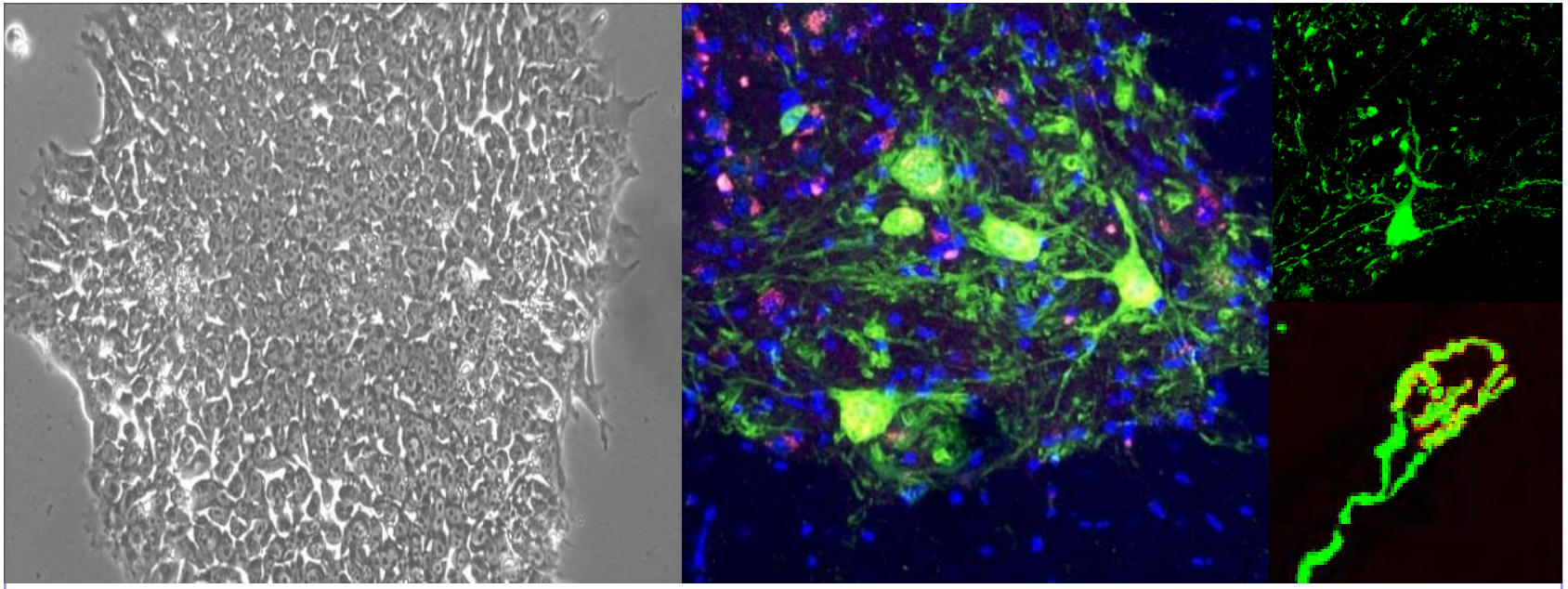


TERAPIE INNOVATIVE
DELL' ATROFIA MUSCOLARE SPINALE

FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO

Prof. Giacomo P. Comi

✧Centro Dino Ferrari, Università di Milano
✧UO Neurologia e UO Malattie neuromuscolari e rare
IRCCS Fondazione Ca' Granda, Ospedale Policlinico di Milano

SMA overview

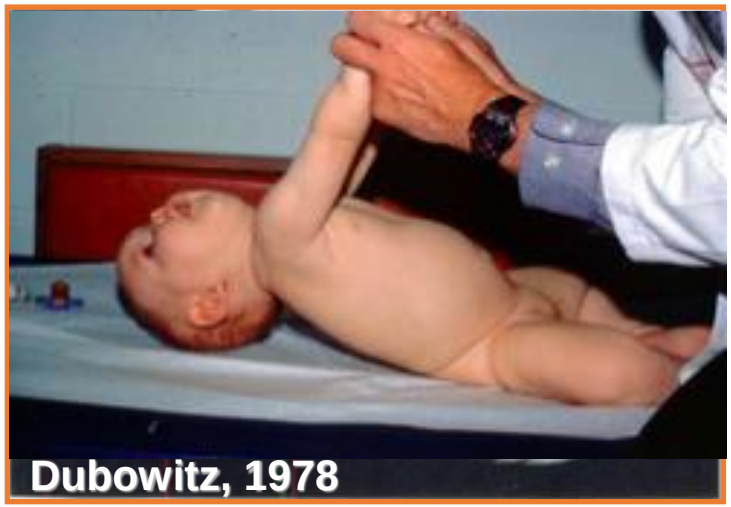
SMA is a neuromuscular disease characterized by lower motor neuron loss, progressive muscle weakness and atrophy.

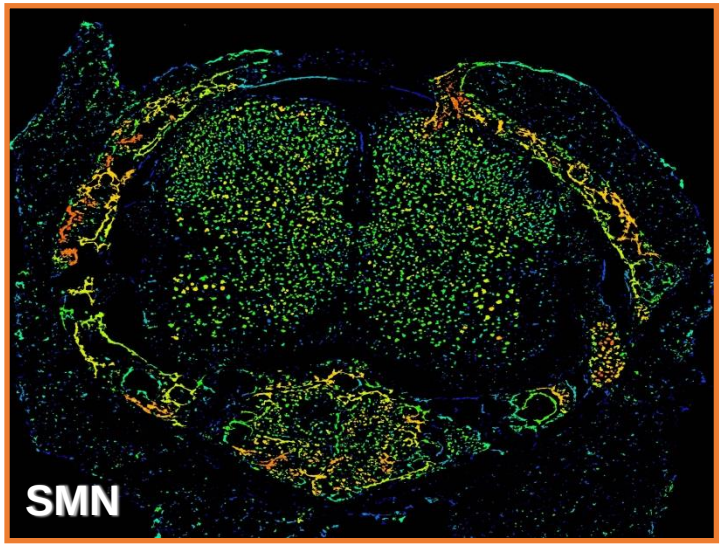
Most of case occurs in the first year of life: SMA type I most common genetic cause of infant death.

Incidence: ~1 in 10,000 live births;
Carrier: 1:40

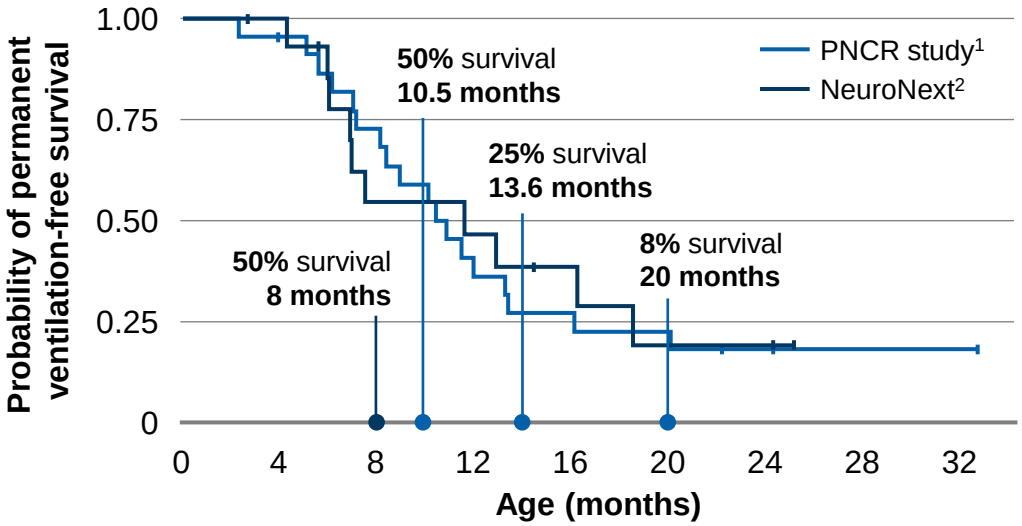
SMA type 0-I-II-III-IV
Autosomal recessive disease
Deletions/mutations ***SMN1* gene**
Deficiency SMN protein

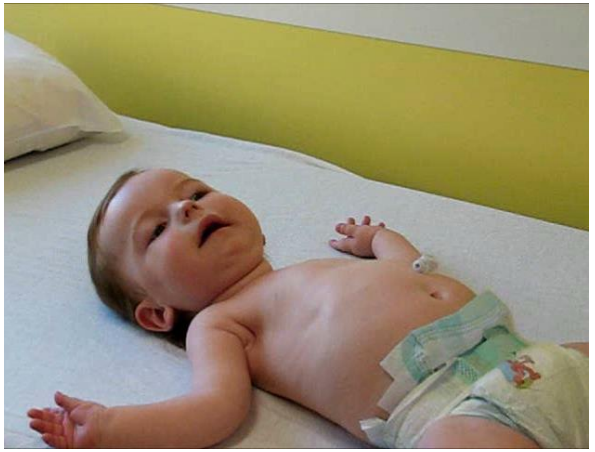
SMN2 back up gene
Severity: number of copies of *SMN2*





Rapid decline in event-free survival leads to death or continuous ventilation in most children with SMA type 1





SMA TYPE I

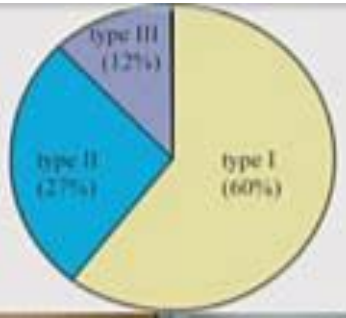
"Floppy baby" syndrome

Survival for PNCR[1] = no death, or no need for ≥16-h/day ventilation continuously for ≥2 weeks, in the absence of an acute reversible illness; n=23 (2 copies of *SMN2*)

Survival for NeuroNext[2] = no death, or no tracheostomy; n=20

Adapted from Finkel RS, et al. 2014
PNCR, Pediatric Neuromuscular Clinical Research; NeuroNext, National Network for Excellence in Neuroscience Clinical Trials; SMA, spinal muscular atrophy; SMN, survival motor neuron.
1. Finkel RS, et al. *Neurology*. 2014;83:810–817 and PNCR matched data set; 2. Kolb SJ, et al. *Ann Neurol.* 2017;82:883–891.

Broad Phenotypic Spectrum of SMA



SMA Type I

Severe form

Never sit

Limited life expectancy

Respiratory failure

Birth Prevalence 60%

SMA Type II

Intermediate form

Sitting or standing

Life expectancy shortened

Skeletal deformities

Birth Prevalence 27%

SMA Type III

Mild form

Walkers at some point

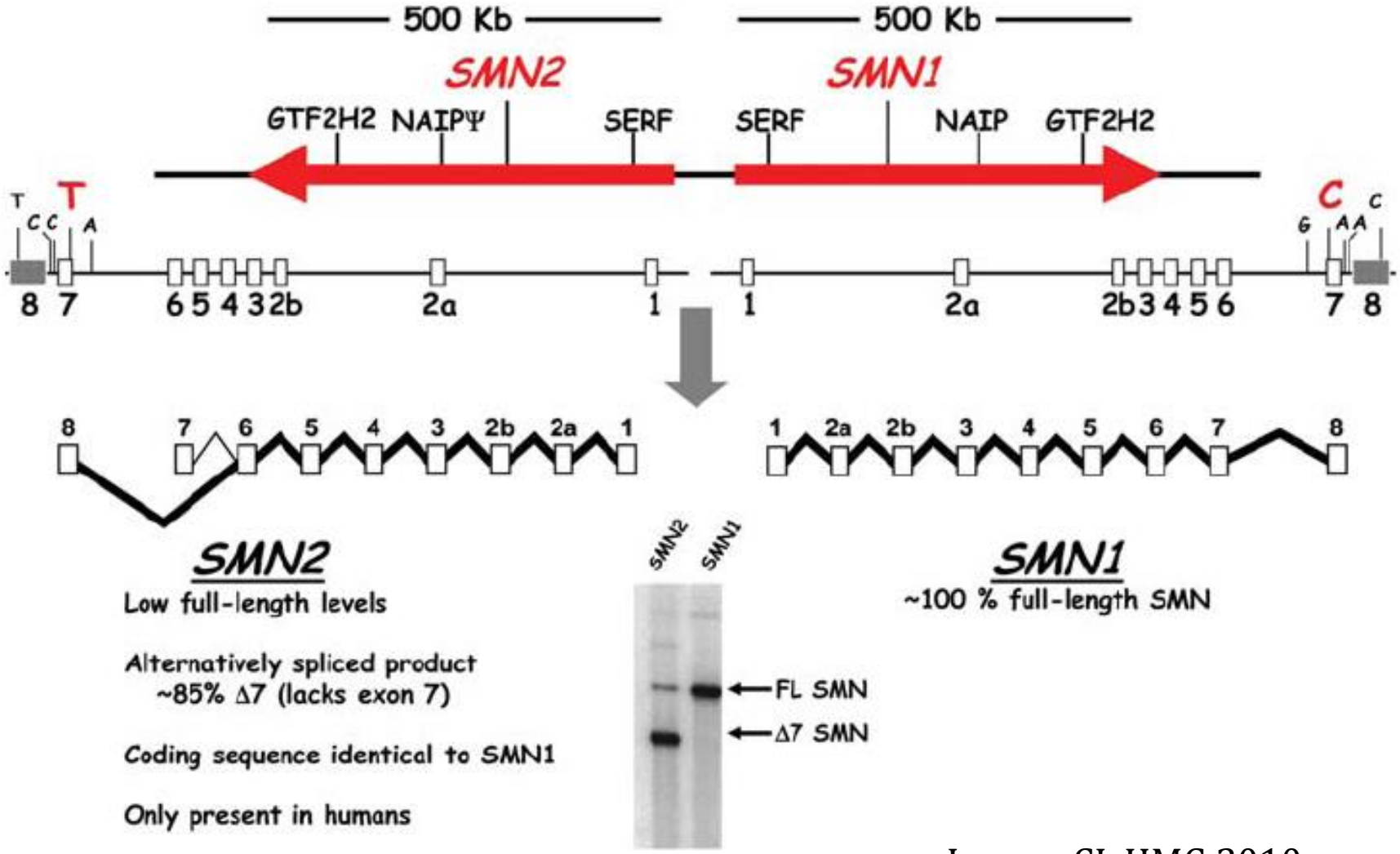
Life expectancy (nearly) normal

Proximal weakness prominent

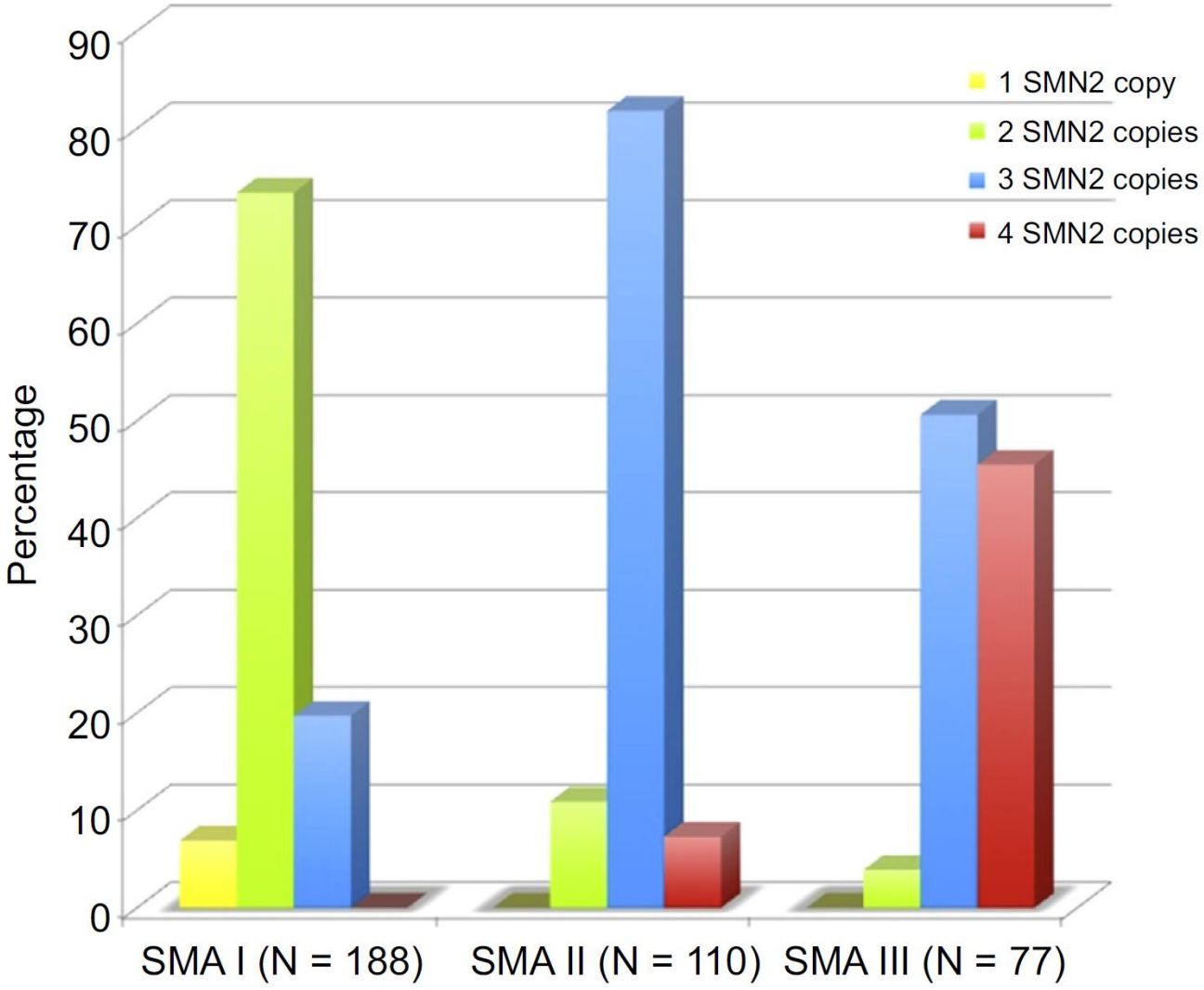
Birth Prevalence 12%

Columbia University Medical Center

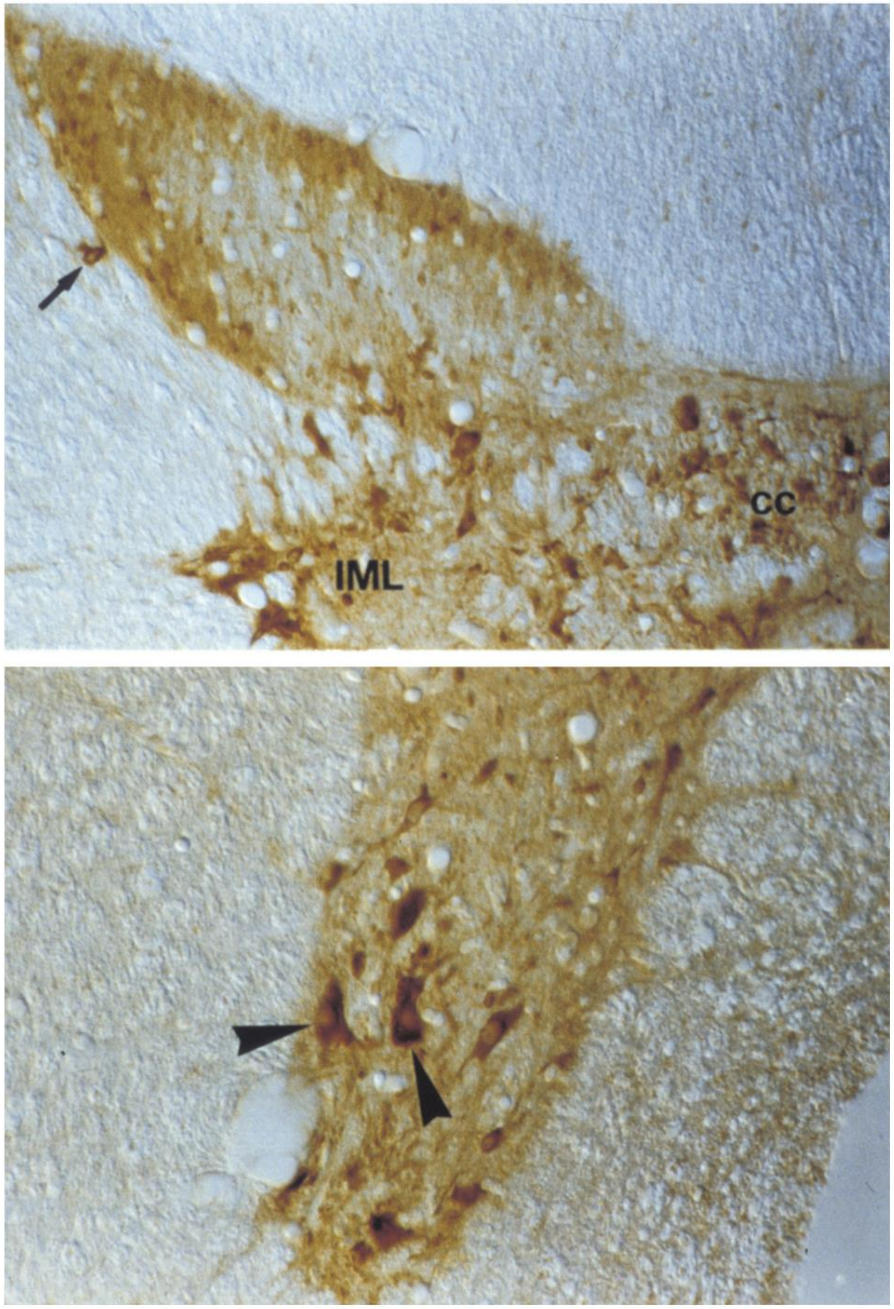
Courtesy of Dr. Darryl De Vivo



Lorson CL HMG 2010



Markus Feldkötter,[1] Verena Schwarzer,[1] Radu Wirth,[2] Thomas F. Wienker,[3] and Brunhilde Wirth[1]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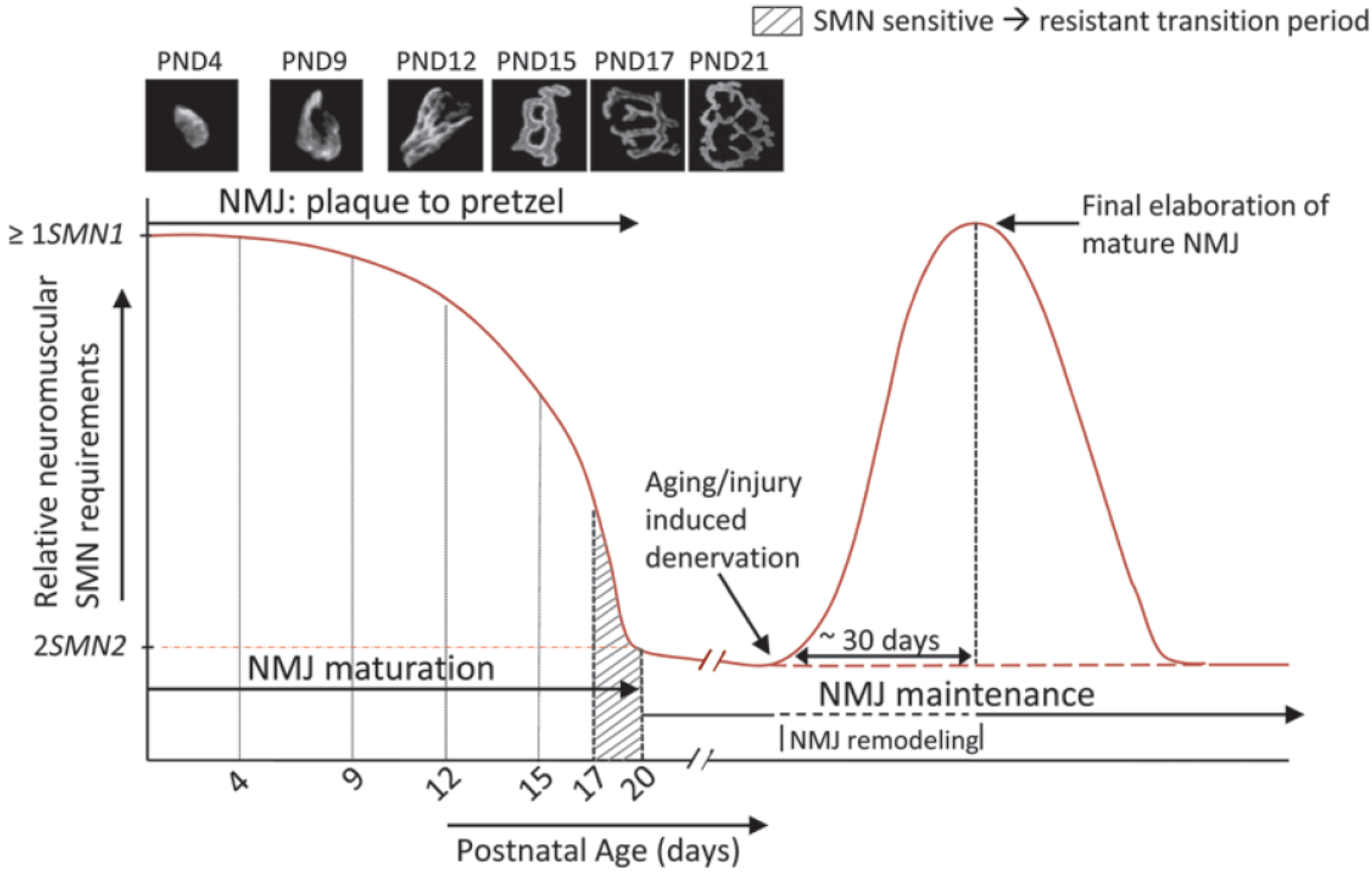
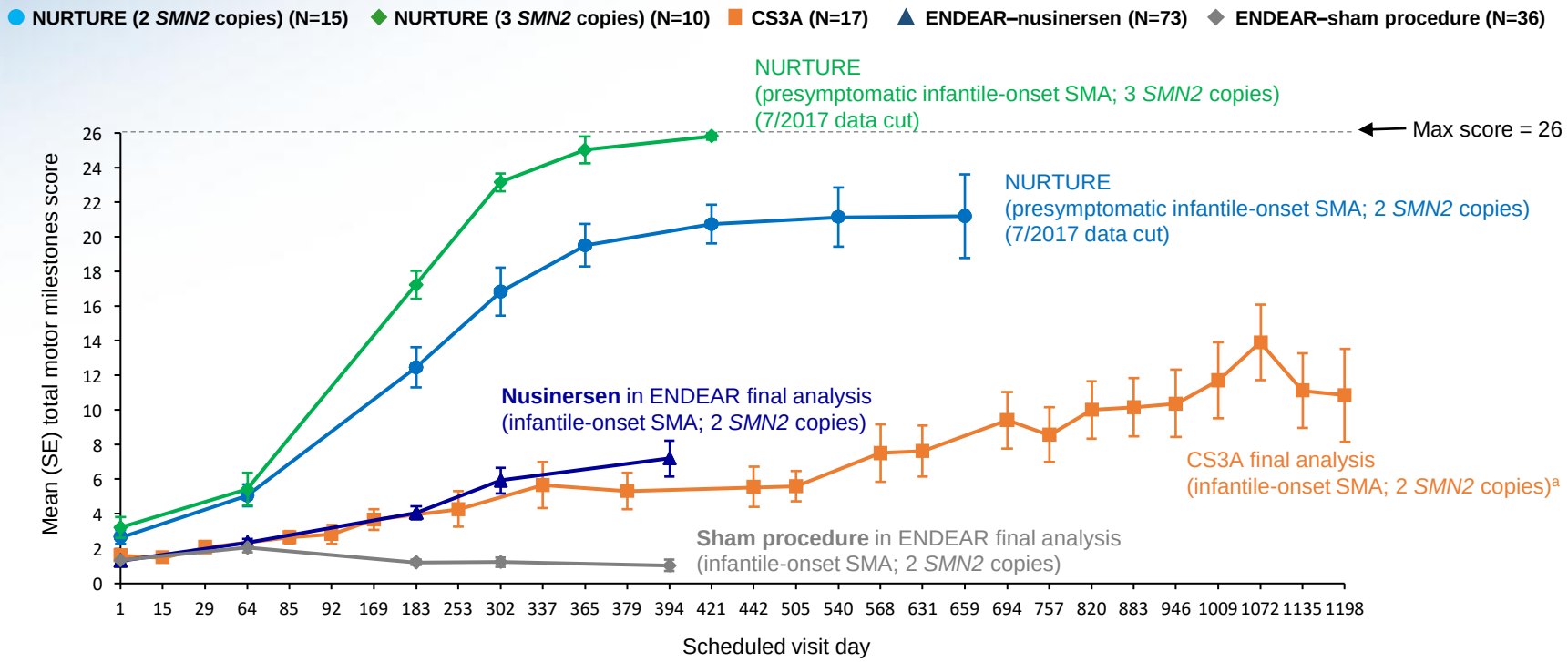


Requirement of Survival Motorneuron protein imposed during neuromuscular junction maturation

Shingo Kariya,[1,2] Teresa Obis,[3] Caterina Garone,[4,5] Turgay Akay,[2,6] Fusako Sera,[7] Shinichi Iwata,[7] Shunichi Homma,[7] and Umrao R. Monani[1,2,4]

HINE Motor Milestone Scores Over Time Across Studies

- The greatest improvements in total HINE Section 2 motor milestones were observed in infants treated with nusinersen in the presymptomatic stage of SMA in NURTURE



NURTURE (2 *SMN2* copies)	15			15			15		12		12		11			7			5												
NURTURE (3 *SMN2* copies)	10			10			9		7		5		5																		
CS3A (2 *SMN2* copies)	17	17	16		17	16	15		15		12		13		11	12		10	13		13	13	13	13	11	10	9	9	6			
ENDEAR–nusinersen	73			66			59		36		26																				
ENDEAR–sham procedure	36			29			22		15		10																				

NURTURE study interim analysis data cutoff date: July 5, 2017. [a]CS3a end of study data for the cohort of infants with 2 *SMN2* copies.

Courtesy Dr. E. Bertini

Table 2 Clinical and molecular data of 10 Spanish SMA patients with homozygous absence of the *SMN1* gene and with the c.859G>C variant in the *SMN2* gene

Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sex	**Male**	**Male**	**Male**	**Male**	**Male**	**Male**	**Male**	**Male**	**Male**	**Female**
Age (years)	65	36	22	59	34	18	30	12	5	3
SMA type	IIIb	IIIb	IIIb	IIIb	IIIb	IIIa	II	II	II	II
Age at onset of weakness (months/years)	15 years	14 years	4 years	14 years	13 years	<3 years	7 months	8–9 months	12 months	14 months
Walked unaided	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Wheelchair bound (age)	Yes (59 years)	No	No	Recently*	Recently*	Yes (6 years)†	Yes‡	Yes‡	Yes‡	–§
SMN2 copies	2	2	2	3	3	2	2	2	2	2
c.859G>C in SMN2	Homoz.	Homoz.	Homoz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.
Telomeric NAIP	+	–	–	+	–	–	–	–	–	–
Parental inheritance	NA	Both	NA	NA	NA	Maternal	Paternal	NA	Paternal	Maternal
C272 alleles	**193**	**193**	**193**	189 191 **193**	181 189 **193**	181 **193**	183 **193**	181 **193**	183 **193**	181 **193**
C212 alleles	**225**	**227**	**227**	**225** **227** 233	219 221 **225**	217 **227**	217 **225**	215 **227**	217 **227**	215 **227**

NAIP + indicates at least one copy of the *NAIP* gene.
Alleles associated with the c.859G>C variant for C272 (Ag1-CA) and C212 markers are in bold.
*Used only to cover long distances.
†This patient had an affected sister who died at the age of 6 due to pneumonia (further clinical data not available).
‡Never walked.
§This patient is not yet using wheelchair because of her age although was never able to walk unaided.
NA, parents were not available for study.

A Positive Modifier of Spinal Muscular Atrophy in the *SMN2* Gene

Thomas W. Prior,[1,4,*] Adrian R. Krainer,[2] Yimin Hua,[2] Kathryn J. Swoboda,[3] Pamela C. Snyder,[1] Scott J. Bridgeman,[1] Arthur H.M. Burghes,[4,5] and John T. Kissel[4]

The American Journal of Human Genetics *85*, 408–413, September 11, 2009

A Rare *SMN2* Variant in a Previously Unrecognized Composite Splicing Regulatory Element Induces Exon 7 Inclusion and Reduces the Clinical Severity of Spinal Muscular Atrophy

Myriam Vezain[1], Pascale Saugier-Veber[1-2], Elisa Goina[3], Renaud Touraine[4], Véronique Manel[5], Annick Toutain[6], Séverine Fehrenbach[1-2], Thierry Frébourg[1-2], Franco Pagani[3], Mario Tosi[1], and Alexandra Martins[1,*]

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

HUMAN MUTATION Mutation in Brief 31: E1110-E1125 (2010)

The c.859G>C variant in the *SMN2* gene is associated with types II and III SMA and originates from a common ancestor

S Bernal,[1] L Alías,[1] M J Barceló,[1] E Also-Rallo,[1] R Martínez-Hernández,[1] J Gámez,[2] E Guillén-Navarro,[3] J Rosell,[4] I Hernando,[5] F J Rodríguez-Alvarez,[6] S Borrego,[7] J M Millán,[8] C Hernández-Chico,[6] M Baiget,[1] P Fuentes-Prior,[9] E F Tizzano[1]

J Med Genet 2010;**47**:640–642.

SMA genetic modifiers

Table 1
Cross-species modifiers of Spinal Muscular Atrophy

Human Gene	*Ce* gene	*Dm* Gene	Modifies *Ce* SMA	Modifies *Dm* SMA	Modifies Human SMA	Function
PLS3	plst-1	Fim	x	x	x	Formation and stabilization of F-actin bundles
NCBP2L	*ncbp-2*	Cbp20	x	x		Nuclear export of mRNA, U snRNA transport, nonsense mediated decay, miRNA maturation
NPVF	*flp-4*	Fmrf	x	x		Activation of neuropeptide gated chloride channels and G-protein coupled receptors
USO1	*uso-1*	p115	x	x		Vesicle tethering during trans-Golgi transport
PPARG	*nhr-85*	Eip75B	x	x		Nuclear hormone receptor, regulation of circadian rhythms
FGFR3	*egl-15*	Btl	x	x		FGF signaling, NMJ function and development
ATF6	*atf-6*	CG3136	x	x		Unfolded protein stress response
PPP1R13	*ape-1*	CG18375	x	x		Prevention of inappropriate apoptosis
NEK2	*nekl-3*	Nek2	x	x		Mitotic regulation
ACTN	*atn-1*	actinin	x	x		Actin-bundling
STRN	*cash-1*	CKA	x	x		Calveolin and calmodulin-binding
DYNLL2	*dlc-1*	ctp	x	x		Intracellular trafficking, regulation of dynamin, F-actin assembly, transport of TGFβ
RNF149	*kcnl-2*	SK	x	x		Potassium channel subunit
BMPR2	*daf-4*	Wit	x	x		TGFβ receptor subunit, cell specification
RXRA	*nhr-25*	Usp	x	x		Ecdysone regulated molting, activation of Smad2 in muscles

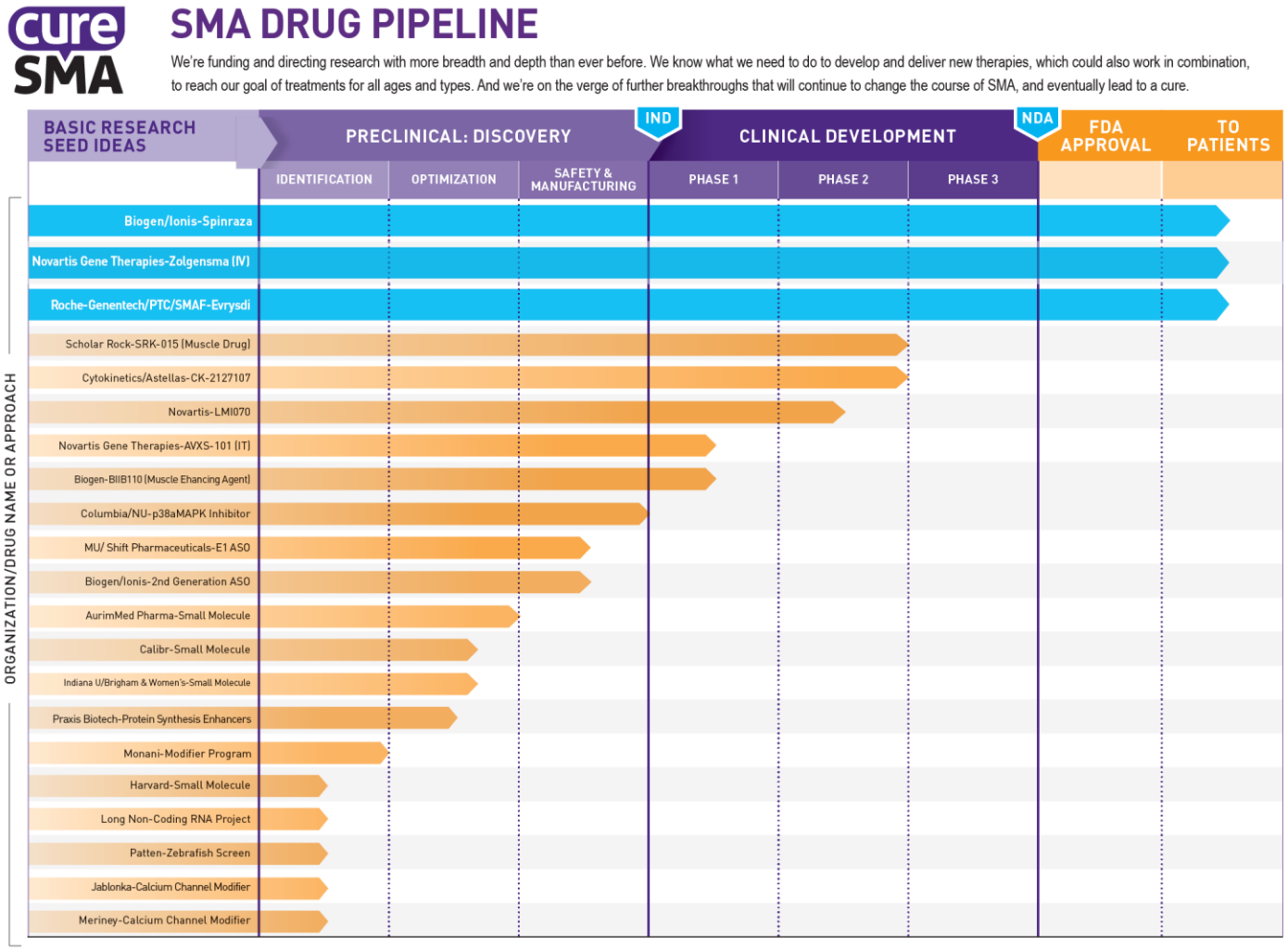
SMA: Spinal muscular atrophy. *Dm: Drosophila melanogaster. Ce: Caenorhabditis elegans.*

Nusinersen

Onasemnogene abeparvovec

Risdiplam

Apitegromab (SRK 015)



Therapeutic pipeline

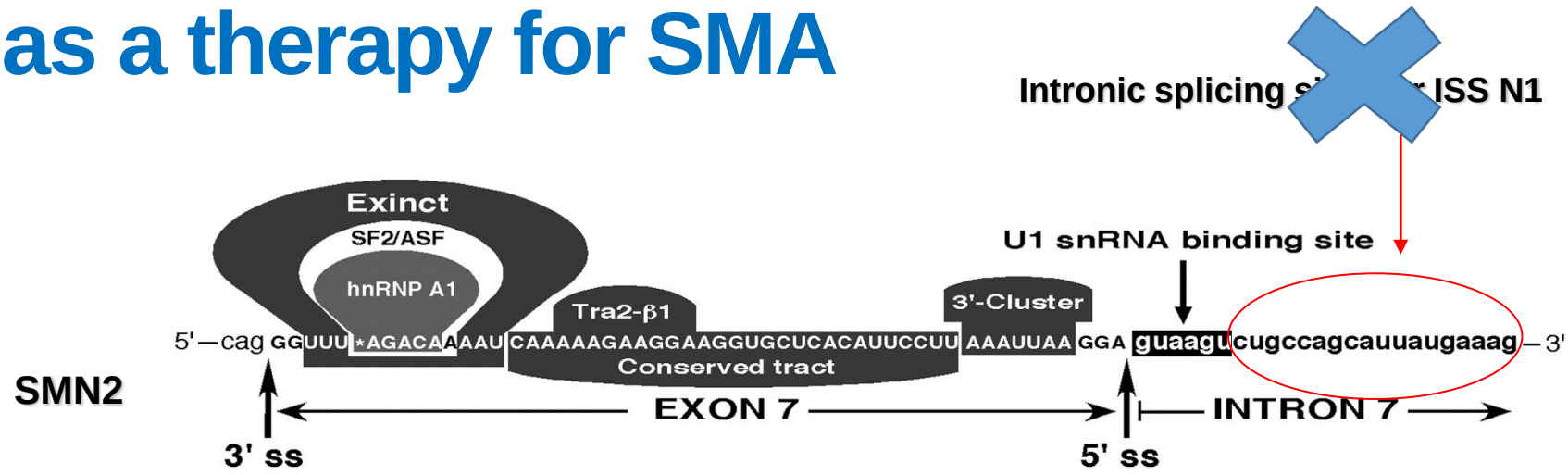
	NOVARTIS Onasemnogene abeparvovec	Biogen Nusinersen	NOVARTIS Branaplam	Roche Risdiplam	Cytokinetics Reldesemtiv	Catalyst pharmaceuticals Amifampridine phosphate	Scholar Rock SRK-015
	Approved for use in US[1], EU[2] & Japan[3]	*Approved for use[8,9]*	*In clinical development[14]*	*Approved in USA[21]*	*In clinical development[26]*	*In clinical development[27]*	*In clinical development[29]*
Drug type	**Gene therapy[1–3]**	**ASO[8]**	**Small molecule[14–19]**			**Small molecule[28]**	**Monoclonal antibody[29]**
Body distribution	**Systemic[1–3]**	**CNS[8]**	**Systemic[14–18]**			**Systemic[27]**	**Systemic[29]**
Delivery method	**IV[1–3]**	**Intrathecal[8]**	**Oral[14–18,26]**			**Oral[27]**	**IV[29]**
Dosing	**One time[1–3]**	**4 loading doses then once every 4 months[8]**	**Once weekly[14]**	**Once daily[18]**	**Twice daily[26]**	**Under investigation[27]**	**Every 4 weeks[29]**
Current target population	**Varies according to region[1–3]**	**Approved all types[8]**	**Type I[20]**	**Type I–III[16–18]**	**Type II–IV[26]**	**Type III[27]**	**Type II or III[29]**
Clinical trials	START[4] SPR1NT[5] STR1VE[6] STR1VE-EU[7]	**ENDEAR[10]** **CHERISH[11]** **NURTURE[12]** **SHINE[13]**	**NCT02268552[14]**	FIREFISH[22] SUNFISH[23] JEWEL FISH[24] RAINBOW FISH[25]	**NCT02644668[26]**	**NCT03781479[27]**	TOPAZ[29]

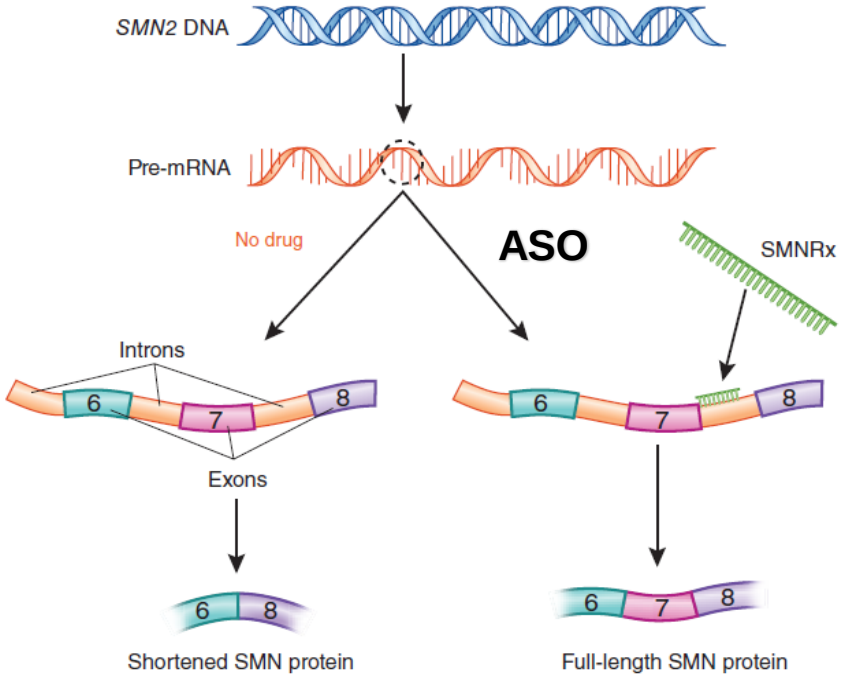
ASO, antisense oligonucleotide; CNS, central nervous system; DNA, deoxyribonucleic acid; FDA, U.S. Food and Drug Administration; IV, intravenous; RNA, Ribonucleic acid; SMA, spinal muscular atrophy; SMN, survival motor neuron.

1. FDA (2019). Onasemnogene abeparvovec US PI. Available at: https://www.fda.gov/media/126109/download. Last accessed: November 2020; 2. AveXis (2020). Onasemnogene abeparvovec Summary of Product Characteristics. Available at: https://www.ema.europa.eu/en/documents/product-information/zolgensma-epar-product-information_en.pdf. Last accessed: November 2020; 3. Reuters (2020). BRIEF-Novartis Receives Approval From Japan For Onasemnogene abeparvovec Gene Therapy. Available at: https://www.reuters.com/article/brief-novartis-receives-approval-from-ja-idUSFWN2BC03D. Last accessed: November 2020; 4. ClinicalTrials.gov Identifier: NCT02122952; 5. ClinicalTrials.gov Identifier: NCT03505099; 6. ClinicalTrials.gov Identifier: NCT03306277; 7. ClinicalTrials.gov Identifier: NCT03461289; 8. FDA (2020). Spinraza US PI. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/209531lbl.pdf. Last accessed: November 2020; 9. Biogen (2017). Spinraza (nusinersen) Summary of Product Characteristics; 10. ClinicalTrials.gov Identifier: NCT02193074; 11. ClinicalTrials.gov Identifier: NCT02292537; 12. ClinicalTrials.gov Identifier: NCT02386553; 13. ClinicalTrials.gov Identifier: NCT02594124; 14. ClinicalTrials.gov Identifier: NCT02268552; 15. Sumner CJ, Crawford TO. *J Clin Invest.* 2018;128(8):3219-3227; 16. American Academy of Neurology Annual Meeting (AAN) 2018 Annual Meeting. Press Release: https://www.aan.com/PressRoom/Home/GetDigitalAsset/12693; 17. Mercuri E, et al. *Neurology.* 2018;90:s15; 18. Chiriboga C, et al. Presented at WMS 2018 [P.257]; 19. Calder AN, et al. *J Med Chem.* 2016.23;59(22):10067-10083; 20. Parente V and Cortis S. *Ther Adv Neurol Disord.* 2018;11:1-13; 21. FDA (2020). FDA Approves Oral Treatment for Spinal Muscular Atrophy. Available at: https://www.fda.gov/news-events/press-announcements/fda-approves-oral-treatment-spinal-muscular-atrophy. Last accessed: November 2020; 22. ClinicalTrials.gov Identifier: NCT02913482; 23. ClinicalTrials.gov Identifier: NCT02908685; 24. ClinicalTrials.gov Identifier: NCT03032172; 25. ClinicalTrials.gov Identifier: NCT03779334; 26. ClinicalTrials.gov Identifier: NCT02644668; 27. ClinicalTrials.gov Identifier: NCT03781479; 28. Adis Insight. Amifampridine - BioMarin. Available at: https://adisinsight.springer.com/drugs/800027190. Last accessed: November 2020; 29. ClinicalTrials.gov Identifier NCT03921528.

Disease-modifying treatment
Intrathecal ASO

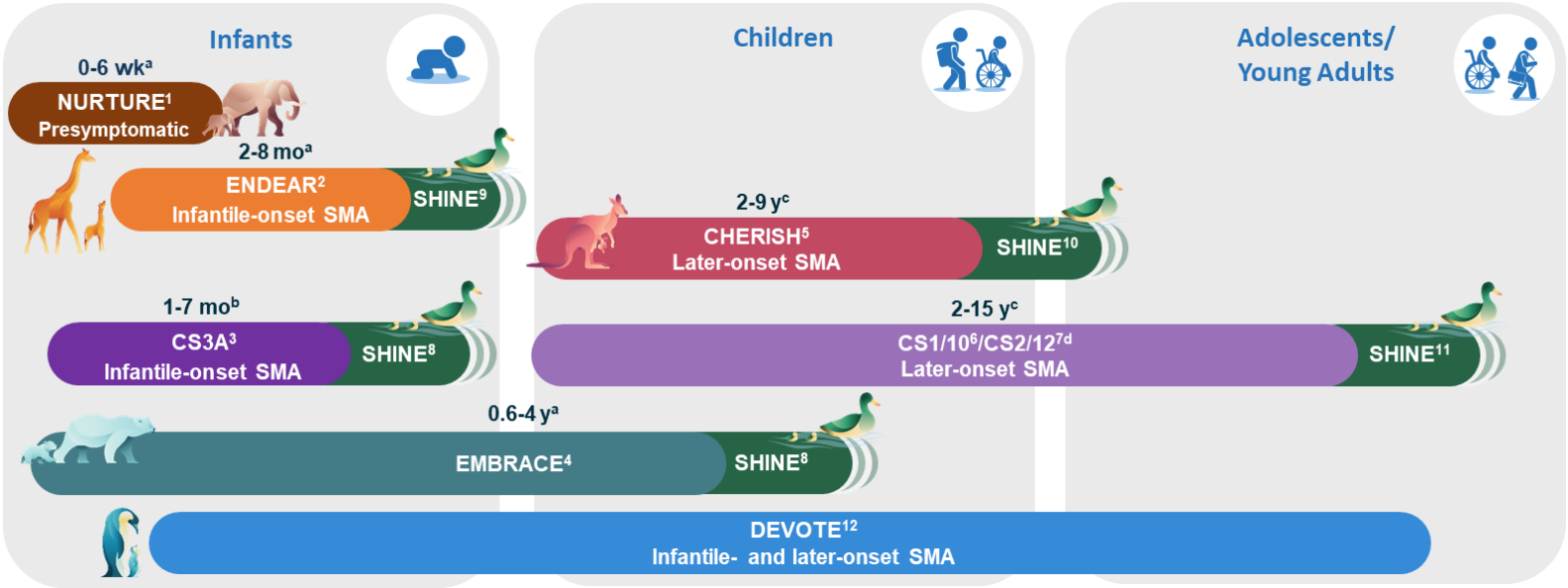
Antisense oligonucleotides as a therapy for SMA





Overview of the Nusinersen Clinical Trial Program

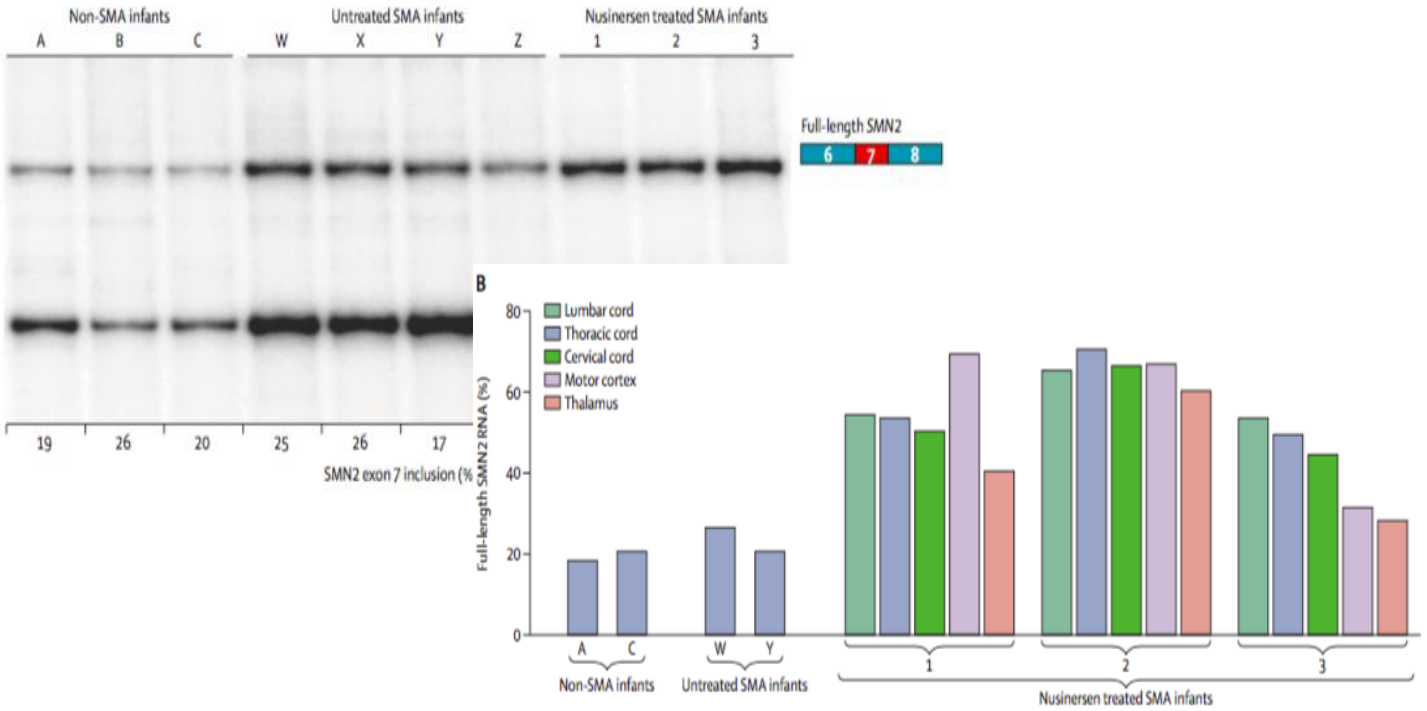
There are robust data on nusinersen efficacy and safety in infants, children, and adolescents/young adults



a Age at first dose. b Age at enrollment. c Age at screening. d Only participants who enrolled in CS2 are included in the CS12 integrated analysis.

1. De Vivo DC, et al; NURTURE Study Group. Neuromuscul Disord. 2019;29(11):842-856. 2. Finkel RS, et al; ENDEAR Study Group. N Engl J Med. 2017;377(18):1723-1732. 3. Finkel RS, et al. Lancet. 2016;388(10063):3017-3026. 4. Castro D, et al. CSMA 2019. P7. 5. Mercuri E, et al; CHERISH Study Group. N Engl J Med. 2018;378(7):625-635. 6. Haché M, et al. J Child Neurol. 2016;31(7):899-906. 7. Darras BT, et al; ISIS-396443-CS2/ISIS-396443-CS12 Study Groups. Neurology. 2019;92(21):e2492-e2506. 8. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT02594124. Accessed June 15, 2020. 9. Castro D, et al. Neurology. 2020;94(15 suppl):1640. 10. Chiriboga CA, et al. Neurology. 2020;94(15 suppl):166. 11. Day JW, et al. Neurology. 2020;94(15 suppl):1132. 12. Finkel RS, et al. Neurology. 2020;94(15 suppl):1169.

Nusinersen Phase II study



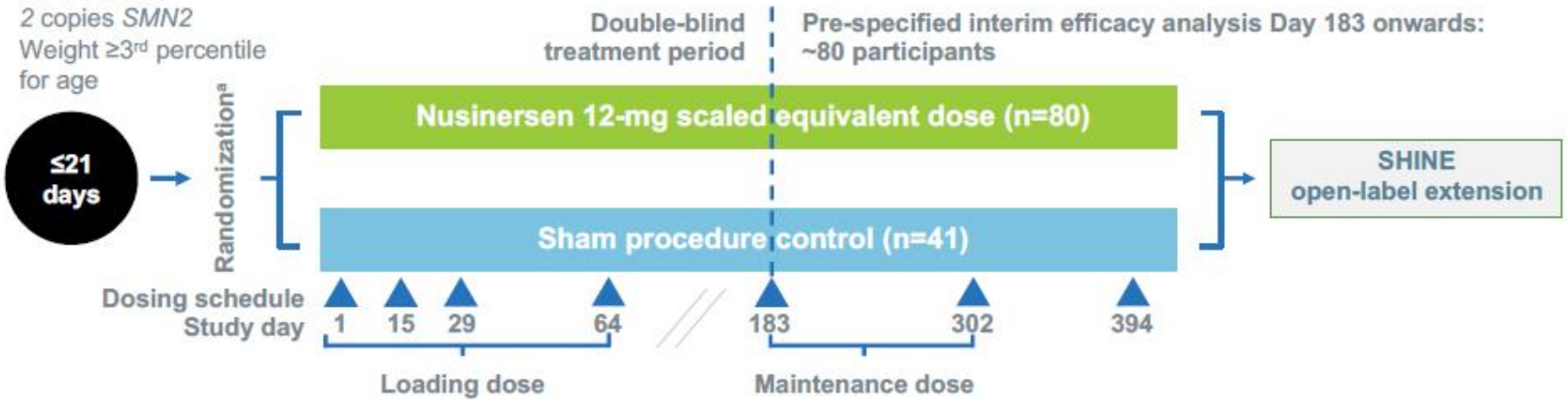
Finkel et al., Lancet Neurol, 2016

Let's start with the label qualifying study: ENDEAR

Phase 3, double blind, sham controlled study in infantile onset SMA[1-3]

Screening

- Gestational age 37–42 weeks
- 2 copies *SMN2*
- Weight ≥3rd percentile for age



[a] Randomization was stratified by disease duration during screening (age at screening minus age at symptom onset): ≤12 vs. >12 weeks

Interim efficacy set: ITT participants who received nusinersen dose/sham procedure control ≥6 months before cut-off date for interim analysis and/or were assessed at any of the Day 183, 302, or 394 visits. Interim efficacy analysis was conducted on June 15, 2016, once ~80 participants had the opportunity to be assessed at the Day 183 visit.

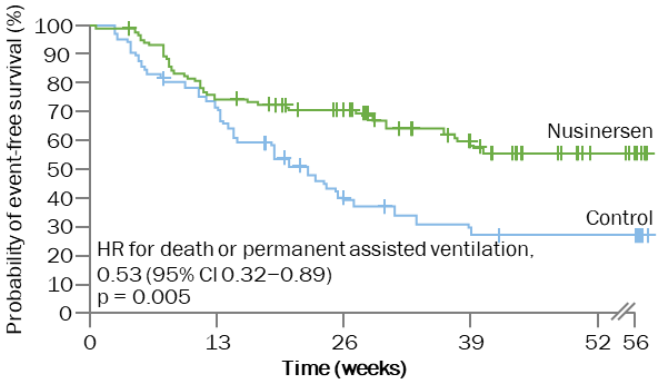
Efficacy set: All infants who received nusinersen dose/sham procedure control ≥6 months before cut-off date for final analysis and/or were assessed at any of the Day 183, 302, or 394 visits.

Figure not to scale. ITT, intention-to-treat; SMA, spinal muscular atrophy, SMN, survival motor neuron.

1. NCT02193074. Available at www.clinicaltrials.gov. 2. Kuntz N, et al. Final Results of the Phase 3 ENDEAR Study Assessing the Efficacy and Safety of Nusinersen in Infants With Spinal Muscular Atrophy (SMA). Presented at 69th Annual Meeting of the American Academy of Neurology, April 22–28 2017, Boston, MA, USA. 3. Finkel R, et al. N Engl J Med 2017;377:1723–1732.

The registration study in early onset: ENDEAR

Phase 3, double blind, sham-controlled study in infantile-onset SMA, 2 *SMN2* (N=121), mean age at dosing 5.4 months



Number at risk:

Nusinersen	80	59	46	29	16 13
Control	41	30	14	9	7 7

ENDEAR[1]
Infantile-Onset SMA
Final Analysis at 9 Months

	Nusinersen No./Total No. (%)	Sham No./Total No. (%)	p Value
Motor-milestone responders[a]	37/73 (51)	0/37	<0.0001
Event-free survival	49/80 (61)	13/41 (32)	0.005
Any AE	77/80 (96)	40/41 (98)	–

Nusinersen significantly improved survival and motor function in infants

Ongoing analyses in SHINE will continue to evaluate patients from to increase understanding of longer-term safety/tolerability and efficacy of repeated nusinersen

a HINE-2; AE = adverse event; HINE-2 = Hammersmith Infant Neurological Examination Section 2

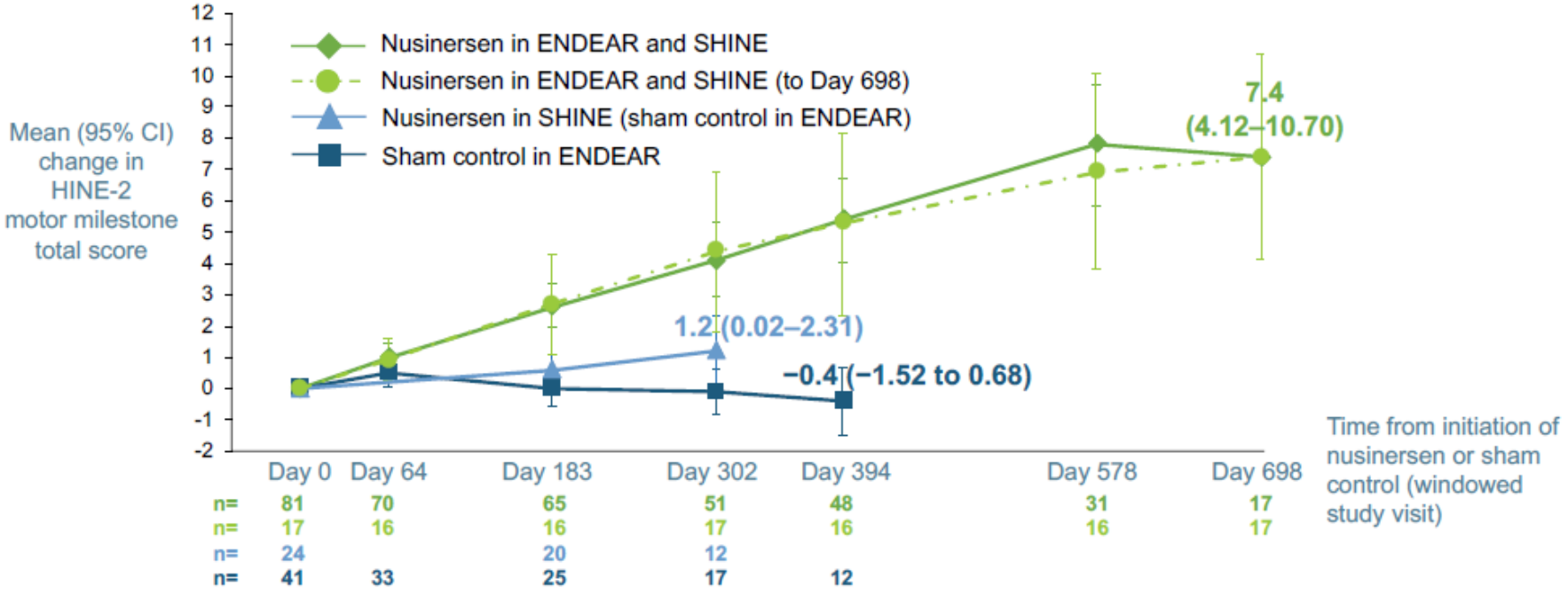
1. Finkel RS, et al; ENDEAR Study Group. N Engl J Med. 2017;377(18):1723-1732

HINE-2 score change over time

ENDEAR

SHINE

ENDEAR (end of study) and SHINE:

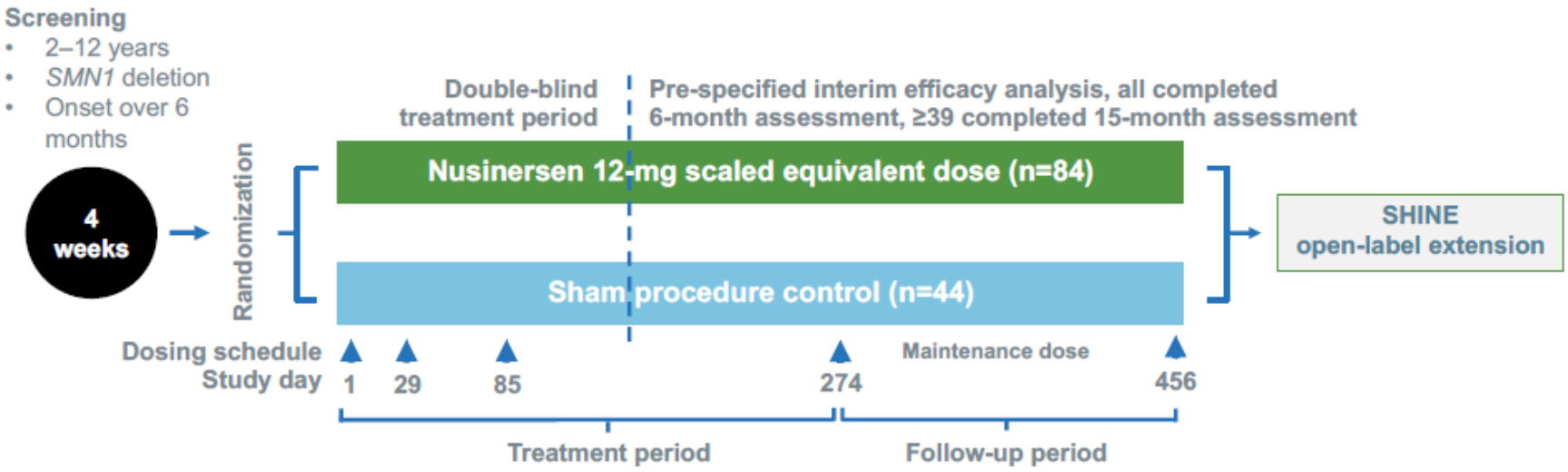


CI, confidence interval; HINE, Hammersmith infant neurological examination.

Finkel RS, et al. Longer-term Assessment of the Safety and Efficacy of Nusinersen for the Treatment of Infantile-Onset Spinal Muscular Atrophy (SMA): An Interim Analysis of the SHINE Study. Presented at 15th International Congress on Neuromuscular Diseases (ICNMD), July 6–10, 2018, Vienna, Austria.

Next up, the older kids: CHERISH

Phase 3, double blind, sham controlled study in later onset SMA[1,2]



ITT set: All children who were randomized and received ≥1 dose of study drug/sham procedure control
Efficacy set: Infants who were assessed at the Month 15 (D456) visit

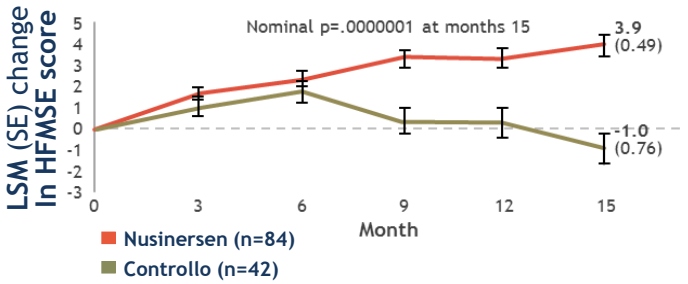
ITT, intention-to-treat; SMA, spinal muscular atrophy, SMN, survival motor neuron.
1. Mercuri E, et al. N Engl J Med. 2018;378:625–635. 2. NCT02292537. Available at www.clinicaltrials.gov.

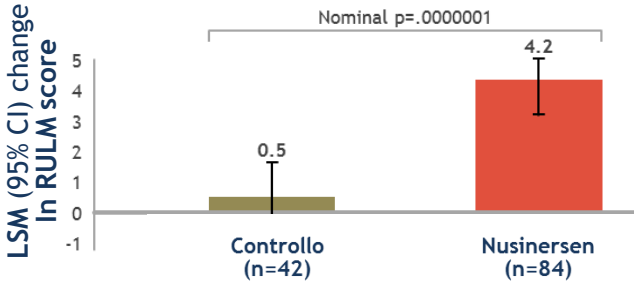
The registration study in later onset: CHERISH

Phase 3, double blind, sham-controlled study in infantile-onset SMA, 2 or 3 SMN2 (N=126)

CHERISH[1]
Later-Onset SMA
Final Analysis at 15 Months

	Nusinersen (N = 84)	Sham (N = 42)	p Value
Improved motor function[b]	+3.9	−1.0	<0.0001
Any AE	93%	100%	–





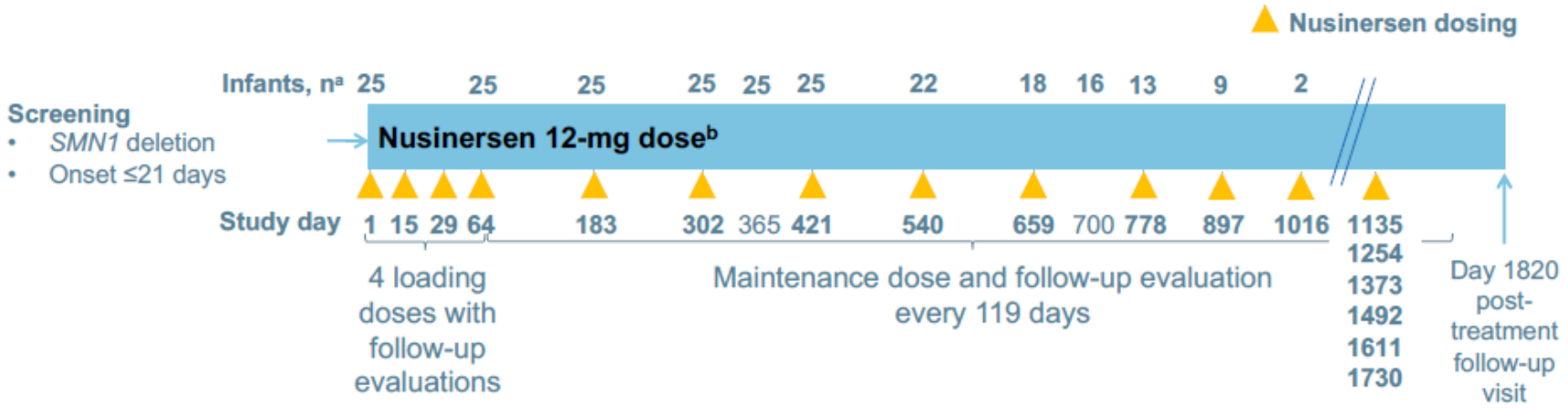
HFMSE = Hammersmith Functional Motor Scale – Extended.

1. Mercuri E, et al; CHERISH Study Group. *N Engl J Med*. 2018;378(7):625-635.

And finally, the youngest…

NURTURE

Phase 2 study in pre-symptomatic newborns with genetic diagnosis of SMA

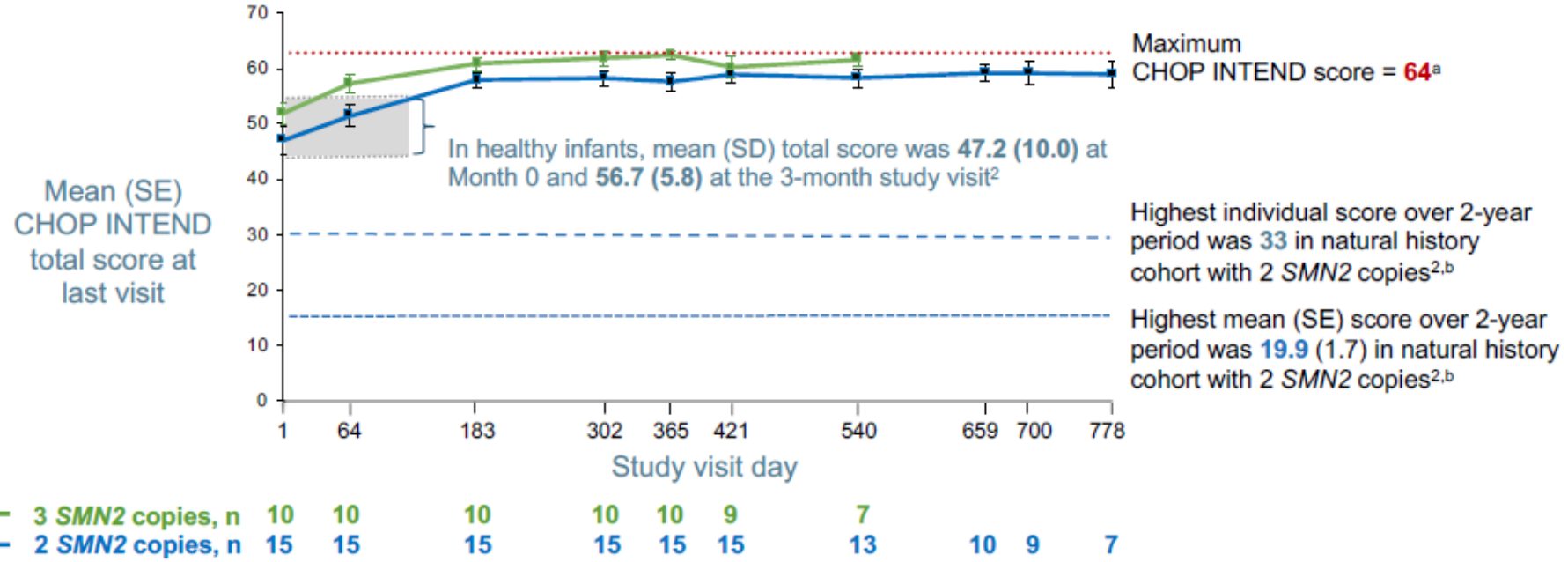


[a] Infants who attended or who had the opportunity to attend the visit.
[b] Infants treated with nusinersen 12 mg; some infants received a 12-mg scaled equivalent dose before the protocol was revised in March 2017.
Figure not to scale. SMA, spinal muscular atrophy.
Swoboda KJ, et al. Nusinersen in infants who initiate treatment in a presymptomatic stage of spinal muscular atrophy (SMA): interim efficacy and safety results from the Phase 2 NURTURE study. Presented at the 23rd International Annual Congress of the World Muscle Society (WMS), Oct 2–6, 2018. Mendoza, Argentina.

Mean CHOP INTEND total score over time

NURTURE

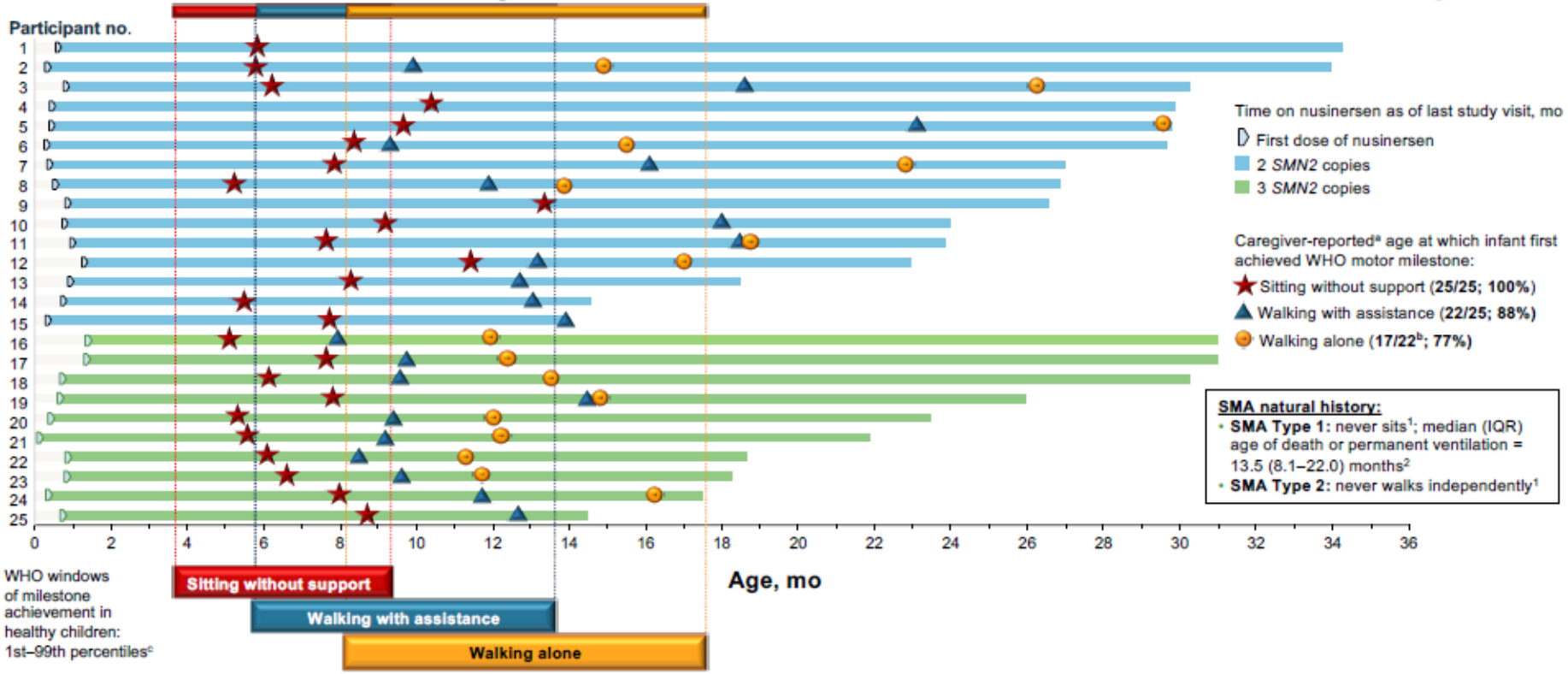
3 *SMN2* copies = 62.6 (58, 64); 2 *SMN2* copies = 61.0 (46, 64)[1]



[a] Per version 6 of the study protocol, CHOP INTEND was assessed in participants until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed.
[b] Infants were aged ≤6 months at enrollment, born between 36 and 42 weeks' gestation, and had genetically confirmed SMA; infants were excluded if they required non-invasive ventilatory support for >12 hours/day, had a comorbid illness, or were enrolled in a SMA clinical trial. NURTURE study interim analysis data cutoff date: May 15, 2018. Time points with n≥5 included.
CHOP INTEND, Children's Hospital of Philadelphia infant test of neuromuscular disorders; Max, maximum; SD, standard deviation, SE, standard error; SMN, survival motor neuron.

1. De Vivo DC, et al. Nusinersen in infants who initiate treatment in a presymptomatic stage of spinal muscular atrophy (SMA): interim efficacy and safety results from the Phase 2 NURTURE study. Presented at Muscular Dystrophy Association Clinical Conference. March 11–14, 2018. Arlington, VA, USA. 2. Kolb SJ, *et al*; NeuroNEXT Clinical Trial Network on behalf of the NN101 SMA Biomarker Investigators. Ann Neurol. 2017;82(6):883–891.

Participants are alive without permanent ventilation and achieving WHO motor milestones – many in timeframes consistent with normal development



NURTURE study interim analysis data cut-off date: May 15, 2018.

[a] caregiver-reported achievement was confirmed by the study site at the next study visit with a yes or no response. [b] Achievement among infants with enough follow-up time = number of infants who achieved milestone divided by number of infants who achieved or were past the expected age of achievement. Infants who did not achieve but were younger than the 99th percentile for expected age of achievement were not included in the denominator. [c] WHO motor milestone windows of achievement were determined based on the WHO Multicenter Growth Reference Study windows of achievement in healthy children. WHO Multicentre Growth Reference Study Group. Acta Paediatr Suppl. 2006;450:86–95.

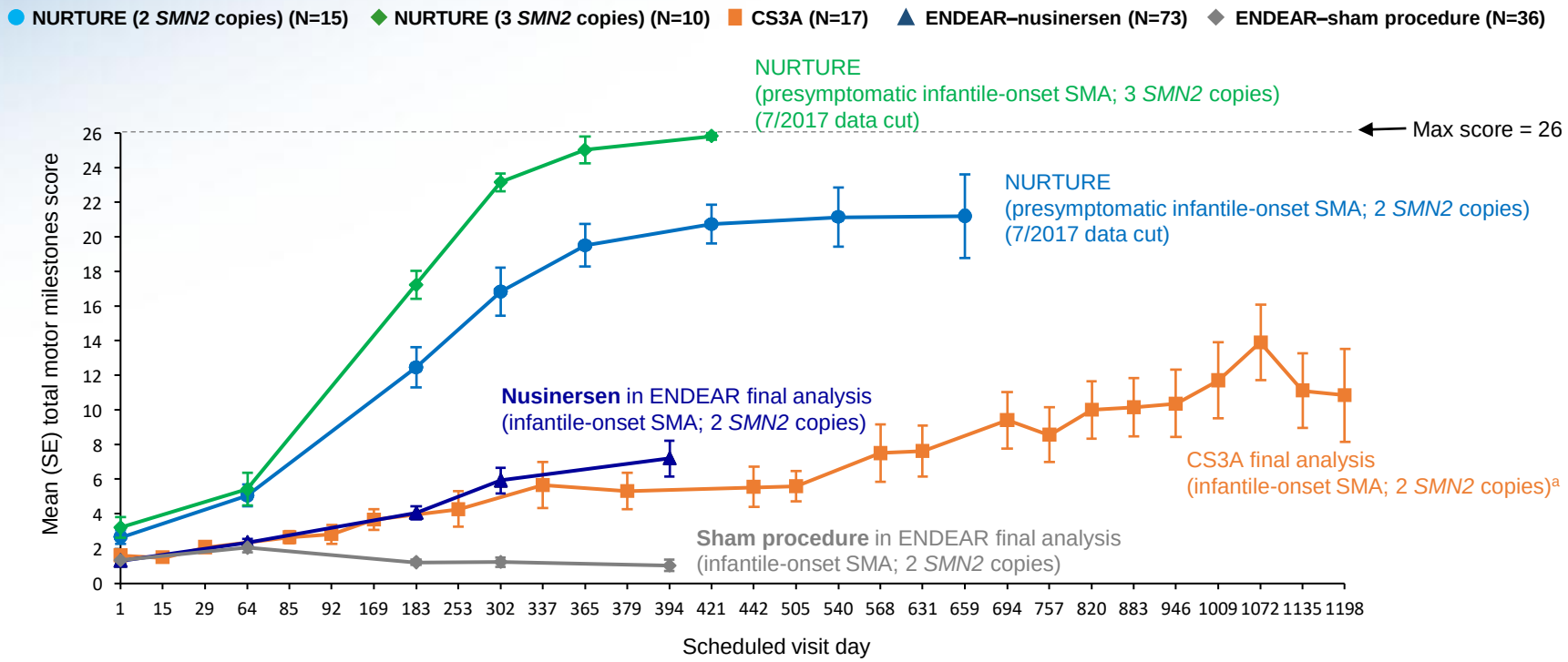
IQR, interquartile range; Mo, month; SMA, spinal muscular atrophy; SMN, survival motor neuron; WHO, World Health Organization

1. Finkel R, et al; ENMC SMA Workshop Study Group. Neuromuscul Disord. 2015;25(7):593–602. 2. Finkel RS, et al. Neurology. 2014;83(9):810–817.

De Vivo, Bertini et al Neruromuscular disorders. 12 Oct 2019 on line

HINE Motor Milestone Scores Over Time Across Studies

- The greatest improvements in total HINE Section 2 motor milestones were observed in infants treated with nusinersen in the presymptomatic stage of SMA in NURTURE

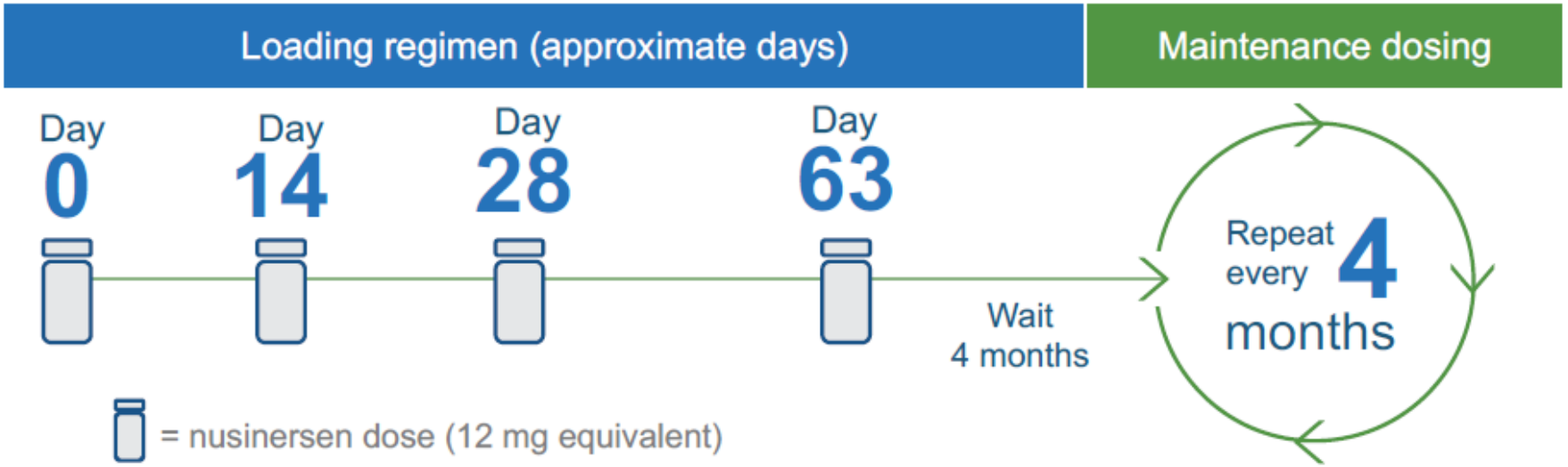


NURTURE (2 *SMN2* copies)	15			15			15		12		12		11			7			5												
NURTURE (3 *SMN2* copies)	10			10			9		7		5		5																		
CS3A (2 *SMN2* copies)	17	17	16		17	16	15		15		12		13		11	12		10	13		13	13	13	13	11	10	9	9	6			
ENDEAR–nusinersen	73			66			59		36		26																				
ENDEAR–sham procedure	36			29			22		15		10																				

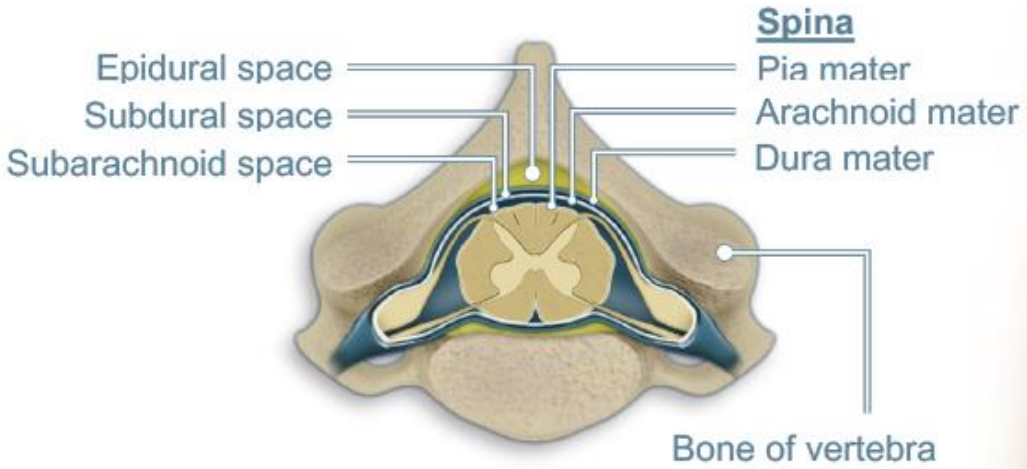
NURTURE study interim analysis data cutoff date: July 5, 2017. [a]CS3a end of study data for the cohort of infants with 2 *SMN2* copies.

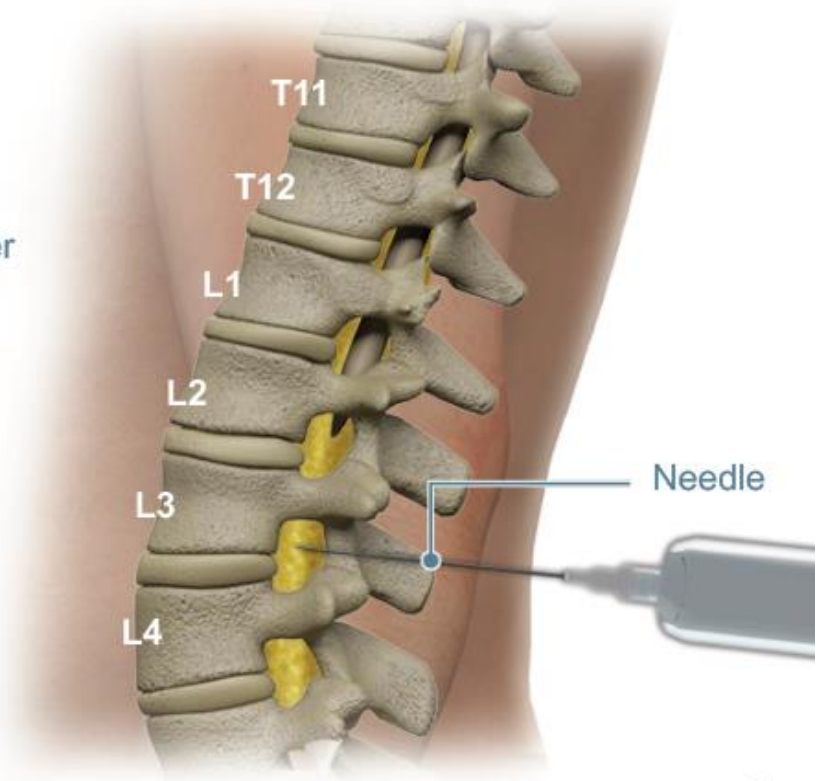
Courtesy Dr. E. Bertini

Nusinersen is administered intrathecally



Injection into the subarachnoid space[1]





Both images adapted from: Marieb & Hoehn, 2016[2]

ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

Type I SMA "new natural history": long-term data in nusinersen-treated patients

Marika Pane[1,2,a], Giorgia Coratti[1,2,a], Valeria A. Sansone[3], Sonia Messina[4], Michela Catteruccia[5], Claudio Bruno[6], Maria Sframeli[4], Emilio Albamonte[3], Marina Pedemonte[6], Adele D'Amico[5], Chiara Bravetti[2], Beatrice Berti[2], Concetta Palermo[2], Daniela Leone[2], Giorgia Brigati[6], Paola Tacchetti[6], Francesca Salmin[3], Roberto De Sanctis[2], Simona Lucibello[1,2], Maria Carmela Pera[1,2], Marco Piastra[7], Orazio Genovese[7], Enrico Bertini[5], Gianluca Vita[4], Francesco Danilo Tiziano[8], Eugenio Mercuri[1,2] & the Italian EAP Working Group

ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

Nusinersen in pediatric and adult patients with type III spinal muscular atrophy

Maria Carmela Pera[1,2,*], Giorgia Coratti[1,2,*], Francesca Bovis[3], Marika Pane[1,2], Amy Pasternak[4], Jacqueline Montes[5,6], Valeria A. Sansone[7], Sally Dunaway Young[8], Tina Duong[8], Sonia Messina[9], Irene Mizzoni[10], Adele D'Amico[10], Matthew Civitello[11,12], Allan M. Glanzman[13], Claudio Bruno[14], Francesca Salmin[7], Simone Morando[14], Roberto De Sanctis[2], Maria Sframeli[9], Laura Antonaci[1,2], Anna Lia Frongia[1], Annemarie Rohwer[15], Mariacristina Scoto[15], Darryl C. De Vivo[5], Basil T. Darras[4], John Day[8], William Martens[16], Katia A. Patanella[17], Enrico Bertini[10], Francesco Muntoni[15,18,†], Richard Finkel[11,12,†], Eugenio Mercuri[1,2,†] on behalf of the iSMAC group

Nusinersen safety and effects on motor function in adult spinal muscular atrophy type 2 and 3

Lorenzo Maggi[1], Luca Bello[2], Silvia Bonanno,[1] Alessandra Govoni,[3,4] Claudia Caponnetto,[5] Luigia Passamano,[6] Marina Grandis,[5,7] Francesca Trojsi,[8] Federica Cerri,[9] Manfredi Ferraro,[10] Virginia Bozzoni,[2] Luca Caumo,[2] Rachele Piras,[11] Raffaella Tanel,[12] Elena Saccani,[13] Megi Meneri,[3] Veria Vacchiano[14], Giulia Ricci,[4] Gianni Soraru'[2], Eustachio D'Errico,[15] Irene Tramacere,[16] Sara Bortolani,[10] Giovanni Pavesi,[17] Riccardo Zanin,[18] Mauro Silvestrini,[19,20] Luisa Politano,[6] Angelo Schenone,[5,7] Stefano Carlo Previtali[9], Angela Berardinelli,[21] Mara Turri,[22] Lorenzo Verriello,[23] Michela Coccia,[20] Renato Mantegazza,[1] Rocco Liguori,[14,24] Massimiliano Filosto[25,26], Gianni Marrosu,[27] Gabriele Siciliano,[4] Isabella Laura Simone,[15] Tiziana Mongini,[10] Giacomo Comi,[3,28] Elena Pegoraro[2]

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Age related treatment effect in type II Spinal Muscular Atrophy pediatric patients treated with nusinersen

Giorgia Coratti[a,b,1], Marika Pane[a,b,1], Simona Lucibello[a,b], Maria Carmela Pera[a,b], Amy Pasternak[c], Jacqueline Montes[d,e], Valeria A Sansone[f], Tina Duong[g], Sally Dunaway Young[g], Sonia Messina[h], Adele D'Amico[i], Matthew Civitello[j], Allan M Glanzman[k], Claudio Bruno[l], Francesca Salmin[f], Paola Tacchetti[l], Sara Carnicella[b], Maria Sframeli[h], Laura Antonaci[a,b], Anna Lia Frongia[a], Darryl C. De Vivo[d], Basil T. Darras[c], John Day[g], Enrico Bertini[i], Francesco Muntoni[m,n], Richard Finkel[j,o,2], Eugenio Mercuri[a,b,2,*], on behalf of the iSMAC group

BRIEF COMMUNICATION

Nusinersen efficacy data for 24-month in type 2 and 3 spinal muscular atrophy

Marika Pane[1,2,†], Giorgia Coratti[1,†], Maria Carmela Pera[2,†], Valeria A. Sansone[3], Sonia Messina[4], Adele d'Amico[5], Claudio Bruno[6,†], Francesca Salmin[3], Emilio Albamonte[3], Roberto De Sanctis[2], Maria Sframeli[4], Vincenzo Di Bella[4], Simone Morando[6], Concetta Palermo[2], Anna Lia Frongia[1], Laura Antonaci[1], Anna Capasso[1], Michela Catteruccia[5], Antonella Longo[5], Martina Ricci[1], Costanza Cutrona[1], Alice Pirola[3], Chiara Bravetti[1], Marina Pedemonte[6], Noemi Brolatti[6], Enrico Bertini[5], Eugenio Mercuri[1] & Italian ISMAC group

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Eugenio Mercuri, Pediatric Neurology, Università Cattolica del Sacro Cuore, Rome, Italy. Tel: 0630155340; Fax: 0630154363; E-mail: eumercuri@gmail.com

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Funding Information
Fondazione Telethon

Received: 30 December 2021; Revised: 14 January 2022; Accepted: 14 January 2022

Annals of Clinical and Translational

Abstract

The study reports real world data in type 2 and 3 SMA patients treated for at least 2 years with nusinersen. Increase in motor function was observed after 12 months and during the second year. The magnitude of change was variable across age and functional subgroup, with the largest changes observed in young patients with higher function at baseline. When compared to natural history data, the difference between study cohort and untreated patients swas significant on both Hammersmith Functional Motor Scale and Revised Upper Limb Module both at 12 months and at 24 months.

DEVOTE (232SM203) Study Design

HYPOTHESIS: Additional efficacy with SPINRAZA may be observed using a new dosing regimen that achieves higher CSF concentrations

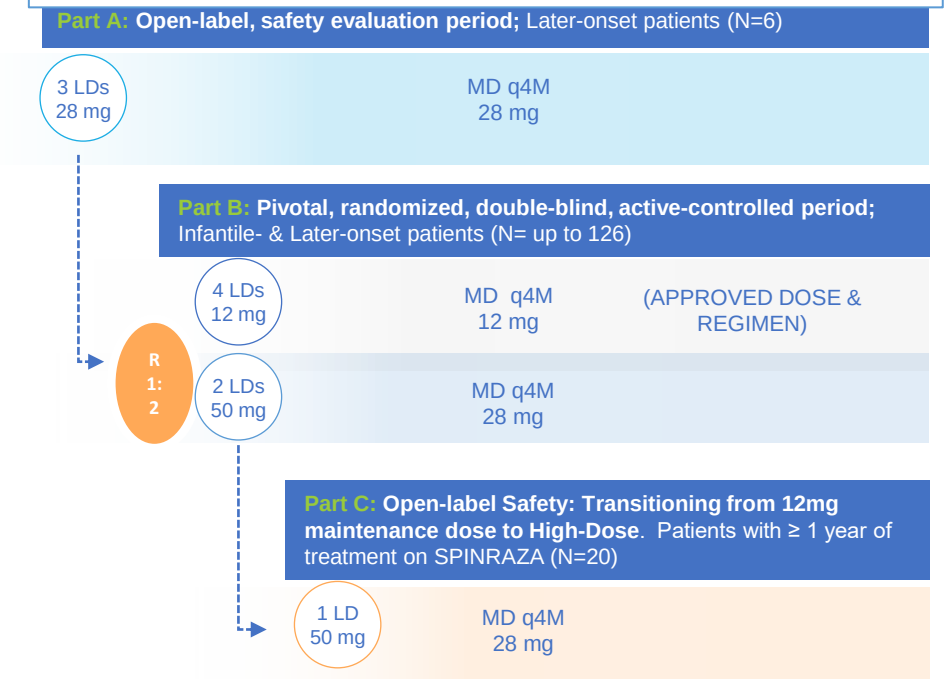
Nusinersen has been studied up to its current approved dose in clinical studies from presymptomatic to later onset patients

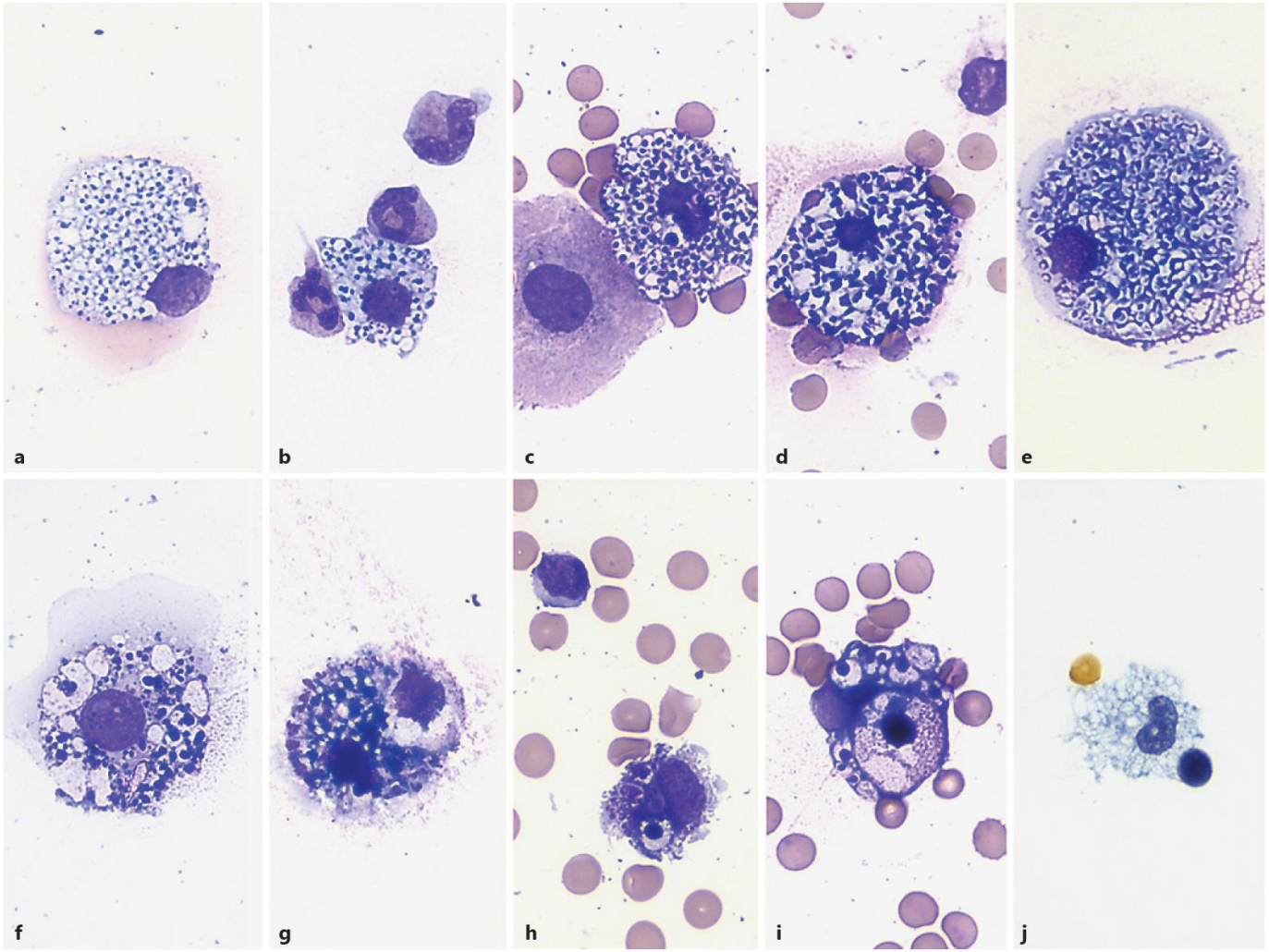
Nusinersen's current benefit/risk profile, mechanism of action and route of administration gives us the opportunity to explore a higher dose to advance the understanding of nusinersen and to study whether a higher dose has the potential to offer additional efficacy while maintaining a favorable benefit/risk profile

Study is currently ongoing; additional details around the study design and endpoints as well as information on participating study sites can be found on www.clinicaltrials.gov **(NCT04089566)**

A Phase 2/3, randomized, controlled, dose-escalating, 3-part study that will be conducted at approximately **50 sites around the world and will enroll up to 152 subjects with infantile and later-onset SMA**

DEVOTE – First Patient In (FPI) was achieved March 2020

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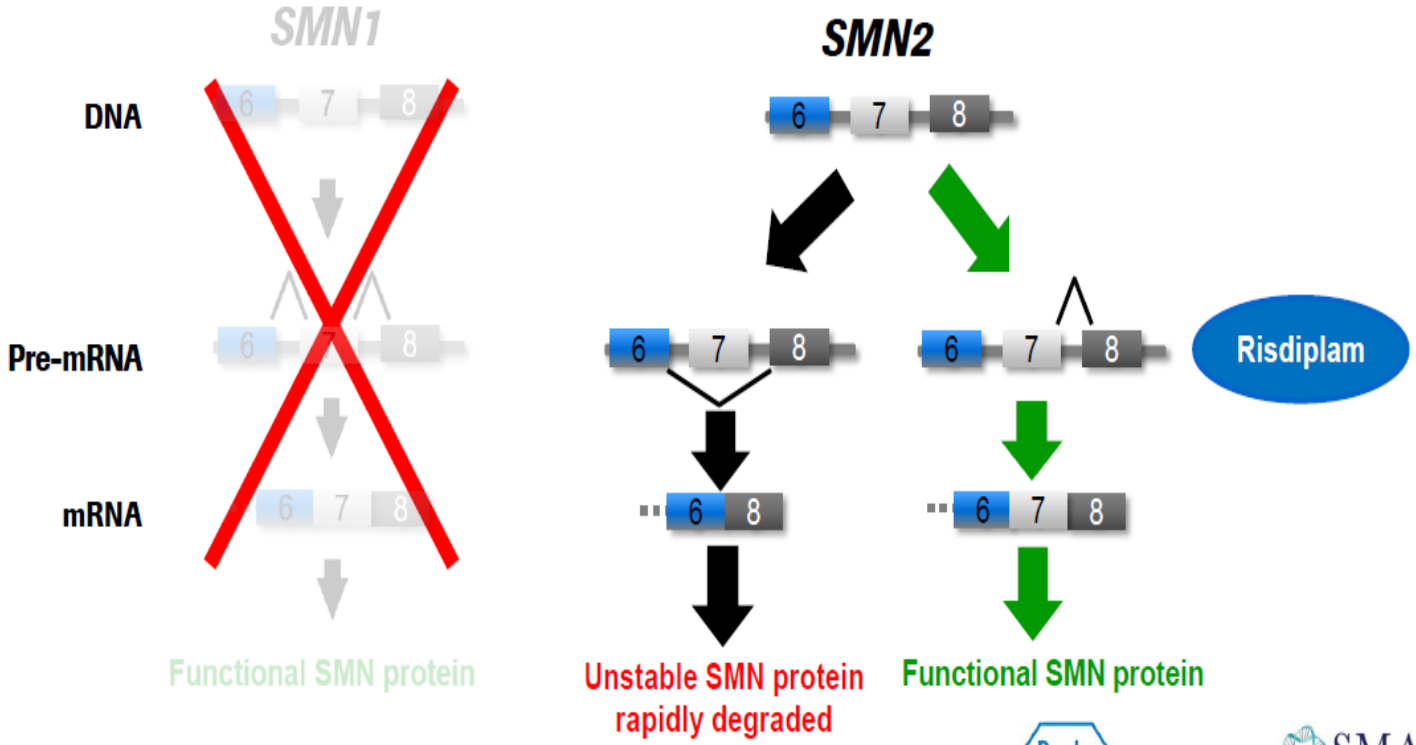
Disease-modifying treatment
Oral molecules: Risdiplam

Risdiplam: an Oral, Centrally and Peripherally Distributed Small Molecule *SMN2* Splicing Modifier

- Risdiplam (RG7916; RO7034067) is an oral, centrally and peripherally distributed small molecule *SMN2* splicing modifier
  - Modulates *SMN2* pre-mRNA splicing towards the production of full-length *SMN2* mRNA and functional SMN protein[1]



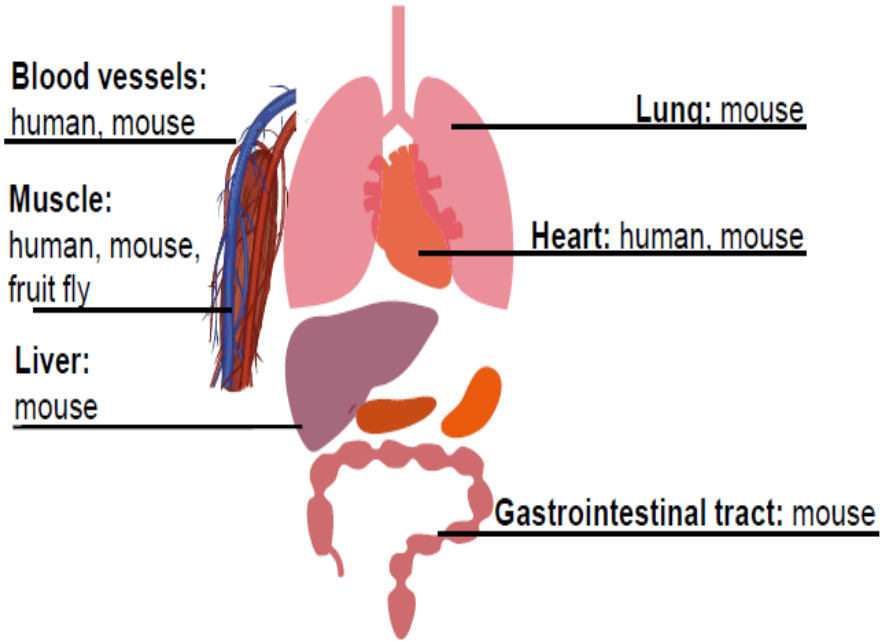
SMN, survival of motor neuron.
1. Farrar MA, *et al. Ann Neurol* 2017; 81:355–368.

Roche in collaboration with SMA FOUNDATION and PTC THERAPEUTICS

Rationale for Risdiplam: Role of SMN Protein Beyond the Motor Neuron

- Increasing evidence suggests SMN depletion directly affects cells and tissues in both the CNS and periphery[1]
  - Growing evidence of NMJ involvement[2]
  - SMN is important for skeletal muscle differentiation and function[1,3]
  - Vascular and cardiac abnormalities reported in patients with severe SMA[1,4]
- SMN protein level increases in both the central and peripheral compartments may benefit patients with SMA[5]

- Animal models: Peripheral and central SMN restoration was required for long-term rescue of a severe SMA mouse model[5]



1. Hamilton G, et al. Trends in Mol Med 2013; 19:40–50; 2. Martínez-Hernández R, et al. J Pathol 2013; 229:49–61; 3. Bricceno KV, et al. Hum Mol Genet 2014; 23:4745–4757; 4. Wijngaarde CA, et al. Orph J Rare Dis 2017; 12:67; 5. Hua Y, et al. Nature 2011; 478:123–126. CNS, central nervous system; NMJ, neuromuscular junction; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

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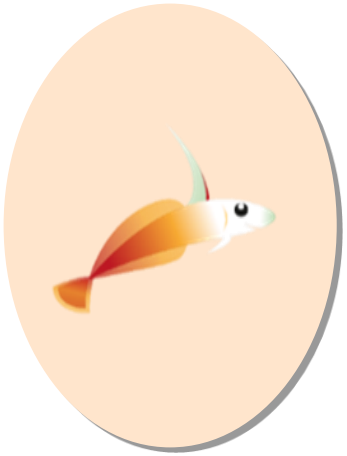
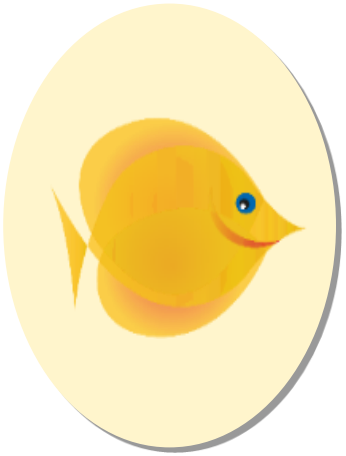
Risdiplam is Being Investigated in Several Clinical Trials

FIREFISH[1]



Type 1 SMA
1–7 months old

SUNFISH[2]

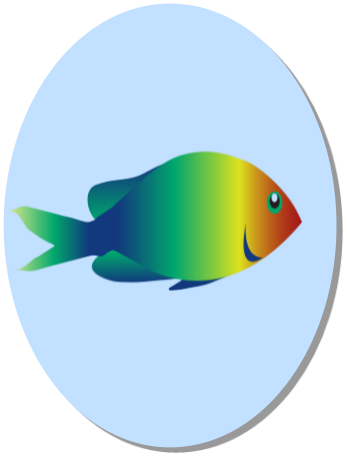


Type 2 or 3 SMA
2–25 years old

JEWELFISH[3]

Type 2 or 3 SMA
12–60 years old
Previously received other *SMN2* splicing agents

RAINBOWFISH



Planned study in pre-symptomatic SMA
Birth–6 weeks old

SMN, survival of motor neuron.
1. NCT02913482; 2. NCT02908685; 3. NCT03032172, clinicaltrials.gov (Accessed June 2018).

FIREFISH: Study overview

- FIREFISH is an open-label, multicenter clinical study
  - Part 1: Dose-finding period followed by open-label extension
    - Cohort A: Low-dose cohort (N=4)
    - Cohort B: High-dose cohort* (N=17)
  - Part 2: Efficacy and safety at the dose selected in Part 1
    - Open-label risdiplam treatment for 24 months

FIREFISH
Type 1 SMA
1–7 months old
Two *SMN2* gene copies

	Part 1 (N=21)†	Part 2 (N=41)†
Primary endpoint	• Safety, tolerability, PK and PD of risdiplam • Dose selection for Part 2	Proportion of infants sitting without support for 5 seconds after 12 months on treatment as assessed by Gross Motor Scale of the BSID-III
Secondary endpoints		Motor function (HINE-2, CHOP-INTEND), PD/PK, safety, time to death or permanent ventilation, RP

*Dose adjusted per protocol. Part 1 included multiple doses. †Actual number of infants enrolled
BSID-III, Bayley Scales of Infant and Toddler development Third Edition; CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HINE-2, Hammersmith Infant Neurological Examination Module 2; PD, pharmacodynamics; PK, pharmacokinetics; RP, respiratory plethysmography; SMA, spinal muscular atrophy.
Clinicaltrials.gov/ct2/show/NCT02913482 (Accessed October 2019).

Roche in collaboration with SMA FOUNDATION and PTC THERAPEUTICS

A multicenter, global, open-label study

12 Months | 24 Months

Risdiplam → Risdiplam → Extension

Part 2[1]

N=41

Genetic diagnosis of 5q SMA symptomatic Type 1 SMA

Two *SMN2* gene copies

1–7 months old

Primary endpoint:

- **proportion of infants sitting without support for 5 seconds at Month 12 (as assessed by the Gross Motor Scale of the BSID-III)**

Key additional endpoints:

- time to death or permanent ventilation
- achievement of motor milestones at Month 12 as measured by the HINE-2
- proportion of infants who achieve an increase of ≥4 points in the CHOP-INTEND at Month 12
- proportion of infants who achieve a score of ≥40 in the CHOP-INTEND at Month 12
- ability to swallow and feed orally at Month 12
- number of nights in hospital per infant by Month 12

Safety of risdiplam

BSID-III, Bayley Scales of Infant and Toddler Development, Third edition; CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HINE-2, Hammersmith Infant Neurological Examination, Module 2; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

1. Clinicaltrials.gov. NCT02913482 (Accessed Mar 2020).

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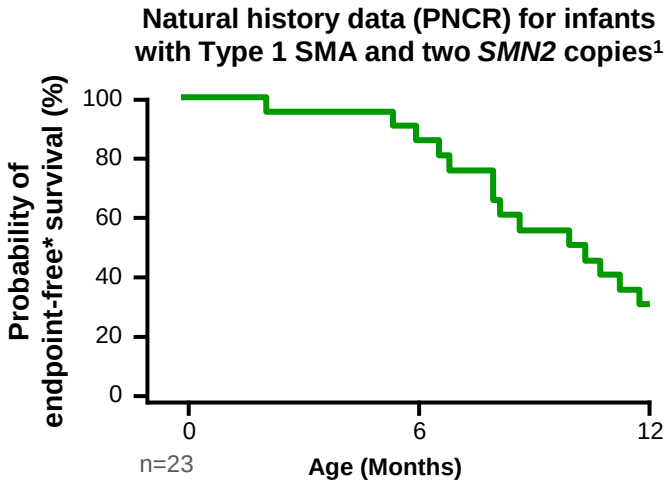
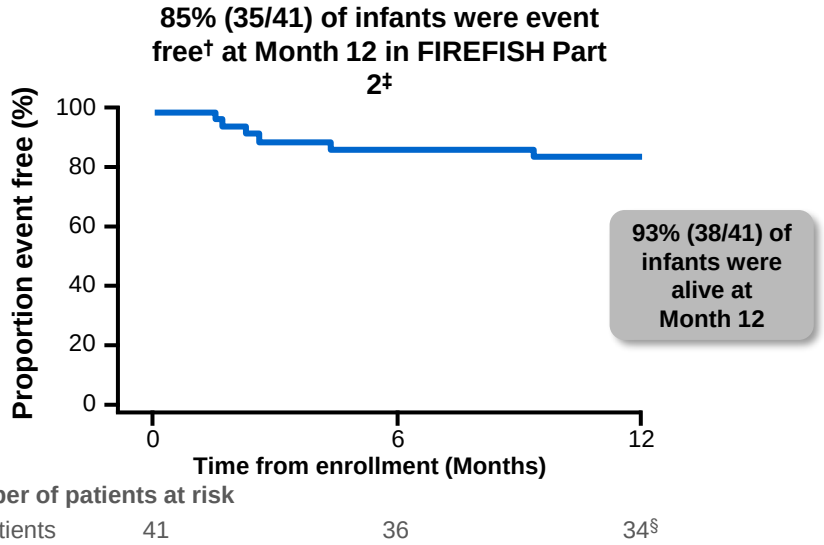
Event-free survival time was greatly improved in infants treated with risdiplam compared with natural history





In natural history, median age (IQR) for reaching death or permanent ventilation for infants with two *SMN2* copies was 10.5 (8.1–13.6) months[1]

In FIREFISH Part 2, median time to reaching death or permanent ventilation was not estimable due to lack of events

*Endpoint-free survival was defined as alive and not requiring at least 16 hours/day non-invasive ventilation support for at least 2 weeks. †Event-free in FIREFISH is defined as alive with no permanent ventilation (i.e. no tracheostomy or BiPAP ≥16 hours per day continuously for >3 weeks or continuous intubation >3 weeks, in the absence of, or following the resolution of, an acute reversible event). ‡Of the six infants who were not 'event-free', three infants met the definition of permanent ventilation and three had died. §One patient performed the Month 12 visit a few days early and therefore had not yet reached 12 months from enrollment as of the data cut-off. Data cut-off: 14 Nov 2019.
BiPAP. Bilevel Positive Airway Pressure; IQR, interquartile range; PNCR, Pediatric Neuromuscular Clinical Research Network; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

1. Finkel R, et al. Neurology. 2014; 83:810–817.

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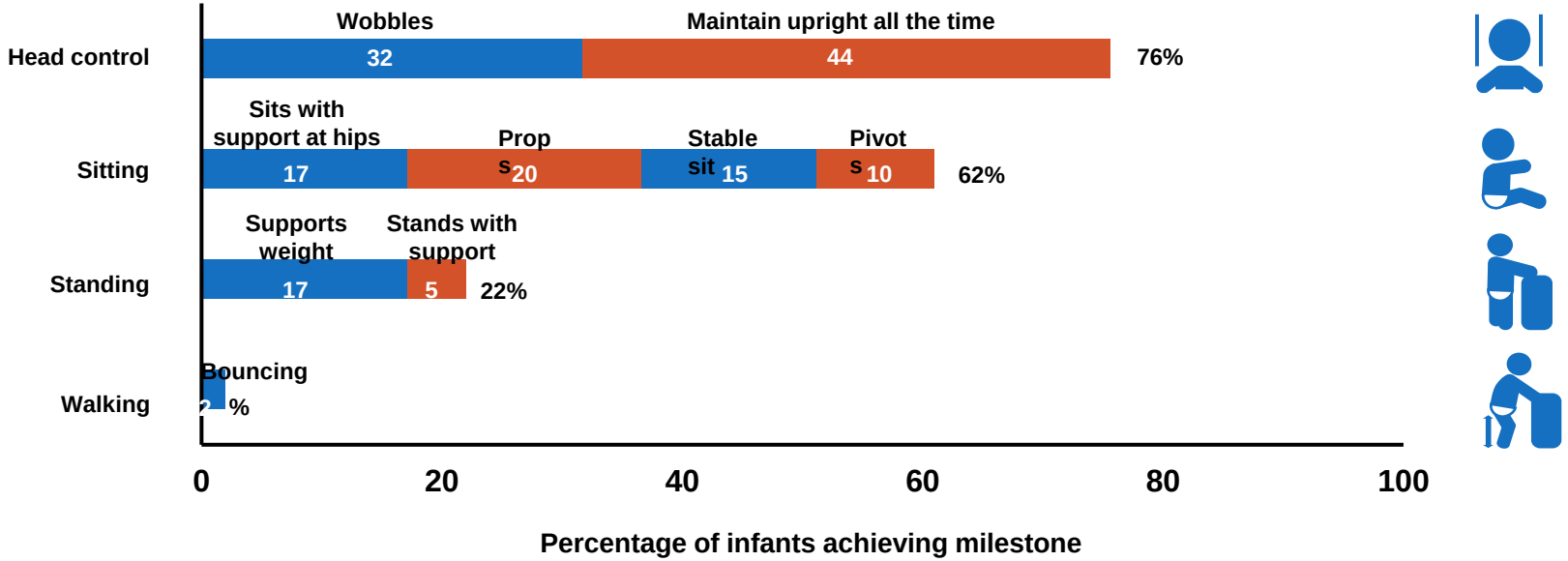
Risdiplam treatment resulted in significant gains in motor milestones after 12 months (HINE-2 items)

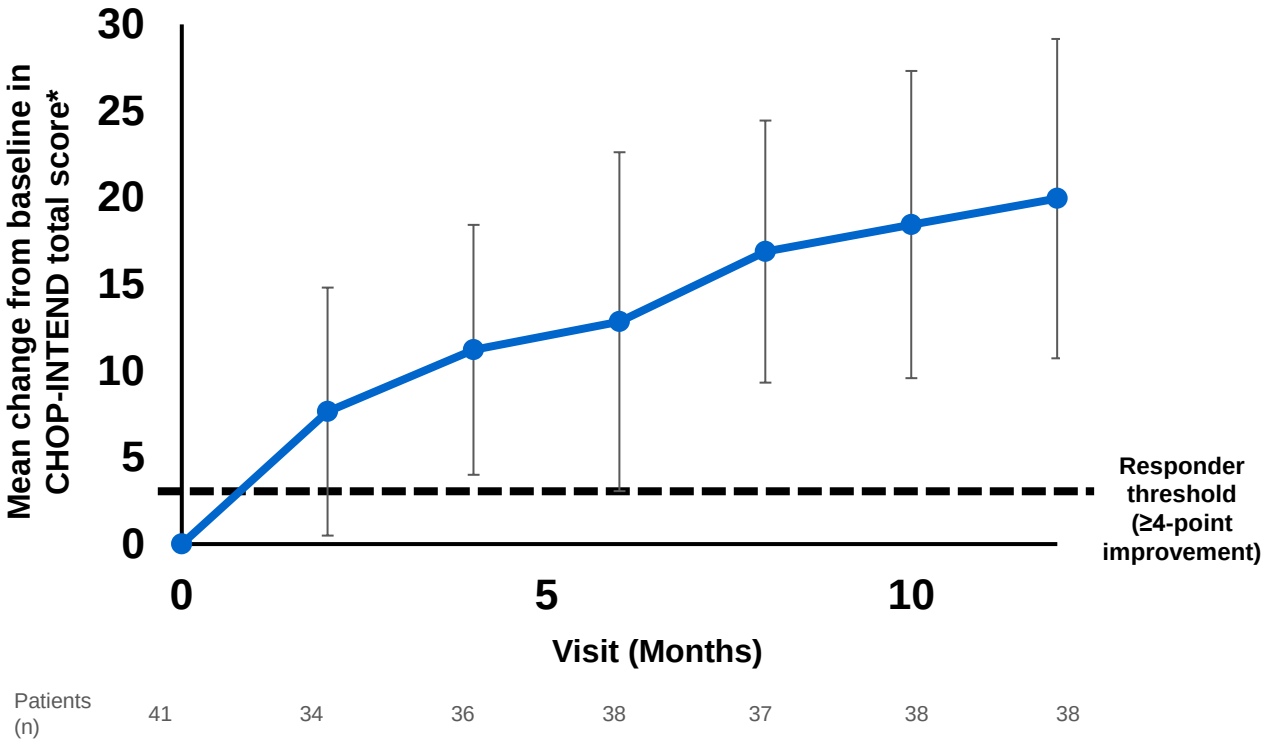


78% of infants (32/41) responded to treatment using the HINE-2 scale and pre-specified response criteria*†

*Infant classed as responder if more motor milestones show improvement than show worsening. Improvement defined as a ≥2-point increase in ability to kick (or maximal score) or ≥1-point increase in head control, rolling, sitting, crawling, standing or walking. Worsening defined as ≥2-point decrease in ability to kick (or lowest score) or ≥1-point decrease in head control, rolling, sitting, crawling, standing or walking. †P<0.0001, performance criterion=12%, exact binomial test. Data cut-off: 14 Nov 2019.
HINE-2, Hammersmith Infant Neurological Examination, Module 2.

Roche in collaboration with SMA FOUNDATION and PTC THERAPEUTICS

CHOP-INTEND total score continued to improve over 12 months



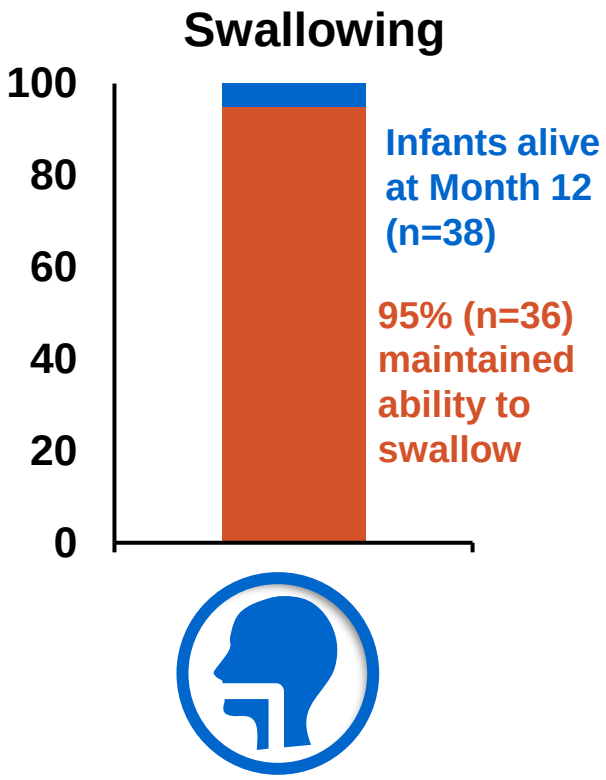
In natural history, children with Type 1 SMA rarely reach a CHOP-INTEND total score of 40 points[1]

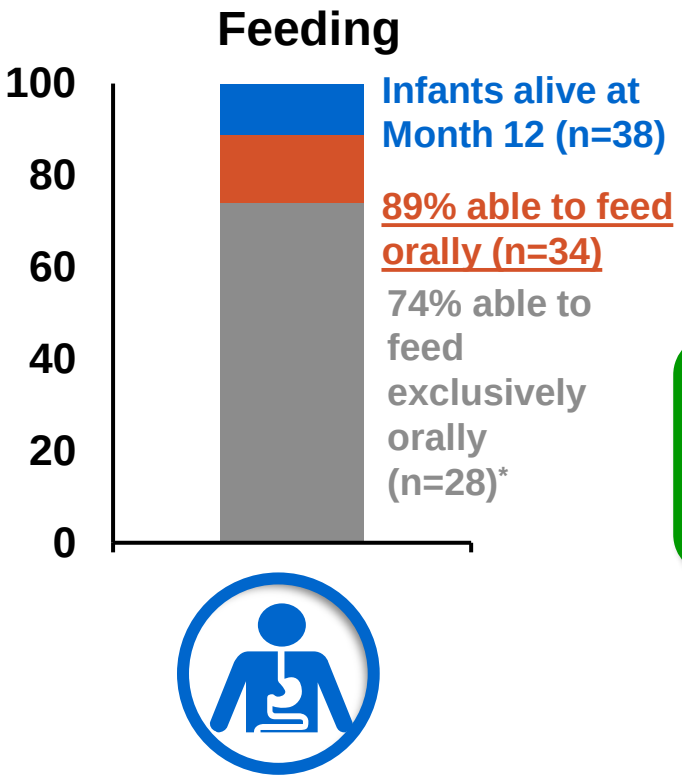
56% (23/41) achieved a CHOP-INTEND score ≥40 at Month 12† in FIREFISH Part 2

*±Standard deviation. †P<0.0001, performance criterion=17%, exact binomial test. Data cut-off: 14 Nov 2019.
CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders.
1. Finkel R, et al. Neurology. 2014; 83:810–817.

Roche in collaboration with SMA FOUNDATION and PTC THERAPEUTICS

Swallowing and feeding ability was maintained by the majority of infants alive at Month 12





In a natural history cohort, all infants with Type 1 SMA older than 12 months required feeding support[1]

*Six infants fed orally in combination with a feeding tube and four fed exclusively via a feeding tube. Data cut-off: 14 Nov 2019.
SMA, spinal muscular atrophy.
1. Finkel RS, et al. Neurology. 2014; 83:810–817.

ORIGINAL ARTICLE

Risdiplam in Type 1 Spinal Muscular Atrophy

Giovanni Baranello, M.D., Ph.D., Basil T. Darras, M.D., John W. Day, M.D., Ph.D., Nicolas Deconinck, M.D., Ph.D., Andrea Klein, M.D., Riccardo Masson, M.D., Eugenio Mercuri, M.D., Ph.D., Kristy Rose, Ph.D., Muna El-Khairi, Ph.D., Marianne Gerber, Ph.D., Ksenija Gorni, M.D., Ph.D., Omar Khwaja, M.D., Ph.D., Heidemarie Kletzl, Ph.D., Renata S. Scalco, M.D., Ph.D., Timothy Seabrook, Ph.D., Paulo Fontoura, M.D., Ph.D., and Laurent Servais, M.D., Ph.D., for the FIREFISH Working Group*

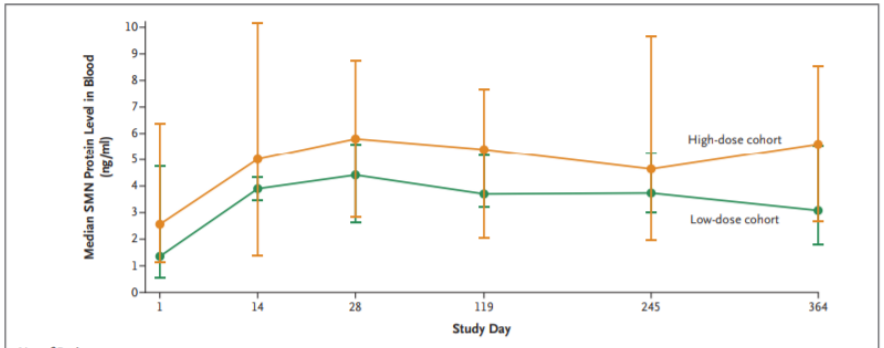


Figure 1. SMN Protein Concentration in Whole Blood.

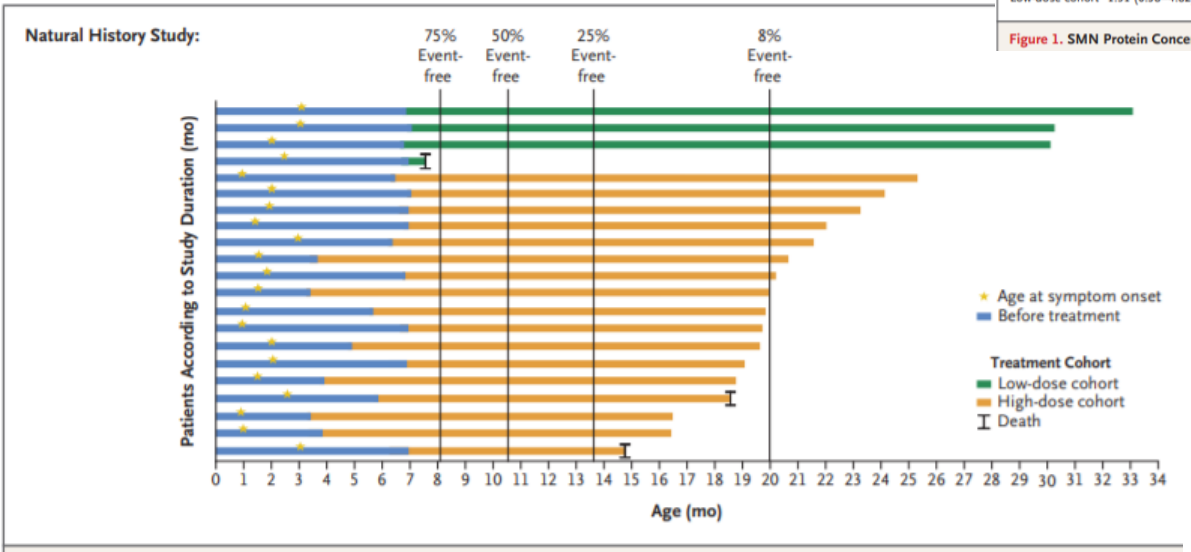
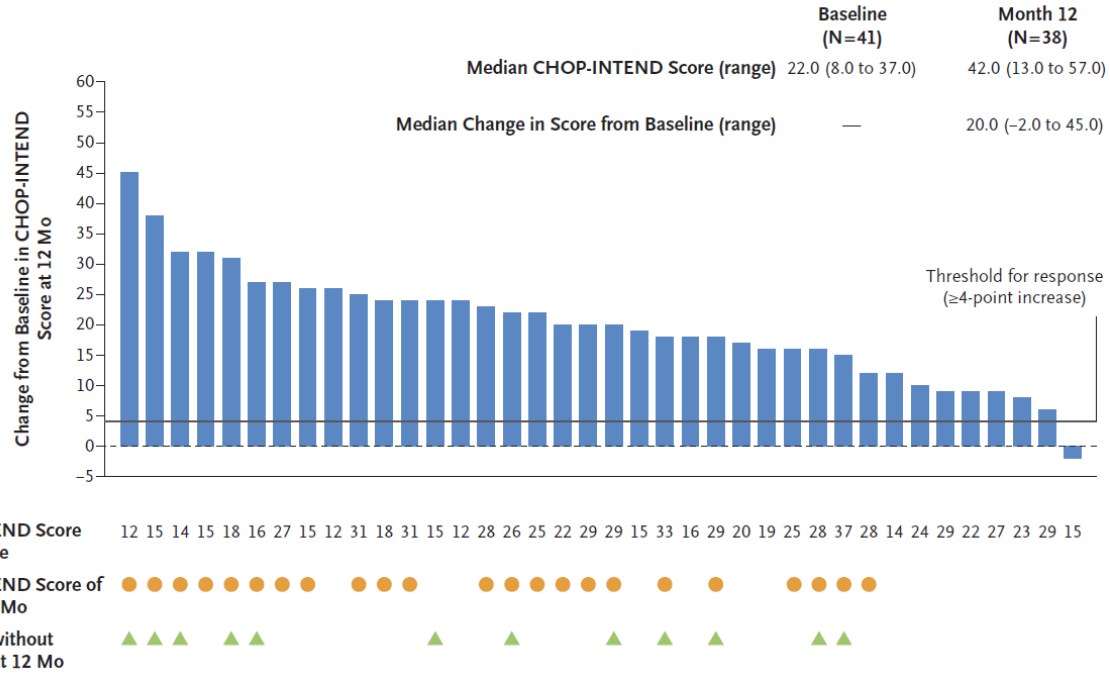


Figure 2. Event-free Survival.

ORIGINAL ARTICLE

Risdiplam-Treated Infants with Type 1 Spinal Muscular Atrophy versus Historical Controls

B.T. Darras, R. Masson, M. Mazurkiewicz-Bełdzińska, K. Rose, H. Xiong, E. Zanoteli, G. Baranello, C. Bruno, D. Vlodavets, Y. Wang, M. El-Khairi, M. Gerber, K. Gorni, O. Khwaja, H. Kletzl, R.S. Scalco, P. Fontoura, and L. Servais, for the FIREFISH Working Group*



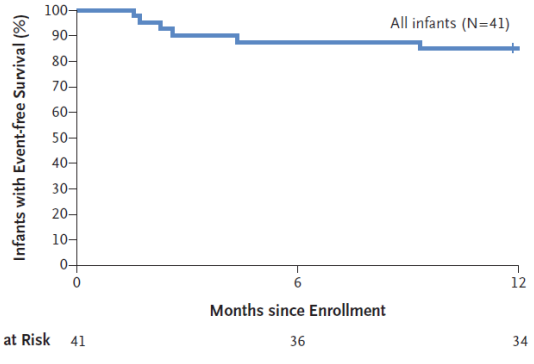


Figure 2. Event-free Survival after Risdiplam Treatment.

The NEW ENGLAND JOURNAL of MEDICINE

Risdiplam Clinical Development Program

FIREFISH[1]

Type 1 SMA
1–7 months old

Part 1 (completed) 21 patients Safety, tolerability and PK/PD	Part 2 40 patients* Safety, efficacy at selected dose from Part 1

SUNFISH[2]

Type 2 or 3 SMA
2–25 years old

Part 1 (completed) 51 patients Safety, tolerability and PK/PD, 2:1 placebo	Part 2 168 patients* Safety, efficacy at selected dose from Part 1

JEWELFISH[3]

Type 2 or 3 SMA
12–60 years old
Previous participation in studies with other *SMN2* splicing agents

24 patients*
12 patients enrolled so far
Safety, tolerability, PK/PD of risdiplam

RAINBOWFISH: Planned study in presymptomatic SMA patients

Birth–6 weeks old

Safety, efficacy and PK/PD of risdiplam

*Target enrollment
PK, pharmacokinetics; PD, pharmacodynamics; SMA, spinal muscular atrophy.
Clinicaltrials.gov; 1. NCT02913482; 2. NCT02908685; 3. NCT03032172. (Accessed June 2018).

Roche in collaboration with SMA FOUNDATION and PTC THERAPEUTICS

SUNFISH: Study overview

SUNFISH (NCT02908685)[1] is a multicenter, two-part, randomized, placebo-controlled, double-blind study

- Part 1: Dose finding – Identify a recommended dose of risdiplam for Part 2 and assess safety, tolerability, PK and PD of risdiplam
- Dose selection for Part 2
  - Risdiplam:placebo (2:1) for a minimum of 12 weeks, followed by open-label extension at pivotal dose with the dose selected for Part 2
- Part 2: Efficacy (MFM32) and safety (enrollment complete)
  - Risdiplam:placebo (2:1) for 12 months, followed by a further 12 months on active treatment and then an open-label extension

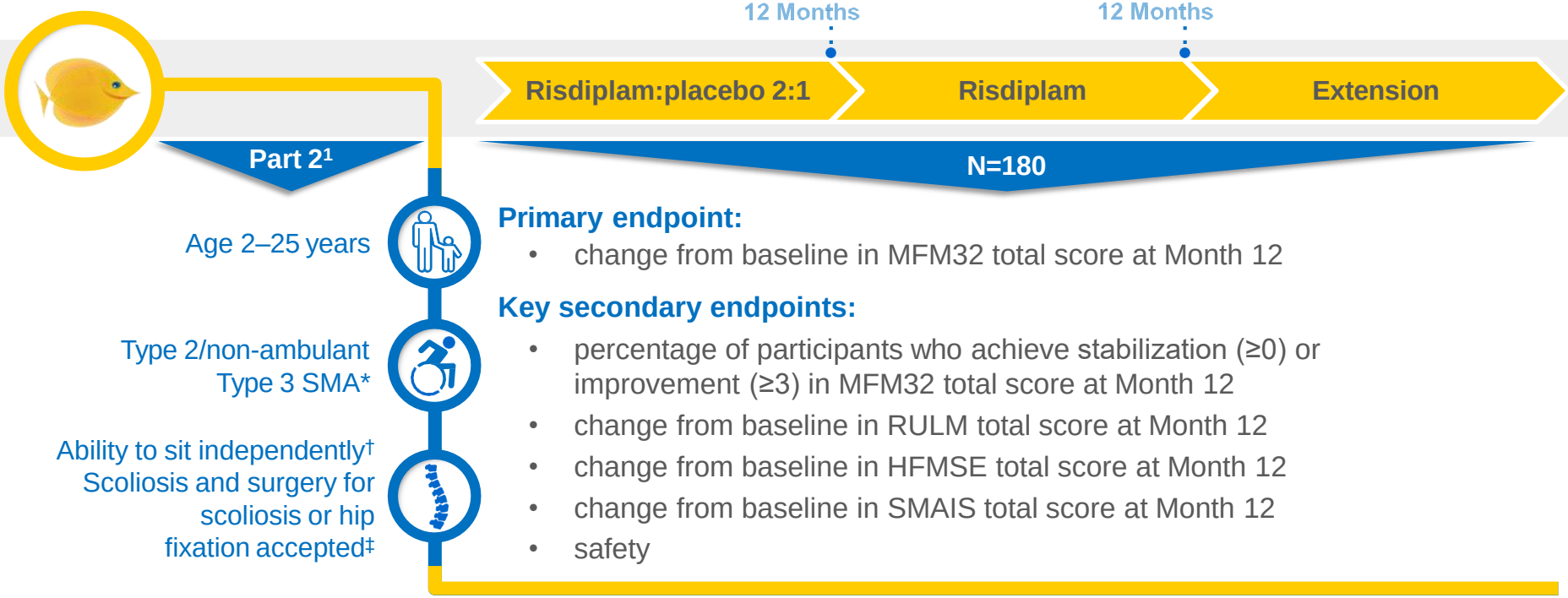
SUNFISH[1]
Type 2 or 3 SMA
2–25 years old

Inclusion/exclusion criteria		
Key inclusion criteria	**Part 1 (N=51)** • Type 2 or ambulatory and non-ambulatory Type 3 SMA. • Confirmed genetic diagnosis of SMA.*	**Part 2 (N=180)** • Type 2 or non-ambulatory Type 3 SMA. • Confirmed genetic diagnosis of SMA.*
Key exclusion criteria	• Previous participation in an *SMN2*-targeting study or gene therapy study. • Planned (within 18 months) or previous (<1 year prior) surgery for scoliosis or hip fixation.	

*5q-autosomal recessive SMA. MFM32, Motor Function Measure (32 items); PD, pharmacodynamics, PK, pharmacokinetics; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

1. Clinicaltrials.gov: NCT02908685. Accessed September 2019.

A randomized, placebo-controlled, double-blind study with broad inclusion criteria and a large dataset



*Non-ambulant is defined as not having the ability to walk unassisted for ≥10m; †RULM entry item A (Brooke score) ≥2; ability to sit independently (≥1 on item 9 of the MFM32). ‡Except in the one year preceding screening or planned within the next 18 months.

HFMSE; Hammersmith Functional Motor Score – Expanded; MFM32, 32-item Motor Function Measure; RULM, Revised Upper Limb Module; SMAIS; SMA Independence Scale.

1. Clinicaltrials.gov. NCT02908685 (Accessed Jan 2020).
Mercuri E. et al. Presented at SMA Europe 2020

Roche in collaboration with SMA FOUNDATION and PTC THERAPEUTICS

Safety and efficacy of once-daily risdiplam in type 2 and non-ambulant type 3 spinal muscular atrophy (SUNFISH part 2): a phase 3, double-blind, randomised, placebo-controlled trial

*Eugenio Mercuri, Nicolas Deconinck, Elena S Mazzone, Andres Nascimento, Maryam Oskoui, Kayoko Saito, Carole Vuillerot, Giovanni Baranello, Odile Boespflug-Tanguy, Nathalie Goemans, Janbernd Kirschner, Anna Kostera-Pruszczyk, Laurent Servais, Marianne Gerber, Ksenija Gorni, Omar Khwaja, Heidemarie Kletzl, Renata S Scalco, Hannah Staunton, Wai Yin Yeung, Carmen Martin, Paulo Fontoura, John W Day, on behalf of the SUNFISH Study Group**

Lancet Neurol 2022; 21: 42–52

Summary

Background Risdiplam is an oral small molecule approved for the treatment of patients with spinal muscular atrophy,

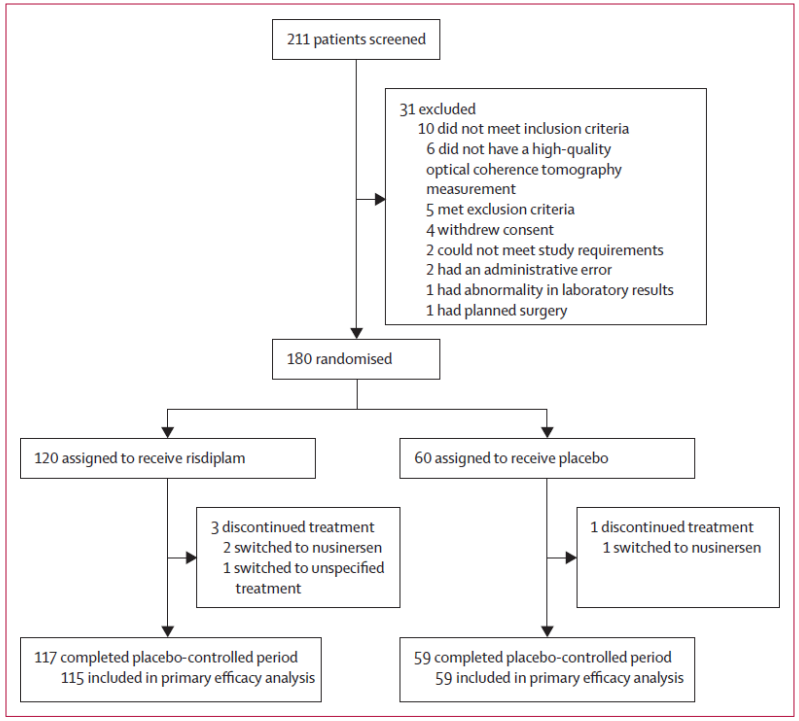


Figure 1: Trial profile

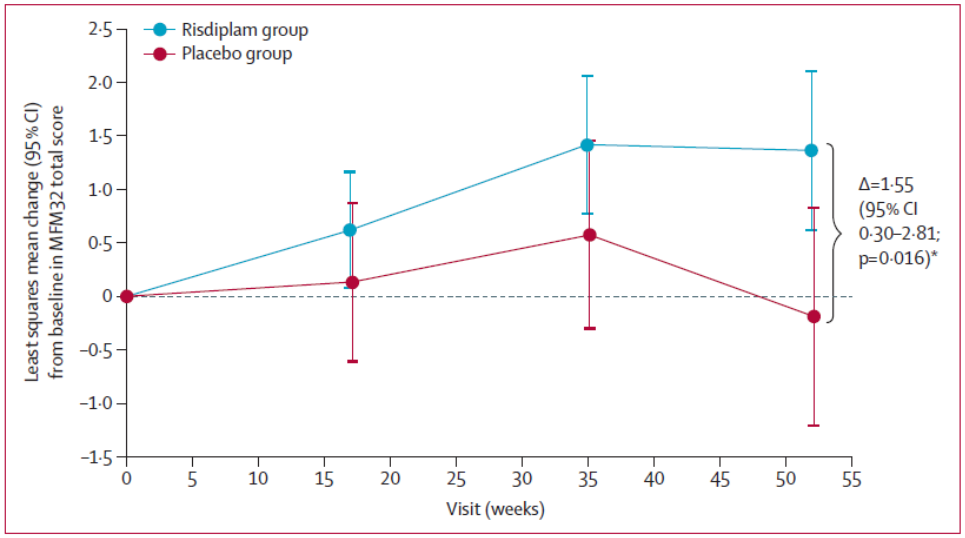
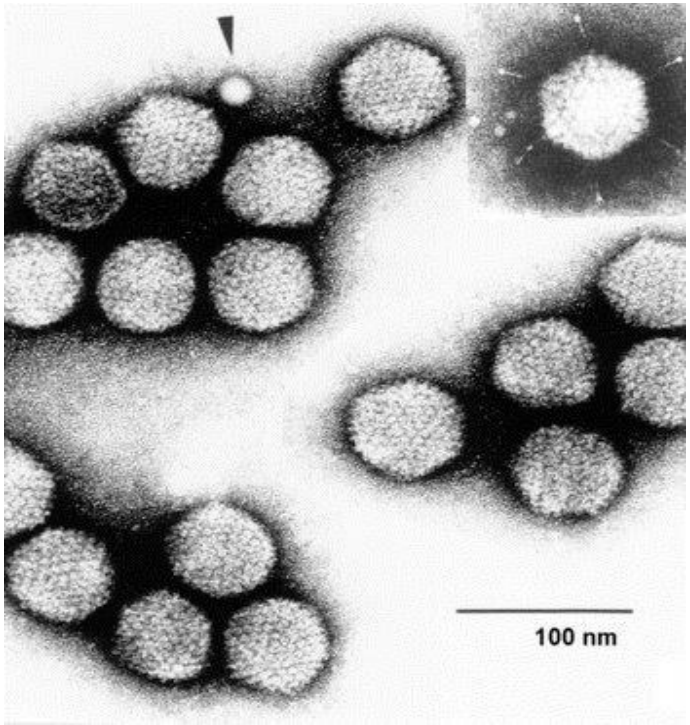


Figure 2: Change in MFM32 total score from baseline to 12 months of treatment

Disease-modifying treatment
Gene therapy: Onasemnogene abeparvovec

Adeno Associated Virus (AAV)



Discovered in mid-1960s
Members of the **Parvoviridae** family.
The viruses belong to the genus Dependovirus, the members of which require a **helper virus**

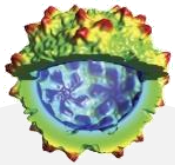
Advantages

- ✓ non-pathogenic, non inflammatory
- ✓ low immunogenicity
- ✓ long term expression (> 10 years in humans in clinical trial)
- ✓ no integration into cell genome, persist as non integrated episomes
- ✓ infect dividing and non dividing cells

AAV-based gene therapy is a novel technique designed to address the genetic route cause of SMA[1]

Able to deliver across the blood-brain barrier and into the spinal cord[2,3]

Designed not to integrate into genome of the patient[4]



Recombinant AAV9 Capsid Shell

Continuous promoter

Hybrid CMV enhancer and CB promoter activates the transgene to allow for continuous and sustained SMN protein expression[3]

Human *SMN* transgene

Full copy of a stable, functioning human *SMN* gene that is introduced into the cell's nucleus[3]

scAAV ITR

The scAAV ITR increases the speed at which the double-stranded transgene is transcribed and the resulting protein is produced[4]

Figure adapted from Powell SK, Rivera-Soto R. Discov Med 2015;19(102):49–57; AAV2, adeno-associated virus serotype 2; AAV9, AAV serotype 9; BGH Poly A, bovine growth hormone polyadenylation; CB, chicken β-actin; cDNA, complementary DNA; CMV, cytomegalovirus; GRT, gene-replacement therapy; ITR, inverted terminal repeat; scAAV, self-complementary AAV; SMA, spinal muscular atrophy; SMN, survival motor neuron gene; SV40, simian virus 40;

1. Kumar SRP, et al. *Mol Ther Methods Clin Dev.* 2016;3:16034 2. Foust KD, et al. Nat Biotechnol. 2010;28(3):271–274;
3. Foust KD & Kaspar BK. Chapter 19 - Gene Transfer in Spinal Muscular Atrophy in Spinal Muscular Atrophy: Disease Mechanisms and Therapy. US: Elsevier Inc; 2017. Available from: http://dx.doi.org/10.1016/B978-0-12-803685-3.00019-7; Last accessed: June 2020;
4. Daya S and Berns KI. Clin microbial Rev. 2008;21:583-593.

Onasemnogene abeparvovec is being studied in both symptomatic and pre-symptomatic patients with SMA

1. European Medicines Agency (2020). Onasemnogene abeparvovec Summary of Product Characteristics (May 2020). Available at: https://www.ema.europa.eu/en/documents/product-information/zolgensma-epar-product-information_en.pdf. Last accessed: November 2020; 2. ClinicalTrials.gov (2019). Gene replacement therapy clinical trial for patients with spinal muscular atrophy type 1 (STR1VE). Available at: https://clinicaltrials.gov/ct2/show/record/NCT03306277. Last accessed: November 2020; 3. ClinicalTrials.gov (2020). Single-dose gene replacement therapy clinical trial for patients with spinal muscular atrophy Type 1 (STRIVE-EU). Available at: https://clinicaltrials.gov/ct2/show/record/NCT03461289?term=ZOLGENSMA&draw=2&rank=9. Last accessed: November 2020; 4. ClinicalTrials.gov (2020). Pre-Symptomatic study of intravenous onasemnogene abeparvovec-xioi in spinal muscular atrophy (SMA) for patients with multiple copies of SMN2 (SPR1NT). Available at: https://clinicaltrials.gov/ct2/show/record/NCT03505099. Last accessed: November 2020; 5. Mendell JR, et al. *New Engl J Med*. 2017;377(18):1713–22; 6. ClinicalTrials.gov (2019). Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1. Available at: https://clinicaltrials.gov/ct2/show/NCT02122952. Last accessed: November 2020.ClinicalTrials.gov (2020); 7. ClinicalTrials.gov (2019). Long-term follow-up study for patients from AVXS-101-CL-101 (START). Available at: https://clinicaltrials.gov/ct2/show/record/NCT03421977. Last accessed: November 2020.ClinicalTrials.gov (2020); 8. Long-term follow-up study of patients receiving onasemnogene abeparvovec-xioi. Available at: https://clinicaltrials.gov/ct2/show/record/NCT04042025. Last accessed: November 2020; 9. ClinicalTrial.gov (2019). NCT04174157. Available from: https://clinicaltrials.gov/ct2/show/record/NCT04174157. Last accessed; November 2020.

Phase I Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1 Delivering the Survival Motor Neuron Gene by Self-Complementary AAV9

Single ev administration

FIRST CLINICAL TRIAL

Inclusion Criteria

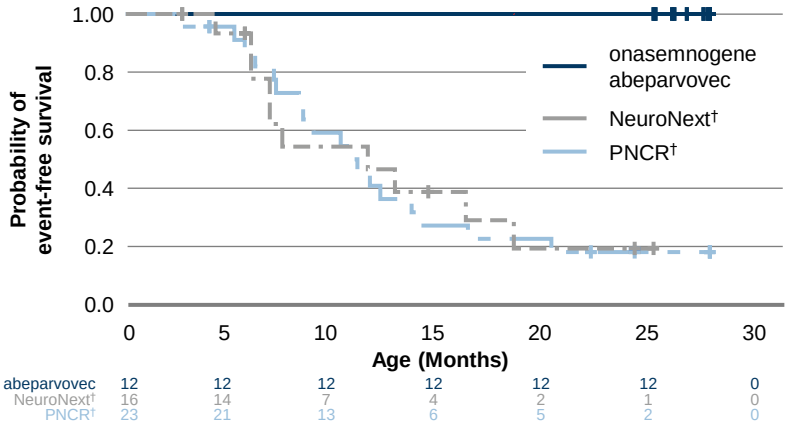
- Diagnosis of SMA 1
- 2 copies of SMN2
- 6 or 9 months of age and younger
- no non-invasive ventilator support

- Cohort 1 (low dose): 6.7×10^{13} vg/kg (n=3)

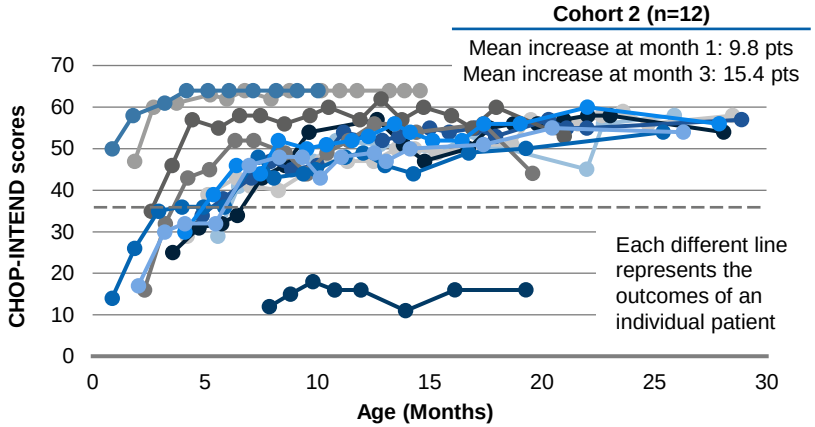
- Cohort 2 (high dose): 2.0×10^{14} vg/kg (n=12)

START: Improvement in outcomes in patients with SMA type 1 treated with onasemnogene abeparvovec[1]

START[1] | **n=15** | ***SMN2* = 2 copies** | **Completed**



Onasemnogene abeparvovec	12	12	12	12	12	12	0
NeuroNext†	16	14	7	4	2	1	0
PNCR†	23	21	13	6	5	2	0



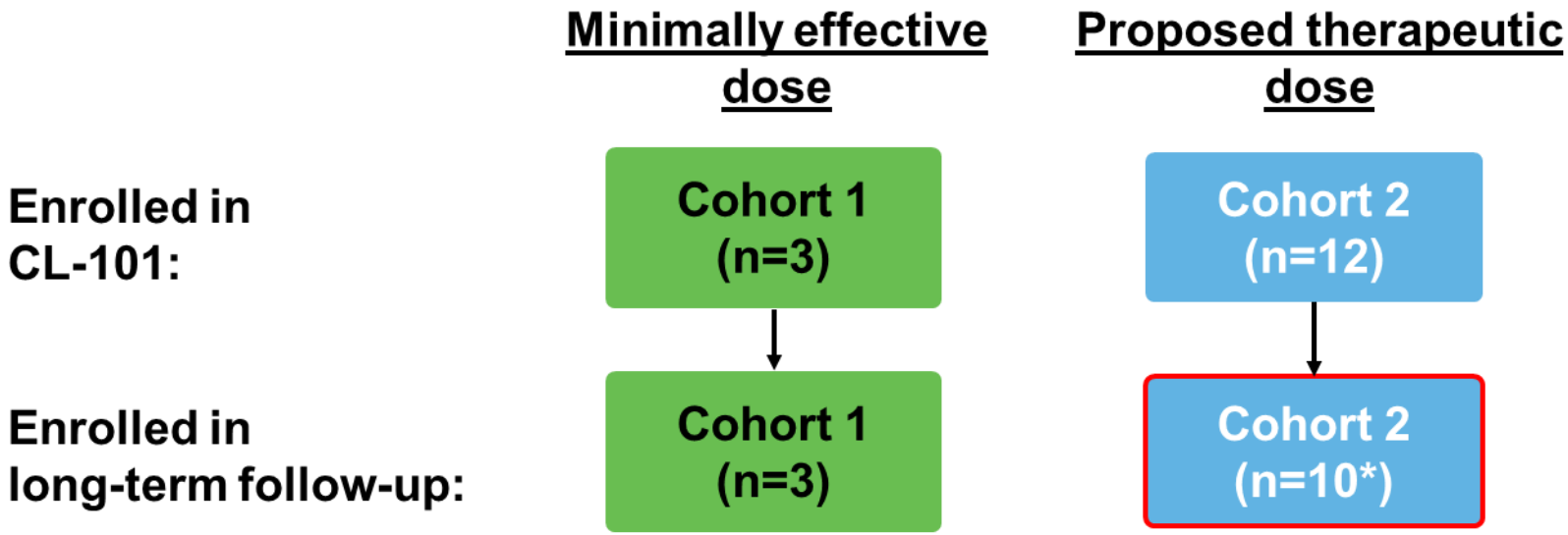
11 **sit alone** — **Eleven** of 12 patients **sat unassisted**[2]

2 **walk alone** — **Two** children crawl, pull to a stand, stand, and **walk independently**[2]

2 **related AEs** — **2 events** were **treatment-related AEs**[2]

AE, adverse event; CHOP INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; PNCR, Pediatric Neuromuscular Clinical Research Network. SMA, spinal muscular atrophy; SMN, survival motor neuron.
*Based on data up to December 31 2019. Ten of 12 patients from Study CL-101 who received the proposed therapeutic dose of onasemnogene abeparvovec continue to be followed in a long-term study (for up to 5.7 years after dosing) and all have survived. Four of the 10 patients received concomitant nusinersen treatment at some point during the long-term study. Claim is based on the 6/6 patients who did not receive nusinersen and remain alive to date. The four patients who have received nusinersen are also alive and free of permanent ventilation, but maintenance of efficacy cannot solely be attributed to onasemnogene abeparvovec in these children.1 †Natural history: the percentage of patients who were event free in two studies of SMA (*SMN2*=2 copies) conducted by the Pediatric Neuromuscular Clinical Research (PNCR, n=23) Network and Network for Excellence in Neuroscience Clinical Trials (NeuroNext, n=16).[1]
1. European Medicines Agency (2020). Onasemnogene abeparvovec Summary of Product Characteristics (May 2020). Available at: https://www.European Medicines Agency.europa.eu/en/documents/product information/zolgensma-epar-product-information_en.pdf. Last accessed: November 2020; 2. Mendell JR et al. *New Engl J Med.* 2017;377(18):1713–22.

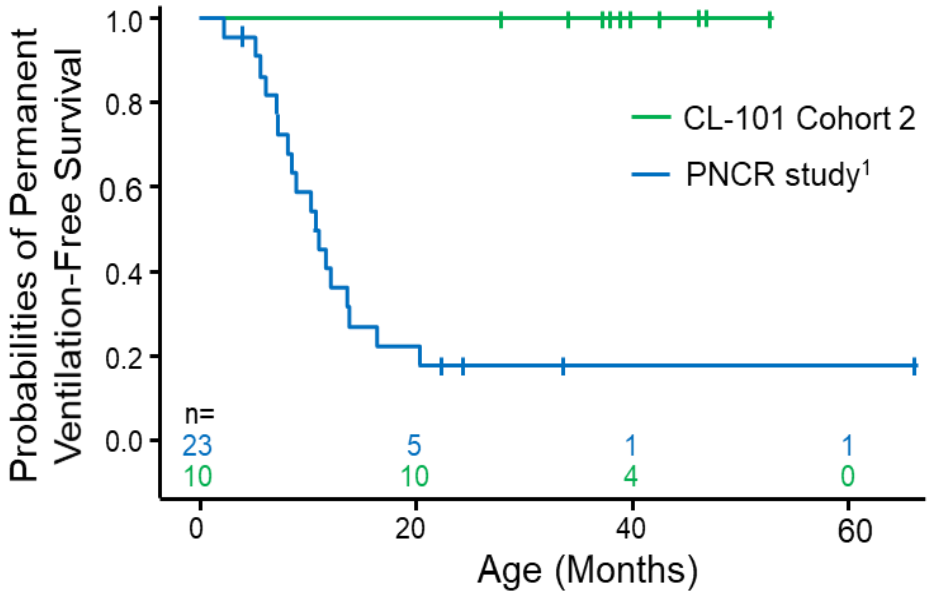
Long-term follow-up from phase 1/2a study of AVXS-101 in patients with SMA1



*Two patients who did not enroll in the long-term follow-up study are being followed at the Nationwide Children's Hospital Spinal Muscular Atrophy Clinic, Columbus, OH.

Long-term follow up study

All patients in cohort 2 Remain Alive andre Free of permanent Ventilation as of March 8, 2019



Mean (range) **age** at last follow-up:
3.9 (3.4–4.8) years

Mean (range) **time** since start of treatment:
3.7 (3.3–4.3) years

- No patient was treated with concomitant nusinersen during the parent study
- As of March 8, 2019, 3 started nusinersen after 24 months study (7 out of 10 are not on concomitant nusinersen)

+ = censored.
1. Finkel RS, et al. *Neurology*. 2014;83:810–7.

Long-term follow up study

Ventilatory

- Patients **maintained or improved** ventilatory status*
  - Of the 4 patients who used **BiPAP** at the start of the LTFU study, **2 no longer require BiPAP regularly**

Nutritional

- **All** patients **maintained** their ability to swallow

*Aside from in the context of acute reversible illness.

Long-term follow up study

Maintenence of Motor Milestones demonstrates the durability of the effect of AVXS-101

At the end of CL-101

Motor milestones achieved, n (%)	Patients in Cohort 2 (n=12)
Head control	11 (92)
Rolls from back to sides	9 (75)
Sitting with assistance	11 (92)
Sitting without assistance	
≥5 seconds[a]	11 (92)
≥10 seconds[b]	10 (83)
≥30 seconds[a]	9 (75)
Standing with assistance	2 (17)
Walking alone	2 (17)

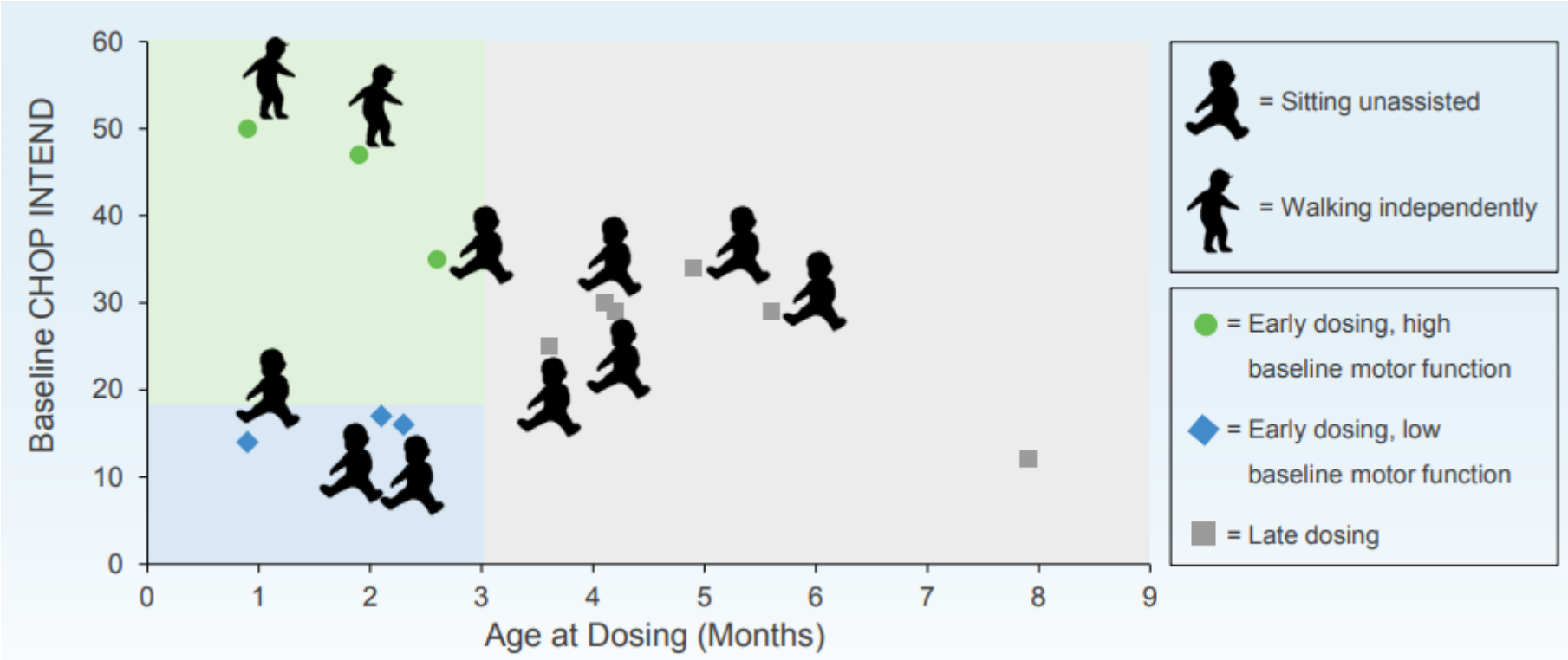
In the LTFU (N=10)

After a mean of 3.7 years post-AVXS-101 infusion

No previously achieved motor milestone has been lost during LTFU amongst the children enrolled in the LTFU

[a]Consistent with the Bayley-III, gross motor subtest. [b]In accordance with the WHO-MGRS criteria.

Achievement of Milestones in CL-101 Highlights the Benefits of Early Treatment



Conclusions from the CL-101 LTFU

Patients have remained alive, without loss of therapeutic benefit, for as long as 4.3 years following AVXS-101 treatment

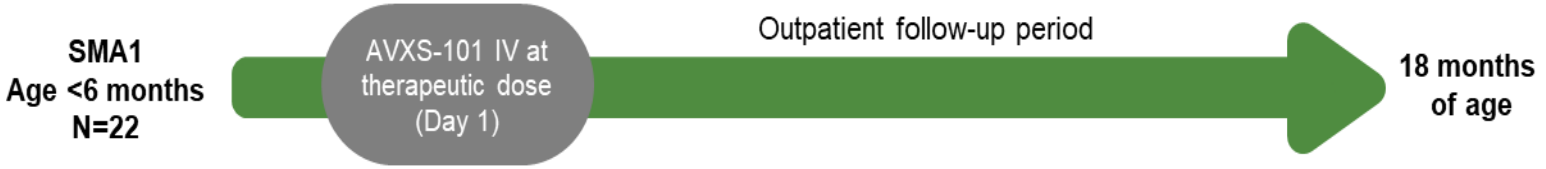
- No loss of developmental milestones
- No additional ventilatory/nutritional support requirements, and reduced ventilatory requirements in 2 patients
- No delayed AEs related to treatment

The strong, ongoing clinical impact following dosing supports that AVXS-101 continues to effectively halt motor neuron loss several years after dosing

The apparent relationship between age at treatment and efficacy emphasizes the importance of screening and early treatment

USA

Open-label, Single-arm, Multicenter Phase 3 study (CL-303, NCT03306277) – STR1VE



Key Inclusion Criteria

- Biallelic *SMN1* mutations
- One or two copies of *SMN2* (inclusive of *SMN2* gene modifier mutation [c.859G>C])
- Age <6 months at time of dosing

Key Exclusion Criteria

- Tracheostomy or current use or requirement for non-invasive ventilatory support averaging ≥6 hours/day
- Anti-AAV9 antibody titer >1:50

All 22 patients met ITT criteria – clinically symptomatic at study entry and genetically confirmed to have biallelic *SMN1* mutations and only 2 copies of *SMN2* without the c.859G>C mutation

Primary Efficacy Outcomes (ITT Population)

1

- Functional independent sitting for ≥30 seconds at 18 months of age
- Event-free survival (avoidance of death or permanent ventilation [tracheostomy or ≥16 hours of non-invasive ventilatory support for ≥14 days]) at 14 months of age

Secondary Efficacy Outcomes (ITT Population)

2

- Ability to thrive at 18 months of age
- Independence from ventilatory support at 18 months of age

Exploratory Efficacy Outcomes (ITT Population)

CHOP INTEND, other motor milestones, Bayley-III, compound motor action potential amplitude

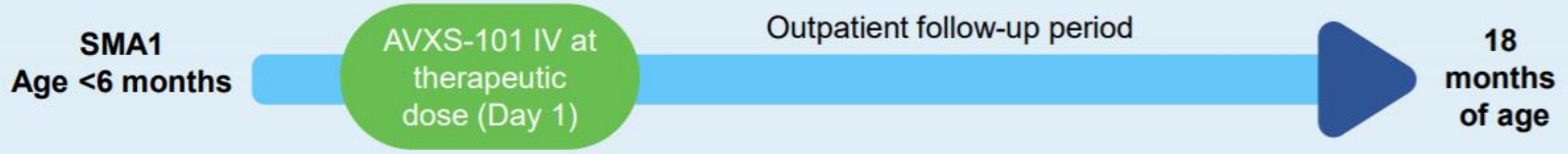
Safety

Unanticipated treatment-related toxicity of CTCAE grade 3 or higher

AAV9, adeno-associated virus serotype 9; AE, adverse event; Bayley-III, Bayley Scales of Infant and Toddler Development, V.3; CHOP INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CTCAE, Common Terminology Criteria for Adverse Events; DSMB, data and safety monitoring board; ITT, intent-to-treat; IV, intravenous; SMA1, spinal muscular atrophy type 1; SMN, survival motor neuron.

EUROPE

Phase 3, STR1VE-EU (CL-302) Study of AVXS-101 in Patients With SMA1



Key Inclusion Criteria

- Biallelic *SMN1* mutations
- One or two copies of *SMN2* (inclusive of *SMN2* gene modifier mutation [c.859G>C])
- Age <6 months at time of dosing
- No tracheostomy or current use or requirement for non-invasive ventilatory support averaging **≥12 hours/day**
- **Patients must have a swallowing evaluation test performed prior to administration of gene-replacement therapy; patients with a gastrostomy tube who pass the swallowing test may enrol**
- Anti-AAV9 antibody titre ≤1:50

Efficacy Outcomes (ITT Population)

- **Primary**: Independent sitting **≥10 seconds** based on the WHO-MGRS at 18 months of age
- **Secondary**: **Event-free survival (absence of death or permanent ventilation[a]) at 14 months of age**

Safety Outcomes

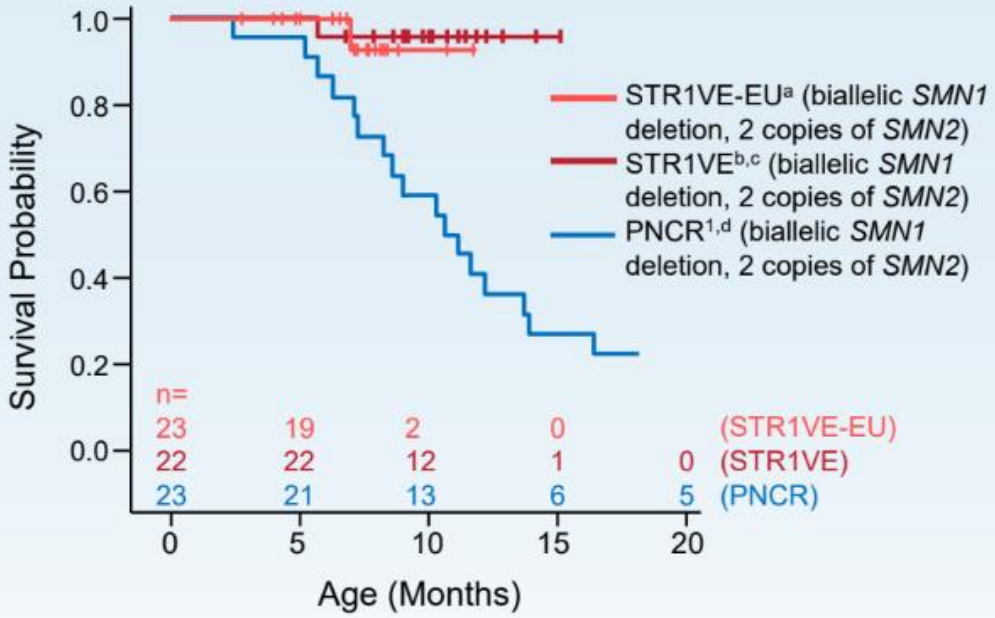
- Unanticipated treatment-related toxicity of CTCAE grade 3 or higher

Boldfaced text denotes differences between US and EU trials

[a]Tracheostomy or ≥16 hours of non-invasive ventilatory support for ≥14 days.
NCT03461289.
AAV9, adeno-associated virus serotype 9; CTCAE, Common Terminology Criteria for Adverse Events; ITT, intent-to-treat; IV, intravenous; SMA1, spinal muscular atrophy type 1; SMN, survival motor neurone; WHO-MGRS, World Health Organization Multicentre Group Reference Study.

Age at Datacut, months		
	STR1VE	STR1VE-EU
Median	14.4	7.9
Mean	13.8	7.5

Survival was improved compared with natural history



Enrolment in STR1VE is complete

- In STR1VE, none of the 25 patients screened for AAV9 antibodies had exclusionary AAV9 antibody titres (>1:50)

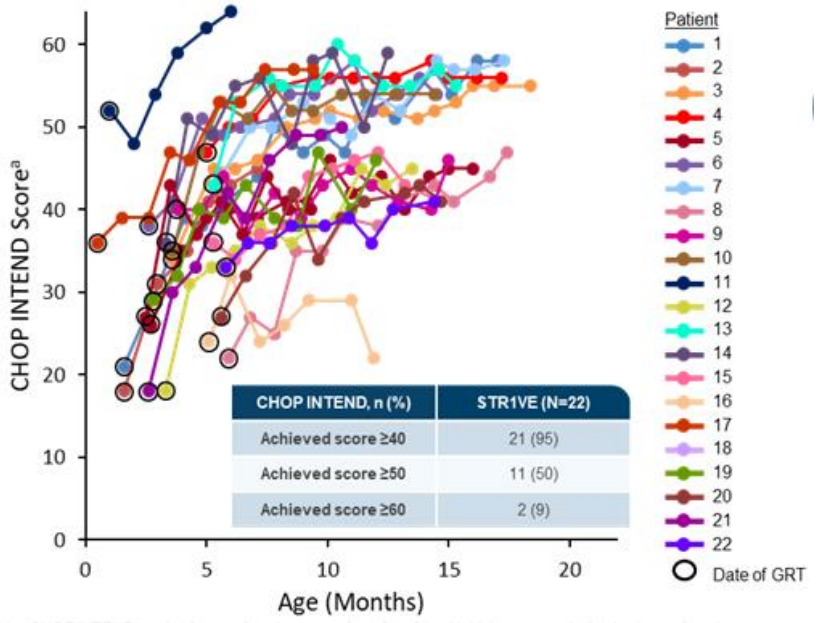
Enrolment in STR1VE-EU is complete as of 21 May 2019, with 33 patient enrolled out of a target of 30 patients

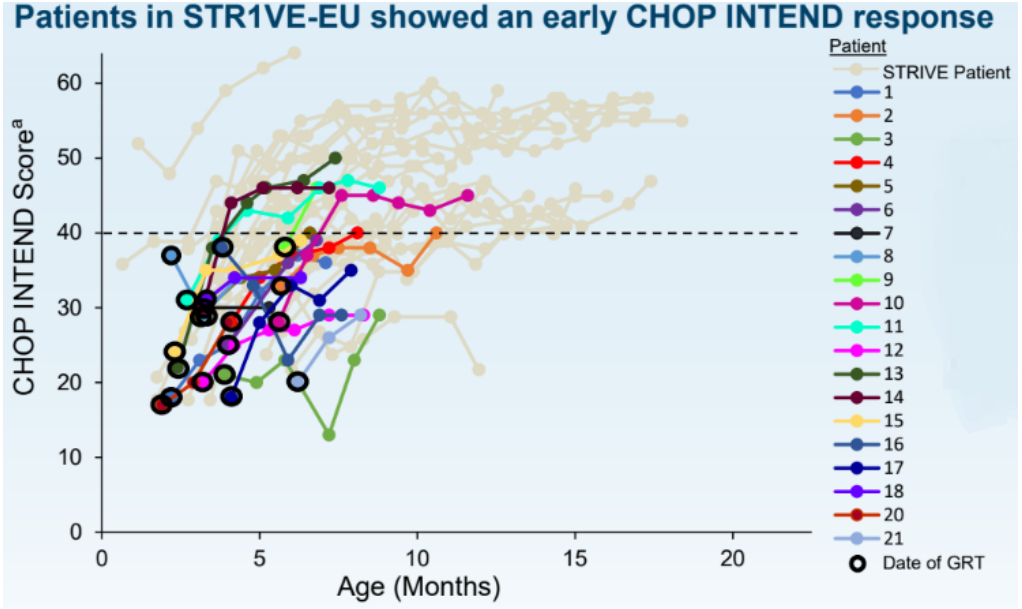
- In STR1VE-EU, 6 of the 40 (15%) patients screened for AAV9 antibodies had titres >1:50
  - Upon rescreening, 1 of these patients was enrolled, while the other 5 were excluded from the study due to elevated AAV9 antibodies

Clinical support and swallowing varied at baseline

[a]One patient died in STR1VE-EU from severe respiratory infection followed by neurological complications; the event was deemed possibly related to AVXS-101. [b]One patient in STR1VE died at the age of 7.8 months due to disease progression. [c]One patient in STR1VE withdrew consent at 11.9 months of age (censored). [d]Survival for PNCR[1] = no death, or no need for ≥16-hours/day ventilation continuously for ≥2 weeks, in the absence of an acute reversible illness; n=23 (2 copies of *SMN2*).
Figure adapted from Finkel RS, et al. *Neurology*. 2014;83:810–817.
PNCR, Pediatric Neuromuscular Clinical Research; SMA1, spinal muscular atrophy type 1; SMN, survival motor neurone.
1. Finkel RS, et al. *Neurology*. 2014;83:810–817 dataset.

Motor Function Improvements in Patients With SMA1in STR1VE and STR1VE-EU





Mean Increase in CHOP INTEND Score From Baseline		
Month	STR1VE (N=22)	STR1VE-EU (N=23)
1	6.9	5.5
3	11.7	9.4
5	14.3	NR

Interim data from the multicentre AVXS-101 phase 3 studies STR1VE / STR1VE-EU

Conclusions

Patients in STR1VE showed rapid and significant improvements in motor function

Patients in STR1VE-EU show significant improvement in motor function, despite more severe disease at baseline

In contrast with the observations from natural history studies,[1,2] these data suggest that a single dose of AVXS-101 has therapeutic benefit in rapidly improving motor function and prolonging survival in patients with SMA1

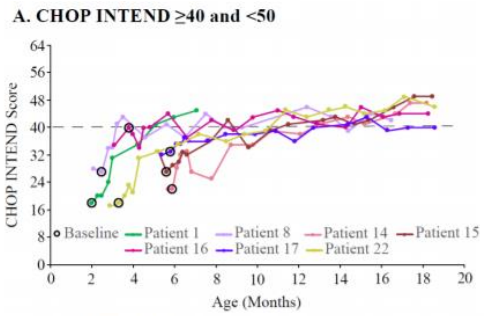
The low levels of AAV9 antibody titres in these studies, together with the results of other AVXS-101 clinical studies[3], suggest that titre levels at screening should not prohibit the vast majority of infants with SMA from receiving an AAV9-based gene-replacement therapy

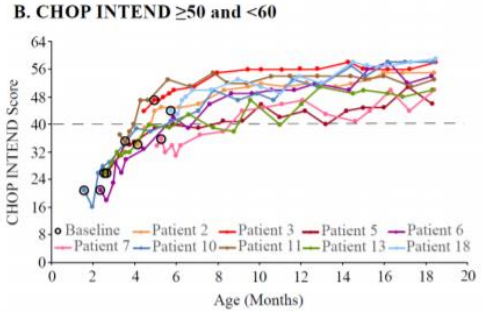
The safety profile is similar between the studies, and AEs of special interest were transient, asymptomatic, and not associated with any sequelae

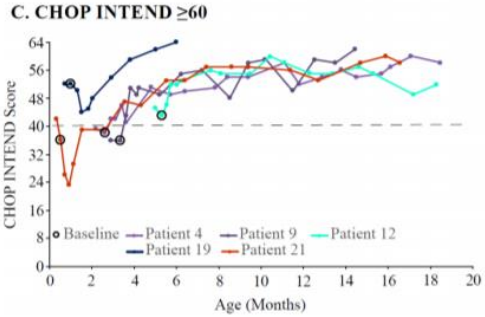
Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of *SMN2* (STR1VE): an open-label, single-arm, multicentre, phase 3 trial

John W Day, Richard S Finkel, Claudia A Chiriboga, Anne M Connolly, Thomas O Crawford, Basil T Darras, Susan T Iannaccone, Nancy L Kuntz, Loren D M Peña, Perry B Shieh, Edward C Smith, Jennifer M Kwon, Craig M Zaidman, Meredith Schultz, Douglas E Feltner, Sitra Tauscher-Wisniewski, Haojun Ouyang, Deepa H Chand, Douglas M Sproule, Thomas A Macek, Jerry R Mendell

Lancet Neurol **2021; 20: 284–93**







	Patients (n=22)
Maintained ability to thrive*	
Number of patients	9 (41%)
97·5% CI†	21–100
p value†	<0·0001
Subitems comprising the ability to thrive	
Ability to tolerate thin liquids‡	12 (55%)
Fed exclusively by mouth§	19 (86%)
Maintains weight consistent with age¶	14 (64%)

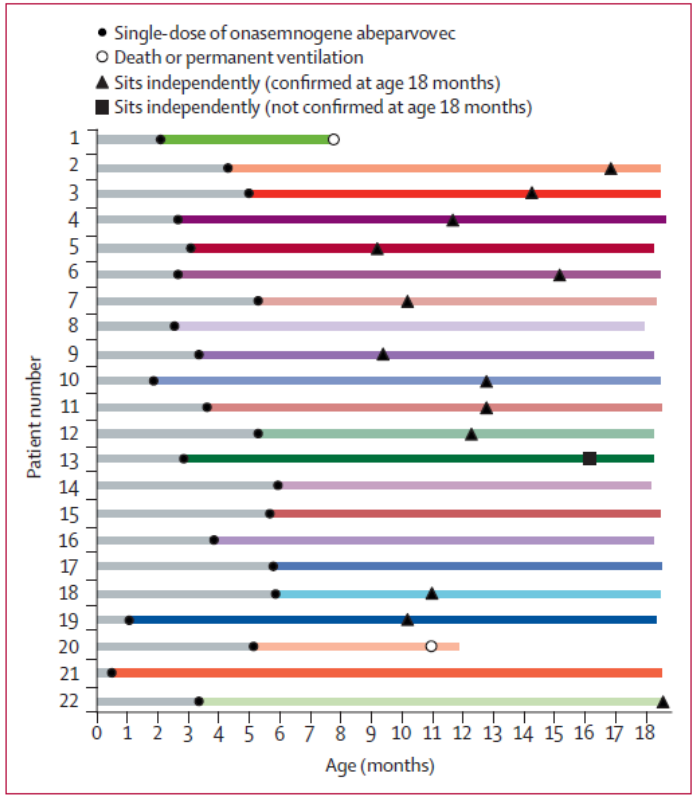
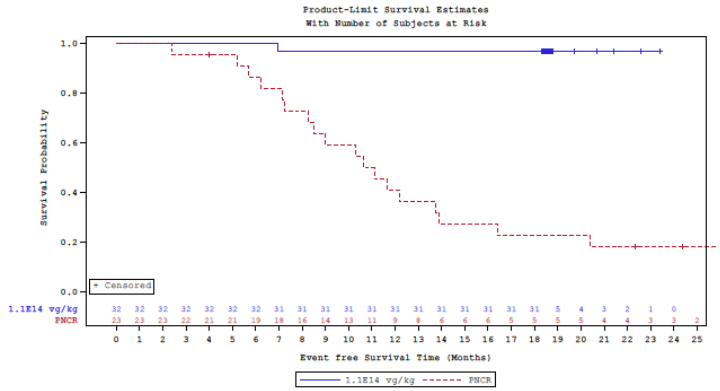
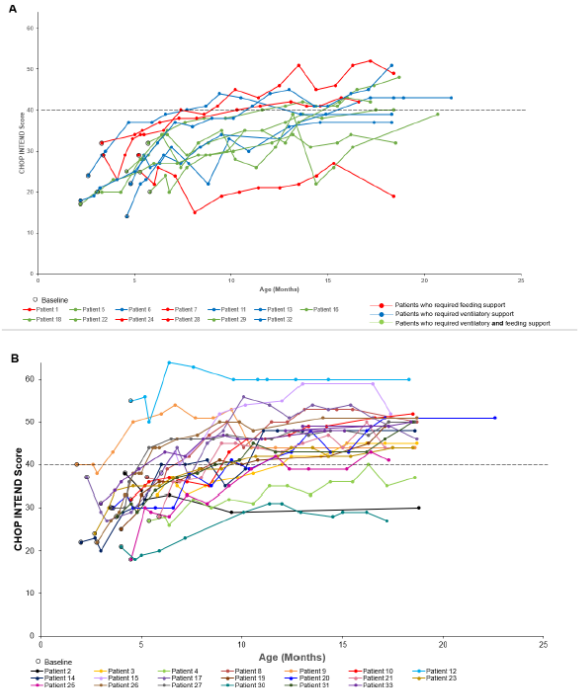


Figure 2: **Survival at age 18 months and independent sitting at age 14 months (coprimary endpoints)**
Patient 1 died and patient 20 required permanent ventilation. Patient 8 withdrew from the study because of an adverse event at age 18 months but was alive and did not require permanent ventilation at the 18 months of age study visit, although there was no end-of-study or early termination visit.
Patient 13 achieved functional independent sitting at age 16 months, but this finding was not confirmed at the 18 months of age study visit.

Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy type 1 (STR1VE-EU): an open-label, single-arm, multicentre, phase 3 trial

*Eugenio Mercuri, Francesco Muntoni, Giovanni Baranello, Riccardo Masson, Odile Boespflug-Tanguy, Claudio Bruno, Stefania Corti, Aurore Daron, Nicolas Deconinck, Laurent Servais, Volker Straub, Haojun Ouyang, Deepa Chand, Sitra Tauscher-Wisniewski, Nuno Mendonca, Arseniy Lavrov, on behalf of the STR1VE-EU study group**





Lancet Neurol 2021

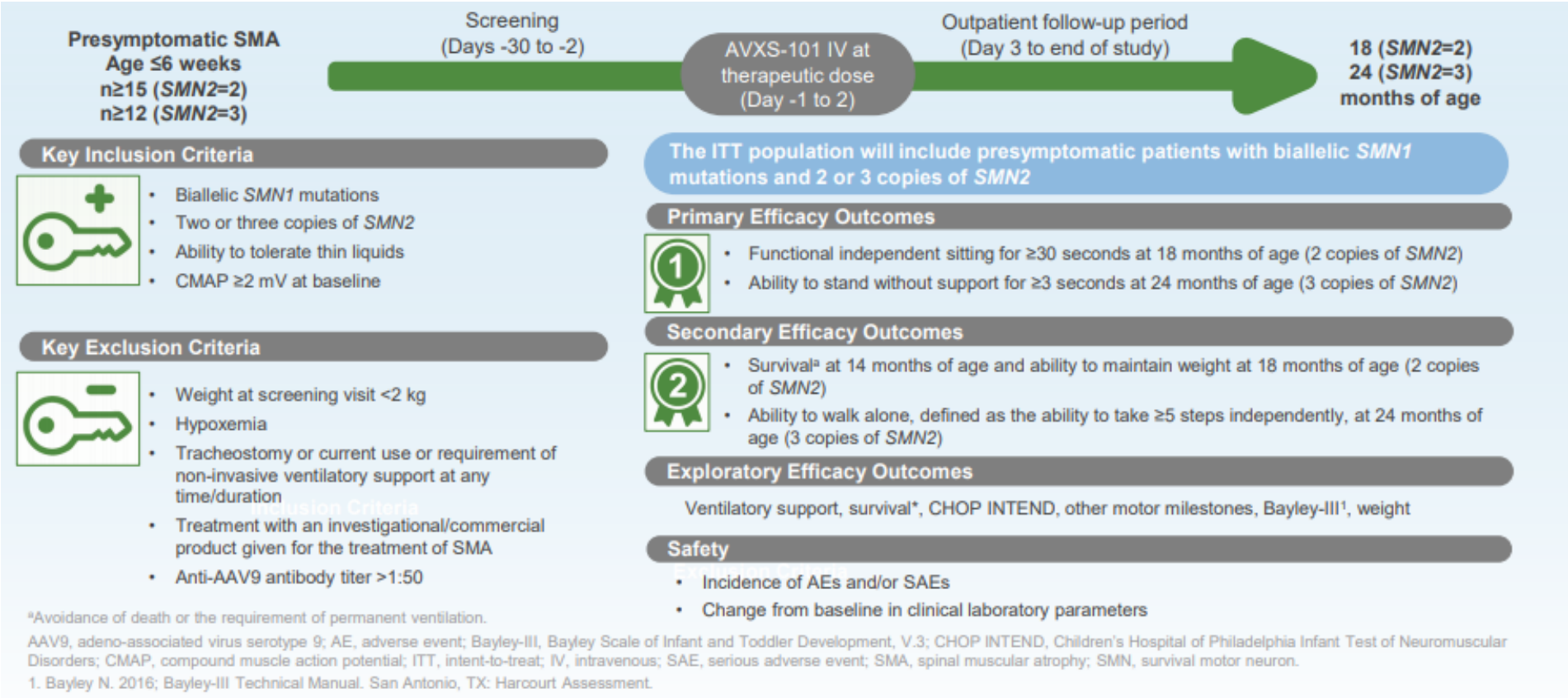
AVXS-101 ongoing studies

STRONG Intrathecal administration in patients with 3 copies of SMN2 (SMA type 2)

SPR1NT Pre-symptomatic patients (2 or 3 copies of SMN2)

START Long-term follow-up in SMA type 1 patients

Phase 3 study (CL-304, NCT03505099) – SPR1NT
Presymptomatic SMA



[a]Avoidance of death or the requirement of permanent ventilation.

AAV9, adeno-associated virus serotype 9; AE, adverse event; Bayley-III, Bayley Scale of Infant and Toddler Development, V.3; CHOP INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CMAP, compound muscle action potential; ITT, intent-to-treat; IV, intravenous; SAE, serious adverse event; SMA, spinal muscular atrophy; SMN, survival motor neuron.

1. Bayley N. 2016; Bayley-III Technical Manual. San Antonio, TX: Harcourt Assessment.

Phase 3 study (CL-304, NCT03505099) – SPR1NT Presymptomatic SMA

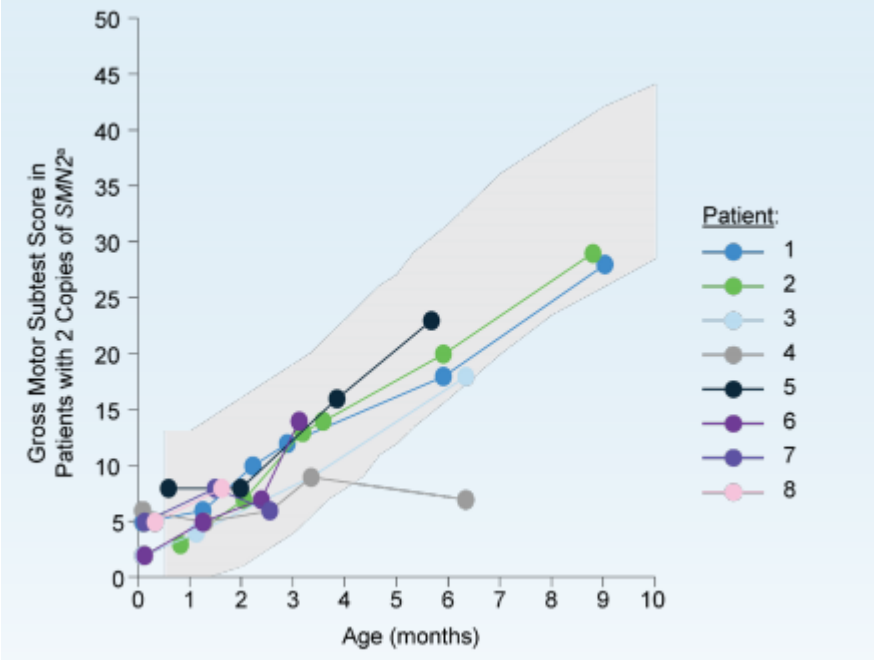
All patients are alive and free of permanent ventilation[a] as of March 8, 2019

	All Enrolled (N=18)[b]	2 Copies of *SMN2* (n=8)	3 Copies of *SMN2* (n=9)
Event-free survival[c], n (%)	18 (100)	8 (100)	9 (100)
Median age (range) at last follow-up, months	3.3 (0.8–9.1)	6.1 (1.7–9.1)	3.2 (0.8–6.0)
Median treatment duration (range), months	2.9 (0.4–8.7)	5.4 (1.1–8.7)	2.2 (0.4–4.8)

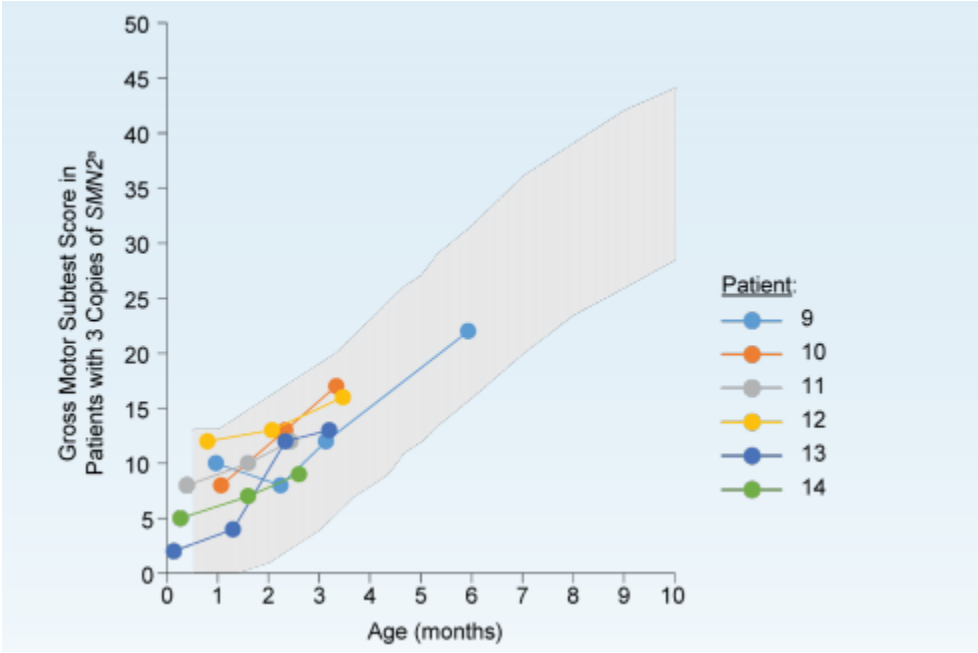
Phase 3 study (CL-304, NCT03505099) – SPR1NT

Bayley-III Motor Scores

in Patients With 2 Copies of SMN2



in Patients With 3 Copies of SMN2



Phase 3 study (CL-304, NCT03505099) – SPR1NT

Conclusions

Preliminary evidence demonstrates a dramatic response when patients are treated presymptomatically with AVXS-101

- Age-appropriate improvements in motor function
  - CHOP INTEND scores are approaching those in unaffected control children from a natural history study[1]
  - Bayley scores[2] are approaching those of healthy children
- Achievement of age-appropriate motor milestones
- Intact swallowing in all patients
- Appears well tolerated with no new safety signals relative to other AVXS-101 trials

There was low prevalence of exclusionary AAV9 antibody titers at screening in SPR1NT, as well as other AVXS-101 clinical trials[3]

Motor neuron loss and disease progression can occur rapidly in newborns with SMA. Prompt diagnosis achieved by newborn screening allows for timely implementation of disease-modifying treatment, which halts neuronal degeneration and enables normal motor development[4]

- Efficiency of newborn screening is greatly improved by carrier testing in healthy adults

Currently, standards of care form an important basis for the management of SMA patients[1,2]

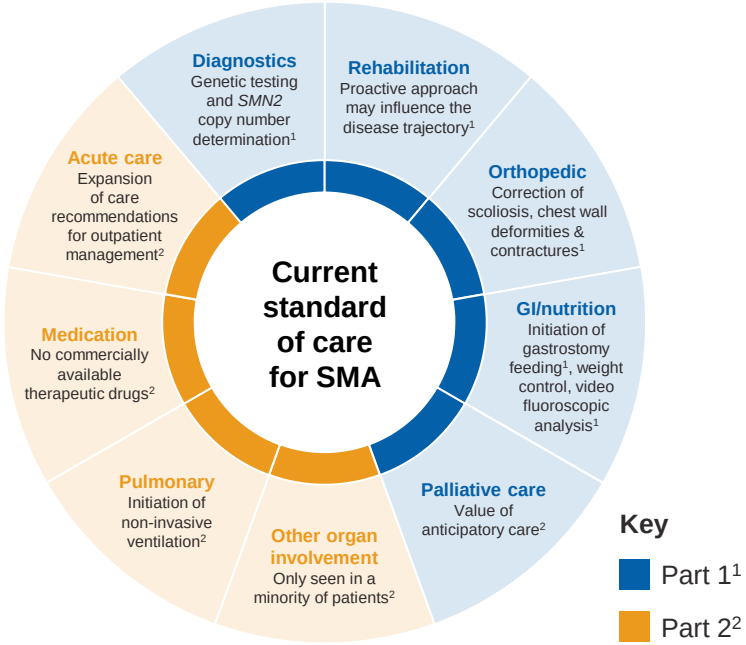
Past

Formerly, it was upon the families to coordinate all appointments, adding to their burden of care[1]

Present

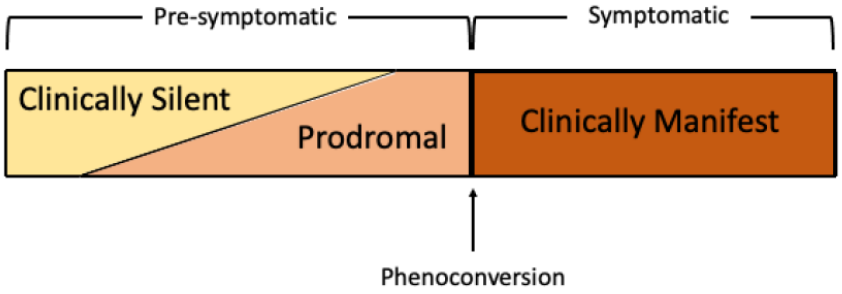
It is recommended that the lead physician coordinates all assessments and visits with the families, to optimize the standard of care[1]

A multi-disciplinary approach is key in enabling patients to achieve the best possible quality of life[1,2]



SMA, spinal muscular atrophy.

1. Mercuri E, et al. *Neuromuscul Disord*. 2018;28(2):103–115;
2. Finkel RS, et al. *Neuromuscul Disord.* 2018;28(3):197–207.



Combined SMN therapies?

Myostatin inhibitors

Changing phenotypes

National neonatal screeening

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