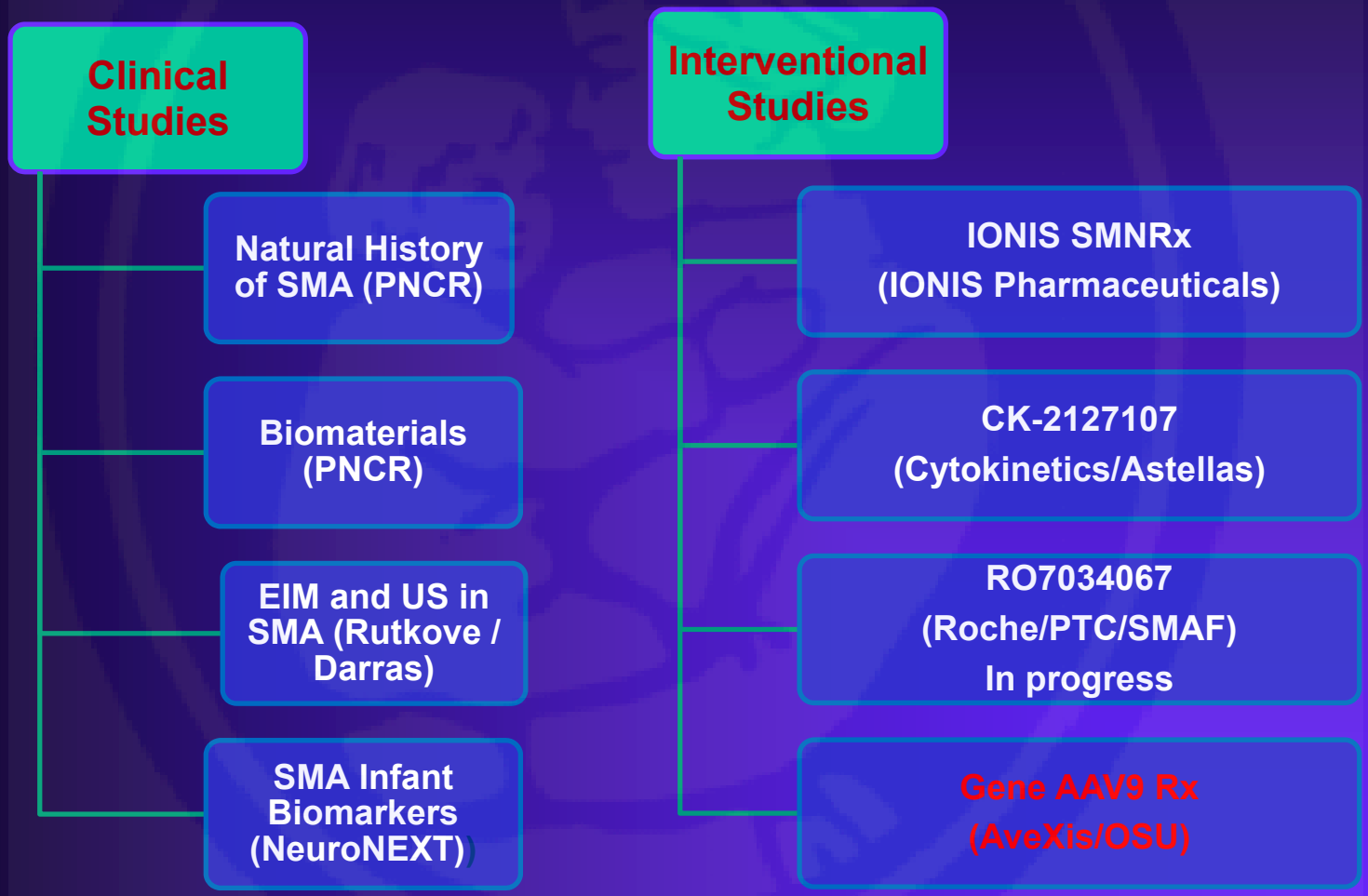
PTC-Roche Pharmaceuticals: Oral SMN2 splicing modifier (2)

- **RG7916 (Risdiplam): A second SMN2 splicing modifier**
  - **Various studies in SMA Types I (FIREFISH) and II/III (SUNFISH, JEWELFISH) are in progress in the US, Europe and elsewhere.**
  - **Advantages: oral administration, systemic distribution.**
  - **It was approved by FDA in August 2020 as Evrysdi for adults and children over 2 months of age.**
  - **Yearly Cost: As high as $340,000!**

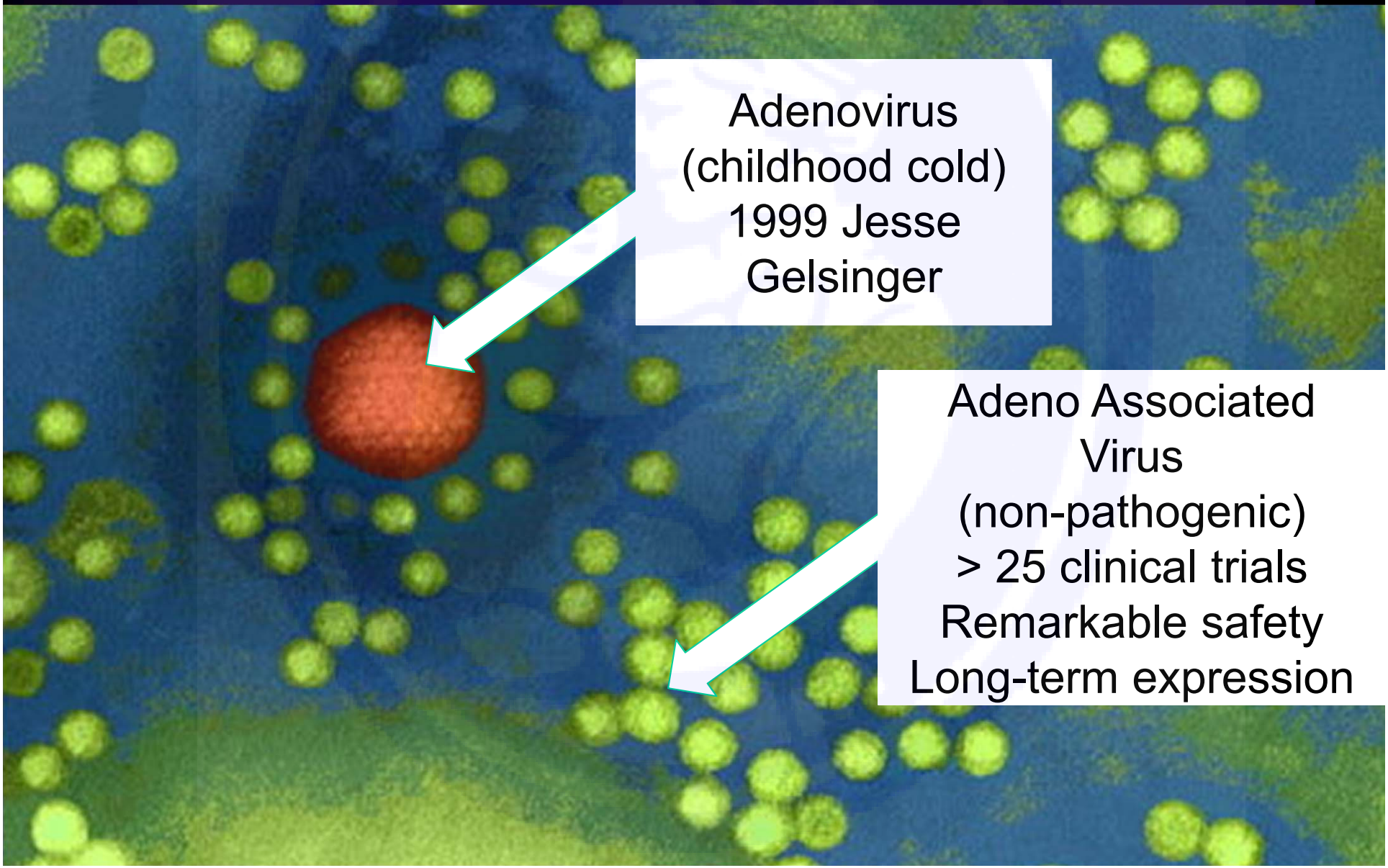
SMA DRUG DEVELOPMENT PROGRAMS



Spinal Muscular Atrophy (SMA) Studies at Boston Children's Hospital



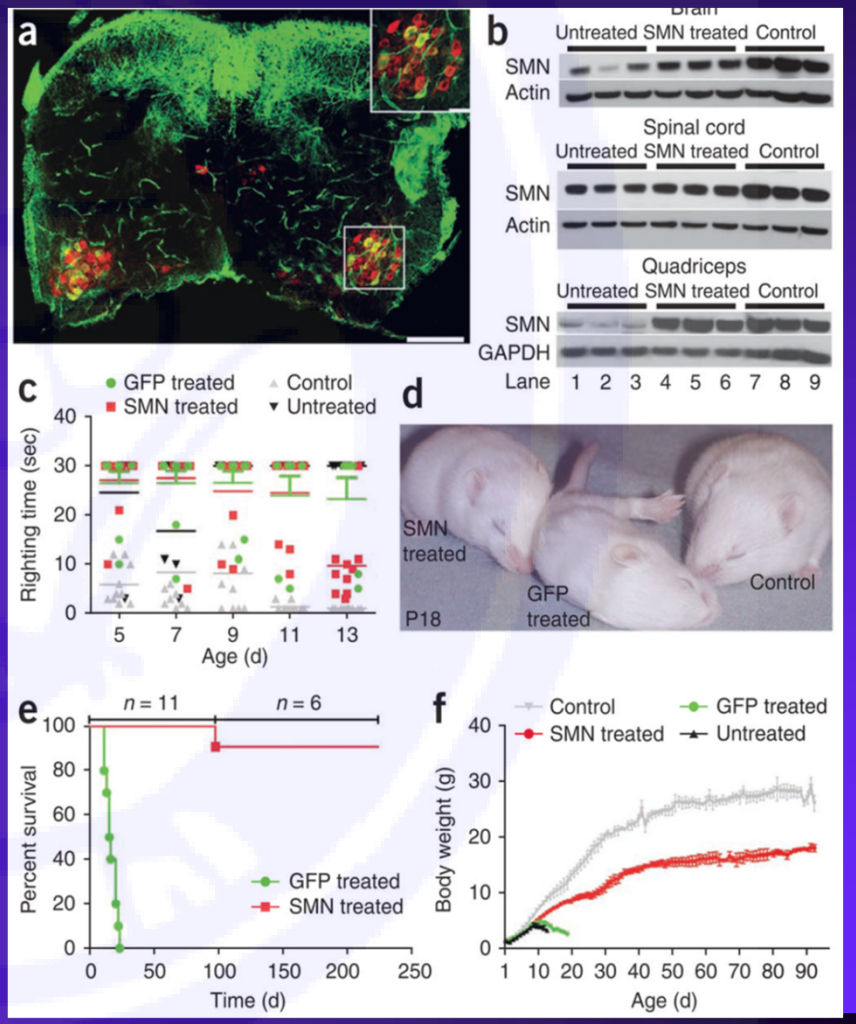
Adenovirus & Adeno-associated Virus



Gene Replacement by Early I.V. Delivery of scAAV9

Foust KD et al
Nature Biotech
February 2010

D1: good effect
D5: partial effect
D10: no effect



Gene Replacement Therapy by Delivery of SMN via scAAV9

- *Ohio State University (Jerry Mendell, MD)*

  In 2013, NINDS award to Brian Kaspar, PhD, in collaboration with FSMA to advance a CNS-directed gene therapy to IND

- **Results on the first treated 15 SMA Type I infants (IV) (START phase 1/2 study) were very encouraging. Similar to Nusinersen or better? But single site study with high SOC**
- **Type I and II gene therapy Phase 3 studies (STRIVE and STRONG) sponsored by AveXis are ongoing or have been completed at BCH and other sites in the US but also in Europe SPRINT is a study for pre-symptomatic infants**

- **Approved for patients under 2 years of age as onasemnogene abeparvovec xioi (Zolgensma) in May 2019 (cost: $2.15 M)**

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

J.R. Mendell, S. Al-Zaidy, R. Shell, W.D. Arnold, L.R. Rodino-Klapac, T.W. Prior, L. Lowes, L. Alfano, K. Berry, K. Church, J.T. Kissel, S. Nagendran, J. L'Italien, D.M. Sproule, C. Wells, J.A. Cardenas, M.D. Heitzer, A. Kaspar, S. Corcoran, L. Braun, S. Likhite, C. Miranda, K. Meyer, K.D. Foust, A.H.M. Burghes, and B.K. Kaspar

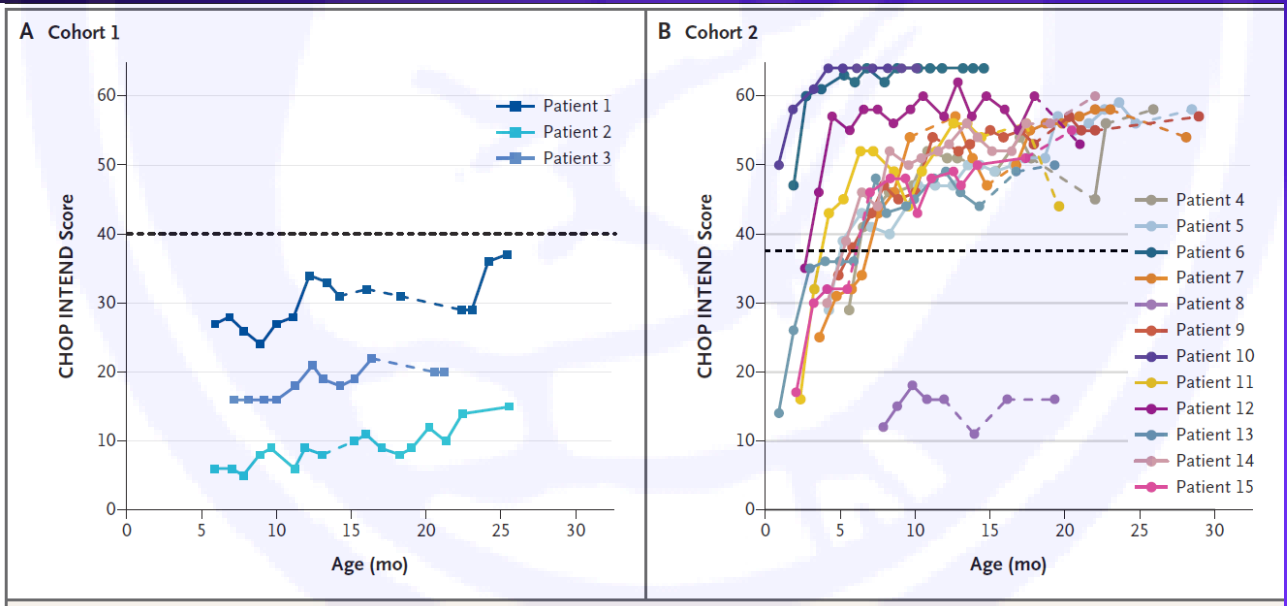


Figure 2. Motor Function after Gene Therapy.

MOTOR MILESTONES IN COHORT 2

A total of 11 of 12 patients in cohort 2 were able to sit unassisted for at least 5 seconds, 10 for at least 10 seconds, and 9 for at least 30 seconds (Table 2). A total of 11 achieved head control, 9 could roll over, and 2 were able to crawl, pull to stand, stand independently, and walk independently. Eleven patients attained the ability to speak. No patients in the historical cohorts had achieved any of these motor milestones and rarely had achieved the ability to speak.[6,23]

Three scAAV9 SMA Studies:

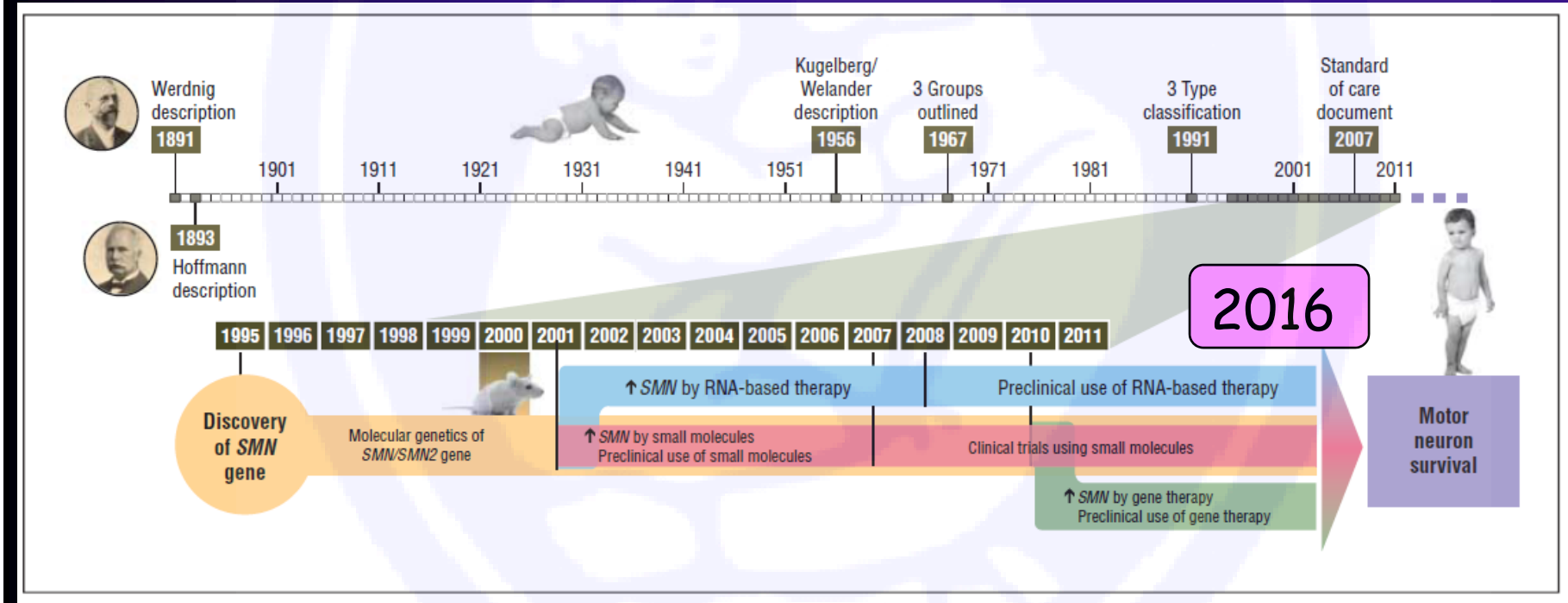
AveXis

STRIVE for Type I (IV)

STRONG for Type II (IT)

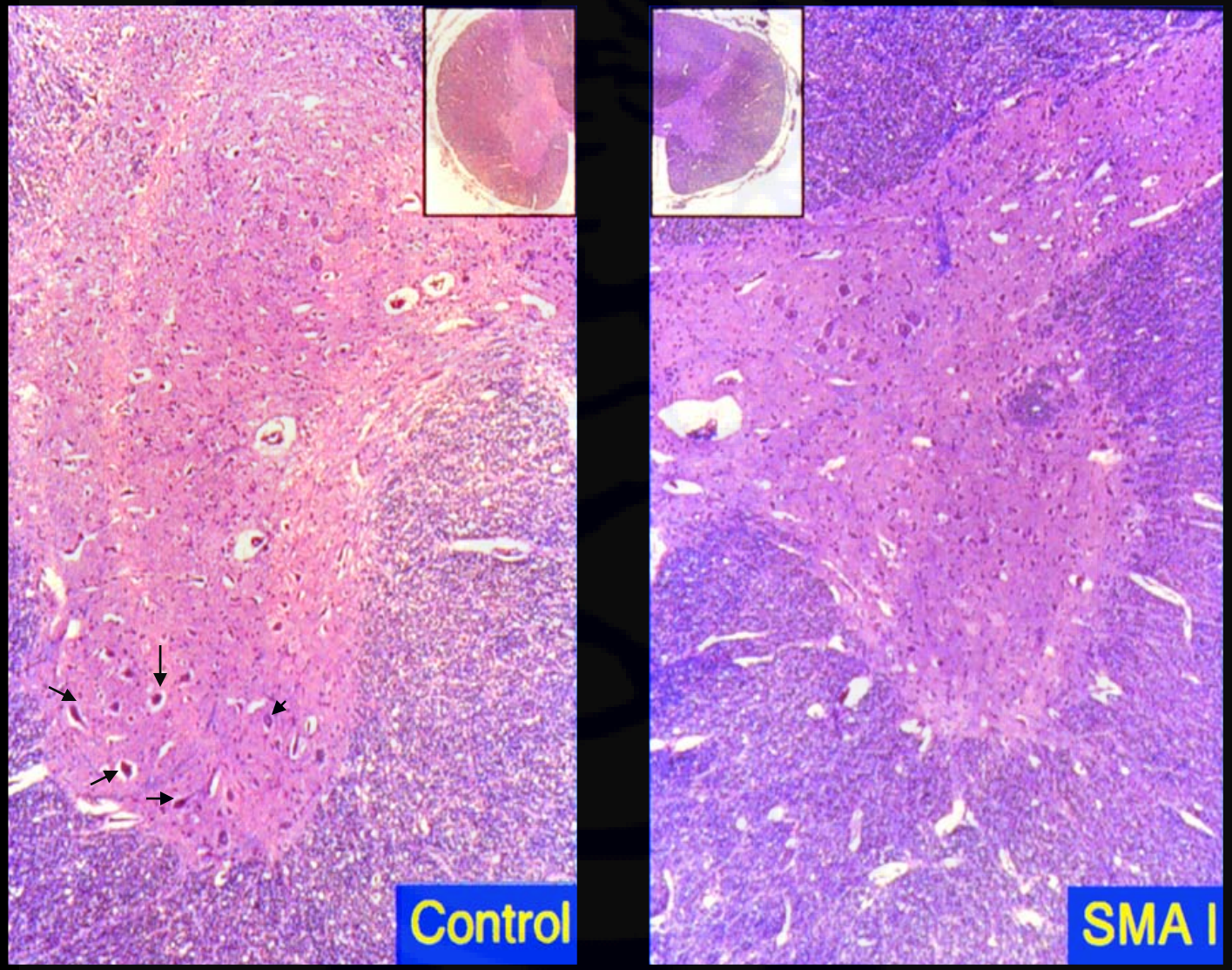
SPRINT (IV) for 1-6 weeks

Spinal Muscular Atrophy: 125 years later the first approved treatment… but can SMA be prevented?



Kolb SJ, Kissel JT. Arch Neurol 2011 Aug;68:979.

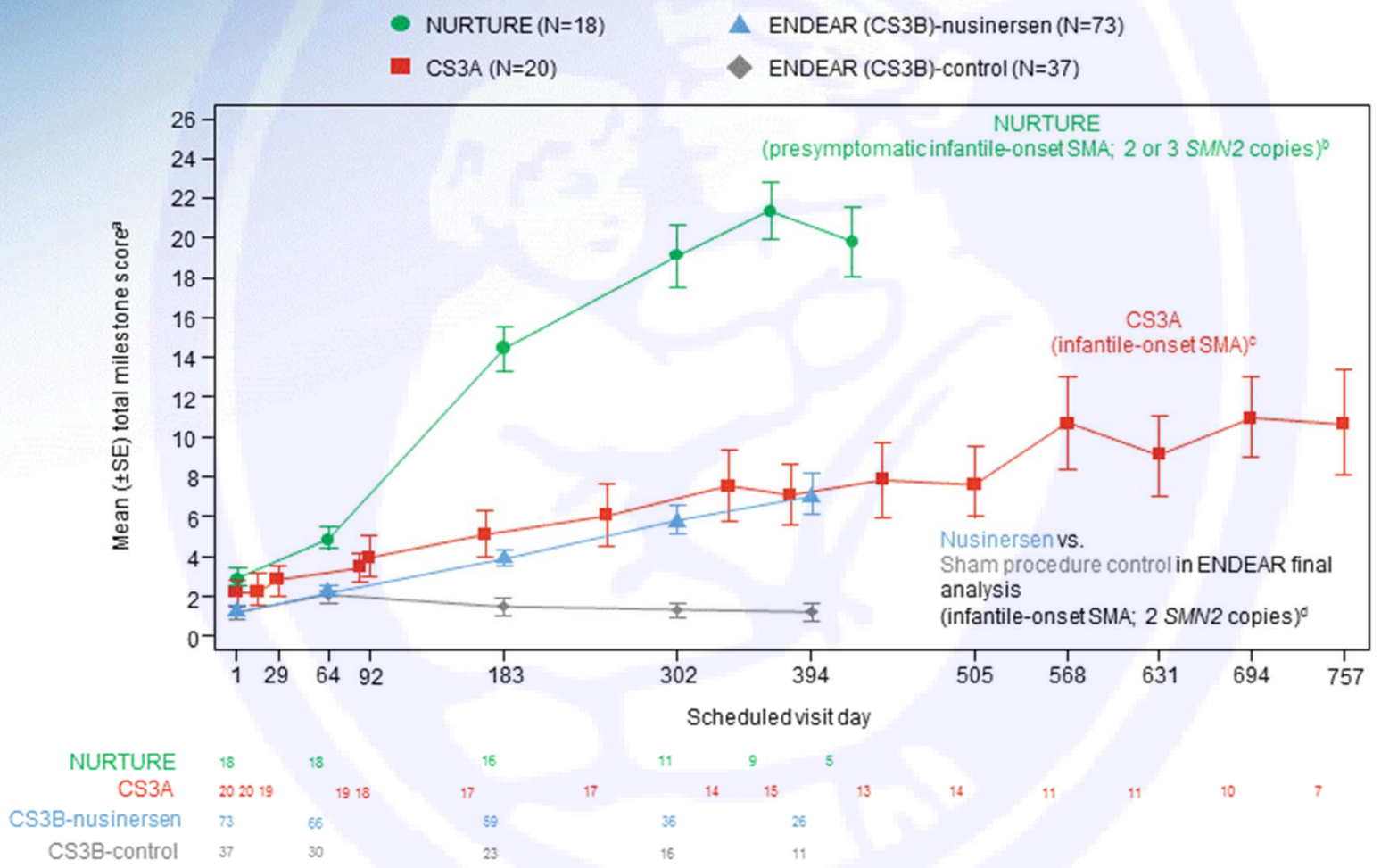
Loss of Anterior Horn Cells in SMA



Can SMA be prevented?

- *If a therapeutic is partially effective in symptomatic patients, it will probably be even more effective or preventive of the disease, if the treatment is started early in life before the onset of symptoms.*
- *Pilot newborn screening program in NY, approved in MA*
- *Patients with less than 4 copies of* SMN2 *may need to be treated presymptomatically ASAP after the diagnosis is made with newborn screening, particularly patients with 2 or 3 copies of* SMN2
- ***Nusinersen NURTURE Study Presymptomatic, for patients with 2 or 3 copies of* SMN2**

Change in HINE Motor Milestone Scores Across Studies

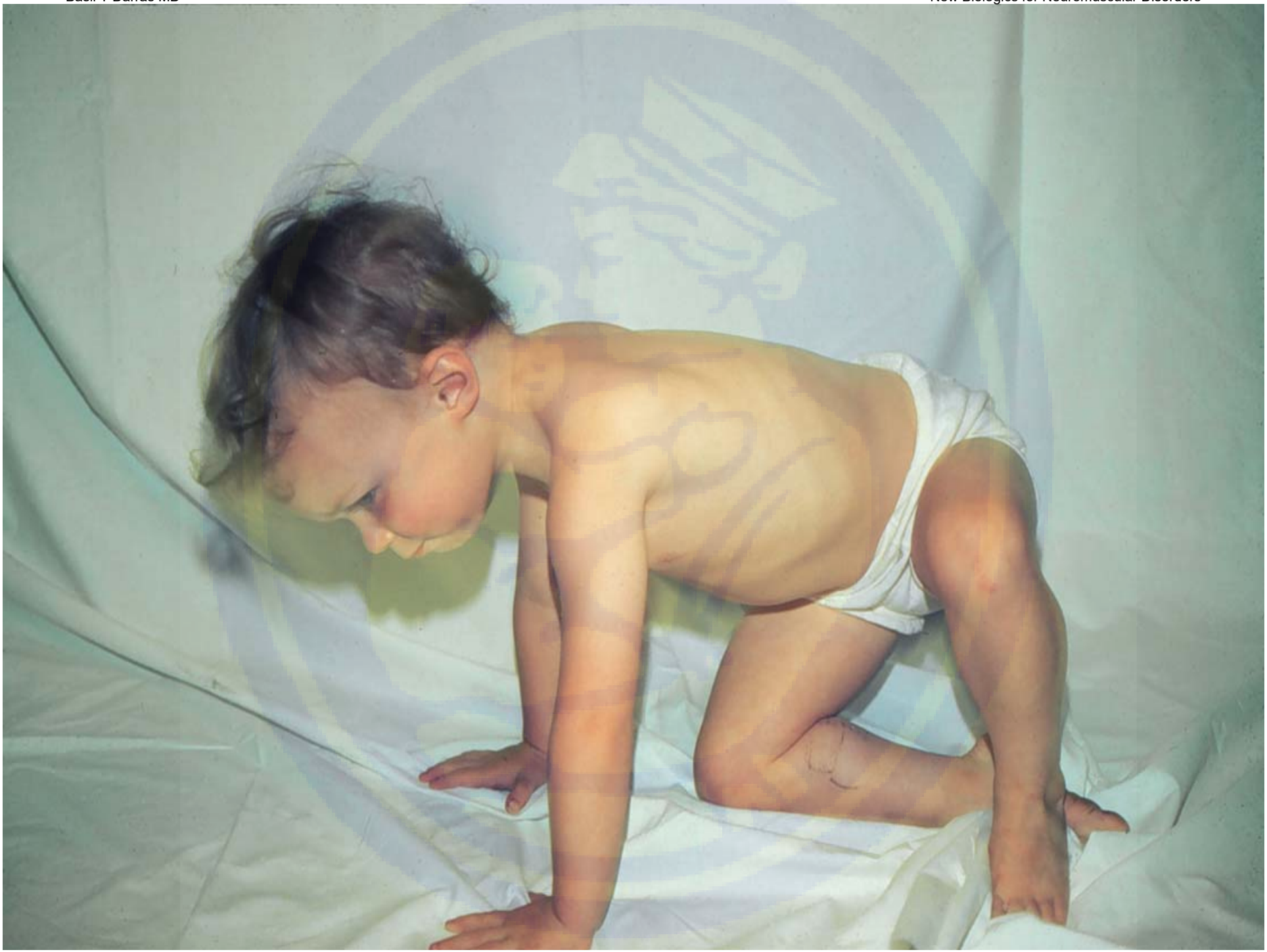


Populations: NURTURE (232SM201) = interim efficacy set, CS3A = all dosed infants; ENDEAR (CS3B) = interim efficacy set. For each study, visits with n<5 are not plotted. [a]Maximum total milestone score = 26. [b]Median (range) age at first dose: 19.0 (3–42) days. [c]Median (range) age at enrolment: = 155 (36–210) days. [d]Median (range) age at first dose: 175.0 (30–262) days.

Spinal Muscular Atrophy: 125 years later the first approved treatment… but can SMA be prevented?

- ***Presymptomatic treatment***
  - Diagnosis at birth with newborn screening but……
    - Treatment very expensive ($125.000/dose)
    - Life-long treatment and invasive (LP)
    - $750.000 (1st year), $375.000 per year
    - Perhaps need for Rx of peripheral tissues
- ***Preconception carrier screening***
  - Tay-Sachs reduced by >90% in 10 years
  - Thalassemia reduced by 50-80% in 3 years

Emerging Therapies in Duchenne Muscular Dystrophy



Dystrophinopathies

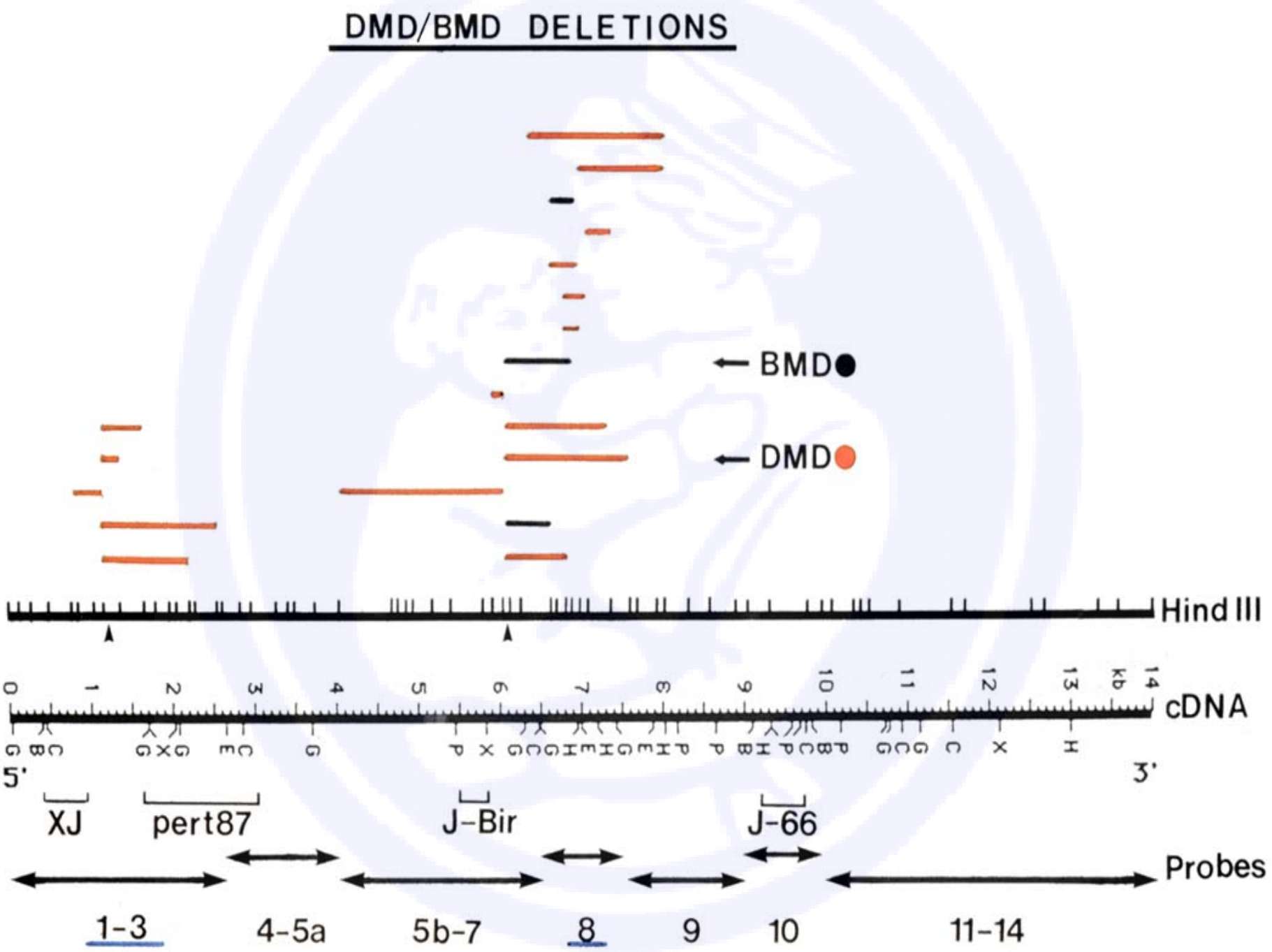
Clinical phenotypes

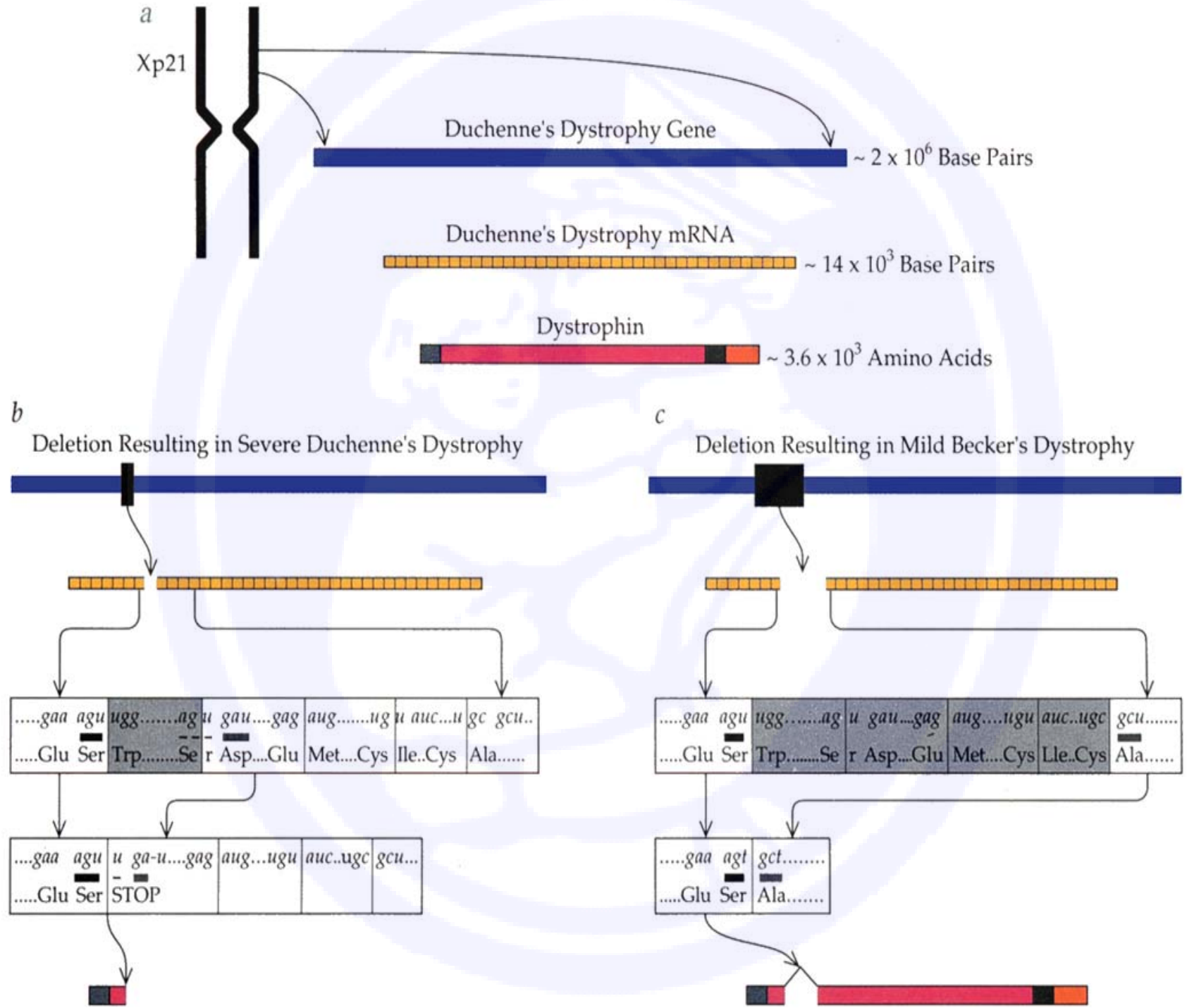
- Duchenne muscular dystrophy
- Becker muscular dystrophy
- Intermediate phenotype / “outliers”
- Manifesting DMD/BMD carrier females
- X-linked dilated cardiomyopathy
- Muscle cramps with myoglobinuria

Dystrophinopathies (DMD)

DMD gene mutations

- Deletions (~60-70%)
- Duplications (~5-10%)
- "Small" mutations [microdel/dupl, point mutations, splicing errors, (~20-30%)]





Reading Frame Hypothesis

- DMD gene - each exon fits together with specific shape
- These shapes represent the reading frame
- Maintaining the reading frame - a partially functional protein
- Milder disease

Emerging therapies for DMD

Gene therapy

After 20 years, no success

Major issues

- Choice of vector (AAV1. AAV8)
- Duration of the effect
- Practicality of administration

  Usually injected, oral administration does not seem practical
- Packaging capacity of vector
- Host immune response

Emerging therapies for DMD

Gene therapy

***After 20 years, no* success till recently…**

Emerging therapies for DMD

- ***Gene therapy (Sarepta, Mendell J., Nationwide Children's)***
  - 3 patients treated with AAVrh/micro-D/MHCK7
  - Robust expression of micro-dystrophin
  - Day 90 biopsies: mean 38.2% dystrophin by WB
  - 1.6 vector copies per nucleus
  - Mean reduction of CK levels of over 87% at Day 60

Emerging therapies

Cell-based therapy

After 25 years, no success

Emerging therapies

Antisense oligonucleotides

for deletion mutations in DMD

Antisense oligonucleotides

- Also known as ***exon skipping***
- 2'-O-methyl antisense oligoribonucleotides (morpholinos)
- Designed to bind to targeted mutant exon
- Induces a frame-preserving skipping of that exon during transcription
- ***Basic idea: Convert DMD into BMD***

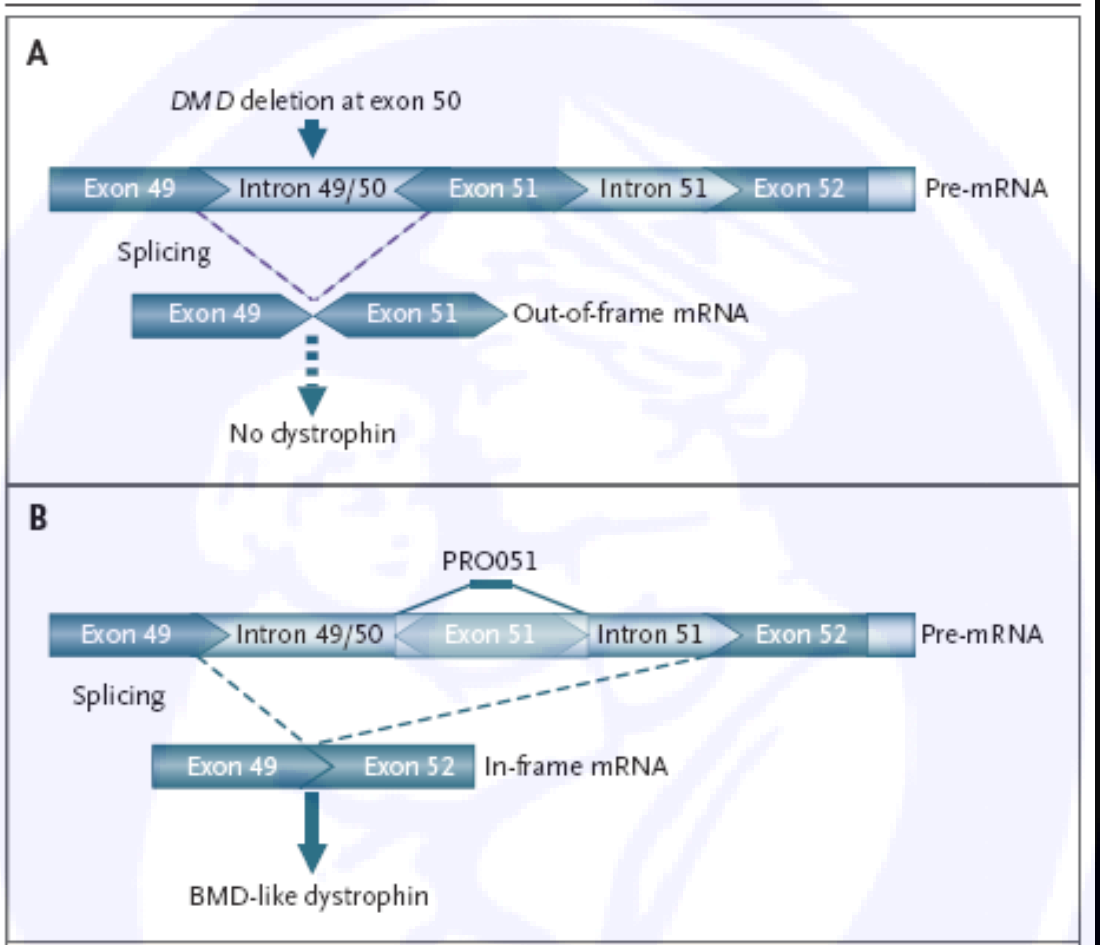


Figure 1. Schematic Representation of Exon Skipping.

In a patient with Duchenne's muscular dystrophy who has a deletion of exon 50, an out-of-frame transcript is generated in which exon 49 is spliced to exon 51 (Panel A). As a result, a stop codon is generated in exon 51, which prematurely aborts dystrophin synthesis. The sequence-specific binding of the exon-internal antisense oligonucleotide PRO051 interferes with the correct inclusion of exon 51 during splicing so that the exon is actually skipped (Panel B). This restores the open reading frame of the transcript and allows the synthesis of a dystrophin similar to that in patients with Becker's muscular dystrophy (BMD).

Exon Skipping

- PRO051 Prosensa/GSK project failed
  - No FDA or EMA approval
  - No efficacy (FDA analysis)
  - Safety issues
    - Injection site reactions
    - Thrombocytopenia
    - Proteinuria

Exon Skipping

- **Eteplirsen, by Sarepta, Cambridge, MA**
  - Approved by FDA as ExonDys51 in Sept. 2016
  - Accelerated approval (conditional)
  - Emotionally charged meeting
  - Approved against the recommendation of an expert advisory panel ("stunning exception")
  - Clinical trials still ongoing. Weekly IV infusion
  - Cost: $300.000 to $500.000 a year!

Eteplirsen/ExonDys51 FDA label

- EXONDYS 51 is an antisense oligonucleotide indicated for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping. This indication is approved under accelerated approval based on an increase in dystrophin in skeletal muscle observed in some patients treated with EXONDYS 51 *[see Clinical Studies (14)].* A clinical benefit of EXONDYS 51 has not been established. Continued approval for this indication may be contingent upon verification of a clinical benefit in confirmatory trials.

- ——————**DOSAGE AND ADMINISTRATION**
- • 30 milligrams per kilogram of body weight once weekly

Eteplirsen/ExonDys51 FDA label

- Dystrophin levels in muscle tissue were assessed by Western blot. In the 12 patients with evaluable results, the pre-treatment dystrophin level was 0.16% ± 0.12% (mean ± standard deviation) of the dystrophin level in a healthy subject and 0.44% ± 0.43% after 48 weeks of treatment with EXONDYS 51 ($p < 0.05$). The median increase after 48 weeks was 0.1%.

Emerging therapies for DMD

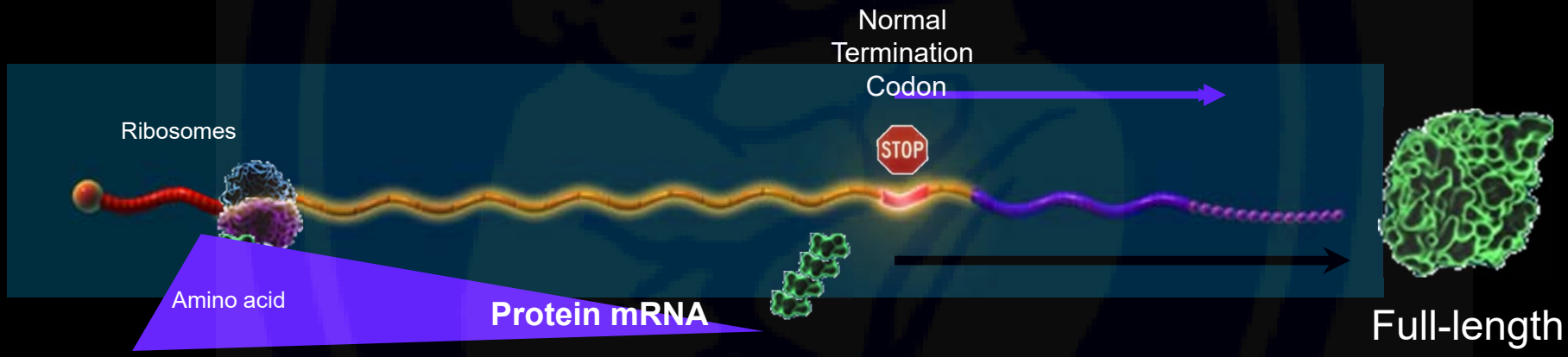
Stop codon read-through

Basics

- Gentamicin too toxic
- PTC124, (3-[5-(2-fluorophenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid, $C_{15}H_9FN_2O_3$
- PTC = post-transcriptional control
- ***Nonsense (stop) mutations***
  - UAA, UAG, UGA in mRNA
  - **~13% of DMD cases**
- Specific for premature stop codons, not for termination codons (UGA>UAG>UAA)
- These compounds cause read-through of the stop codon and preserve normal transcription

Normal flow of genetic information results in full-length protein production

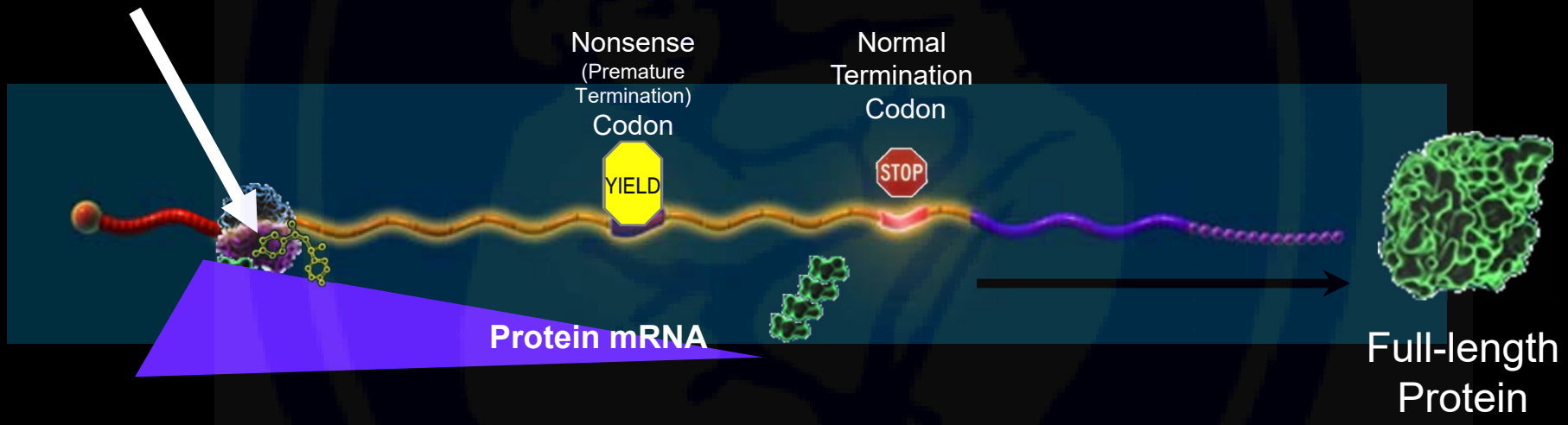
Normal Translation



PTC Therapeutics, Inc. © 2009 – Slide 84

Ataluren (PTC124) was designed to overcome nonsense mutations

***Ataluren* facilitated translation**



- ***A nonsense mutation must be present for ataluren to be active***
- ***Full-length gene sequencing can determine if a nonsense mutation is present***

PTC Therapeutics, Inc. © 2009 – Slide 85

PTC124 clinical trials

- **Phase 2a**
- **26 DMD patients treated orally for 28 days at low, medium and high doses**
- **Muscle enzymes decreased significantly**
- **Increased dystrophin production in cultured myotubes and *in vivo* at low and mid doses**
- **However, no significant change in muscle strength and timed function tests**

PTC124 (Ataluren) clinical trials

- Phase 2b
- *Ataluren* is safe but not effective, Phase 2b data analysis, 3/3/2010
- ACT-DMD, Phase 3 (medium dose)
  - Failed to meet endpoint (30 m on 6MWT)
  - A subset met the endpoint (300-400 m 6MWT)
  - Approved by EMA as *Translarna* (conditional)
  - *Ataluren* not approved by FDA (10/11 experts)
  - Clinical trials continue (also 2-5 years)

Other Emerging therapies

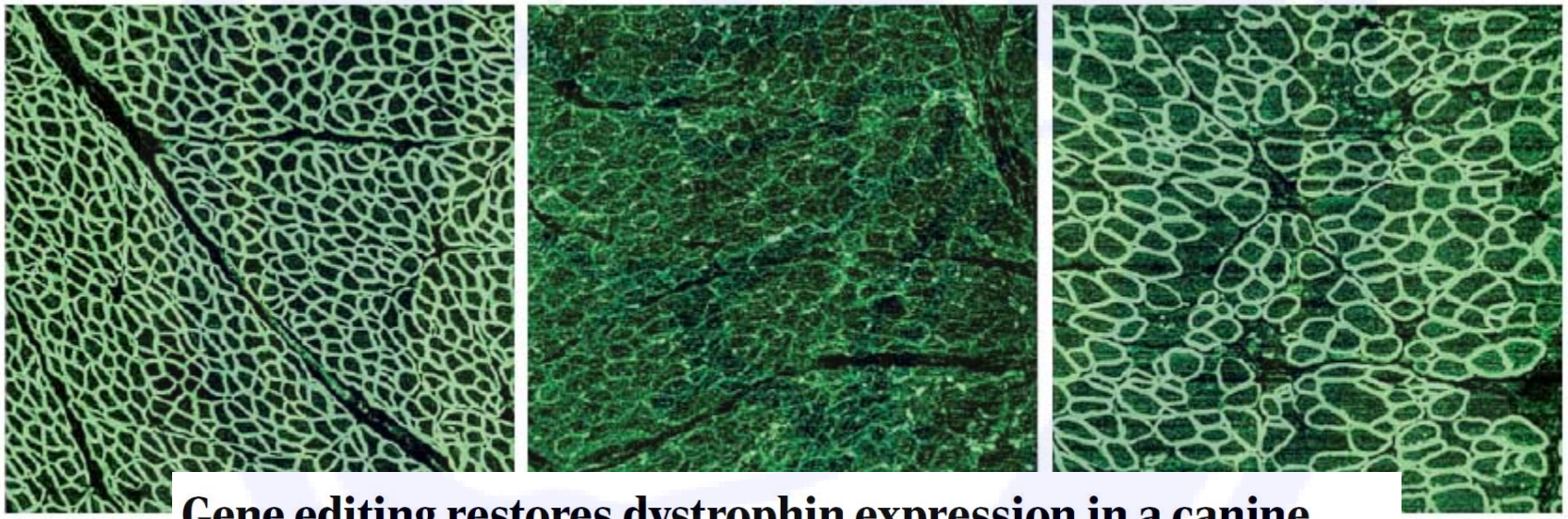
- Myostatin inhibition (BMS)
- Utrophin upregulation (Summit) *D/Ced*
- Idebenone/Raxone (Santhera)
- FB-3019 (anti-CTGF Ab) (FibroGen)
- Catabasis (anti-NF-κB pathway)
- CRISP-Cas9

Science

BIOMEDICINE

In dogs, CRISPR fixes a muscular dystrophy

Treatment repairs gene in beagles by further mutating it, but human trials are far off



Dystrophin

a muscular

eagle with

R (right).

Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy

Leonela Amoasii[1,2], John C.W. Hildyard[3], Hui Li[1], Efrain Sanchez-Ortiz[1], Alex Mireault[1], Daniel Caballero[1], Rachel Harron[3], Thaleia-Rengina Stathopoulou[4], Claire Massey[3], John M. Shelton[5], Rhonda Bassel-Duby[1], Richard J. Piercy[3], Eric N. Olson[1]*

[1]Department of Molecular Biology, Hamon Center for Regenerative Science and Medicine, Sen. Paul D. Wellstone Muscular D
University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA. [2]Exonics Therapeutic
[3]Department of Clinical Science and Services, Comparative Neuromuscular Diseases Laboratory, Royal Veterinary College, L
and Analgesia, Royal Veterinary College, London NW1 0TU, UK. [5]Department of Internal Medicine, University of Texas Southv
Boulevard, Dallas, TX 75390, USA.

L. Amoasii *et al.*, *Science* 10.1126/aau1549 (2018).

Emerging therapies for DMD

- Despite the huge investment the course has been a rocky one
- ~10 companies in Cambridge, MA, working on DMD drug development

Economics of genetic disease treatment

Robert ("Berch") Griggs—International Congress of Neuromuscular Diseases, Naples 2010, was the first person I heard warn about the costs of therapies for genetic disorders.

"If a therapy costs $500,000 per year and you extend survival to a lifespan of 70 years, then the therapy will total $35m per patient. Health services simply cannot afford this."

Insurers Battle Families Over Costly Drug for Fatal Disease

By Katie Thomas, June 22, 2017

Asian Ocean Myology Center (AOMC) Annual Meeting, August 2017, Singapore. Singaporean health economist Jeremy Lim:

- **"It has to be cost effective."**
- **"It must fit in with the health budget of the country."**

From: Nigel Laing, PhD, University of Western Australia
World Muscle Society Meeting, St. Malto, France, 2017

Thank you