The Advisory Committee on Heritable Disorders in Newborns and Children

HRSA Headquarters - 5600 Fishers Lane, Rockville, MD 20852
February 8, 2018

TIME	TOPIC	PRESENTER
8:30 – 8:50 AM	• Welcome • Roll Call • Opening Remarks • November 2017 Minutes	**Joseph Bocchini, MD** Committee Chair Professor and Chairman, Department of Pediatrics, Louisiana State University **Catharine Riley, PhD, MPH** Designated Federal Official Health Resources and Services Administration
8:50 – 9:10 AM	An Overview of Cutoff Determinations and Risk Assessment Methods Used in Dried Blood Spot Newborn Screening	**Joe Orsini, PhD** Wadsworth Center, New York State Department of Health Co-Chair, APHL Newborn Screening Quality Assurance Quality Control Subcommittee
9:10 – 9:25 AM	Laboratory Standards and Procedures Workgroup: Review of the Overview of Cutoff Determinations Document	**Kellie Kelm, PhD** Ex-Officio Committee Member Chair, Laboratory Standards and Procedures Workgroup
9:25 – 9:50 AM	Committee Discussion and Vote	**Joseph Bocchini, MD** Committee Chair
9:50 – 10:30 AM	Public Comment	
10:30 – 10:45 AM	Break	

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TIME	TOPIC	PRESENTER
10:45 – 11:45 AM	Newborn Screening for Spinal Muscular Atrophy (SMA): A Systematic Review of Evidence (Part 1)	**Alex R. Kemper, MD, MPH, MS** Lead, Evidence-Based Review Group **Jelili Ojodu, MPH** Member, Evidence-Based Review Group **Lisa A. Prosser, Ph.D.** Member, Evidence-Based Review Group
11:45 – 12:30 PM	Lunch	
12:30 – 1:30 PM	Newborn Screening for Spinal Muscular Atrophy (SMA): A Systematic Review of Evidence (Part 2)	
1:30 – 2:00 PM	Committee Report: Newborn Screening for Spinal Muscular Atrophy (SMA)	**Dietrich Matern, MD, PhD** Committee Member **Beth Tarini, MD, MS, FAAP** Committee Member
2:00 – 2:30 PM	Committee Discussion and Vote on SMA	**Joseph Bocchini, MD** Committee Chair
2:30 – 2:40 PM	Follow-Up and Treatment Workgroup Report: The Role of Quality Measures to Promote Long-Term Follow-Up of Children Identified by Newborn Screening Programs	**Jeffrey P. Brosco, MD, PhD** Committee Member Chair, Follow-Up & Treatment Workgroup
2:40 – 2:55 PM	Committee Discussion	**Joseph Bocchini, MD** Committee Chair
2:55 – 3:00 PM	New Business	**Joseph Bocchini, MD** Committee Chair
3:00 PM	Adjourn	

Recommendation to ACHDNC regarding Newborn Screening for Spinal Muscular Atrophy

Beth Tarini M.D., M.S.

Dietrich Matern M.D., Ph.D.

Committee Representatives to the Condition Review Workgroup

ACHDNC Meeting
February 8, 2018

Decision Matrix

- Magnitude and certainty of the net benefit of screening for SMA to the population of affected newborns.
- Feasibility of newborn screening for SMA.
- Readiness of states to implement population-wide screening for SMA.

Secretary's Discretionary Advisory Committee Decision Matrix for Nominated Conditions for the Recommended Uniform Screening Panel (Approved January 31, 2013)

NET BENEFIT/ CERTAINTY			READINESS			FEASIBILITY	
			Ready	Developmental	Unprepared	Feasibility	HIGH or MODERATE / LOW
SIGNIFICANT Benefit	Certainty	HIGH	**A1** Screening for the condition has a high certainty of significant net benefits, screening has high or moderate feasibility. Most public health departments are ready to screen.	**A2** Screening for the condition has a high certainty of significant net benefits and screening has high or moderate feasibility. Public health departments have only developmental readiness.	**A3** Screening for the condition has a high certainty of significant net benefits and screening has high or moderate feasibility. Public health departments are unprepared for screening.		
			A4 There is high certainty that screening would have a significant benefit; however, most health departments have low feasibility of implementing population screening.				LOW
		MOD	**B 1-4** There is moderate certainty that screening would have a significant benefit.			----	
Small to ZERO Benefit		MOD/HIGH	**C 1-4** There is high or moderate certainty that adoption of screening for the targeted condition would have a small to zero net benefit.			----	
NEG Benefit		MOD/HIGH	**D 1-4** There is high or moderate certainty that adoption of screening for the targeted condition would have a negative net benefit.			----	
--	Certainty	LOW	**L 1-4** There is low certainty regarding the potential net benefit from screening.			----	

NET BENEFIT/
CERTAINTY

Spinal Muscular Atrophy
(SMA)

- Autosomal recessive inheritance.
- Majority of SMA patients are homozygous for a deletion of exon 7 of *SMN1* independent of severity (5% of cases are compound heterozygotes).
- Incidence estimated at 1 in 10,000 live births.
- Carrier frequency of 1 in 40 – 80 live births.
- *SMN2* copy number modifies the severity of disease.

NET BENEFIT/ CERTAINTY

Spinal Muscular Atrophy (SMA)

	Alternate name	Age at onset	Max. muscular activity achieved	Life expectancy (palliative care)	*SMN2* copies	% of all SMA cases
SMA type 0	Congenital	Prenatal	None	< 6 months	1	Rare
SMA type I	Severe infantile acute; Werdnig-Hoffmann disease	< 6 months	Never sit without support, problems sucking and swallowing	Median: 24 months	1 – 3	40 – 60%
SMA type II	Infantile chronic; Intermediate; Dubowitz disease	6 – 12 months	Sit independently, lose this ability by mid-teens	70% alive at 25 years	2 – 4	30 – 40%
SMA type III	Juvenile; Wohlfart-Kugelberg-Welander disease	> 18 months	Walk independently, lose this ability with time	Normal	3 – 4	~10%
SMA type IV	Adult onset	20 - 30 years	Mild to moderate muscle weakness; typically only proximal muscles affected	Normal	4 – 5	Possibly often undetected
SMA other	variable	variable	variable	n/a	n/a	variable

NET BENEFIT/ CERTAINTY

Spinal Muscular Atrophy (SMA)

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SMA type IV	Adult onset	20 - 30 years	Mild to moderate muscle weakness; typically only proximal muscles affected	Normal	4 – 5	Possibly often undetected
SMA other	variable	variable	variable	n/a	n/a	variable

NET BENEFIT/ CERTAINTY

Spinal Muscular Atrophy (SMA)

	Age at onset	Max. muscular activity achieved	Life expectancy (palliative care)	*SMN2* copies	% of all SMA cases	Delay of Diagnosis
SMA type 0	Prenatal	None	< 6 months	1	Rare	
SMA type I	< 6 months	Never sit without support, problems sucking and swallowing	Median: 24 months	1 – 3	40 – 60%	3.6 months
SMA type II	6 – 12 months	Sit independently, lose this ability by mid-teens	70% alive at 25 years	2 – 4	30 – 40%	14.3 months
SMA type III	> 18 months	Walk independently, lose this ability with time	Normal	3 – 4	~10%	43.6 months
SMA type IV	20-30 years	Mild to moderate muscle weakness; typically only proximal muscles affected	Normal	4 – 5	Possibly often undetected	
SMA other	variable	variable	variable	n/a	n/a	variable

NET BENEFIT/ CERTAINTY

Spinal Muscular Atrophy (SMA)

	Age at onset	Max. muscular activity achieved	Life expectancy (palliative care)	*SMN2* copies	% of all SMA cases	Delay of Diagnosis
Focus of Evidence Review						
SMA type I	< 6 months	Never sit without support, problems sucking and swallowing	Median: 24 months	1 – 3	40 – 60%	3.6 months
SMA type II	6 – 12 months	Sit independently, lose this ability by mid-teens	70% alive at 25 years	2 – 4	30 – 40%	14.3 months
SMA type III	> 18 months	Walk independently, lose this ability with time	Normal	3 – 4	~10%	43.6 months
SMA type IV	20-30 years	Mild to moderate muscle weakness; typically only proximal muscles affected	Normal	4 – 5	Possibly often undetected	
SMA other	variable	variable	variable	n/a	n/a	variable

NET BENEFIT/
CERTAINTY

Treatment

- **Palliative/symptomatic (ventilator, gastrostomy feeding, physical therapy).**
- **Nusinersen (only FDA approved SMA-specific treatment).**
- **Gene therapy (ongoing trial).**

NET BENEFIT/ CERTAINTY

Treatment

Nusinersen (Spinraza™):

- The only FDA approved SMA-specific treatment.
- Intrathecal administration (6 doses in 1st year, then 1 dose every 4 months).
- Expensive (reported cost: $125,000 per vial/dose).
- Limited data available suggest that treatment effect is greater when:
  - initiated before symptoms develop,
  - *more SMN2* copies are present (likely because later onset and milder phenotype).

NET BENEFIT/ CERTAINTY

Treatment

Nusinersen (Spinraza™):

Limitations of treatment studies:

- No data on long term outcomes (follow up limited to ≤2 years).
- Small study populations (20 presymptomatically treated infants).
- Anecdotally, 1 patient with two *SMN2* copies had normal development at 12 months old (treatment started at 13 days old following positive NBS in NY; Kraszewski JN et al. *Genet Med*. doi:10.1038/gim.2017.152).
- No peer reviewed publications available on presymptomatically treated patients.

NET BENEFIT/
CERTAINTY

Treatment

Nusinersen (Spinraza™):

- Peer reviewed treatment guideline not (yet) published.
- Draft guideline has been developed by an "SMA NBS Multidisciplinary Working Group" using a modified version of the Delphi technique to reach consensus

Treatment Algorithm for Infants Diagnosed with Spinal Muscular Atrophy through Newborn Screening

Glascock J[1], Sampson J[2], Haidet-Phillips A[3], Connolly A[4], Darras B[5], Day J[2], Finkel R[6], Howell R[7], Klinger K[8], Kuntz N[9], Prior T[10], Shieh P[11], Crawford T[12], Kerr D[13], Jarecki J[1].

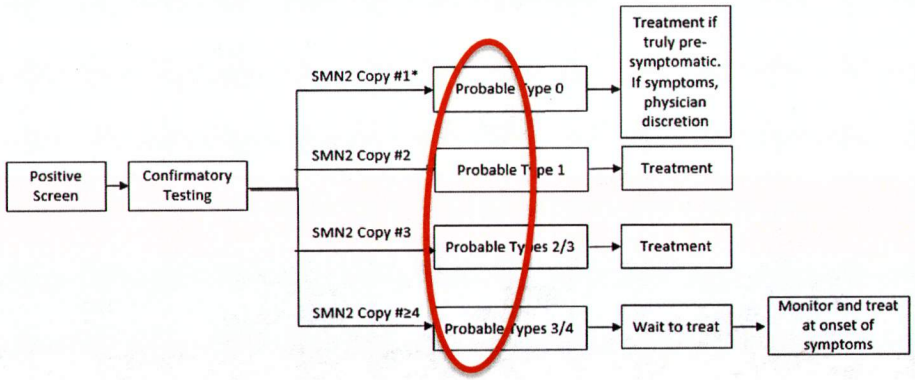
1 Cure SMA, Elk Grove Village, Illinois, USA.
2 Stanford University, Stanford, CA 94304, USA.
3 Muscular Dystrophy Association, Chicago, Illinois, USA
4 Washington University School of Medicine, St. Louis, MO.
5 Department of Neurology, Boston Children's Hospital, Boston, MA.
6 Nemours Children's Hospital, University of Central Florida College of Medicine, Orlando, FL, USA.
7 Miller School of Medicine, University of Miami, Miami, FL.
8 Genzyme Corporation, a Sanofi Company, Framingham, MA.
9 Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL.
10 Department of Molecular Pathology, Ohio State Wexner Medical Center, Columbus, OH.
11 University of California-Los Angeles, Los Angeles, CA.
12 Department of Neurology, Johns Hopkins University, Baltimore, MD, USA.
13 Generation Bio, Cambridge, MA.

NET BENEFIT/ CERTAINTY

Treatment

Nusinersen (Spinraza™):

- Peer reviewed treatment guideline not (yet) published.
- Summary of draft guideline by Glascock J et al.:



"Probable" because SMA types cannot be reliably differentiated by *SMN2* copy number. Presymptomatic treatment outcomes can therefore not be reliably assigned to SMA type.

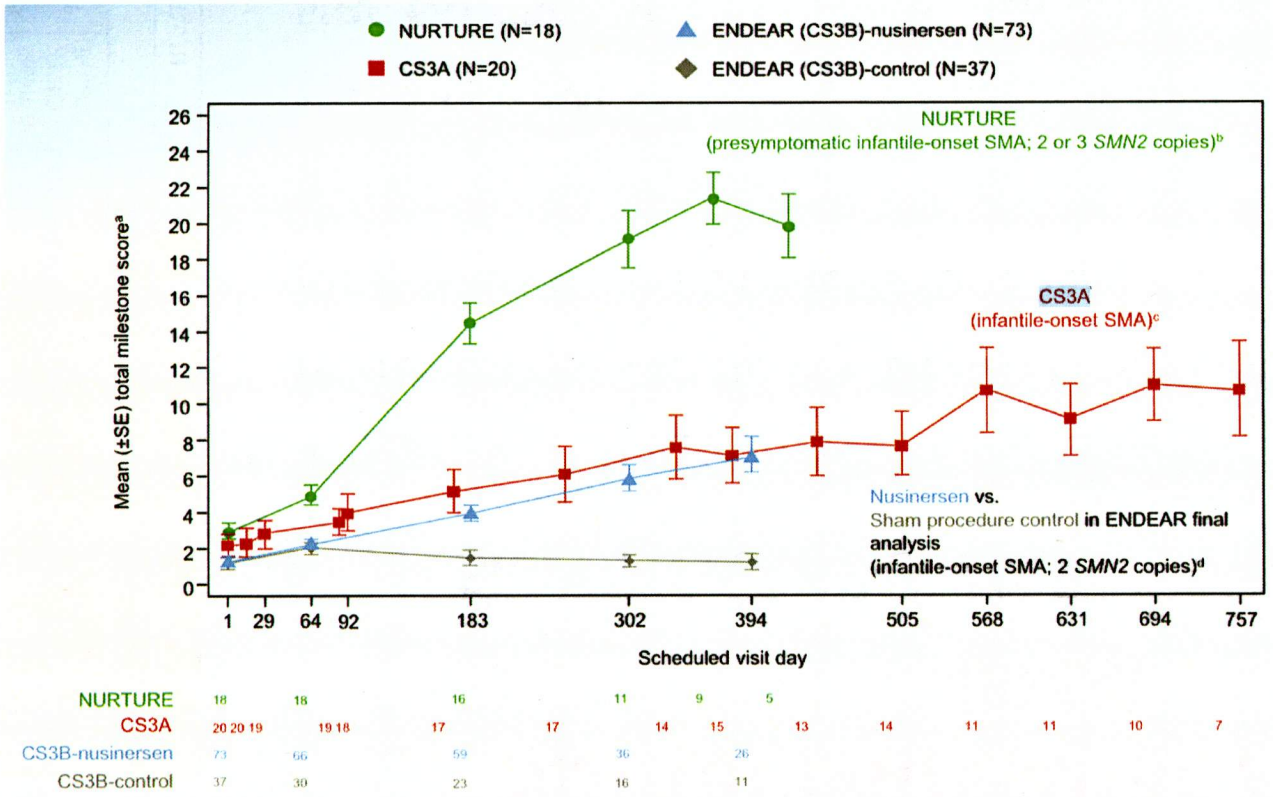
NET BENEFIT/ CERTAINTY

Spinal Muscular Atrophy (SMA)

	Age at onset	Max. muscular activity achieved	Life expectancy (palliative care)	*SMN2* copies	% of all SMA cases	Life Expectancy & Outcome (Nusinersen)
Focus of Evidence Review						
SMA type I	< 6 months	Never sit without support, problems sucking and swallowing	Median: 24 months	1 – 3	40 – 60%	**>12 months, improved development**
SMA type II	6 – 12 months	Sit independently, lose this ability by mid-teens	70% alive at 25 years old	2 – 4	30 – 40%	**>12 months, improved development**
SMA type III	> 18 months	Walk independently, lose this ability with time	Normal	3 – 4	~10%	>12 months, better vs. untreated
SMA type IV	20-30 years	Mild to moderate muscle weakness; typically only proximal muscles affected	Normal	4 – 5	Possibly often undetected	No data
SMA other	variable	variable	variable	n/a	n/a	n/a

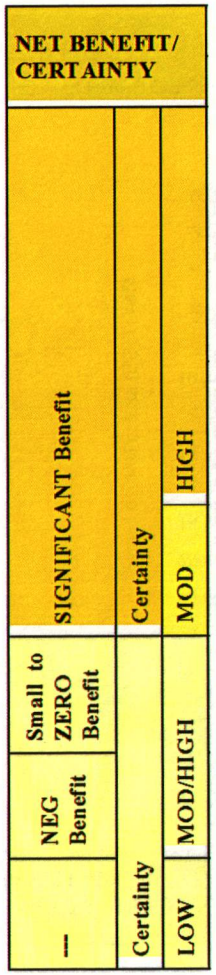
NET BENEFIT/ CERTAINTY

SMA Treatment with Nusinersen



- **Suggests early treatment allows for more normal development.**
- **Does not allow comparison to normal development**

from page 40 of Evidence Review report



Spinal Muscular Atrophy (SMA)

What is SIGNIFICANT Benefit?

- if ***improved*** neuromuscular development and survival, then there appears to be MODERATE certainty of SIGNIFICANT long-term benefit of NBS for SMA;
- if ***normal*** neuromuscular development and survival, then there is LOW certainty of SIGNIFICANT long-term benefit of NBS for SMA given the limited available data, in particular of ***peer reviewed data on presymptomatic treatment*** with Nusinersen which is the only treatment available outside of clinical trials.

Secretary's Discretionary Advisory Committee Decision Matrix for Nominated Conditions for the Recommended Uniform Screening Panel (Approved January 31, 2013)

NET BENEFIT/ CERTAINTY			READINESS			FEASIBILITY	
			Ready	Developmental	Unprepared	Feasibility	HIGH or MODERATE
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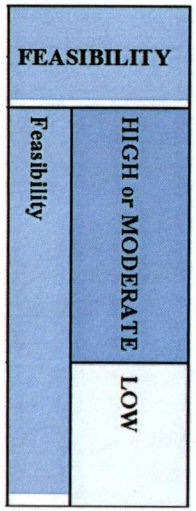
			A4 There is high certainty that screening would have a significant benefit; however, most health departments have low feasibility of implementing population screening.				
		MOD	**B 1-4** There is moderate certainty that screening would have a significant benefit.			----	
Small to ZERO Benefit		MOD/HIGH	**C 1-4** There is high or moderate certainty that adoption of screening for the targeted condition would have a small to zero net benefit.			----	
NEG Benefit			**D 1-4** There is high or moderate certainty that adoption of screening for the targeted condition would have a negative net benefit.			----	
—	Certainty	LOW	**L 1-4** There is low certainty regarding the potential net benefit from screening.			----	

Newborn Screening for SMA



Newborn screening test is available:

- **real-time PCR assay specific for exon 7 deletion in *SMN1*,**
- **expected to identify at least 95% of SMA cases,**
- **will miss ca. 5% of SMA cases that are not homozygous for exon 7 deletion unless:**
  - **carriers for the deletion will be reported (most will not harbor a 2nd mutation),**
  - **a 2nd tier test is performed to rule out a 2nd pathogenic (!) mutation.**

Newborn Screening for SMA

Recommendation to ACHDNC

Newborn Screening for SMA due to homozygous deletion of exon 7 *in SMN1* should be added to the RUSP as a Core Condition under matrix category B2 to the benefit of most patients with SMA.

Newborn Screening for SMA

Considerations

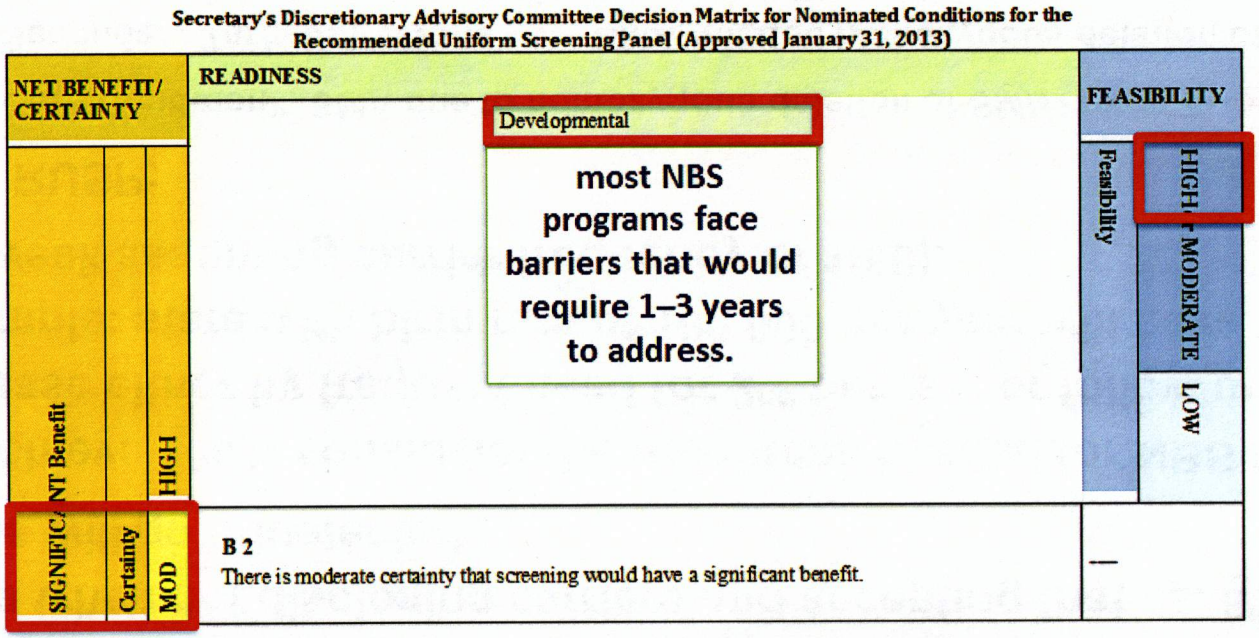
- **NBS would likely show that the majority of SMA cases have SMA type II, III or IV**
- **SMA types II and III are likely to benefit the most from early treatment**

most patients with SMA will benefit from early treatment!

Newborn Screening for SMA

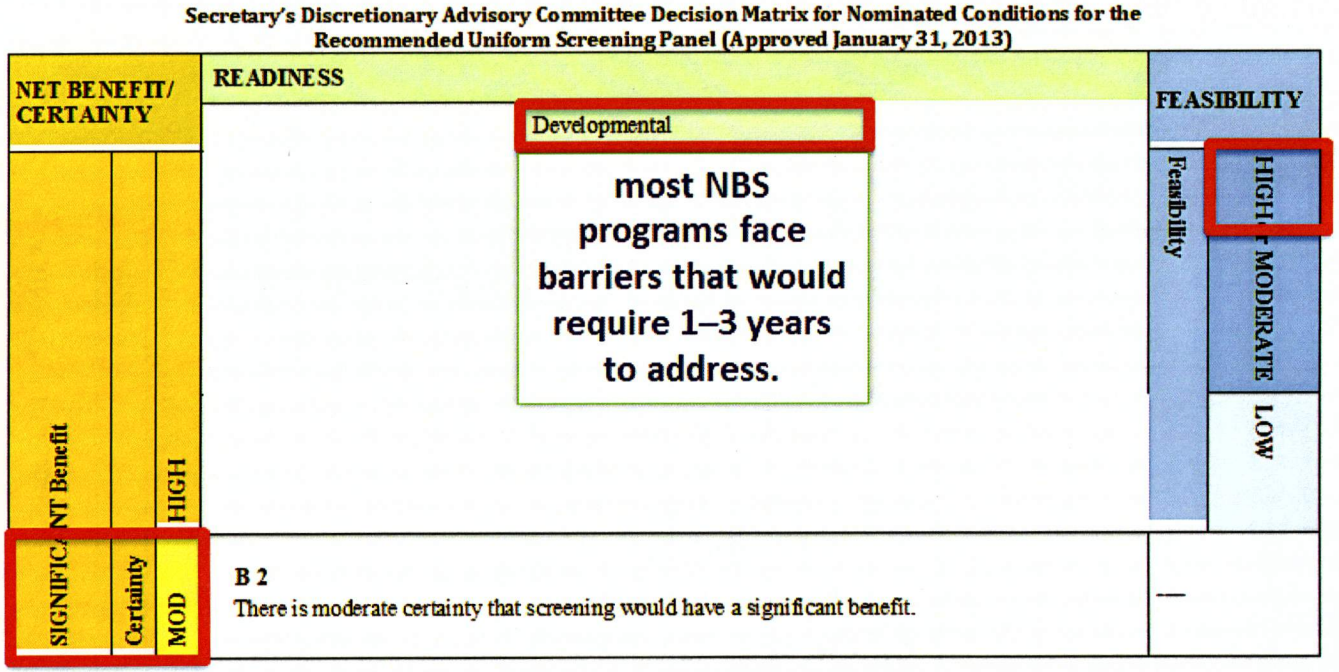
Considerations

- **NBS for SMA is possible at low cost and with high positive predictive value when not disclosing carriers and accepting that ca. 5% of SMA cases will go undetected.**
- **To achieve 100% sensitivity the resources needed for NBS for SMA will increase either by frequent need for 2nd tier test or follow up of carriers (example: state with birth rate of 100,000 per year will have 32 carriers per week assuming carrier frequency of 1:60).**
- **If on RUSP:**
  - **"Core Condition": SMA due to homozygous deletion of *SMN1* exon 7 *or* all of SMA?**
  - **"Secondary Target(s)": None *or* SMA not due homozygous deletion of *SMN1* exon 7 (needs 2nd tier test or reporting of carriers!)**



- **Do we need to wait for peer reviewed guidelines for the management of specific SMA types?** NO

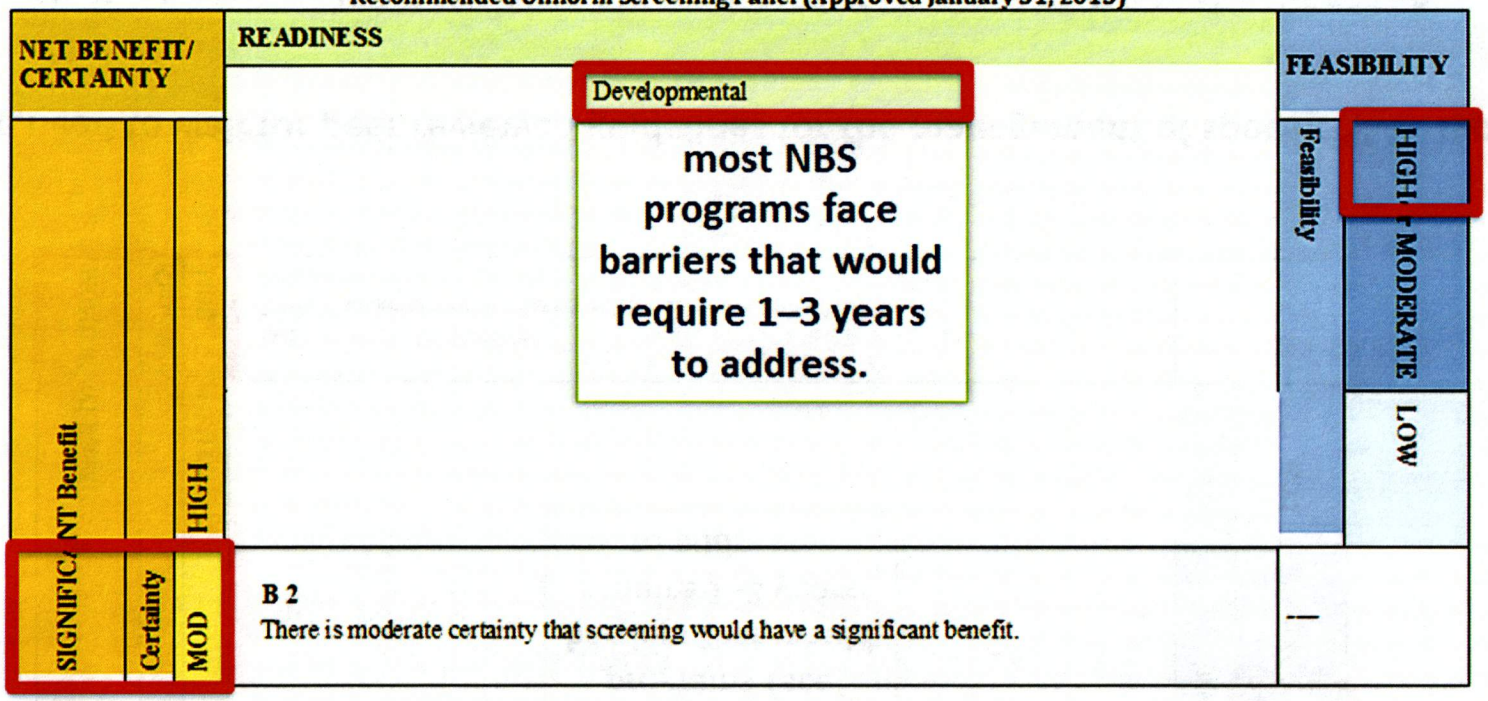
- **What role do disclosure (or not) of carriers and cost of treatment play in the decision?** NONE



- **Do we need to wait for peer reviewed guidelines for the management of specific SMA types?**

- **What role do disclosure (or not) of carriers and cost of treatment play in the decision?**

Secretary's Discretionary Advisory Committee Decision Matrix for Nominated Conditions for the Recommended Uniform Screening Panel (Approved January 31, 2013)



NBS Programs with Mandates/Pilots

State	SMA added to NBS panel	Start	Funds	Time from decision to add to NBS panel to start
MA	12/5/2015 (Adv. Cmte.)	1/29/2018	N/A	**Could have been < 1 year if not for a physical lab move**
MN	12/27/2018	3/5/2018	NBS fee	**< 1 year**
MO	7/11/2017 (Senate Bill 50)	Must start by 1/1/2019	NBS fee	**1.5 years**
NC	-	Apr 2018	NICHD contract	**No decision made**
NY	-	Jan 2016	Biogen	**No decision made**
UT	August 2017 (Rule R438-15)	1/29/2018	NBS fee	**< 1 year**
WI	Expected for July 2018	TBD (likely before 7/2019)	Cure SMA as bridge funding	**< 1 year**

modified from APHL PHSI report

NBS Programs with Mandates/Pilots

State	SMA added to NBS panel	Start	Select or Whole Population	Funds	Carriers	Costs
MA	12/5/2015 (Adv. Cmte.)	1/29/2018	Whole, consent, PILOT	N/A	Not identified	n/a
MN	12/27/2018	3/5/2018	Whole	NBS fee	Not identified	< $1.00
MO	7/11/2017 (Senate Bill 50)	Must start by 1/1/2019	Whole, likely no reporting initially	NBS fee	Decision expected 4/2018	~$1.00
NC	-	Apr 2018	Select, consent, PILOT	NICHD contract	Not reported or identified	n/a
NY	-	Jan 2016	3 hospitals, consent, PILOT	Biogen	Reporting for pilot; undecided future	0.15-$1.00
UT	August 2017 (Rule R438-15)	1/29/2018	Whole	NBS fee	Not identified	TBD
WI	Expected for July 2018	TBD (likely before 7/2019)	Whole	Cure SMA as bridge funding	Not identified	$1.00

modified from APHL PHSI report

Newborn Screening for SMA

READINESS		
Ready	Developmental	Unprepared

Newborn screening tests are available and used already:

- pilot study with consent in 3 hospitals in New York City (1:72 carriers);
- MA began in January 2018 (consent; no carriers identified; not multiplexed);
- Utah began 1/29/2018 (no consent; no carriers identified; multiplexed with SCID);
- Minnesota to begin in March 2018 (no consent; no carriers will be identified; multiplexed with SCID);
- WI to begin in 2018; MO to begin in 2019; NC to begin pilot study in April 2018;
- APHL's PHSI Assessment found:
  - majority of states can implement within 1-3 years;
  - addition of SMA to RUSP would "bolster implementation activities."

Newborn Screening for SMA

READINESS		
Ready	Developmental	Unprepared

Newborn screening test is available

- test can be multiplexed with SCID screening test;
- CDC's Newborn Screening Quality Assurance Program (NSQAP) can provide training, quality control and reference materials;
- incremental cost is small when multiplexed with SCID screening assay;
- higher incremental cost if 100% sensitivity is expected because:
  - 2nd tier test needed on ca. 1 in 60 newborns heterozygous for exon 7 deletion, or
  - ca. 1 in 60 newborns will require follow up but are only carriers.

Newborn Screening for SMA

READINESS		
Ready	Developmental	Unprepared
most NBS programs could implement screening within 1 year after the state makes the decision to include the condition and funding is made available.	most NBS programs face barriers that would require 1–3 years to address.	most NBS programs would take longer than 3 years to implement, even with the decision to add the condition and the availability of funding to begin comprehensive screening.

Decision-making process for conditions nominated to the Recommended Uniform Screening Panel: statement of the US Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children

Alex R. Kemper, MD, MPH[1], Nancy S. Green, MD[2], Ned Calonge, MD, MPH[3], Wendy K.K. Lam, PhD[1], Anne M. Comeau, PhD[4], Aaron J. Goldenberg, PhD, MPH[5], Jelili Ojodu, MPH[6], Lisa A. Prosser, PhD[7], Susan Tanksley, PhD[8] and Joseph A. Bocchini Jr, MD[9]

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	A4 There is high certainty that screening would have a significant benefit; however, most health departments have low feasibility of implementing population screening.			LOW
Certainty MOD	**B 1-4** There is moderate certainty that screening would have a significant benefit.			----

DACHDNC Form for Nomination of a Condition for Inclusion in the Uniform Screening Panel	
DATE	
NAME OF NOMINATOR AND ORGANIZATION (include professional degrees)	**INDICATE AFFILIATION (i.e., Health Professional, Subject Matter Expert, Researcher, Clinician, Advocate, etc.)**
Cure SMA	Advocacy Organization
CO-SPONSORING ORGANIZATIONS (include professional degrees)	**INDICATE AFFILIATION (i.e., Health Professional, Subject Matter Expert, Researcher, Clinician, Advocate, etc.)**
Muscular Dystrophy Association	Advocacy Organization
SMA NBS Working Group (see cover letter)	Subject Matter Experts

Note: Please reference each statement/answer with the corresponding reference number listed in Section III – Key References.

SECTION I – CONDITION INFORMATION AND TREATMENT

SECTION I, PART A

CONDITION	STATEMENT
Nominated Condition	Spinal Muscular Atrophy
Type of Disorder	Autosomal recessive neuromuscular disease
Screening Method	Newborn blood spot screening test using multiplexed real-time PCR[1]
Gene	Survival of Motor Neuron T (SMN1)
Locus	Include ClinVar link if applicable. 5q12.2-13.3, https://www.ncbi.nlm.nih.gov/clinvar/?term=SMN1[all]
OMIM or other names for condition	Include Genetics Home Reference link if applicable. 253300 (SMA1), 253550 (SMA2), 253400 (SMA3), 271150 (SMA 4) http://www.omim.org/entry/600354#0007
Case Definition	NA
Incidence	Determined by what method(s): pilot screening or clinical identification? In the United States, the pan-ethnic disease incidence of SMA, calculated using the measured carrier frequency of SMA of 1/54 and a detection rate of 91.2%, is calculated to be 1/11,000.[2]
Timing of Clinical Onset	Relevance of the timing of newborn screening to onset of clinical manifestations. There are four main clinical subtypes of SMA caused by mutations in the SMN1 gene. While the same disease with the same genetic cause, each subtype has a different

	timing of clinical onset.[3] Infants with the severe variant called SMA Type I, which accounts for 50 to 60% of all cases, are normal at birth. They manifest onset of weakness and respiratory or bulbar insufficiency within the first few months of life. A very small subset of infants are already weak from birth, or are born with congenital arthrogryposis (SMA Type 0). SMA Type II, comprising 30-40% of all cases, has onset of symptoms typically between 6-18 months. SMA Type III patients, comprising about 10% of cases, typically present after 18 months of age through the teen years. Another very small subgroup present in adulthood, and this is called SMA Type IV.[3]
Severity of Disease	Morbidity, disability, mortality, spectrum of severity. As summarized above, according to the consensus care guidelines for SMA, four main clinical sub-types are distinguished.[3] These include the acute infantile type, or Werdnig-Hoffmann disease (SMA Type I; affected infants are never able to sit independently), the intermediate type (SMA Type II; affected children are able to sit but never walk), the mild type (SMA Type III; affected individuals are ambulatory and typically manifest weakness after 18 months of age), and the adult onset form (SMA Type IV; affected individuals are ambulatory and typically manifest weakness as adults). The most severe form, SMA Type I, occurs during infancy and accounts for 50-60% of all cases; these children never sit, and 100% suffer bulbar and respiratory insufficiency with early mortality.[3] Two recent natural history studies in infants with SMA Type I have shown that the median age to reach the combined endpoint of death or requiring at least 16 hours/day of ventilation support is 13.5 and 8 months, respectively.[4,5] In these natural history studies, requirements for nutritional support preceded ventilation support, and the mean rate of decline in motor function as measured by The Children's Hospital of Philadelphia Infant Test for Neuromuscular Disorders scale was 1.27 points/year.[4] SMA Type II and III are slowly progressive with little change in motor function observed in most patients over a twelve-month period. Functional declines are observed over periods exceeding one-year.[6] Survival probabilities at 2, 4, 10, and 20 years of age have been reported to be 100%, 100%, 98%, and 77% in children with SMA Type II. For patients with SMA Type III, life expectancy has not been reported to be significantly less than in the unaffected population, although a significant portion lose the ability to walk by 40 years of age.[7,8] A very small subgroup of individuals present in adulthood, and this is called SMA Type IV. Regardless of clinical severity, 95% of all SMA patients have the same homozygous SMN1 gene deletion, and detection of the SMN1 gene deletion is used as the primary diagnostic assay. All patients possess a low-functioning analog to the SMN1 gene called SMN2. The SMN2 copy number is predictive of clinical severity. Humans have a variable copy number of the *SMN2* gene (0-8 copies), which correlates with SMA disease severity. Importantly, in the context of NBS, 80% of patients with SMA Type I carry one or two SMN2 copies, and 82% of patients with SMA Type II carry three SMN2 copies, whereas 96% of patients with Type III SMA carry three or four SMN2 copies.[9] *SMN2* is a key determinant of disease phenotype and is routinely determined after initial diagnosis to help predict the clinical phenotype. Thus, it is highly likely an infant identified by NBS with subsequent testing showing 3 or fewer copies of SMN2 will present with Type I or Type II SMA, which are associated with substantial early morbidity and/or mortality. Therefore, the identification of homozygous SMN1 deletion and determination of SMN2 copy number allows confident prediction that an infant will develop SMA.

SECTION I, PART B

TREATMENT	STATEMENT
Modality	Drug(s), diet, replacement therapy, transplant, other. Include information regarding regulatory status of treatment. **On December 23, 2016, the FDA approved the first disease-modifying therapy for SMA called Spinraza (nusinersen) for the treatment of SMA patients of all types and ages.** Spinraza, marketed by Biogen, is an antisense oligonucleotide drug that alters splicing of the SMN2 pre-mRNA to increase the amount of full-length SMN2 mRNA. Full-length SMN2 mRNA is translated into mRNA to increase the amount of functional SMN protein. (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm534611.htm). In addition to Spinraza, a number of clinical care approaches have been shown to improve survival and quality of life in SMA Type I, including: 1) nutritional support and careful monitoring of nutritional intake and swallow function, typically resulting in additional supplementation orally at first and then placement of nasogastric, nasojejunal or gastrostomy tube as needed, and prevention of fasting/catabolic state given their severe sarcopenia, and 2) respiratory support including techniques to mobilize and clear lower airway secretions such as chest physiotherapy devices, cough assist devices and pulse oximetry monitoring, and also the use of respiratory support devices including bi-level positive airway pressure via face/nose mask or tracheostomy tube to treat sleep disordered breathing.[3,10] There are also five additional therapies in development for the treatment of SMA, including SMN1 gene replacement therapy, small molecules designed to alter SMN2 mRNA splicing, and additional small molecule approaches aimed at motor neuron protection and muscle enhancement. These include Olesoxime sponsored by F. Hoffman - La Roche, which is a small molecule designed to prevent neuronal cell death (clinical trial identifiers: NCT02628743, NCT01302600, contact: Sangeeta Jethwa Schnetzler MD at sangeeta.jethwa@roche.com). There is also AVXS-101 sponsored by AveXis, which is a gene therapy to replace the SMN1 gene (clinical trial identifier: NCT02122952, contact: Douglas M. Sproule MD MSc at dsproule@avexis.com). LMI070 is sponsored by Novartis Pharmaceutical and is a small molecule designed to alter splicing of SMN2 mRNA and increase the amount of functional SMN protein (clinical trial identifier: NCT02268552, contact: Lawrence Charnas MD PhD at lawrence.charnas@novartis.com). RO7034067 and RO6885247 are sponsored by F. Hoffmann – La Roche and are small molecules designed to alter splicing of SMN2 mRNA and increase functional SMN protein (clinical trial identifiers: NCT02633709, NCT02240355, NCT02908685, NCT02913482, contact: Sangeeta Jethwa Schnetzler MD at sangeeta.jethwa@roche.com). Finally, there is CK-2127107, which is sponsored by Cytokinetics and is a small molecule to enhance muscle contraction (clinical trial identifier: NCT02644668, contact: Stacy A. Rudnicki MD at srudnicki@cytokinetics.com).
Urgency	How soon after birth must treatment be initiated to be effective? Both human natural history data and animal model data suggest that early drug intervention is required for greatest efficacy in the most common and severe form of SMA Type I. **In fact, in human SMA Type I, there is strong evidence that the irreplaceable loss of motor neurons begins early in the perinatal period, with severe denervation in the first 3 months of life and loss of more than 90% of motor units within 6 months of age.**[1] Moreover, a recent multi-center natural history study conducted by the NINDS NeuroNEXT clinical trial network in infants under six

	months of age with genetically confirmed SMA has shown significant differences between the SMA and control infants at the baseline visit in motor function tests, ulnar compound muscle action potential, and electrical impedance myography (EIM).[5] Moreover, studies looking at the timing of drug delivery in mouse models of SMA Type I have strongly suggested that early administration of SMN-based drug therapies is more effective than post-symptomatic delivery. **The results have been remarkably consistent across modalities including genetic means, gene therapy vectors, and antisense oligonucleotides to increase SMN levels. All have demonstrated the best results when the drugs are given as early as possible before significant motor weakness or loss in severe mouse models of SMA.[12, 13]** In addition, supportive treatment in the first few weeks to months of life prolongs survival and improves quality of life. In fact, the increases in survival of the type I infants over the past decade have been documented to correlate specifically to proactive respiratory and nutritional care.[10] However, in the current environment in the absence of newborn screening, these interventions remain predominantly reactive to medical crises. Many SMA Type I infants' initial presentation is in crisis with acute respiratory failure or bulbar insufficiency with aspiration prior to diagnosis and often associated with common viral respiratory infections. **In fact, diagnostic delay is very common in SMA. A recent systematic literature search conducted from 21 reports in PubMed and Web of Science databases for studies published between 2000 and 2014 showed that the mean ages of onset were 2.5, 8.3, and 39.0 months for SMA Types I, II, and II, respectively, while the weighted mean ages of confirmed spinal muscular atrophy genetic diagnosis were 6.3, 20.7, and 50.3 months, respectively, for Types I, II, and III.[14]** Better clinical outcomes are possible simply with the use of the currently available proactive care options, such as gastrostomy tube surgery prior to an aspiration event, and proactive respiratory care including use of the cough assist device to mobilize respiratory secretions and nocturnal bi-level positive airway pressure support via mask or nasal interface.[3] **A comprehensive rationale for the urgency for SMA newborn screening has been delineated in the review article, "Newborn screening for spinal muscular atrophy: Anticipating an imminent need".[12]**
Efficacy (Benefits)	Extent of prevention of mortality, morbidity, disability. Treatment limitations, such as Difficulty with acceptance or adherence. *Spinraza in Symptomatic Infants:* The efficacy of Spinraza was demonstrated in the ENDEAR Phase III randomized, double-blinded, sham-controlled clinical trial in 121 patients with infantile-onset SMA with two copies of SMN2 who were diagnosed before 6 months of age and who were less than 7 months old at the time of their first dose. Results were reported at the 2016 International Congress of the World Muscle and at the 43rd Annual Congress of the British Paediatric Neurology Association Meeting (see both slide decks appended to the references and at http://media.corporate-ir.net/media_files/IROL/22/222170/Kuntz_ENDEAR_study_design_WMS_LB_podium_Draft2v2_PIPE14989.pdf; http://newsroom.biogen.com/press-release/rare-and-genetic-diseases/new-data-show-spinraza-nusinersen-significantly-reduces-risk). Patients were randomized to receive an injection of Spinraza, into the fluid surrounding the spinal cord, or undergo a mock procedure without drug injection (sham). The trial assessed two primary endpoints: 1) percentage of patients with improvement in motor milestones, such as head control, sitting, ability to kick in supine position, rolling, crawling, standing and walking by measuring the proportion of motor milestone responders with the Hammersmith Infant Neurological Examination (HINE) and 2)

percentage of patients reaching the combined endpoint of death or greater than 16 hours per day of ventilatory support.

At a pre-specified interim analysis, 78 of 121 patients had the opportunity to be on treatment/sham for at least 6 months and were eligible for analysis (data available in the appended slides). Forty-one percent of patients treated with Spinraza (n=51) achieved improvement in motor milestones, whereas none of the control patients did (n=27, p<0.0001). Spinraza met the pre-specified primary endpoint for event-free survival, demonstrating a statistically significant 47% reduction in the risk of death or permanent ventilation (p<0.01). In the analysis, a greater percentage of untreated infants (68%) died or required permanent ventilation compared to infants treated with Spinraza (39%). Spinraza also demonstrated a favorable safety profile. The commonly reported adverse events include respiratory events and constipation, consistent with those expected in the general population of infants with SMA. The interim analysis represents 44.89 patient years of exposure to Spinraza treatment.

Open label trial results of Spinraza in both infants and children have been recently published.[15,16,17]

Spinraza in Pre-symptomatic Infants:
Biogen is currently conducting a Phase 2, open-label, multicenter study in 10 countries for pre-symptomatic infants with SMA termed NURTURE. The study objective is to evaluate the efficacy and safety profile of Spinraza in infants with genetically diagnosed and pre-symptomatic SMA. The planned enrollment is up to 25 infants, with key inclusion criteria of 1) less than 6 weeks of age at first dose, 2) pre-symptomatic, 3) genetic diagnosis of 5q SMA gene deletion or mutation, 4) 2 or 3 SMN2 copies, and 5) Ulnar CMAP amplitude ≥1 mV at baseline. The primary study endpoints are time to respiratory intervention (invasive or non-invasive ventilation for ≥ 16 hours/day continuously for ≥7 days or tracheostomy) or death. The secondary endpoints include: safety, tolerability, pharmacokinetics, motor function milestones, survival (proportion of patients alive), and growth parameters.

The results of an interim analysis were presented at the 2016 International Congress of the World Muscle Society (see slides at appended here and link to media.corporate-ir.net/media_files/IROL/22/222170/Bertini_NURTURE_interim_WMS_LB_podium_draft%202_PIPE-14990_3Oct16.pdf) and at the 43rd Annual Congress of the British Paediatric Neurology Association (BPNA) Meeting (see appended slides). At the interim analysis on June 8, 2016, 13 infants had reached their first efficacy assessment. All were still alive and did not require invasive ventilation at all or non-invasive ventilation for greater than 6 hours per day continuously for more than 7 days. 10 of 13 infants demonstrated increased motor milestones from baseline to last evaluation as measured by HINE and CHOP INTEND tests of motor function. In infants with 2 copies of SMN2 (likely to present with SMA Type I and having the same genotype of those enrolled in the ENDEAR Phase III trial) motor milestone development based on HINE Motor Milestone Achievements were as follows: 1) head control in 55% (5 of 9 infants), 2) sitting independently in 44% (4 of 9 infants), 3) standing in 22% (2 of 9 infants), and walking in 11% (1 of 9 infants). This data can be seen in slide 9 of the appended slide deck on NURTURE at from the WMS meeting. In comparison, a recent natural history study of developmental milestone achievement in 33 Type 1 SMA infants confirmed that no infants in the study achieved a major milestone such as rolling over, or sitting independently.[18] **More importantly, comparing the NURTURE trial data to the ENDEAR trial data of symptomatic infants clearly demonstrates greater attainment of milestones with pre-**

symptomatic treatment. In the interim analysis of the ENDEAR trial, the following age appropriate motor milestone development based on HINE Motor Milestone Achievements were met: 1) head control in 18% infants with treatment (n=51) and 0% in the sham (n=27), 2) sitting independently in 10% of infants with treatment and 0% in the sham, 3) standing in 2% of infants with treatment and 0% in the sham.

Table 1. Summary of HINE motor milestone achievements of infants receiving Spinraza in NURTURE[a] versus infants receiving Spinraza in ENDEAR.[b, c]

MILESTONE	Total no. of infants achieving milestone (%)	
	NUTURE (open-label, n=9)	ENDEAR (treated infants, n=51)
Head control (Full)	5/9 (55%)	9/51 (18%)
Sitting (Independent: stable, pivot)	4/9 (44%)	5/51 (10%)
Standing (Stands with support, unaided)	2/9 (22%)	1/51 (2%)
Walking (Cruising, walking)	1/9 (11%)	0/51 (0%)

[a]Only infants with 2 copies of SMN2 were included in this table (no 3 copy SMN2 patients were included from the NUTURE trial). All infants who enrolled in ENDEAR had 2 copies of SMN2.

[b]The ENDEAR interim was performed when 51 subjects who received Spinraza had the opportunity to be treated and observed for at least 183 days and up to 394 days.

[c]The data included in this chart are taken from a June 8, 2016 interim analysis of NUTURE and a June 15th, 2016 interim analysis of ENDEAR. An updated data set for NUTURE is expected to be presented at the American Academy of Neurology Annual Meeting April 22-28, 2017.

The greater attainment of motor milestones in NUTURE versus ENDEAR is also demonstrated by the mean total HINE score. This data can be seen in slide 11 of appended slide deck from BPNA meeting. The following was observed around 300 days of treatment: ~12 point total mean improvement in NURTURE (n=5), ~4 point mean total improvement in the treatment group of ENDEAR (n=51), and less than a 2 point total mean improvement in the ENDEAR sham group (n=27). This data can be seen on slide 12 of an appended presentation from the recent BPNA meeting. **Thus, the total mean HINE score improvement was substantially higher in the pre-symptomatically-treated infants.**

Spinraza in Children and Teens:
In addition, a placebo controlled Phase III trial in children called CHERISH has been ongoing at over 30 sites worldwide. CHERISH was a fifteen-month study investigating Spinraza in 126 non-ambulatory patients with later-onset SMA (consistent with Type 2), including patients with the onset of signs and symptoms at greater than 6 months and an age of 2 to 12 years at screening. The trial has been recently stopped due to positive results from an interim analysis. Results from the primary endpoint of the pre-specified interim analysis demonstrated a difference of 5.9 points (p= 0.0000002) at 15 months between the treatment (n=84) and sham-controlled (n=42) study arms, as measured by the Hammersmith Functional Motor Scale Expanded (HFMSE). From baseline to 15 months of treatment, patients who received Spinraza achieved a mean improvement of 4.0 points in the HFMSE, while patients who were not on treatment declined by a mean of 1.9 points. See http://media.biogen.com/press-

	release/corporate/biogen-and-ionis-pharmaceuticals-announce-spinraza-nusinersen-meets-primary- for more details. *Phase 1 Gene Therapy Trial of AVXS-101 in Symptomatic Infants:* At the 2016 International Congress of the World Muscle Society, AveXis presented information on their Phase 1/2 trial of AVXS-101 for SMA (see slides at http://investors.avexis.com/phoenix.zhtml?c=254285&p=irol-calendar). The open-label study is designed to evaluate safety and efficacy of AVXS-101 in infants with two copies of SMN2 less than nine months of age. The primary outcome in the study is safety and tolerability. The secondary outcome measure is an efficacy measure as defined by the time from birth to an "event." Exploratory outcome measures include the CHOP-INTEND score, a motor function scale used in infants with SMA. There were two dosing cohorts, consisting of three patients in a low-dose cohort (6.7 $X10^{13}$ vg/kg) and six patients in a mid-dose cohort (2.0 $X10^{14}$ vg/kg). As of September 15, 2016, AVXS-101 appeared to have a favorable safety profile and to be generally well tolerated. Four patients experienced treatment related elevation in serum transaminase levels, which were clinically asymptomatic and resolved with prednisolone treatment. Observed increases in motor function appear to be dose dependent, with the low dose cohort increasing an average of 9.0 points from an average baseline CHOP INTEND score of 16.3 points and the high dose cohort increasing an average of 24.8 points from an average baseline CHOP INTEND score of 28.2 points. Comparative natural history data for similar patients with SMA Type I has shown that none have been observed scoring above 40 points by 6 months of age, with one transient exception. In this study, 11 out of 12 patients in the high dose cohort reached a CHOP INTEND score ≥40 points, 9 out of 12 patients reached a CHOP INTEND score ≥50 points, and 3 out of 12 patients reached a CHOP INTEND score ≥60 points. In addition, all but one patient in the high dose cohort gained a milestone: -11 out of 12 patients could sit with assistance -8 out of 12 could sit unassisted, including one patient who achieved the milestone after September 15, 2016 -7 of 12 patients could roll -2 of 12 were walking independently; these two patients each achieved earlier and important developmental milestones such as crawling, standing with support, standing alone and walking with support. In contrast, data from a recently reported natural history study in 33 Type I SMA infants, shows that none achieved a major milestone such as rolling over, or sitting independently.[18] All patients in Cohort 2 (proposed therapeutic dose) were alive and event free. The median age at last follow-up for Cohort 2 is 17.3 months, with the oldest patient at 27.4 months. Natural history data shows a 25% survival rate at 13.6 months.[4] AveXis announced recently that it plans a pivotal study of AVXS-101 in SMA Type I infants starting in the second quarter of 2017. It will use a single-arm design with natural history of the disease as a comparator and is expected to enroll 20 patients.
Availability	Limits of availability? Spinraza, which is marketed by Biogen, is FDA approved for the treatment of SMA patients of all ages and types. The FDA approved Spinraza on December 23rd, 2016. (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm534611.htm).

SECTION I, PART B

	In addition to Spinraza, interventions including bi-level pressure support (BIPAP) and placement of a gastrostomy feeding tube are widely available at major medical centers. They are currently recommended as the standard care options of choice for infants with SMA Type I in the "Consensus Statement for Standard of Care in Spinal Muscular Atrophy".[3]
Potential Harms of Treatment	Potential medical or other ill effects from treatment Spinraza is a therapy administered into the intrathecal space and in some cases, anesthesia is required for administration. Therefore, the established risks of routine lumbar puncture procedure exist, which include headache, nausea, bleeding and CSF leak. In addition, there are standard risks of anesthesia, which depend upon the anesthetic used. Specifically in the SMA population, there is the respiratory risk for general anesthesia, but for whom local anesthesia would not be sufficient. Also, SMA patients who have undergone scoliosis surgery may have complicated intrathecal access, potentially requiring fluoroscopic guidance, which would include cumulative radiation risk. Finally, children responding to therapy may have improving, but still weak motor skills, and may roll or fall resulting in respiratory compromise or injury. In regard to Spinraza, the most common side effects found in participants in the clinical trials were upper respiratory infection, lower respiratory infection and constipation. Warnings and precautions include low blood platelet count and toxicity to the kidneys (renal toxicity). In the randomized Phase III ENDEAR clinical trial, no patient had a platelet count less than 50,000 cells per microliter and no patient developed a sustained low platelet count despite continued drug exposure. Toxicity in the nervous system (neurotoxicity) was observed in animal studies. The FDA USPI for Spinraza can be accessed at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/209531lbl.pdf

SECTION II – EVIDENCE-BASED INFORMATION

For a nominated condition to be considered there are 3 core requirements:

1. Validation of the laboratory test (see Section II, Part A)
2. Widely available confirmatory testing with a sensitive and specific diagnostic test (see Section II, Part B)
3. A prospective population based pilot study (see Section II, Part C)

SECTION II, PART A

TEST	STATEMENT
Screening test(s) to be used	Description of the high volume method, instrumentation and if available as part of multi-analyte platform. There have been two pilots conducted using the assays described below. They are referred to by the location in which they took place, Taiwan and New York state. Also listed is an assay in development by PerkinElmer for which R&D studies are currently being conducted and pilot studies are being planned. It is being adapted from the assay described in reference number 1. TAIWAN: Real-time PCR TaqMan® single nucleotide polymorphism (SNP)

SECTION I, PART B

	genotyping assay on a StepOnePlus™ RT-PCR 96-well System (Applied Biosystems). The assay targets a SNP in SMN1 intron 7 to distinguish SMN1 from SMN2 using a Taqman probe.[1] NY State: A custom TaqMan real-time polymerase chain reaction (PCR) assay targeting the SMN1 exon 7 deletion and a fragment of RNaseP (used as an internal control gene) are run on a real-time PCR platform such as an ABI 7900 or QuantStudio™ 12K Flex Real-Time PCR System (ThermoFisher™ Scientific). Testing can be conducted in 96-well or 384-well format. The screen can be conducted using the same instrumentation and platform as used for molecular SCID screening, allowing the tests to be multiplexed as described previously.[1] PERKINELMER: Real-time PCR assay targeting SMN1 and SMN2 SNPs in exon 7 using dual-labeled lock nucleic acid Taqman® probes. The 5-plex assay detects SMN1, SMN2, TREC (to detect SCID), KREC (to detect XLA), and RNaseP (internal control). Preliminary testing has been conducted using a 96-well or 384-well format on a ThermoFisher™ QuantStudio 5 or a QuantStudio™ DX real time PCR platform.
Modality of Screening	(Dried blood spot, physical or physiologic assessment, other) TAIWAN: DNA extracted from 3-mm dried blood spot punch to detect homozygous deletions in SMN1 intron 7. NY State: DNA extracted from 3-mm dried blood spot punch to detect homozygous and heterozygous deletions in SMN1 exon 7. PERKINELMER: DNA extracted from 3-mm dried blood spot punch to detect homozygous and heterozygous (if desired) deletions in SMN1 exon 7 and SMN2 copy number.
Does the screening algorithm include a second tier test? If so, what type of test and availability?	(Dried blood spot, physical or physiologic assessment, other) TAIWAN: A feasibility assessment for a second tier test has been completed. The proposed second tier test is a digital droplet PCR (ddPCR) to exclude false positives and to detect SMN2 copy number. NY State: Second tier testing includes: 1. Targeted sequencing in infants positive for SMN1 deletion for quality assurance, to rule out allelic dropout due to polymorphisms in the SMN1 assay primer and probe binding sites. 2. SMN2 copy number testing can be performed in affected infants using a custom TaqMan assay and run on the same platform as the SMN1 assay. Alternatively, a digital droplet PCR kit (ddPCR SMN2 Copy Number Determination Kit) is commercially available from BioRad and can be run on the BioRad platform (for example, QX200 Auto DG Digital Droplet PCR system). PERKINELMER: No second tier test is needed as the primary assay detects both SMN1 and SMN2 copy number.

TEST	STATEMENT
Clinical Validation	Location, duration, size, preliminary results of past/ongoing pilot study for clinical validation, positive predictive value, false positive rate, analytical specificity, sensitivity. TAIWAN: The screening method was validated by testing the DBS samples of 2,937 anonymous newborns and 9 DNA samples with known SMN1 and SMN2 copy numbers. From November 2014 to September 2016, 120,267 infants have been tested in a consented pilot study at the National Taiwan University Hospital with: PPV=100% FPR=0% (First tier screen identified 15 positives out of 120,000 screened. Eight of the 15 positives were ruled false positives during second tier testing.) Analytical SP=100% Analytical SS=100% NY State: From January 2016 to December 2016, 3,269 infants have been tested in an ongoing consented pilot study at three hospitals in New York City with: PPV= 100% FPR= 0% Analytical SP= 100% Analytical SS= 100% PERKINELMER: An R&D study is being conducted to screen over 3,000 DBS samples. Thus far, 1,080 DBS samples, along with characterized reference samples and controls, have been screened. From those: PPV=100% FPR=0% Analytical SP=100% Analytical SS=100%
Analytical Validation	Limit of detection/quantitation, detection rate, reportable range of test results, reference range. Include regulatory status of test, information about reference samples and controls required for testing and availability of or potential for external quality assurance system, e.g., QC and PT for both screening and confirmatory tests. TAIWAN: LOD/LOQ= Provided that DNA can be extracted from a given blood spot, the screen is valid. The presence of DNA is assessed by ensuring amplification of the internal control gene. Detection rate=100%. All specimens with homozygous deletions of SMN1 have screened positive with this method. Reportable range: Results are reported as: No SMN1 homozygous deletion; SMN1 homozygous deletion. This screening method does not detect point mutations in the SMN1 gene, which are present in around 5% of SMA. It does detect a hybrid SMN1 allele present in the Taiwanese population resulting in identification of some false positives. Quality controls in each 96-well plate included a water blank, a filter paper blank, and 3 DNA samples with known SMN1:SMN2 copy numbers, 0:2 (affected), 1:2 (carrier), and 2:2 (normal). This test is intended as a LDT (laboratory developed test) and is not FDA approved. NY State: LOD/LOQ= Provided that DNA can be extracted from a given blood spot, the screen is valid. The presence of DNA is assessed by ensuring amplification of the internal control RNaseP gene, where amplification at Ct

	<=37 indicates the presence of DNA. Detection rate= 100%. All specimens with known homozygous deletions of SMN1 exon 7 have screened positive using the screening assay. This screening method does not detect point mutations in the SMN1 gene. Reportable range= Results are reported as: No SMN1 exon 7 deletion; Heterozygous SMN1 exon 7 deletion; Homozygous SMN1 exon 7 deletion. The NYS newborn SMA screening tests are LDTs and are not FDA-cleared/approved. The SMN1 screen underwent NYS regulatory approval and is an approved clinical test. Specimens from infants with known SMA genotypes (including affected and carriers) obtained from outside collaborators are used as positive controls and for proficiency testing. PERKINELMER: LOD/LOQ= Provided that DNA can be extracted from a given blood spot, the screen is valid. The presence of DNA is assessed by ensuring amplification of the internal control RNaseP gene. Detection rate= 100%. All specimens with known homozygous deletions of SMN1 exon 7 have screened positive using the screening assay. Reportable range= Results are reported as: No SMN1 exon 7 deletion; Heterozygous SMN1 exon 7 deletion; Homozygous deletion. SMN2 copy number is also reported. PerkinElmer offers a Customer Contracted Manufacturing Service (CCMS) where laboratories with an existing LDT may source their raw materials from, achieving a greater level of control over the quality.
Considerations of Screening and Diagnostic Testing	False positives, carrier detection, invasiveness of method, other. TAIWAN: False positives: A hybrid allele of SMN1 present in the Taiwanese population gives rise to a false positive result. 8 false positives have been reported. Carrier detection: Carriers are not detected. Invasiveness of method: Minimal, results obtained from routine newborn DBS acquired via heel stick. NY State: False positives: None reported. Carrier detection: Carriers are detected and reported (heterozygous SMN1 exon 7 deletion). 48 carriers have been detected thus far. Invasiveness of method: Minimal, results obtained from routine newborn DBS acquired via heel stick. PERKINELMER: False positives: None reported. Carrier detection: Carriers are detected (SMN1 heterozygous). SMN2 copy number (a determinant of disease severity) is detected. Invasiveness of method: Minimal, results obtained from routine newborn DBS acquired via heel stick.
Potential Secondary Findings	Detection or suggestion of other disorders. TAIWAN: None NY State: None PERKINELMER: None

SECTION II, PART B

CONFIRMATORY	

TESTING	STATEMENT
Clinical and Analytical Validity	Quantitative or qualitative? Include sensitivity, specificity, etc. The diagnostic test for SMA detects the presence or absence of the SMN1 gene, due to deletion or gene conversion of the SMN1 gene to the SMN2 gene, using DNA analysis of both SMN1 alleles. It is an allele specific real-time PCR assay. It is estimated to be 99% analytically sensitive for the presence of the SMN1 gene and approximately 95% clinically sensitive for patients with features of SMA. Approximately 5% of affected individuals with SMA have compound heterozygosity for a rare intragenic point mutation within the SMN1 gene on one chromosome and a deletion/gene conversion of SMN1 exon 7 on the other chromosome. The standard molecular diagnostic test is unable to detect those individuals.[19] However, there are clinical tests available, although not widely utilized, which can detect SMN1 point mutations in cases in which a patient shows clinical signs of SMA but the carrier genotype: www.ncbi.nlm.nih.gov/gtr/all/tests/?term=C0026847. The standard diagnostic test does not identify patients who are heterozygous carriers for the SMN1 deletion, and a distinct diagnostic test is routinely utilized to detect carriers.[19, 20]
Type of test and/or sample matrix (blood, radiology, urine, tissue sample, biophysical test)	The SMA diagnostic test is conducted from a whole blood sample.[18] It can also be conducted prenatally from amniotic fluid or amniocytes or chorionic villus (CVS) culture.[18]
Is test FDA cleared/approved	Include availability information, sole source manufacturer, etc. No
List all CLIA certified labs offering testing in the US	Link to GeneTests and Genetic Test Reference if applicable. The SMA diagnostic test is available at CLIA certified labs throughout the United States. A comprehensive list can be found at: https://www.genetests.org/search/tests.php?locations[]=USA&user_submitted=1&search=SPINAL+MUSCULAR+ATROPHY&filter_status=1

SECTION II, PART C

POPULATION-BASED PILOT STUDY	STATEMENT
Location of Prospective Pilot	Two prospective pilots have been ongoing in SMA. They are described below. TAIWAN: National Taiwan University Hospital newborn screening center, Taiwan (manuscript under review at time of submission, Dr. Yin-Hsiu Chien personal communication). Contact: Dr. Yin-Hsiu (Nancy) Chien, National Taiwan University: chienyh@ntu.edu.tw NY STATE: New York, New York (unpublished, Dr. Wendy Chung personal communication). Contact: Dr. Wendy Chung, Columbia University (New York): wkc15@cumc.columbia.edu PERKINELMER: No prospective pilots had begun using this assay at the time of submission, but plans are in development for such a study in Wisconsin, once the retrospective study on de-identified dried blood spots described in the section above is completed. Dr. Mei Baker, Wisconsin Laboratory of Health: mei.baker@slh.wisc.edu Additional pilots are proposed in Massachusetts and North Carolina. -MA Contact: Dr. Anne Comeau: New England Newborn Screening Lab (Massachusetts): Anne.Comeau@umassmed.edu -NC Contact: Dr. Don Baily at RTI: dbailey@rti.org Plan to begin Spring 2018. Legislation to add SMA to statewide NBS in Missouri (HB 66) has been proposed and is currently undergoing the approval process. As of 2/16/17, the bill had passed the Missouri House of Representatives and will be moved to the Senate shortly.
Number of Newborns Screened	TAIWAN: From November 2014 to September 2016, 120,267 newborns were screened. NY STATE: From January 2016 to December 2016, 3,269 newborns have been screened (pilot ongoing at the time of submission).
Number of Screen Positive Results	Positive by primary test vs. 2nd tier test if applicable. TAIWAN: 15 by primary test, 7 by 2nd tier test, with a measured incidence of 1 in 17,181 infants screened. NY STATE: One infant screened positive in both the primary and secondary test.
False Positive Rate; False Negative Rate (if known)	False positive by primary test vs. 2nd tier test if applicable. TAIWAN: 8 false positives by primary test, zero false positives by 2nd tier test (false negatives unknown) NY STATE: False positive rate = 0% False negative rate = unknown (none have been reported to the newborn screening program). *The qPCR test targeting the SMN1 exon 7 deletion will only

SECTION II, PART C

	identify infants with the exon 7 deletion. Infants with SMA who are compound heterozygous for the deletion and a different mutation could be incorrectly classified as carriers, and infants with SMA and two mutations not tested could be incorrectly classified as screen negative
Number of Infants Confirmed with Diagnosis	How is diagnosis confirmed [clinical, biochemical, molecular]? TAIWAN: 7 confirmed by molecular confirmation of homozygous SMN1 exon 7 deletion. For these 7 cases, the following is known: Patient #1- Normal at age 25 months Patient #2- Sibling has SMA, refused further contact Patient #3- Clinically followed, SMA onset at 13 months of age Patient #4- Respiratory failure at birth, death at 3 months of age Patient #5- SMA onset at age 2 months Patient #6- Enrolled in the Biogen NURTURE Trial. Patient #7- Enrolled in the Biogen NURTURE Trial. NY STATE: One infant with molecular confirmation of homozygous SMN1 exon 7 deletion. The patient was enrolled in Biogen's NURTURE trial.

cure SMA

Make today a breakthrough.

February 28, 2017

Dr. Joseph A. Bocchini, Jr., M.D., *Chairman*
Advisory Committee on Heritable Disorders in Newborns and Children
5600 Fishers Lane, Room 18W68
Rockville, MD 20857

Dear Dr. Bocchini:

On behalf of all children born with spinal muscular atrophy (SMA), Cure SMA and the Muscular Dystrophy Foundation (MDA) are submitting this application for the Advisory Committee's consideration to nominate SMA as a condition to be listed on the Recommended Uniform Screening Panel (RUSP). The timing of this submission follows the December 23, 2016 FDA approval of the first-ever disease modifying therapy approved to treat SMA patients.

SMA is the number one genetic cause of death for infants, and affects approximately 1 in 11,000 babies. SMA is a progressive neurodegenerative disease that robs people of physical strength by affecting the motor nerve cells in the spinal cord, taking away the ability to walk, eat, and breathe. SMA can affect any race or gender. The disease is an autosomal recessive genetic disease caused by a mutation in the survival motor neuron gene 1 (*SMN1*). In a healthy person, this gene produces a protein that is critical to the function of the nerves that control muscles. Without it, those nerve cells cannot function properly and eventually die, leading to debilitating and often fatal muscle weakness.

As many on the committee know, Cure SMA is the largest organization in the United States dedicated to the treatment and cure of spinal muscular atrophy, and to supporting families affected by the disease. Cure SMA's reach includes 14,000 households in the SMA community, representing all four types of SMA as well as researchers and healthcare providers working in the neuromuscular fields—plus over 115,000 additional supporters. These stakeholders represent all 50 states as well as dozens of countries. In addition, Cure SMA has funded just over $62 million in research, with another $3 million pledged for the next 12 months.

MDA is a national 501(c)(3) organization dedicated to improving and saving the lives of people living with neuromuscular diseases, including SMA, muscular dystrophy, and ALS. All of the disorders under MDA's umbrella are classified as rare diseases and are progressive in nature, with life expectancy varying by disease. For more than 65 years, MDA has been committed to helping bring safe and effective treatments and cures to families as quickly as possible. To that end, MDA has funded over $1 billion in research grants and hosts a scientific conference in alternating years that brings together the leaders in neuromuscular disease from around the world. MDA is also committed to ensuring expert clinical care, and to that end, MDA supports more than 150 clinics (MDA Care Centers) nationwide that provide coordinated care to people with over 40 different kinds of neuromuscular disease, including SMA.

Cure SMA and MDA have joined together in this important effort—to ensure that all babies born in the United States are tested for SMA—as both organizations are committed to ensuring that those living with SMA are identified and treated as early as possible. In our combined effort to see NBS for SMA, we are pleased to be joined by the experts engaged in the SMA Newborn Screening Working Group (list enclosed). Together we have welcomed the opportunity to work with and testify before this Committee in advance of our submission and we appreciate the interest and attention of this

body over the months. As we submit this nomination, we offer our continued support throughout the committee's review process, and request and encourage you to reach out to us in any way we can be helpful.

The materials included in this application have been assembled and reviewed by the aforementioned Working Group, comprised of experts in the SMA field. The materials clearly demonstrate the importance of newborn screening for SMA as evidenced by (1) the substantial need for and benefit of pre-symptomatic treatment for SMA patients; (2) the availability of validated laboratory tests used to screen for SMA; (3) the availability of an FDA-approved treatment for SMA; and (4) the ability to treat successfully, once diagnosed, positive patients detected through newborn screening pilot studies.

An examination of the natural history, pathology, and continuing clinical trials for treatment development has shown that time has a clear and profound effect on the outcome for SMA patients. Just a few weeks can mean the difference between life and death for an infant with type I SMA, where the median survival is between 8 and 11 months in recent natural history studies. Preliminary results of the continuing NURTURE study conducted during the development and approval of SPINRAZA™, the first disease-modifying drug approved by the FDA, demonstrated a clear benefit for the pre-symptomatic treatment of SMA patients. Infants who were diagnosed before the onset of symptoms – either through the successful newborn screening pilots or because of a previously affected sibling – and who received SPINRAZA™ have achieved unprecedented motor milestones of sitting, standing, and walking, never before seen in Type I infants with SMA.

It is with great urgency and also excitement that we submit this application to the Committee for its consideration. To assist the Committee in its review enclosed please find: 1) member list of the SMA Newborn Screening Working Group and 2) table of contents setting out the content of the application and additional resource materials included.

We welcome all inquiries from the committee and look forward to working with your members, staff, and external advisory review group throughout the review process. Thank you for your time and dedication to these efforts and a special thank you to Debi Sarkar for her guidance and support as we worked to bring forth this application to the committee.

Sincerely,

Kenneth Hobby
President
Cure SMA

Valerie Cwik MD
Executive Vice President
Chief Medical & Scientific Officer
Muscular Dystrophy Association

CC: Ms. Debi Sarkar, MPH – ACHDNC Designated Federal Official

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Newborn Screening Multidisciplinary Working Group

Kenneth Hobby
President
Cure SMA

Jackie Glascock, PhD
Scientific Program Manager
Cure SMA

Spencer Perlman
Cure SMA Board of Directors

Katherine Klinger, PhD
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Scientific Program Officer
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Rodney Howell, MD
Chair of the Board, Muscular Dystrophy Association (MDA)
Chair Emeritus Pediatrics, Professor of Pediatrics
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Kristin Stephenson
Vice President Policy & Advocacy
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Tom Crawford, MD
Professor of Neurology
John Hopkins School of Medicine

Tom Prior, PhD
Professor, Department of Pathology
Ohio State University

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Nomination Form Table of Contents

UnitedHealthcare Community Plan

UnitedHealthcare® Community Plan

SPINRAZA™ (NUSINERSEN)- ARIZONA

Policy Number: CS2017D0059A **Effective Date**: May 1, 2017

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Commercial Policy

- Spinraza™ (Nusinersen)

INSTRUCTIONS FOR USE

This Drug Policy provides assistance in interpreting UnitedHealthcare benefit plans. When deciding coverage, the federal, state or contractual requirements for benefit plan coverage must be referenced. The terms of the federal, state or contractual requirements for benefit plan coverage may differ greatly from the standard benefit plan upon which this Drug Policy is based. In the event of a conflict, the federal, state or contractual requirements for benefit plan coverage supersedes this Drug Policy. All reviewers must first identify member eligibility, any federal or state regulatory requirements, and the contractual requirements for benefit plan coverage prior to use of this Drug Policy. Other Policies and Coverage Determination Guidelines may apply. UnitedHealthcare reserves the right, in its sole discretion, to modify its Policies and Guidelines as necessary. This Drug Policy is provided for informational purposes. It does not constitute medical advice.

UnitedHealthcare may also use tools developed by third parties, such as the MCG™ Care Guidelines, to assist us in administering health benefits. The MCG™ Care Guidelines are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.

BENEFIT CONSIDERATIONS

Before using this policy, please check the federal, state or contractual requirements for benefit coverage.

COVERAGE RATIONALE

Spinraza™ (nusinersen) is proven and medically necessary for:[1]

1. **The treatment of Spinal Muscular Atrophy (SMA) in patients who meet ALL of the following criteria**:
   a. **For initial therapy**, ALL of the following:
      (1) ONE of the following:
         (a) Diagnosis of spinal muscular atrophy type I, II, or III by a neurologist with expertise in the diagnosis of SMA
         (b) Diagnosis of spinal muscular atrophy type I, II, or III by a physician in consultation with a neurologist with expertise in the diagnosis of SMA;
      and
      (2) Submission of medical records (e.g., chart notes, laboratory values) confirming BOTH of the following:
         (a) The mutation or deletion of genes in chromosome 5q resulting in ONE of the following:
            i. Homozygous gene deletion or mutation (e.g., homozygous deletion of exon 7 at locus 5q13);[1,2]
            or
            ii. Compound heterozygous mutation (e.g., deletion of SMN1 exon 7 [allele 1] and mutation of SMN1 [allele 2]);
         and
         (b) Patient has at least 2 copies of SMN2;

and

(3) Patient is not dependent on either of the following:
   (a) Invasive ventilation or tracheostomy
   (b) Use of non-invasive ventilation beyond use for naps and nighttime sleep;

   and

(4) Submission of medical records (e.g., chart notes, laboratory values) of the baseline exam of at least ONE of the following exams (based on patient age and motor ability) to establish baseline motor ability:
   (a) Hammersmith Infant Neurological Exam (HINE)[1,8,12] (infant to early childhood)
   (b) Hammersmith Functional Motor Scale Expanded (HFMSE)[1,9,13-14]
   (c) Upper Limb Module (ULM) Test (Non ambulatory)[1,9]
   (d) Children's Hospital of Philadelphia Infant Test of Neuromucular Disorders (CHOP INTEND);[1,8]

   and

(5) ONE of the following:
   (a) Spinraza is prescribed by a neurologist with expertise in the treatment of SMA
   (b) Spinraza is prescribed by a physician in consultation with a neurologist with expertise in the treatment of SMA;

   and

(6) Spinraza is to be administered intrathecally by, or under the direction of, healthcare professionals experienced in performing lumbar punctures; **and**

(7) Spinraza dosing for SMA is in accordance with the United States Food and Drug Administration approved labeling: maximum dosing of 12mg for each loading dose; **and**

(8) **Initial authorization will be for no more than 4 loading doses (2 months).**

b. **For continuation therapy**, ALL of the following:

(1) ONE of the following:
   (a) Diagnosis of spinal muscular atrophy type I, II, or III by a neurologist with expertise in the diagnosis of SMA
   (b) Diagnosis of spinal muscular atrophy type I, II, or III by a physician in consultation with a neurologist with expertise in the diagnosis of SMA;

   and

(2) Submission of medical records (e.g., chart notes, laboratory values) confirming BOTH of the following:
   (a) The mutation or deletion of genes in chromosome 5q resulting in ONE of the following:
      i. Homozygous gene deletion or mutation (e.g., homozygous deletion of exon 7 at locus 5q13);[1,2]

      or

      ii. Compound heterozygous mutation (e.g., deletion of SMN1 exon 7 [allele 1] and mutation of SMN1 [allele 2]);

      and

   (b) Patient has at least 2 copies of SMN2;

   and

(3) Patient is not dependent on either of the following:
   (a) Invasive ventilation or tracheostomy
   (b) Use of non-invasive ventilation beyond use for naps and nighttime sleep;

   and

(4) Submission of medical records (e.g., chart notes, laboratory values) with the most recent results (< 1 month prior to request) documenting a positive clinical response **from pretreatment baseline status** to Spinraza therapy as demonstrated by at least ONE of the following exams:
   (a) HINE milestones:
      i. One of the following:
         (i) Improvement or maintenance of previous improvement of at least 2 point (or maximal score) increase in ability to kick
         (ii) Improvement or maintenance of previous improvement of at least 1 point increase in any other HINE milestone (e.g., head control, rolling, sitting, crawling, etc.), excluding voluntary grasp;

         and

      ii. One of the following:
         (i) The patient exhibited improvement or maintenance of previous improvement in more HINE motor milestones than worsening, from pretreatment baseline (net positive improvement)
         (ii) Achieved and maintained any new motor milestones when they would otherwise be unexpected to do so (e.g., sit unassisted, stand, walk);

      or

   (b) HFMSE: One of the following:
      i. Improvement or maintenance of previous improvement of at least a 3 point increase in score from pretreatment baseline

ii. Patient has achieved and maintained any new motor milestone from pretreatment baseline when they would otherwise be unexpected to do so;

or

(c) ULM: One of the following:

i. Improvement or maintenance of previous improvement of at least a 2 point increase in score from pretreatment baseline

ii. Patient has achieved and maintained any new motor milestone from pretreatment baseline when they would otherwise be unexpected to do so;

or

(d) CHOP INTEND: One of the following:

i. Improvement or maintenance of previous improvement of at least a 4 point increase in score from pretreatment baseline

ii. Patient has achieved and maintained any new motor milestone from pretreatment baseline when they would otherwise be unexpected to do so;

and

(5) ONE of the following:

(a) Spinraza is prescribed by a neurologist with expertise in the treatment of SMA

(b) Spinraza is prescribed by a physician in consultation with a neurologist with expertise in the treatment of SMA;

and

(6) Spinraza is to be administered intrathecally by, or under the direction of, healthcare professionals experienced in performing lumbar punctures; **and**

(7) Spinraza dosing for SMA is in accordance with the United States Food and Drug Administration approved labeling: maximum dosing of 12mg every 4 months, starting 4 months after the last loading dose; **and**

(8) **Reauthorization will be for no more than 3 maintenance doses (12 months).**

Spinraza is not proven or medically necessary for spinal muscular atrophy without chromosome 5q mutations or deletions.[1]

U.S. FOOD AND DRUG ADMINISTRATION (FDA)

Spinraza is a survival motor neuron-2 (SMN2)-directed antisense oligonucleotide indicated for the treatment of spinal muscular atrophy (SMA) in pediatric and adult patients.

BACKGROUND

Spinal muscular atrophy (SMA) is a rare, autosomal recessive neuromuscular disease that affects the survival of motor neurons of the spinal cord.[2] SMA is caused by the deletion/mutation of the SMN1 gene.[2] The estimated annual incidence of SMA is 5.1 to 16.6 cases per 100,000 live births. Approximately 1/40 to 1/60 people are SMA carriers, equating to 3.5 to 5.2 million and 12 to 18 million individuals in the United States and Europe, respectively.[3-6] SMA is characterized by the degeneration of motor neurons of the spinal cord, resulting in hypotonia and muscle weakness. Five subtypes of SMA (0-IV) have been described based on age of symptom onset and motor function achieved.[7]

Spinraza™ (nusinersen) is a modified antisense oligonucleotide designed to treat SMA caused by mutations in chromosome 5q that lead to SMN protein deficiency. Nusinersen binds to a specific sequence in the intron downstream of exon 7 of the SMN2 transcript. Using in vitro assays and studies in transgenic animal models of SMA, nusinersen was shown to increase exon 7 inclusion in SMN2 messenger ribonucleic acid (mRNA) transcripts and production of full-length SMN protein.[1]

APPLICABLE CODES

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by federal, state or contractual requirements and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Coverage Determination Guidelines may apply.

ICD-10 Diagnosis Code	Description
G12.0	Infantile spinal muscular atrophy, type I [Werdnig-Hoffmann]
G12.1	Other inherited spinal muscular atrophy

CLINICAL EVIDENCE

A Phase III, multicenter, randomized, double-blind, sham-procedure controlled study assessed the clinical efficacy and safety of nusinersen, administered intrathecally in 121 symptomatic infants, ≤ 7 months of age at the time of first dose, diagnosed with SMA (symptom onset before 6 months of age). Patients were randomized 2:1 to receive either nusinersen or sham injection. A planned interim efficacy analysis was conducted based on patients who died, withdrew, or completed at least 183 days of treatment. Of the 82 patients included in the interim analysis, 44% were male and 56% were female. Age at first treatment ranged from 30 to 262 days (median 181). Eighty-seven (87%) of subjects were Caucasian, 2% were Black, and 4% were Asian. Length of treatment ranged from 6 to 442 days (median 261 days). Baseline demographics were balanced between the nusinersen and control groups with the exception of age at first treatment (median age 175 vs. 206 days, respectively). The nusinersen and control groups were balanced with respect to gestational age, birth weight, disease duration, and SMN2 copy number (2 copies in 98% of subjects in boths groups). Median disease duration was 14 weeks. There was some imbalance in age at symptom onset with 88% of subjects in the nusinersen group and 77% in the control group experiencing symptoms within the first 12 weeks of life. The primary endpoint assessed at the time of interim analysis was the proportion of responders: patients with an improvement in motor milestones according to Section 2 of the Hammersmith Infant Neurologic Exam (HINE). A treatment responder was defined as any patient with at least a 2-point increase (or maximal score of 4) in ability to kick (consistent with improvement by at least 2 milestones), or at least a 1-point increase in the motor milestones of head control, rolling, sitting, crawling, standing or walking (consistent with improvement by at least 1 milestone). To be classified as a responder, patients needed to exhibit improvement in more categories of motor milestones than worsening. Of the 82 patients who were eligible for the interim analysis, a statistically significantly greater percentage of patients achieved a motor milestone response in the nusinersen group compared to the sham-control group.[1, 8]

A Phase III multicenter, double-blind, randomized, sham-procedure controlled study assessed the clinical efficacy and safety of nusinersen in patients with later-onset SMA consistent with Type II SMA. Subjects were randomized 2:1 to receive intrathecal nusinersen or a sham procedure control, respectively. Inclusion criteria included diagnosis with SMA, have clinical signs and symptoms consistent with SMA at greater than 6 months of age, be able to sit independently, but never able to walk independently and have a HFMSE score greater than or equal to 10 and less than or equal to 54 at Screening. The primary endpoint is change from baseline in HFMSE score (at 15 months). Secondary Endpoints are (at 15 months): proportion of subjects who achieve a 3-point increase from baseline in HFMSE score, proportion of subject that achieve any new motor milestone, number of motor milestones achieved per subject, change from baseline in Upper Limb Module Test, proportion of subjects that achieve standing alone, proportion of subject that achieve walking with assistance. In a pre-planned interim analysis, a significant difference ($p = 0.0000002$) of 5.9 points in HFMSE was observed at 15 months between patients given nusinersen (n=84) compared to the sham-procedure control (n=42). Patients receiving nusinersen experienced a mean improvement of 4.0 points in the HFMSE compared to a mean decrease of 1.9 points in the sham procedure control group (5). A change of ≥ 3 points in the HFMSE has previously been determined to be clinically important. Results for other endpoints were consistent with a favorable response to nusinersen compared to sham-procedure control. Adverse events were mostly considered to be related to SMA disease, common events found in the general population, or events related to the lumbar puncture procedure. No patients discontinued the study. Nusinersen was well tolerated with a favorable safety profile.[9]

The results of the controlled trial in infantile-onset SMA patients were supported by open-label uncontrolled trials conducted in symptomatic SMA patients who ranged in age from 30 days to 15 years at the time of first dose, and in presymptomatic patients, who ranged in age from 8 days to 42 days at the time of first dose. The patients in these studies had or were likely to develop Type 1, 2, or 3 SMA. Some patients achieved milestones such as ability to sit unassisted, stand, or walk when they would otherwise be unexpected to do so, maintained milestones at ages when they would be expected to be lost, and survived to ages unexpected considering the number of SMN2 gene copies of patients enrolled in the studies.[10,11]

CENTERS FOR MEDICARE AND MEDICAID SERVICES (CMS)

Medicare does not have a National Coverage Determination (NCD) for Spinraza™ (nusinersen). Local Coverage Determinations (LCDs) do not exist at this time.

Medicare covers outpatient (Part B) drugs that are furnished "incident to" a physician's service provided that the drugs are not usually self-administered by the patients who take them. See the Medicare Benefit Policy Manual (Pub. 100-2), Chapter 15, §50 Drugs and Biologicals at http://www.cms.hhs.gov/manuals/Downloads/bp102c15.pdf. (Accessed January 4, 2017)

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POLICY HISTORY/REVISION INFORMATION

Date	Action/Description
10/01/2017	• New policy. This policy applies to only the Arizona line of business.

SEMINARS IN PERINATOLOGY 39 (2015) 217–229

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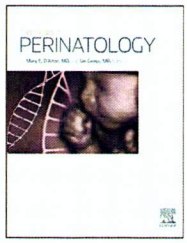

Newborn screening for spinal muscular atrophy: Anticipating an imminent need

Han C. Phan, MD[a,*], *Jennifer L. Taylor, PhD*[b], *Harry Hannon, PhD*[c], *and Rodney Howell, MD, FAAP, FACMG*[d]

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ARTICLE INFO

Keywords:
Spinal muscular atrophy
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ABSTRACT

Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality. Children with type I SMA typically die by the age of 2 years. Recent progress in gene modification and other innovative therapies suggest that improved outcomes may soon be forthcoming. In animal models, therapeutic intervention initiated before the loss of motor neurons alters SMA phenotype and increases lifespan. Presently, supportive care including respiratory, nutritional, physiatry, and orthopedic management can ameliorate clinical symptoms and improve survival rates if SMA is diagnosed early in life. Newborn screening could help optimize these potential benefits. A recent report demonstrated that SMA detection can be multiplexed at minimal additional cost with the assay for severe combined immunodeficiency, already implemented by many newborn screening programs. The public health community should remain alert to the rapidly changing developments in early detection and treatment of SMA.

Introduction

Spinal muscular atrophy (SMA), the leading cause of heritable infant mortality,[1] is detectable by newborn blood spot screening (NBS).[2–7] SMA is presently an incurable condition characterized by degeneration of alpha motor neurons in the spinal cord. Over time, SMA results in progressive and symmetrical proximal weakness, followed by paralysis and ultimately, premature death. It is disheartening to examine SMA infants with advancing disease, their faces often bright and happy, and their full facial strength apparent because their eye and facial muscles are spared.

Abbreviations: AAV, adeno-associated virus; ASO, antisense oligonucleotides; DBS, dried blood spot(s); DcPS, human scavenger decapping enzyme; DHPLC, denaturing high-performance liquid chromatography; HRMA, high-resolution melting analysis; LNA, lock nucleic acid; MLPA, multiplex ligation probe amplification; NBS, newborn blood spot screening; NBSTRN, Newborn Screening Translational Research Network; RFLP, restriction fragment length polymorphism; scAAV, self-complimentary-associated adenovirus; SACHDNC, Secretary of Health and Human Services' Advisory Committee on Heritable Diseases in Newborns and Children; SCID, severe combined immunodeficiency; SMA, spinal muscular atrophy; SMN, survivor motor neuron; SNP, single nucleotide polymorphism(s); SSCP, single-strand conformation polymorphism; TREC, T-cell receptor excision circle(s)

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0146-0005/

Among autosomal recessive disorders, SMA is the most common fatal disease and is second in birth prevalence only to cystic fibrosis.[1] The incidence of SMA is estimated at 1 in 6000–10,000, and the carrier frequency is 1/40–1/60.[8] SMA is a pan-ethnic disorder and overall represents the most widespread lethal genetic disease of children.[9]

The first article describing SMA was published in 1891 by Guido Werdnig, who meticulously detailed the clinical presentation, symptomatic progression, and pathological findings of a patient with infantile muscular atrophy relative to the gastrocnemius muscles and the spinal cord. He described the patient as a strong child, with lively movements, who succumbed to progressive weakness after a respiratory illness. Muscle pathology revealed a fatty tissue infiltrate, and the spinal cord showed atrophy of the anterior horn cells.[10] This report was followed by Johann Hoffmann's article in 1893 describing what he termed infantile progressive muscular atrophy.[11]

The molecular basis of SMA was recognized in 1990, when Gilliam et al.[12] showed that childhood-onset SMA is monogenic and linked to chromosome 5q, specifically 5q11.2–q13.3. At 5 years later, the site of the molecular lesion was identified as the *SMN1* gene encoding the survival motor neuron (SMN) protein.[13] This discovery was followed by an extraordinary scientific collaboration that untangled the complex genetics underlying SMA and revealed a unified theory of molecular pathogenesis. SMA became understood as a single-gene disorder with a closely related gene serving as a major phenotypic modifier. As investigators overcame the technical challenges that made SMA etiology so obscure, they focused on early identification, recognizing that the rapid onset and dire consequences of infantile SMA would require immediate intervention for any potential therapeutic actions to be effective. The development of methods that could detect SMA from newborn dried blood spots (DBS) was an integral part of the collaborative research.

These research efforts have led to more than a dozen potential therapies to treat SMA, including small molecules, antisense oligonucleotides, vector-based gene replacement, and stem cell transplantation. As this review goes to press, two such therapeutic treatments are in FDA Phase III clinical trials, which have been approved for evaluation in pre-symptomatic infants[14–17] (Table 1). Results from Phase I safety studies and Phase II efficacy trials in symptomatic children have been promising for both the therapies, suggesting stabilization and even modest improvement of symptoms.

This report reviews the current understanding of SMA pathogenesis, its clinical spectrum and pathognomonic features, the laboratory methods that have been used for detection and diagnosis, the current approaches to clinical management, and the promise of emerging therapeutic treatments. The current and future role of SMA-NBS is discussed, including the need for pilot studies that will allow the public health community to prepare for the availability of therapies with proven benefit in pre-symptomatic newborns.

Clinical presentation

Childhood SMA is commonly divided into three clinical groups—type I (OMIM 253300), type II (OMIM 253550), and type III (OMIM 253400)—based upon age of onset, maximum motor milestone attained, and age at death.[18–20] Adult-onset SMA, referred to as type IV (OMIM 271150), occurs at a much lower frequency.

SMA type I (Werdnig–Hoffmann disease) is the most severe phenotype, representing about 60–70% of all cases. Affected patients have severe and generalized weakness with hypotonia at birth or within the first few months of life, most definitely before the age of 6 months. All SMA type I patients are unable to sit unsupported. Because of the degree of weakness, most children die before 2 years of age, unless they receive substantial nutritional and respiratory support. The age of onset in SMA broadly correlates with age at death, indicating that the earlier onset of symptoms correlates with the severity of the condition.[21,22] The mortality rate is 75–95% by the second birthday.[18,22] These patients have normal cognition, and a somewhat higher average intelligence has been reported in comparison to other neuromuscular disorders such as Duchenne Muscular Dystrophy.[23,24]

SMA type II is characterized by the ability to sit unsupported; however, patients may lose this skill over time.

Table 1 – Therapies for spinal muscular atrophy in clinical trials (Accessed January 2015).

Therapy/phase	Title	URL link
Antisense oligonucleotides ISIS Pharmaceuticals Phase III	Randomized, double-blind, sham-procedure controlled study to assess the clinical efficacy and safety of ISIS 396443 administered intrathecally in patients with infantile-onset *spinal muscular atrophy*	https://clinicaltrials.gov/ct2/show/NCT02193074?term=sma&rank=26
Small molecule Trophos Phase III	Multicenter, randomized, adaptive, double-blind, placebo controlled study to assess safety and efficacy of *Olesoxime (TRO19622)* in 3–25 Year old *spinal muscular atrophy (SMA)* patients.	https://clinicaltrials.gov/ct2/show/NCT01302600?term=olesoxime+and+sma&rank=1
Gene therapy AveXis Inc Phase I	Gene transfer clinical trial for *spinal muscular atrophy* Type 1 delivering the survival motor neuron gene by self-complementary AAV9	https://clinicaltrials.gov/ct2/show/NCT02122952?term=sma&rank=39
Small molecule Roche Phase I	A multicenter, randomized, double blind, placebo controlled, multiple dose study to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of RO6885247 following 12 weeks of treatment in adult and pediatric patients with *spinal muscular atrophy* (MOONFISH).	https://clinicaltrials.gov/ct2/show/NCT02240355?term=sma&rank=51

Symptoms present before 18 months of age, and the affected children are never able to walk or stand. Life expectancy is beyond 4 years of age. SMA type III (Kugelberg–Welander disease) is the mildest childhood-onset phenotype, with symptoms presenting after 18 months of age. Type III patients are able to walk with assistance and potentially can have a normal life span.[18,20–22,25]

Further characterizations of the different types of SMA are based upon the copy numbers of the *SMN2* gene, a paralog of *SMN1* that produces SMN protein at much lower levels than *SMN1*. SMA type I patients typically have 2–3 copies of *SMN2*, SMA type II patients have 3–4 copies, and SMA type III patients have at least 4 copies of *SMN2*[19] (Table 2). Classification and determination of the various types of SMA are important for two reasons: first, to correlate clinical symptoms with genotype and second, to establish the natural history as a baseline for measuring the efficacy of therapeutic interventions.

Molecular genetics and pathogenesis

In humans, there are two nearly identical *SMN* genes present in 5q13: telomeric *SMN1* and centromeric *SMN2*. The two inverted sequences both code for the SMN protein and are more than 99% identical. Both genes have equivalent promoters and nine exons with a stop codon near the end of exon 7.[13,26,27] Homozygous absence of *SMN1* is found in about 95% of SMA patients, regardless of the type. The remaining 5% of the affected patients are compound heterozygotes for *SMN1* exon 7 deletions or small intragenic mutations.[5] The primary difference between *SMN1* and *SMN2* is a single nucleotide alteration in exon 7 (840C > T). This transition does not alter the amino acid sequence of the SMN protein produced by *SMN2*, but it affects the splicing process by disrupting the exonic splicing enhancer, resulting in exclusion of exon 7 in most mature *SMN2* mRNA transcripts[28,29] (Fig. 1). The lack of exon 7 disrupts the SMN protein domain that enables oligomerization, which prevents the protein's ability to interact with other proteins.[30] While the *SMN1* gene produces full-length transcripts and functional SMN protein, 90% of *SMN2* transcripts are translated into truncated SMN proteins (SMNΔ7).[26,28,31]

Interestingly, only the human genome contains *SMN2*, while all other animals have only one SMN protein-coding gene. In a transgenic mouse model, homozygous *Smn* knockout ($Smn^{-/-}$) results in lethality, indicating that SMN protein is necessary for cellular survival and function.[32] Inserting *SMN2* transgenically into a $Smn^{-/-}$ mouse results in a viable mouse pup that becomes SMA symptomatic soon after birth. The phenotype can vary depending on the copy number of *SMN2* inserted. Higher numbers reduce the severity of the condition, indicating that *SMN2* has a protective role in the disease phenotype.[33,34] These findings indicate that SMA results from inadequate production of SMN protein and that the *SMN2* gene is the phenotypic modifier.

Multiple studies have shown that *SMN2* copy number can alter the disease phenotype.[35–37] The small quantity of *SMN2* transcripts helps to explain the clinical observation that increased copy numbers of *SMN2* correlate with milder disease.[33–35] Interestingly, about 10–15% of the general population does not have any *SMN2*; however, this subpopulation has at least one intact *SMN1* gene and appears phenotypically normal. Conversely, three SMA carriers each with 5 copies of *SMN2* were unaffected, even though they had confirmed *SMN1* homozygous deletion. These carriers show that the expression of SMN protein from five *SMN2* copies is sufficient to compensate for the loss of *SMN1*.[38,39]

The *SMN2* copy number may not be a reliable indicator of disease severity if the *SMN2* genes have other mutations. In three adult patients with homozygous *SMN1* deletion and mild type III phenotype, a mutation of c.859G > C in exon 7 of the *SMN2* gene was found, resulting in increased number of full-length SMN protein production.[40] Conversely, a higher number of *SMN2* copies may not be beneficial if one or more contain mutations that reduce their ability to express a functional SMN protein.

SMN protein function

Full-length SMN protein is a 38-kDa polypeptide that is found in the cytoplasm and nucleus of all cells.[41,42] This protein is ubiquitously expressed at high levels during embryonic development. In most tissues, SMN protein expression diminishes in the postnatal period, but it remains highly expressed in the spinal motor neuron even in adulthood.

It has been well established that a deficiency of SMN protein leads to selective motor neuron loss, but the mechanism is unclear. SMN protein self-associates into oligomers, and it also associates with other proteins to form a larger macromolecule called the SMN complex. The SMN complex is known to interact with a variety of other proteins involved in pre-RNA splicing and assembly of the Sm-class spliceosomal small nuclear ribonucleoprotein (snRNP).[43–47] SMN protein also has an important role in axonal transport by forming a complex with beta-actin and profilin that regulates and stabilizes actin function.[48–51] Different hypotheses have been proposed to explain the importance of SMN protein in specific sets of neurons.[52,53]

Medical issues in SMA

Currently, there is no approved treatment that can effectively ameliorate the disease progress of SMA. However, there are

Table 2 – Types of SMA.

Type	Age at onset	Highest function	Age at death	SMN2 copies
I	Birth to 6 months	Never sit	<2 years	2
II	Birth to 18 months	Sit unsupported	>2 years	3–4
III	>18 months	Stand unsupported	Adult	3–4
IV	>21 years	Stand unsupported	Adult	4–8

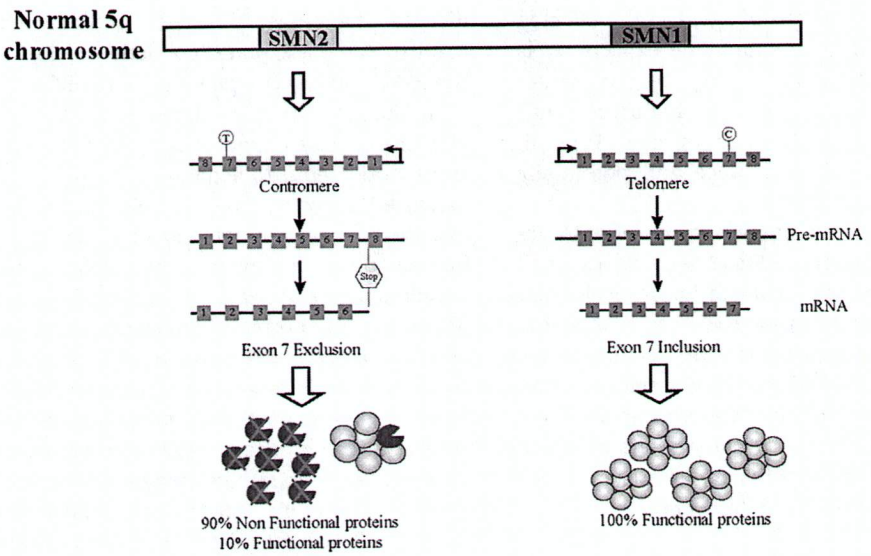

Fig. 1 – The human SMN1 and SMN2 genes are located on chromosome 5. Each gene encodes identical protein product; however, the substitution C to T in exon 7 of SMN2 gene alters exonic splicing enhancer, resulting in exclusion of exon 7 during splicing. Approximately 90% of the mature mRNA lacks exon 7 and consequently translate truncated SMN protein that is defective and degraded rapidly.[63] Schematic of the *SMN1* and *SMN2* genes and their proteins.

guidelines for clinical management based on evidence that the appropriate comprehensive pulmonary, orthopedic, rehabilitation, and nutritional care for patients with SMA reduces disease burden and improve natural history.[54] A team of multiple subspecialists is crucial in implementing these guidelines.[55] Early education and counseling for the family of patients is paramount in preventing SMA-associated complications.

The International Standard of Care Committee for Spinal Muscular Atrophy was formed in 2005 to establish necessary guidelines for the clinical care of patients with SMA. A consensus statement for the standard of care in spinal muscular atrophy in 2007 noted the need for multidisciplinary care for all affected patients. Further, it provided evidence-based recommendations for the common related medical problems in SMA patients, diagnostic strategy, assessment and monitoring requirements, and therapeutic intervention.[55,56]

Pulmonary complication is the major cause of mortality in SMA patients. Aspiration pneumonia results from bulbar dysfunction and from a weak cough and poor clearance. Hypoventilation can be prevented if anticipatory planning with a pulmonologist occurs as soon as the diagnosis is made. Family should be aware of the role of non-invasive ventilator support from weakened muscle strength as the disease progresses. General health maintenance to prevent pulmonary complications such as immunization against influenza, pneumococcus, and respiratory syncytial virus is highly recommended.[57] Those with recurrent pulmonary infection and poor secretion clearance could benefit from tracheostomy; however, this option needs to be weighed against the patient's quality of life.

Gastrointestinal complications such as gastro-esophageal reflux, delayed gastric emptying, and constipation can be present in those who are non-ambulatory. Gastrostomy tube placement is an option to promote adequate nutritional intake and prevent aspiration pneumonia; however, there is not a consensus in terms of the timing of its placement. Nutritional support is very important in SMA patients.

Routine rehabilitation assessment is important to maintain function and improve patient's quality of life. For those who are non-ambulatory, frequent stretching and bracing prevent contractures. Physical therapy can assist in safely attaining maximal mobility.

Scoliosis is largely unavoidable due to the nature of generalized muscle weakness in SMA, particularly in non-ambulatory type I and type II patients. When feasible, surgery is the treatment of choice, and with early anticipatory planning, appropriate timing of surgical intervention can be determined to prevent complications postoperatively.

Therapeutic progress and future promise

With greater understanding of the molecular genetics of SMA over the past 2 decades, therapeutic developments have focused on increasing the full-length SMN protein by enhancing *SMN2* gene expression, increasing the inclusion of exon 7 in *SMN2* transcripts, stabilizing the SMN protein, or replacing the *SMN1* gene. Figure 2 outlines the current progress of therapeutic strategies for the potential treatment of SMA.[58] Some of the more promising therapeutic modalities include antisense oligonucleotides, small-molecule drugs, gene therapy, and stem cell transplantation.

(1) Antisense oligonucleotides (ASO)

Increase in *SMN2* copy number correlates with a milder phenotype primarily because the higher number of *SMN2* (even with only 10% production efficiency by each copy) will result in more full-length SMN protein. The ASO therapeutic approach aims to alter the splicing process, thereby

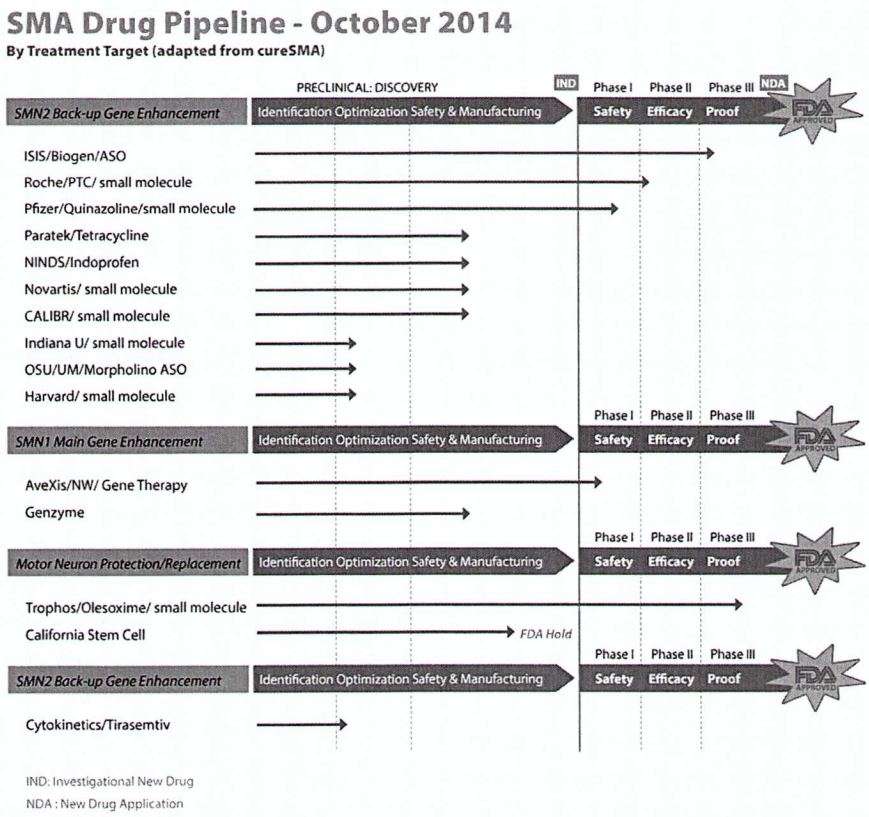

Fig. 2 – This chart outlines the current progress of therapeutic strategies for the potential treatments of SMA. With several therapies that have progressed from preclinical to clinical phase, an FDA-approved therapy is promising. IND, Investigational New Drug; NDA, New Drug Application. (Adapted with permission from SMA Drug Pipeline[58].)

increasing the inclusion of exon 7 in the *SMN2* transcripts, resulting in higher levels of functional SMN protein. ASO are therapeutic RNA molecules that block the cis-acting splice modifier or intron splice enhancer; either mechanism can enhance or disrupt the targeted splicing event. Specifically for SMA, hybridization of ASO-10-27 to the intronic splicing silencer N1 in *SMN2* intron 7 has been shown to increase the level of SMN protein *in vivo*.[59] As a proof of concept, interventricular administration of ASO-10-27 in the SMA mouse model has shown promising results. Treated mice showed an increase in the long-lasting *SMN2* exon 7 inclusion, as measured at both the mRNA and the protein levels in spinal cord motor neurons.[60] Moreover, treated mice had improved muscle structure, function, and ultimately, survival.[61]

In 2012, a Phase I human trial was launched with ASO-10-27 (renamed as ISIS-SMN_{RX}) in which 15 patients with SMA type II and 13 patients with SMA type III received a single intrathecal administration at varying dosages. The result established the drug to be safe and well tolerated in children with SMA. Additionally, treated children showed a dose-dependent improvement in the Hammersmith functional motor scale of muscle function. In a subsequent Phase II study, a total of 20 SMA infants received intrathecal administration of ISIS-SMN_{RX} at one of two different doses: four patients received a 6-mg dose and 16 patients received a 12-mg dose.[62] Results showed that the lumbar punctures were well tolerated and feasible. Also, there were no drug-related serious adverse events and no changes in safety profiles with repeated dosing. In this study, one accidental death and one permanent ventilation were recorded from the 6-mg cohort, whereas in the 12-mg cohort, three patients died due to respiratory infection. None of these events were considered drug related. Analysis of spinal cord tissue at autopsy showed that ISIS-SMN_{RX} was distributed throughout the central nervous system and was correlated with higher levels of full-length *SMN2* mRNA and proteins when compared to untreated infants.[63] Following these promising results, a Phase III clinical trial has begun to recruit patients for a randomized, double-blind, sham-procedure controlled study to assess the clinical efficacy and safety of ISIS-SMN_{RX}.[54]

(2) Small-molecule drugs

Certain low-molecular-weight drugs can promote increased levels of full-length SMN protein by activating the *SMN2* promoter, increasing its expression, and altering the splicing pattern of *SMN2* transcripts so as to favor the inclusion of exon 7.[64,65] Histone deacetylase inhibitor and quinazoline compounds increase *SMN2* mRNA levels and improve the disease phenotype in mouse models of SMA.[66–69] Other small-molecule drugs such as

aminoglycosides promote reading of the stop codon of SMNΔ7 transcripts, enabling the translation of the full-length functional SMN protein.[70] These molecules had shown promising results in mouse models and cell lines derived from SMA patients; however, when they were tested in clinical trials, SMA patients showed little benefit.[71–73]

An alternative class of small molecules including riluzole, olesoxime, and ceftriaxone function as neuroprotective compounds. Originally approved for use to treat patients with amyotrophic lateral sclerosis (ALS), riluzole blocks the sodium channels and inhibits glutamate, and it promotes the expression of neurotrophic factors that enhance motor neuron survival. A Phase I clinical trial showed this approach to be safe in SMA type I patients but without motor function improvement. A Phase II clinical trial of riluzole in children and young adults with SMA (ASIRI)[74] has been completed in France, but results have not been published. Olesoxime promotes neurite outgrowth and communication with the mitochondrial permeability transition pore.[75] A Phase II multicenter, randomized, and double-blinded study to assess safety and efficacy for SMA type II and type III has been completed, and the result of this trial was presented at the American Academy of Neurology in April 2014. Ceftriaxone prevents glutamate toxicity in a cell culture model of motor neuron degeneration, and it delayed motor neuron loss and improved muscle strength in an ALS mouse model. This compound is currently in clinical trials only for ALS. Another small-molecules member—quinazoline—increases full-length mRNA by inclusion of exon 7 in *SMN2* gene transcript, resulting in an increase in SMN protein levels. Its primary function is in the inhibition of human scavenger decapping enzyme (DcpS). The role of DcpS is best understood in the degradation of the 5′ cap during the 3′ to 5′ mRNA decay; it may have additional functions in nuclear–cytoplasm transportation and first intron pre-mRNA splicing.[76] Quinazoline is anticipated to progress to Phase I clinical trials (Table 1).

(3) Gene therapy

As a monogenic disease, SMA is a good target for vector-based gene replacement therapy to restore a normal form of the *SMN1* gene in SMA patients. The overall goal of gene therapy is to replace and permanently restore the deleted *SMN1* gene in the genome of the affected patients. As a result, more full-length SMN protein will be produced to promote normal function survival motor neurons, thereby improving phenotype and survivability.

In selecting a potential vector to deliver the *SMN1* gene, adeno-associated virus vectors (AAV) 8 and 9 appeared to be excellent contenders due to their ability to cross the blood–brain barrier through the vascular system in mouse models.[77–79] Additionally, self-complementary AAV (scAAV), a recombinant virus with a double-stranded DNA genome, results in early onset of gene expression. Several groups have shown this capability with the use of scAAV9, which appeared to have tropism toward motor neurons in the neonatal animals when injected intravascularly.[77,78] However, intracerebroventricular injection with SMN-scAAV8 has also been successfully used. Both modes of administration increased the median survival period; however, mice that received intravascular administration had a greater survival benefit of 250 days, compared to the 157 days in the intracerebroventricular injection group.[80–83]

With evidence that AAV9 could penetrate the blood–brain barrier, resulting in diffuse gene delivery via intravascular administration in animal models, this route of administration has been favored. But the potential side effects of this treatment modality are unknown, since a large vector load is given to increase the probability of penetration into the central nervous system. Alternatively, intrathecal administration of AAV9 has been accomplished in pigs and adult mice, with less vector load and improved gene expression.[84,85]

In 2014, FDA approved a Phase I clinical trial of a systemic AAV9-delivered human SMN gene to SMA type I infants up to 9 months old. The infantile-onset phenotype is considered the most appropriate candidates for immediate therapy.[86] This trial is currently recruiting patients.[15]

Even though the gene therapy approach offers great promise, its limitations are multiple. Similar to many neurodegenerative disorders, SMA is not diagnosed until a substantial amount of motor neuron loss and muscle atrophy has occurred. Like many other therapies, there is a critical therapeutic window in which treatment can increase SMN protein levels, leading to an improved or milder phenotype.[87–89] In the SMA mouse model, there is a crucial period in which a sufficient amount of SMN protein is required for motor neuron development.[8] In a study by Foust et al.,[90] administration of scAAV in SMNΔ7 mice on postnatal day 1 showed much more benefit as compared to postnatal day 5, and there was no benefit in postnatal day 10. The early death of SMA mice could be avoided with embryonic restoration of SMN protein expression, indicating that early administration of therapy can also prevent premature fatality.[91,92] Furthermore, the blood–brain barrier is more permeable during the perinatal period than later in infancy.[93]

(4) Stem cell therapy

Among the therapeutic approaches, stem cell transplantation also holds great potential for SMA patients. The primary goal for transplanted stem cells is to support endogenous motor neurons through the delivery of neuroprotective agents and to partially restore neuronal and non-neuronal cells.[94–96]

Neural stem cells obtained from the spinal cord administered intrathecally to SMA mice showed appropriate migration into the parenchyma and the

capability to generate a small proportion of motor neurons. These treated mice exhibited improved motor unit and neuromuscular function and showed a 38% increase in life expectancy. Furthermore, gene expression in these mice modified its SMA phenotype toward a wild-type pattern.[97]

Despite the positive results of neural stem cell transplantation in mice, its translational value in human is unclear due to limited availability of central nervous system tissues along with technical and ethical dilemmas.[98] These challenges led to the use of alternative stem cell therapy protocols, which includes the use of embryonic stem cells or induced pluripotent stem cells for transplantation, as their sources is vast and easily obtained. These cells have the ability to differentiate *in vitro* and *in vivo* into neural stem cells and motor neurons.[99–101] The findings of improved SMA phenotype in mice following the intrathecal transplantation of embryonic stem cell-derived neural stem cells included proper migration to target tissue in the spinal cord, neuroprotective function, and a 58% increase in lifespan.[102] A protocol to test neuronal stem cells in SMA patients is currently on hold by the FDA. In a similar neurodegenerative disorder such as amyotrophic lateral sclerosis (ALS), Phase I clinical trials of intraspinal stem cell transplantation have been completed and results have been published indicating that this method is feasible and well tolerated.[103] However, as of early 2015, no clinical trials of stem cell therapy in SMA patients are being conducted.

Need for early identification and treatment

The major obstacle in assessing the efficacy of any potential therapeutics for SMA is the recruitment of affected individuals in the early stages of the disease, prior to the loss of irreplaceable motor neuron units. For SMA type 1, such loss typically begins in the perinatal period, with severe denervation in the first 3 months of life and loss of more than 90% of motor units within 6 months of age.[104] This rate of neuronal loss means that the benefit of potential therapeutics can be assessed only by prompt treatment of affected newborns.

Newborn blood spot screening for spinal muscular atrophy

NBS began in the 1960s as a public health initiative to identify newborns with phenylketonuria and intervene with dietary restrictions before they suffered cognitive deficits. Since then, it has been expanded to detect over 30 congenital disorders for which newborns benefit from pre-symptomatic identification.[105] In the case of SMA, early diagnosis through SMA-NBS could assist overall medical care, ultimately reducing morbidity and mortality with the currently available tools, analogous to the benefits of newborn screening for cystic fibrosis.[56] Looking forward, the identification of therapeutic interventions that are effective pre-symptomatically would create a compelling need for SMA-NBS.

Molecular methodologies for the detection and diagnosis of SMA

No biochemical marker has been identified for SMA; however, several approaches to detecting *SMN1* deletion and intragenic mutations have been developed. Some methods are used for diagnostic purposes or carrier testing to detect the presence or absence of *SMN1* as well as quantification of *SMN1* and *SMN2* copy number. Other methods are used for screening purposes and identifying the majority of the affected individual by detecting *SMN1* deletions. All SMA cases identified through SMA-NBS methods must be confirmed with a reliable diagnostic test. A summary of current diagnostic and screening methods is listed in Table 3. These methodologies include single-strand conformation polymorphism (SSCP),[13] restriction fragment length polymorphism (RFLP),[106] denaturing high-performance liquid chromatography (DHPLC),[107–109] multiplex ligation probe amplification (MLPA),[110–112] and competitive PCR.[36,113–115] Only a few methods such as DHPLC and high-resolution melting analysis (HRMA) are useful for identifying intragenic mutations in *SMN1*. Digital PCR, a recently introduced methodology with a growing range of applications, is being used to identify SMA individuals and to quantify *SMN1* and *SMN2* copy number.[116–118]

Several methodologies have been utilized for screening applications with dried blood spot (DBS) specimens and for diagnostic applications; including liquid microbead suspension arrays,[5,6] high-resolution DNA melting analysis,[3,119,120] and real-time PCR.[7,37,121–125] Most screening assays will detect all individuals with the complete deletion of *SMN1* or mutations around exon 7; however, these assays will miss approximately 5% of affected individuals with other intragenic *SMN1* mutations.[4,126] Conversely, all of these methods would probably yield a screen-positive result in the rare phenotypically unaffected individuals with homozygous *SMN1* deletions but four or more copies of *SMN2*.

In order for an SMA detection method to be suitable for public health NBS programs, it must be cost-efficient, capable of high-throughput, and easy to implement in NBS laboratories. Screen-positive results should be confirmed as quickly as possible and reported routinely within 5 days of specimen receipt. Results should be comparable between laboratories.[127] Quality assurance must be established to ensure assay performance. A major component of quality assurance is the use of DBS reference materials from the Centers for Disease Control and Prevention (CDC).[128] Such materials for SMA screening have been developed at CDC and are currently undergoing multi-laboratory validation.

High carrier frequency for SMA (1/40–1/60) is also an issue to be considered in NBS. Identifying and reporting carriers from screening tests could potentially overwhelm follow-up activities; therefore, it may be disadvantageous to identify carriers through the initial screen.

The following sections describe three methods that have potential for use in traditional NBS laboratories.

Liquid microbead assays

Liquid microbead suspension arrays have been used for several disorders such as cystic fibrosis and hemoglobinopathies.[129,130] These methods involve PCR of the target

Table 3 – Molecular methods for SMA

Method	Application	Year[a]	Reference
Single-strand conformation polymorphism (SSCP)	*SMN1* deletion	1995	13
Restriction fragment length polymorphism (RFLP)	*SMN1* deletion	1995	106
Completive PCR	*SMN1* and *SMN2* gene quantitation	1997	36,113–115
Real-time PCR[b]	*SMN1* deletion	2002	2,7,37
Absolute quantitative PCR	*SMN1* and *SMN2* gene quantitation		37,121–124,138
Relative quantitative PCR			
Denaturing high-performance liquid chromatography (DHPLC)	*SMN1* deletion	2002	108–110
	SMN1 and *SMN2* gene quantitation		
	Intragenic mutation detection		
High-resolution melting analysis[b]	*SMN1* deletion	2009	3,119,120
	SMN1 and *SMN2* gene quantitation		
	Intragenic mutation detection		
Multiplex ligation probe amplification (MLPA)	*SMN1* and *SMN2* gene quantitation	2006	110–112,139
Liquid microbead assay[b]	*SMN1* deletion	2007	5,6
Digital PCR[b]	*SMN1* and *SMN2* gene quantitation	2011	116–118

[a] Year of earliest publication.
[b] Molecular methods have been validated on dried blood spots.

sequence with synthetic nucleobases, allele-specific target extension of the unique base in *SMN1* exon 7, microbeads that hybridize to tags on the extension region, and fluorescent signal detection. An advantage of using these assays is the large multiplex capability of 80–100 targets.

An initial study was done on 367 DBS samples using two different liquid bead assays: the MultiCode-PLx protocol and the Tag-it protocol.[6] Scatter plot distribution plots showed distinct clusters that separated the SMA-affected samples from the carrier and unaffected samples. This method was then adapted to the high-throughput protocol that was able to screen 400–500 samples daily and used to test DNA extracted from 40,103 anonymized DBS.[5] Results from this study showed 4 samples were detected with homozygous *SMN1* deletions and deletion was confirmed using competitive PCR. The estimated incidence of the general population affected by SMA was 1 in 10,026, consistent with previous literature. Carriers are not distinguished from unaffected individuals in these assays, and it is not useful for *SMN1* or *SMN2* copy number quantification. The reported cost of reagents and consumable for these methods were less than $10 for the MultiCode-Plx assay and less than $5 for the Tag-it assay. The time to run these assays from postextraction to final detection is about 3 h for the MultiCode-Plx assay and about 6 h for the Tag-it assay. The repeat rate for the high-throughput protocol was less than 0.02%.[4]

High-resolution DNA melting analysis (HRMA)

HRMA has been used to evaluate individuals for SMA in screening and diagnostic applications.[3,119,120] The difference in *SMN1* and *SMN2* copy number present in the specimens will create various unique HRMA profiles, distinguishing a heterozygous PCR product from a homozygous product. Therefore, the *SMN1* homozygous deletions can be readily identified since these samples will generate the same profile independent of *SMN2* copy number. Dobrowolski et al.[3] analyzed 1000 residual newborn blood spots and showed that unaffected individuals have distinct HRMA profiles from those of SMA individuals with *SMN1* homozygous deletion. This study also showed that *SMN2* copy number can be determined by mixing DNA from a homozygous *SMN1*-deleted patient with DNA that has a single copy of *SMN1* and *SMN2*, then matching the HRMA profiles with the corresponding profile of a carrier sample with a known *SMN2* copy number. Therefore, this method has the ability to identify *SMN1* and *SMN2* deletions and also to quantify copy number for both the genes. SMA carriers will be identified using the method, since they will generate different profiles dependent on the *SMN2* copy number. As reported, the assay takes under 3 h, and the cost of reagents and consumables is under $3 per sample. Preliminary results using HRMA were reported from a pilot study in nine hospitals in Utah, Colorado, and Illinois; 16,736 specimens were tested, but no affected individuals were identified.[131]

Real-time PCR

Several real-time quantitative PCR assays have been developed for SMA diagnosis, some of which have been applied to DBS specimens.[7,125] Some assays can only identify *SMN1* deletion (Fig. 3), while others can identify and quantify *SMN1* and *SMN2* copy numbers. The main challenge in developing a PCR assay for *SMN1* absence is the cross-reactivity with the nearly identical *SMN2* sequences; both genes are amplified, so the probe must be highly specific for *SMN1*.[26,132] A multiplex, real-time PCR designed to detect homozygous deletions of *SMN1* and a reference sequence in an RNaseP subunit gene was tested on DNA extracted from 153 DBS specimens.[7] Results showed 100% concordance with clinical status, identifying all of 57 affected individuals, while excluding 39 carriers and 56 unaffected individuals. Successful reduction in cross-reacting fluorescent signals due to probe binding to *SMN2* target was achieved by using an unlabeled *SMN2* probe as a blocker, in combination with a labeled *SMN1* probe with a minor grove binder group at the 3′ end.

Real-time PCR is also used to identify newborns with severe combined immunodeficiency (SCID) by measuring T-cell receptor excision circles (TREC). The TREC assay is currently used by many NBS laboratories in the United States and

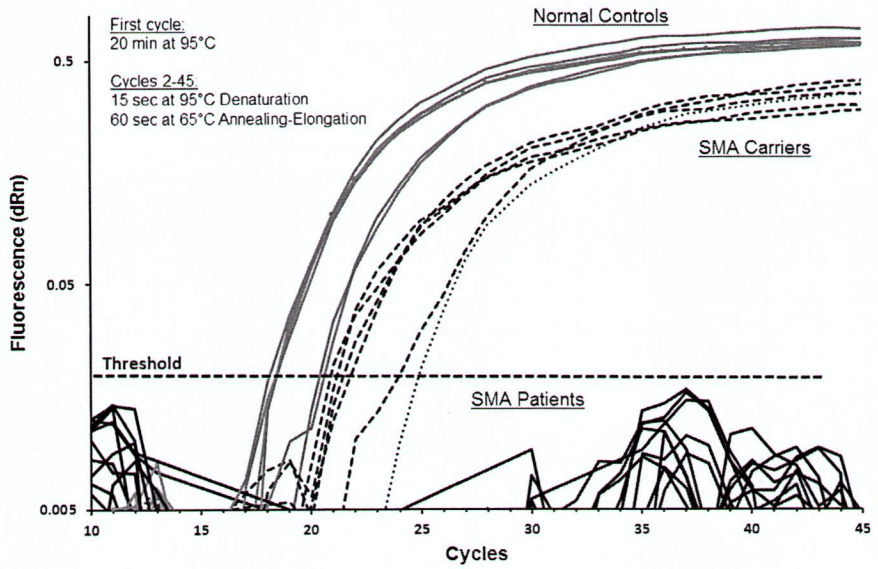

Fig. 3 – Real-time qPCR method for *SMN1* deletion detection. PCR amplification curves from normal controls, SMA parental carriers, and SMA patients with homozygous *SMN1* deletion. No amplification of *SMN1* above cycle threshold was observed in SMA patients. Real-time qPCR method for *SMN1* deletion detection. (Adapted with permission from Tayloret al.[2])

globally. A recent publication established that *SMN1* genotyping could be multiplexed with the TREC assay to simultaneously detect SMA and SCID.[2] The assay described does not require DNA extraction, and the real-time PCR reaction is carried out with the DBS punch in the PCR tube with the PCR reagents. A reference sequence in the *RPP30* gene is used as a control for amplification. The multiplex TREC–*SMN1*–*RPP30* assay has the advantage of using an existing NBS platform, which enhances its robustness, assures adequate throughput, and minimizes incremental costs. Detecting SMA by adding *SMN1*-specific PCR reagents to the TREC assay increases the cost by less than five cents per test; the cost of consumables and reagents to screen for both diseases is the less than $1 per specimen. The turnaround time from sample preparation to data analysis is less than 3 h. This assay reduces the cross-reactivity between *SMN1* and *SMN2* sequences by increasing the stringency of probe hybridization using a novel locked nucleic acid (LNA) technology. LNA oligonucleotides increase assay specificity through restriction of the ribose conformation in the oligonucleotide backbone, allowing shorter probe sequences and the use of higher annealing temperatures.[133] In the TREC–*SMN1*–*RPP30* assay, an LNA probe was used to discern the single nucleotide polymorphism (SNP) in intron 7 of *SMN1* from *SMN2*. Specimens from SMA patients with homozygous *SMN1* deletion produced no signal above the cycle threshold, making the analysis of results very quick and easy to interpret. Samples from carriers are essentially indistinguishable from unaffected individuals in this assay; all samples produce robust signals when at least one copy of the *SMN1* gene is present. The double-blinded study of this method with 26 residual DBS specimens showed 100% concordance with the clinical status.

Confirmatory and follow-up testing

After initial screening, a second-tier test may be used to quantify the number of *SMN1* and *SMN2* genes in the screen-positive sample to further characterize the SMA genotype and provide prognostic information. The second-tier assay may be based on lower-throughput platforms such as digital PCR,[118] since the number of the samples to be tested will be smaller. Second-tier testing will confirm screen-positive samples as well as identify newborns who are at the highest risk for infantile onset.

Legal, ethical, and social issues

Studies have been conducted to examine the legal, ethical, and social issues with population-based pilot studies for SMA. A study focused on the parental decision-making process in population-based pilots by engaging a focus group in public acceptability in an opt-out research pilot study.[134] Parental attitudes toward NBS for Duchenne Muscular Dystrophy and particularly SMA in a recent survey also showed overwhelming parental support for NBS, even without further development in treatments.[135] The Newborn Screening Translational Research Network (NBSTRN) Bioethics and Legal Issues Work Group has assessed questions related to parental consent for NBS pilot studies and concludes that a signed consent document is appropriate in some circumstances for pilot screening.[134] Furthermore, with known diagnosis, genetic counseling can be useful and educational; however, the approach and timing of the counseling will need to be refined.[86]

In 2008, SMA was submitted for formal consideration to the US Health and Human Services Secretary's Advisory Committee on Heritable Diseases in Newborns and Children by a collaborative group of physicians, researchers, the SMA community, and the Families of SMA. The consensus of this committee's internal Nomination and Prioritization Workgroup was that the addition of SMA to the uniform screening panel was premature at that time.[136] The Workgroup recommended the implementation of prospective pilot studies of the screening method by one or more traditional public health laboratories to validate the reproducibility of the preliminary findings in research settings. The Workgroup

also noted that the time required for such validation would allow for an assessment of potential therapies of drugs and other treatment benefits rather than just relying on the nutritional support and respiratory care options. Since then, clinical trials have made considerable progress, and the validation of screening methods in public health NBS laboratories is just beginning. As of this publication, an initial pilot has been completed by the Wadsworth Center of the New York State Department of Health[137] with further validation planned, and a population-based pilot study in Taiwan that began in November 2014 had detected two cases within 4 months (personal communication: Yin-Hsiu Chien, National Taiwan University Hospital, February 2015).

Summary and future directions

The molecular basis for SMA, the leading genetic cause of death in children, was uncovered some 20 years ago. Since then, scientific progress has spurred the development of innovative therapies that are just beginning clinical trials in pre-symptomatic patients. NBS pilot studies to establish technical reliability and operational feasibility in the context of public health programs will be essential to prepare for the prospect of effective pre-symptomatic treatment. Such pilot studies will provide the information needed for evidence-based review, both by NBS programs and their advisory committees, when considering the inclusion of SMA in routine NBS panels. As clinical trials progress and public health programs prepare, the vision of preventing the burden of death and disability caused by SMA will become a reality.

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Newborn Bloodspot Screening Test Using Multiplexed Real-Time PCR to Simultaneously Screen for Spinal Muscular Atrophy and Severe Combined Immunodeficiency

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BACKGROUND: Spinal muscular atrophy (SMA) is a motor neuron disorder caused by the absence of a functional survival of the motor neuron 1, telomeric (*SMN1*) gene. Type I SMA, a lethal disease of infancy, accounts for the majority of cases. Newborn bloodspot screening (NBS) to detect severe combined immunodeficiency (SCID) has been implemented in public health laboratories in the last 5 years. SCID detection is based on real-time PCR assays to measure T-cell receptor excision circles (TREC), a byproduct of T-cell development. We modified a multiplexed real-time PCR TREC assay to simultaneously determine the presence or absence of the *SMN1* gene from a dried blood spot (DBS) punch in a single reaction well.

METHOD: An *SMN1* assay using a locked nucleic acid probe was initially developed with cell culture and umbilical cord blood (UCB) DNA extracts, and then integrated into the TREC assay. DBS punches were placed in 96-well arrays, washed, and amplified directly using reagents specific for TREC, a reference gene [ribonuclease P/MRP 30kDa subunit (*RPP30*)], and the *SMN1* gene. The assay was tested on DBS made from UCB units and from peripheral blood samples of SMA-affected individuals and their family members.

RESULTS: DBS made from SMA-affected individuals showed no *SMN1*-specific amplification, whereas DBS made from all unaffected carriers and UCB showed *SMN1* amplification above a well-defined threshold. TREC and *RPP30* content in all DBS were within the age-adjusted expected range.

CONCLUSIONS: SMA caused by the absence of *SMN1* can be detected from the same DBS punch used to screen newborns for SCID.

Spinal muscular atrophy (SMA),[5] the most common genetic cause of death in infancy *(1)*, is an α-motor neuron disorder caused by insufficient concentrations of the survival of motor neuron (SMN) protein. In about 95% of SMA cases, the reduction in SMN concentrations is due to deletions involving the survival of motor neuron 1, telomeric (*SMN1*)[6] gene *(2)*. The nearly identical survival of the motor neuron 2, centromeric (*SMN2*) gene, a paralog of *SMN1*, also produces SMN protein, but at much lower concentrations. *SMN2* copy numbers vary widely between individuals, ranging from complete absence to 5 or more copies per genome *(3)*. Because some SMN protein is essential for fetal development, all babies born with SMA have at least 1 *SMN2* gene. Higher *SMN2* copy numbers in SMA patients are associated with later onset and milder disease, including juvenile onset (type III; OMIM 253400) and adult onset (type IV; OMIM 271150). However, the majority of SMA newborns become symptomatic as infants (type I; OMIM 253300) or toddlers (type II; OMIM 253550). Type 1 children will never sit unsupported, often require

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[5] Nonstandard abbreviations: SMA, spinal muscular atrophy; SMN, survival of motor neuron; DBS, dried blood spot; NBS, newborn bloodspot screening; SCID, severe combined immunodeficiency; TREC, T-cell receptor excision circles; UCB, umbilical cord blood; Cq, quantification cycle value; LNA, locked nuclei acids.

[6] Human genes: *SMN1*, survival of motor neuron 1, telomeric; *SMN2*, survival of motor neuron 2, centromeric; *RPP30*, ribonuclease P/MRP 30kDa subunit.

ventilatory support in the first year of life, and usually die before 2 years of age. Type II children can survive to early adulthood but will never walk. Cognitive abilities are not impaired by SMA and affected children are generally bright and sociable.

Several potential therapies for SMA are in development *(1, 4)*, 2 of which are undergoing clinical trials in symptomatic children with some encouraging results *(5, 6)*. Because of the early onset and rapid progression of infantile SMA, evaluation of these therapies in presymptomatic infants will require prompt detection. Such early detection would be possible in about 95% of SMA cases by screening newborn dried blood spots (DBS) for the homozygous absence of *SMN1* sequences around exon 7 *(7, 8)*.

The most recent condition added to the US Recommended Uniform Screening Panel for newborn bloodspot screening (NBS) is severe combined immunodeficiency (SCID) *(9)*, a congenital disorder with severe impairment of cellular and humoral immune function due to a profound deficiency in T cells *(10)*. The assay most commonly used for SCID-NBS is real-time PCR to measure T-cell receptor excision circles (TREC), extrachromosomal DNA byproducts of somatic recombination in T cells *(10)*. SCID is the first NBS condition for which DNA analysis is the primary (first-tier) screening method. Since the initial pilot experiences in 2 US state public health laboratories *(11, 12)*, SCID-NBS has expanded to many other state programs *(13, 14)* and now covers the majority of newborns in the US as well as many newborns globally *(15)*. SCID-NBS prevents infant death through early medical intervention and is highly cost-effective *(16, 17)*.

Because of similarities in methods, we reasoned that both SCID and SMA could be detected in the same real-time PCR reaction-well by modifying existing high-throughput TREC PCR assays to include *SMN1* genotyping. The combined assay had to be specific for *SMN1* to avoid cross-reactivity with the *SMN2* gene. Here we show that homozygous *SMN1* absence can be reliably detected from the same DBS punch used to measure TREC at minimal incremental cost.

Materials and Methods

SOURCES OF SAMPLES

DBS and DNA extracts with TREC values in the expected range for typical term newborns were made from residual excluded umbilical cord blood (UCB) units collected at the Duke University Stem Cell Laboratory. SCID-like DBS were prepared from peripheral blood containing no measurable TREC obtained from adults above age 50 years. Before spotting, the blood was depleted of mononuclear cells by layering on Histopaque (Sigma-Aldrich), centrifuging 30 min at 2100*g*, aspirating the fluid above the buffy coat, and reconstituting to 50% hematocrit with pooled serum. Immortalized B lymphocyte and fibroblast cell lines from patients with SMA (GM 23689, GM 10684, GM 03813, GM 00232, and GM 09677) and carriers (GM 23688, GM 23687, GM 03814, and GM 03815) were obtained from the Coriell Institute for Medical Research. DBS with SMA-affected or -carrier genotypes were made from residual peripheral blood samples obtained with informed consent *(3)* from 11 SMA-affected individuals (age range, 1–50 years) and from 15 unaffected parents (age range, 25–57 years). DBS were stored in low-permeability ziplock bags with silica gel desiccant packs (Poly Lam Products) up to one month at room temperature, up to 6 months at 4 °C, and up to 2 years at −20 °C. The CDC laboratory staff was blinded to sample status and had no access to personal identifiers. The study was therefore classified as human subjects research for which the CDC was not engaged.

REAL-TIME PCR ASSAYS

All primers and probes (Table 1) were custom synthesized by Integrated DNA Technologies. Real-time PCR assays were conducted in PCR plates (96-well formats; Agilent Technologies) using a scanning photofluorometric thermal cycler (Stratagene MxPro 3000p). Cycle thresholds were initially determined by inspection of amplification curves and then retained in fixed positions. Quantification cycles (Cq) were reported by instrument software.

TREC QUANTIFICATION IN DBS

The real-time PCR assay of in situ DBS was performed on 2-mm discs punched from DBS samples directly into 96-well PCR arrays. After 125 μL wash buffer was added (DNA elution solution, Qiagen) to each well, the PCR array was incubated at room temperature for 15 min on a microtiter plate shaker set at 1200 rpm. The wash buffer was then removed, and 15 μL of the complete real-time PCR master mix (PerfecTa Toughmix, Quanta Biosciences) containing primers and probes (Table 1) was added to each sample well. The PCR plate was sealed with optical film and processed using the following amplification conditions: 45 °C for 3 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. A reagent blank and a blank filter paper punch (no-template control) were included in the analytical runs.

SMN1 ANALYSIS IN DNA EXTRACTS

DNA was extracted from cell lines or UCB samples using the Qiagen QIAamp® Mini DNA kit according to the manufacturer's protocol. DNA extracts were analyzed immediately or stored at −20 °C for up to 6 months. Extracts from tissue culture cells contained 3–50 ng/μL

Table 1. PCR primers and probes for the triplex real-time PCR assay to amplify sequences in the δRec-ΨJα signal joint (TREC) and in *RPP30* and *SMN1*.

Target/reagent	Sequence	Concentration (nmol/L)[a]
TREC		
Forward primer	5`-TTT GTA AAG GTG CCC ACT CCT-3`	800
Reverse primer	5`-TAT TGC AAC TCG TGA GAA CGG TGA AT-3`	800
Probe	5-FAM/CGGTGATGCATAGGCACCT/Iowa Black quencher-3`	120
RPP30		
Forward primer	5`-TTT GGA CCT GCG AGC G –3`	60
Reverse primer	5`-GAG CGG CTG TCT CCA CAA GT-3`	150
Probe	5`-HEX/TTCTGACCTGAAGGCTCTGCGCG/Iowa Black quencher-3`	200
SMN1		
Forward primer	5`-GTGGAAAACAAATGTTTTTGAACATT-3`	900
Reverse primer	5`-GTAGGGATGTAGATTAACCTTTTATCTAATAGTTT-3`	900
LNA probe[b]	5`-Cy5/CAA***C***TT***T***TAA***C***AT***C***T/3IAbRQSp-3`	100

[a] Final concentration in PCR reaction.
[b] Bases in bold italic font denote LNA nucleotides; the underlined nucleotide indicates the position of the A>G transition in intron 7 that distinguishes *SMN1* from *SMN2*.

DNA and extracts from UCB contained 100–150 ng/uL DNA. Real-time PCR was conducted in 20-μL reaction volumes containing 5 μL of the DNA extract, a commercial real-time PCR premix (PerfecTa, Toughmix, Quanta), the *SMN1* forward primer and reverse primer, the *SMN1* locked nucleic acid (LNA) probe overlying the A>G transition at position 100 of intron 7 to distinguish *SMN1* from *SMN2*, and the *RPP30* forward primer, reverse primer, and probe (Table 1). Primers and probe sequences for *SMN1* were designed using Primer Express (Life Technologies) and confirmed for in silico specificity. Probe modification with LNA bases was designed with software from IDT Biophysics. To determine the optimal annealing temperature, reactions were carried out in a Bio-Rad Laboratories CFX96 real-time PCR instrument with the following amplification conditions: 45 °C for 3 min and 95 °C for 10 min, followed by 45 cycles of melting at 95 °C for 15 s and annealing/extension between 60 °C and 67 °C for 1 min.

Results

SMN1 ASSAY DEVELOPMENT

Prototype assays were initially explored using extracted DNA from cell lines of patients with SMA, cell lines of SMA carriers, and UCB samples. We first used a temperature gradient to determine the optimal PCR annealing temperature for maximal specificity to discriminate *SMN1* and *SMN2* amplification. The results (Fig. 1) showed increasing discrimination between the patients and unaffected controls with increased annealing temperature. At 65 °C, no *SMN1* amplicon was detected by the LNA probe in patients with SMA, whereas healthy individuals and carriers showed clear amplification. This annealing temperature of 65 °C provided the highest analytical specificity and was chosen for all subsequent experiments.

The working parameters for a duplex assay that included *SMN1* and a genomic sequence of *RPP30* as an internal control reference for PCR amplification were then optimized. This duplex assay was applied to DNA extracts prepared from commercially available cell lines from 5 SMA patients and 4 parental carriers as well as cellular DNA from 5 UCB samples. Results (Fig. 2) demonstrated a 100% concordance with the clinical status of the donors. No amplification was seen with DNA extracted from any of the SMA patient cell lines, indicating no detectable cross-reactivity with *SMN2*. In contrast, DNA extracted from cell lines from the unaffected carriers and UCB produced robust amplification, with Cq values ranging from 18 to 25 cycles. *RPP30* in all samples showed amplification in the expected Cq range.

Having established the working conditions for the *SMN1* assay, we incorporated it into the DBS assay for TREC with the annealing temperature raised to 65 °C. Reference DBS samples from a SCID-like positive control with no TREC, a positive control from an SMA infant with no *SMN1*, and a normal control from a UCB sample showed clear discrimination between the amplification patterns of the corresponding targets (Figs. 3). Varying the SMN primer concentration from 50 nmol/L to 900 nmol/L exerted no effect on the other multiplexed

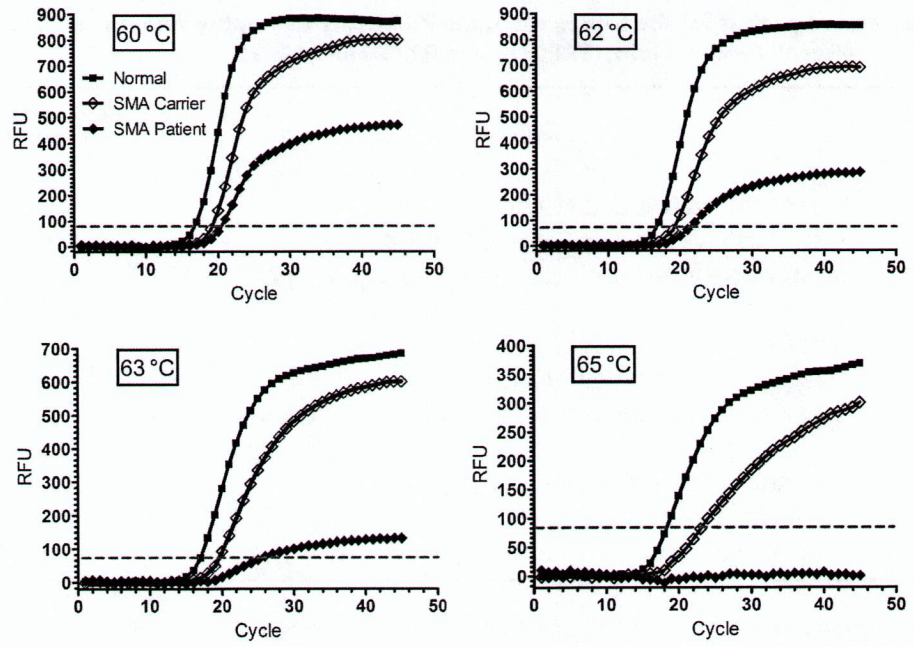

Fig. 1. Seven temperatures (60 °C to 67 °C) were tested to determine the appropriate annealing temperature for the *SMN1* gene; results are shown for 4 selected temperatures. The dashed grey horizontal line denotes the fluorescence threshold for positive results and Cq determination. RFU, relative fluorescence units.

targets (TREC and *RPP30*; see Fig. 1 in Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue2). Therefore primer limitation was not necessary, and the maximum primer concentration (900 nmol/L) in the range tested was used for both forward and reverse *SMN1* primers in all subsequent experiments.

To determine whether the inclusion of the *SMN1* target and the higher (65 °C) annealing temperature would alter the TREC assay, we first compared results on DNA extracts (see online Supplemental Fig. 2). The TREC Cq values were highly correlated ($r^2 = 0.87$) and showed no significant difference between the original duplex (TREC and *RPP30* at 60 °C annealing temperature) and the modified triplex assay ($P > 0.14$ by paired *t*-test). Next we tested 150 DBS made from UCB with both the original duplex assay and the modified triplex assay. All samples showed the typical amplification curves expected for TREC, *SMN1*, and *RPP30*. TREC Cq values obtained by the in situ DBS triplex assay showed a slight decrease (mean 0.4 Cq) compared to those from the duplex assay but were consistent with the overall ranges for term newborns reported by public health newborn screening programs *(11–14)*. The Cq values for *SMN1* showed a near-gaussian distribution (Fig. 4A). Cq values for *SMN1* and *RPP30* (Fig. 4B) were significantly correlated ($r^2 = 0.66$, $Sy/x = 0.47$, $P < 0.01$), suggesting that the variation between samples was due mostly to differences in leukocyte content and thus in the total amount of genomic DNA.

VALIDATION IN CLINICAL SAMPLES

The clinical validity of the TREC-*RPP30-SMN1* triplex assay was examined in a double-blind testing of DBS made from 26 blood samples from SMA patients and their carrier parents. This sample set was analyzed independently at the 2 collaborating laboratories (CDC and Biogen Idec) using the triplex in situ DBS method. The *SMN1* categorical genotypes obtained by both laboratories were identical and showed 100% concordance with the clinical status of the patient (Table 2). All samples showed typical amplification of the internal reference control *RPP30*, and the TREC Cq values were within the expected range for the age of the donor. Reagent blank and blank filter paper punches (no template controls) showed no amplification for any of the 3 targets.

Discussion

The early onset and precipitous clinical course of infantile SMA make it a prime target for early detection through NBS *(7)*. Compared with other conditions currently recommended for NBS in the US *(18)*, SMA has a high birth prevalence, ranking among the 3 NBS primary target conditions presently on the Recommended Uni-

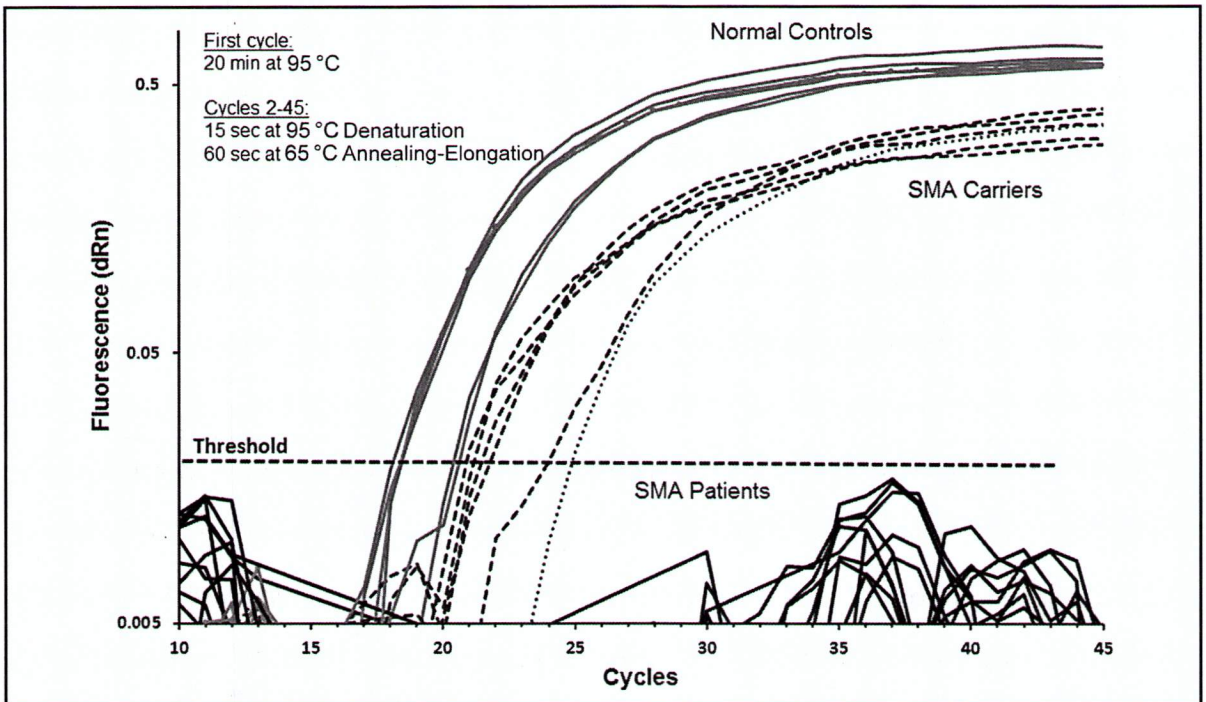

Fig. 2. PCR amplification curves from DNA extracts of 5 randomly selected umbilical cord blood samples (normal controls), 6 cell lines from SMA parental carriers, and 5 cell lines from SMA patients with homozygous *SMN1* deletion. dRn, Δ normalized reporter.

form Screening Panel (congenital hypothyroidism, cystic fibrosis, and hemoglobinopathy). As with SCID, early detection of SMA is likely to provide the best opportunity for effective treatment. With multiple therapeutics currently in clinical trials, and more in development *(1, 3, 19, 20)*, NBS laboratories are in an ideal position to facilitate presymptomatic intervention that could maximize the benefits of potential therapies.

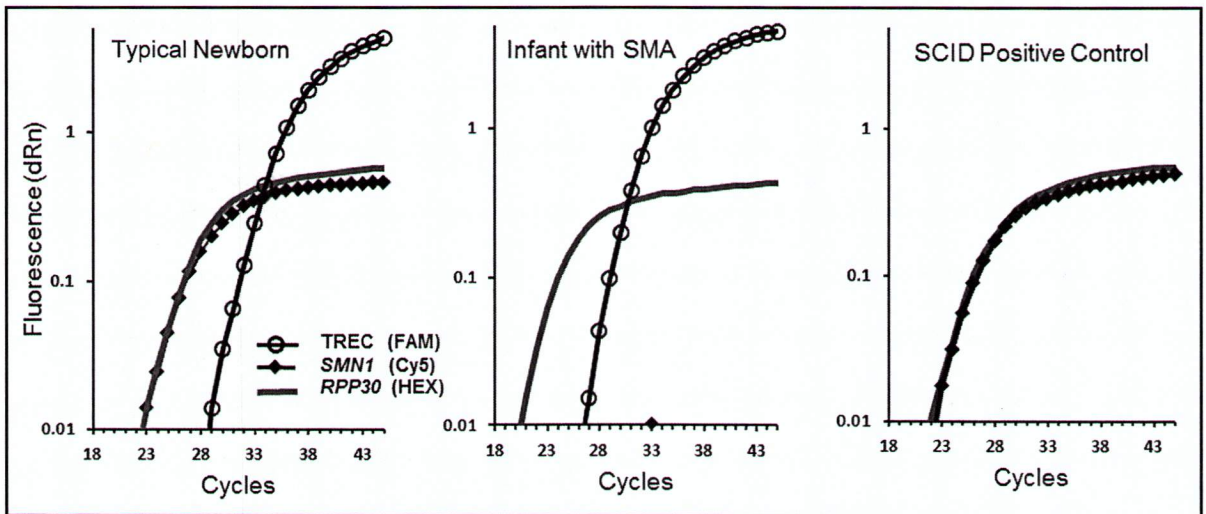

Fig. 3. Triplex TREC-SMN1-RPP30 DBS real-time PCR amplification curves.

DNA targets were amplified on each DBS punch without DNA extraction. Green line: *RPP30* (present in all samples); orange line: *SMN1* (absent in SMA infant); blue line: TREC (absent in SCID-positive control). dRn, Δ normalized reporter; FAM, 6-carboxyfluorescein; HEX, β-hexosaminidase; Cy5, cyanine 5.

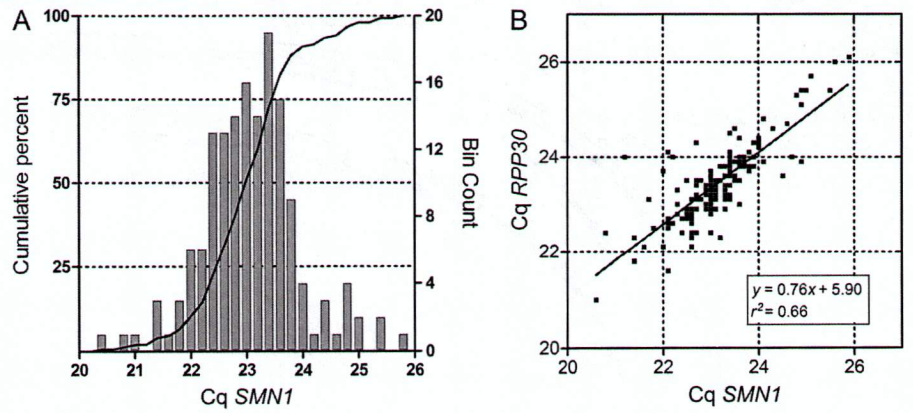

Fig. 4. (A) Distribution of *SMN1* Cq results on dried blood spots made from 150 umbilical cord blood samples analyzed at the CDC laboratory. (B) Correlation between Cq results for *SMN1* and *RRP30* ($r = 0.66$, $P < 0.01$).

Over the last 2 decades, the complex biology and natural history of SMA have been largely delineated *(2, 5)*. Although several factors influence pathogenesis, about 95% of diagnosed SMA cases are caused by the homozygous absence of a functional *SMN1* gene through either deletion or gene conversion. Various approaches to detecting the *SMN1* null genotype have been developed, and some have been validated in DBS samples, including real-time PCR *(8)*, high-resolution melting *(21)*, and microbead suspension arrays *(22)*.

Two major considerations determined our approach to developing an NBS assay for SMA. First, it would be preferable to use an assay platform that is well established in public health newborn screening. Although genotype-based sickle cell disease screening had been performed previously *(23)*, until recently none of the primary screening assays used in public health NBS programs used molecular DNA methods. The incorporation of a DNA-based assay has been prompted by the recommended inclusion of SCID-NBS, which is based on measuring TREC *(24)*. The preferred platform for measuring TREC is real-time PCR, and this technology is now routinely used in screening the majority of newborns in the US for SCID. Second, it would be advantageous to multiplex the *SMN1* assay with an existing routine NBS assay, thereby assuring its robustness and minimizing the incremental cost for detecting SMA. We therefore explored the possibility that *SMN1* genotyping could be multiplexed with the TREC assay developed at CDC, which is used to characterize DBS reference materials for global distribution to participants in the CDC Newborn Screening Quality Assurance Program *(25)*.

The main challenge in developing a PCR assay for *SMN1* gene absence is caused by the near identity of the *SMN1* and *SMN2* gene sequences. Conventional real-time PCR probes to any targeted *SMN1* sequence could cross-react with the corresponding *SMN2* sequence and produce false amplification signals when the *SMN1* sequence is absent, as in an affected SMA sample. Other investigators have previously reported successful reduction of cross-reacting fluorescent signals by using unlabeled *SMN2* probes as a blocker, in combination with a labeled *SMN1* probe with a minor grove binder group at the 3′ end. We opted for an alternative approach to achieve the required analytical specificity by increasing the stringency of probe hybridization using a novel LNA probe. LNA oligonucleotides increase assay specificity through restriction of the ribose conformation in the oligonucleotide backbone, allowing the use of higher annealing temperatures. These shorter probes are known to improve the ability to discern single-nucleotide polymorphisms *(26)*, such as those that distinguish *SMN1* from *SMN2*. LNA probes therefore simultaneously increase both the specificity and sensitivity of the assay.

Another important concern in multiplexing PCR is the potential for different amplicons to compete for PCR reagents. Since TREC is present in a much lower concentration than *SMN1*, the effect of competition would be a decreased amplification for TREC. A serial dilution series for the *SMN1* primers was analyzed to explore this possibility and showed no effect on the other multiplexed targets (TREC and *RPP30*). However, with DNA extracts of normal UCB samples and carrier cell lines, higher primer concentrations did increase the plateau fluorescence for *SMN1*, increasing visual discrimination from the baseline fluorescence of *SMN1*-absent SMA patients.

In addition to familiar instrumentation and multiplexing capability, another factor in developing an approach to SMA-NBS was the high prevalence of SMA carriers. In our assay, both normal and carrier *SMN1* genotypes showed robust *SMN1* amplification, and the difference in Cq was not sufficiently precise for reliable

Table 2. Results of blinded testing of DBS samples prepared from peripheral blood samples of 11 SMA patients and 15 parental carriers by the multiplex TREC-*SMN1-RPP30* DBS real-time PCR assay.

SMA status	SMA type	Age, years	*SMN1* result[a]	*SMN1*, Cq[b]	*RPP30*, Cq[b]	TREC, Cq[b]
Patient	III	4	Absent	No Cq	23.8	30.2
Patient	III	2	Absent	No Cq	24.2	31.7
Patient	II	50	Absent	No Cq	25.1	34.6
Patient	III	3	Absent	No Cq	23.5	29.6
Patient	II	1	Absent	No Cq	24.5	30.0
Patient	II	22	Absent	No Cq	23.7	30.4
Patient	III	13	Absent	No Cq	23.2	31.3
Patient	III	3	Absent	No Cq	23.2	29.5
Patient	II	1	Absent	No Cq	21.6	28.7
Patient	III	4	Absent	No Cq	22.6	28.5
Patient	III	2	Absent	No Cq	22.5	29.1
Carrier[c]	NA[d]	45	Present	24.2	23.3	34.3
Carrier	NA	33	Present	26.9	25.0	34.3
Carrier	NA	34	Present	25.6	24.8	33.9
Carrier	NA	29	Present	25.0	23.8	34.0
Carrier	NA	32	Present	24.2	23.2	34.4
Carrier	NA	43	Present	23.3	22.2	34.8
Carrier	NA	43	Present	22.7	21.9	33.0
Carrier	NA	41	Present	23.0	22.4	35.0
Carrier	NA	57	Present	25.5	24.9	34.4
Carrier	NA	48	Present	22.7	22.3	35.3
Carrier	NA	48	Present	22.5	22.8	34.4
Carrier	NA	44	Present	25.8	25.1	36.7
Carrier	NA	35	Present	21.1	21.1	31.3
Carrier	NA	33	Present	22.6	22.6	No Cq
Carrier	NA	25	Present	21.8	22.0	31.8

[a] Concordant categorical results from CDC and Biogen-Idec laboratories.
[b] Cq results from CDC laboratory.
[c] Unaffected parent of SMA patient.
[d] NA, not appicable.

carrier identification. In contrast, all of the homozygous *SMN1*-negative samples failed to cross the cycle threshold, and they were clearly discriminated from samples with one or more *SMN1* copies.

The determination of *SMN2* copy number is an essential second-tier assay for following up screen-positive samples. It is the most informative prognostic marker and will guide selection criteria for clinical trials. *SMN2* copy number may be determined by real-time qPCR *(27)* as well as other methods *(28)*. Ideally, public health NBS laboratories could perform a single second-tier assay that would both confirm the absence of *SMN1* and quantify the *SMN2* copy number, thereby identifying newborns at high risk for infantile onset. Preliminary studies in our laboratories suggest that digital droplet PCR *(29)* will also be a useful platform for independent confirmation and characterization of SMA screen-positive samples.

The rapid and increasingly widespread implementation of SCID-NBS has ushered real-time PCR technology into the repertoire of NBS laboratories. Since its inception in 2008, SCID-NBS has been implemented in 23 US state public health NBS programs, collectively identifying SCID at twice the previously estimated birth prevalence and achieving high survival rates in treated infants *(11)*. This experience strongly suggests that SMA-NBS will be technically feasible and cost-efficient.

In conclusion, we developed a multiplexed real-time PCR assay to simultaneously measure TREC and screen for the absence of *SMN1* in a single reaction-well. The addition of *SMN1* genotyping to the TREC assay does

not require new equipment or any changes in sample processing or the overall testing procedure. The additional reagent and supply costs beyond those of the current TREC assay are limited to the *SMN1* primers and probe, which amount to less than 5 cents per test. The assay allows clear identification of the *SMN1* null genotype without quantifying copy number, thereby avoiding carrier detection. NBS programs screening for SCID will require evidence of effective presymptomatic intervention in newborns with SMA before combining the 2 tests into routine screening. However, as soon as an effective therapy becomes available, SMA-NBS could be readily implemented alone or in combination with TREC measurements by public health programs already using real-time PCR to screen for SCID.

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