1

Antisense Oligonucleotides (ASOs):

Versatile Tools for Precision Neurology?

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Disclosure

Sponsored research, clinical trials, consultation:

Ionis Pharmaceuticals, Biogen, Genzyme

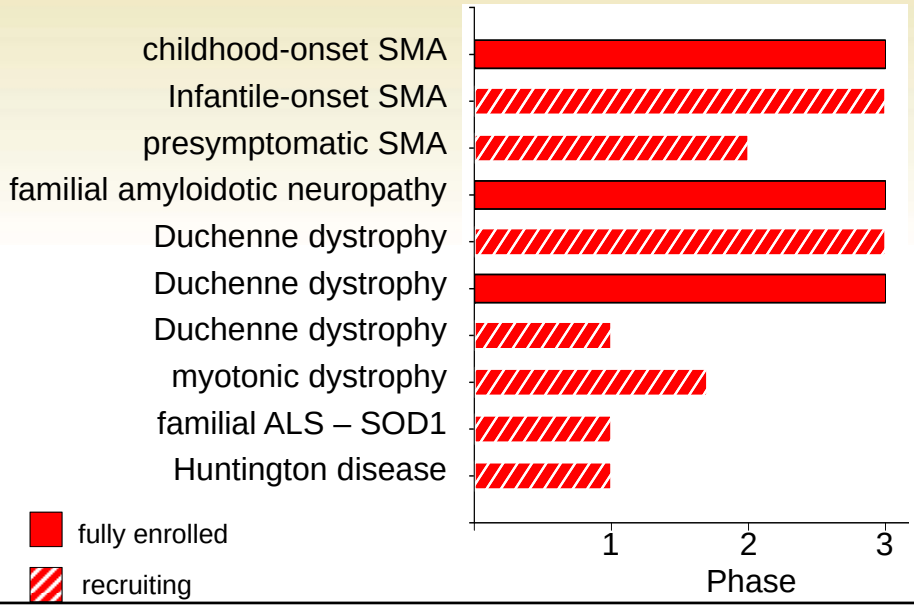
Slide 1

1 Charles Thornton, 3/1/2012

Antisense oligonucleotides (ASOs)

1. short synthetic analogues of DNA or RNA
2. chemically modified to resist enzymatic degradation
3. act by hybridization to a specific RNA target
4. change the expression of a specific gene
5. first proposed in 1978
6. by 2000, 19 ASO drugs in clinical trials, 18 failed

ASO Trials for Neurological Disorders



Historical challenges in developing antisense drugs

1. rapidly degraded
2. filtered by the kidney
3. undesirable binding
   - serum proteins
   - cell surface
4. off target activity

chemical modification

sequence predictions
empirical testing

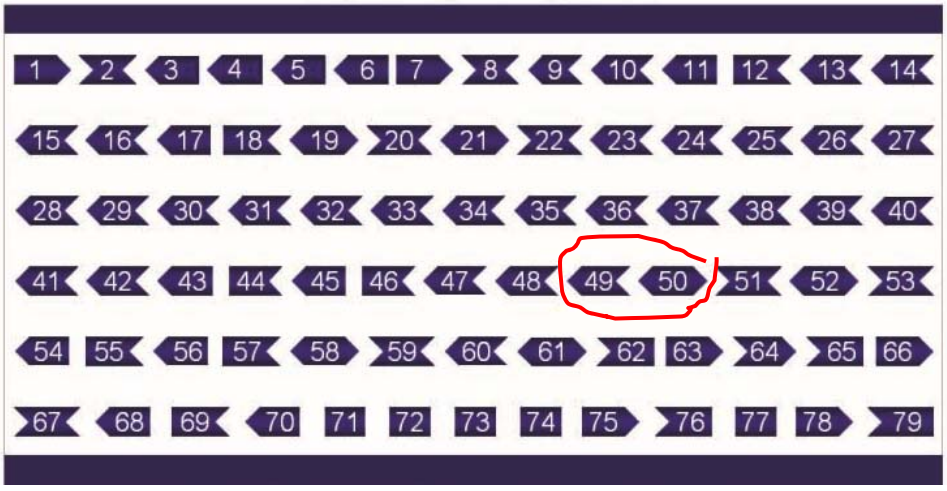
Biodistribution with systemic administration

- liver, kidney >> bone marrow, lymph nodes
- 40-fold less in muscle and heart
- none in brain

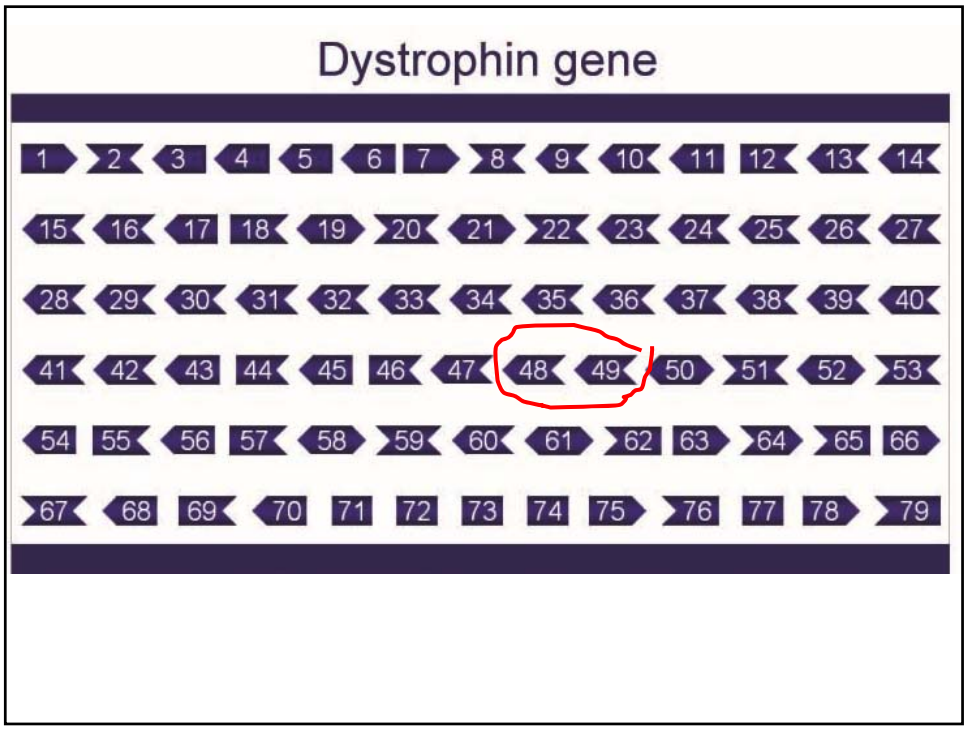
Two kinds of ASO action

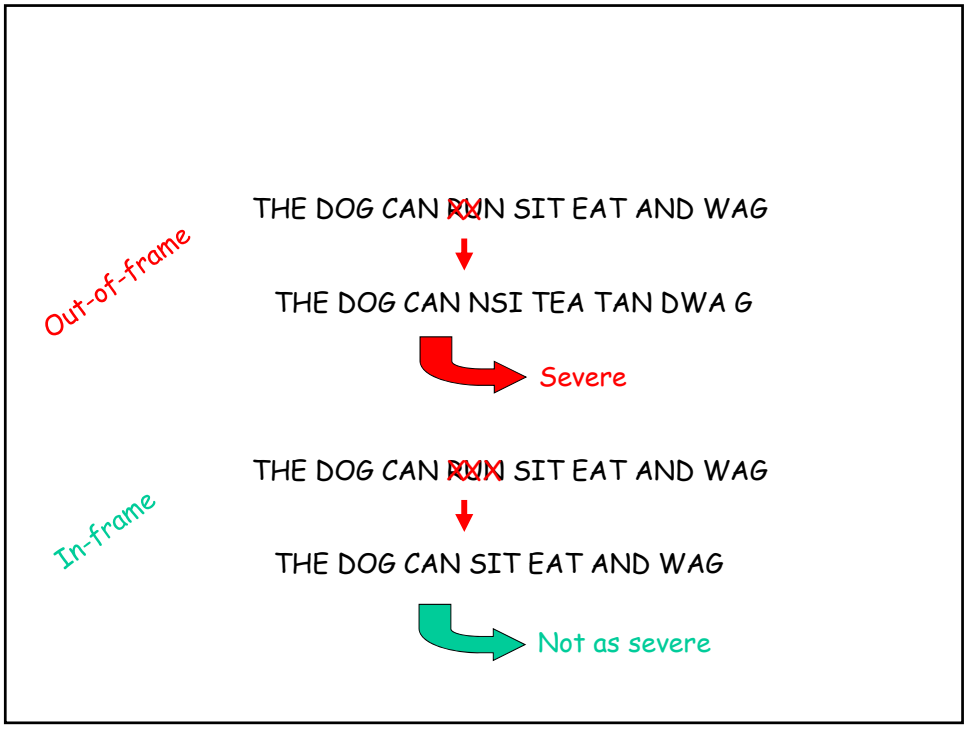
1. induce cleavage of target RNA
    - silence expression of a single gene
2. blocker ASO
    - bind RNA before it is spliced
    - affect how it is spliced: “splice shifting”
    - exon skipping, exon inclusion

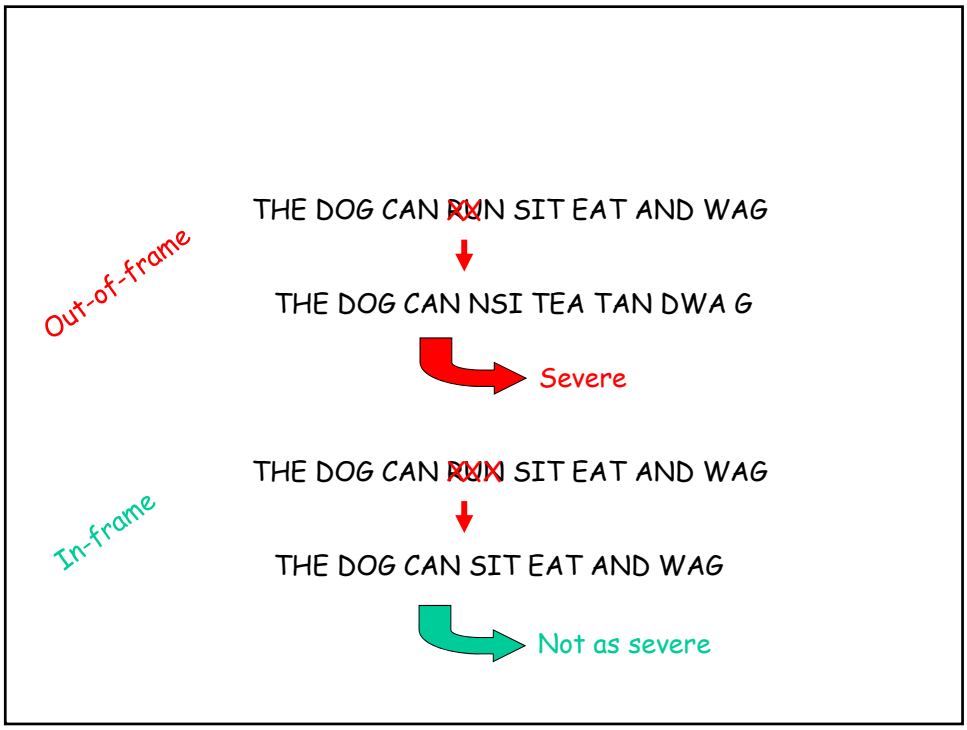
Dystrophin gene




THE DOG CAN RUN SIT EAT AND WAG
Out-of-frame
THE DOG CAN NSI TEA TAN DWA G
Severe


Dystrophin gene
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24 25 26 27
28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50 51 52 53
54 55 56 57 58 59 60 61 62 63 64 65 66
67 68 69 70 71 72 73 74 75 76 77 78 79


Out-of-frame
THE DOG CAN RUN SIT EAT AND WAG
THE DOG CAN NSI TEA TAN DWA G
Severe
In-frame
THE DOG CAN RUN SIT EAT AND WAG
THE DOG CAN SIT EAT AND WAG
Not as severe


Out-of-frame
THE DOG CAN RUN SIT EAT AND WAG
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Severe
In-frame
THE DOG CAN RUN SIT EAT AND WAG
THE DOG CAN SIT EAT AND WAG
Not as severe

Exon skipping: convert out-of-frame deletion to in-frame deletion

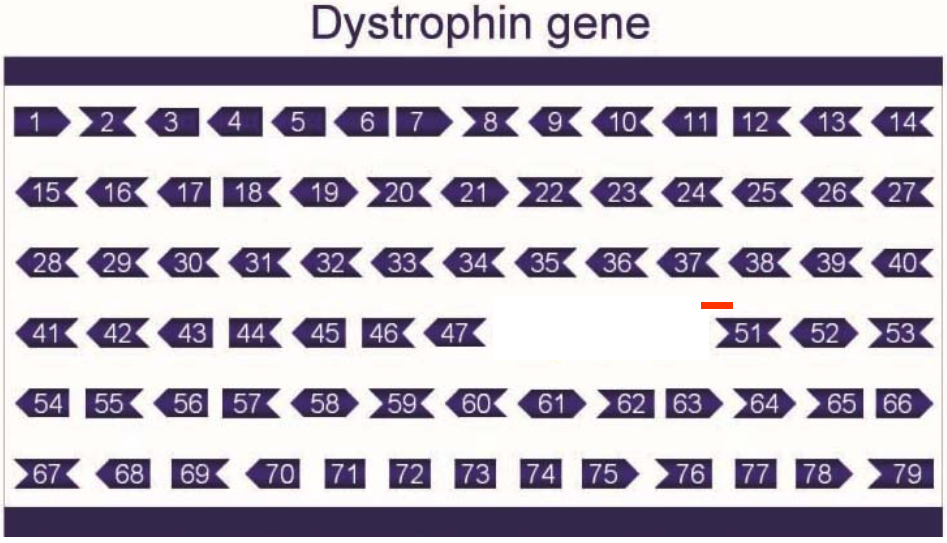
ASO

THE DOG CAN RUN SIT EAT AND WAG

THE DOG CAN SIT EAT AND WAG

In-frame

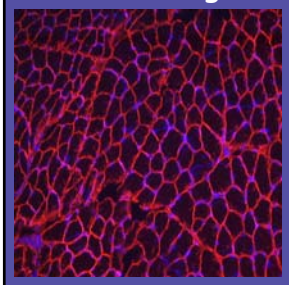
Not as severe



antisense oligonucleotide for skipping exon 51

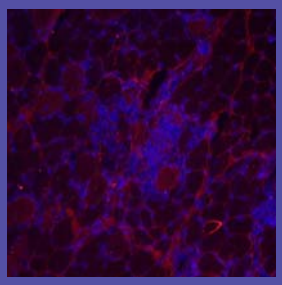
Recovery of dystrophin expression after systemic treatment with exon skipping ASOs (canine X-linked muscular dystrophy)

normal beagle



dog runs, etc.

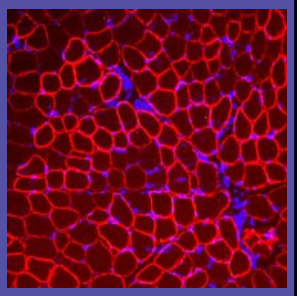
CXMD un-treated



dog sits

Leaky fibers

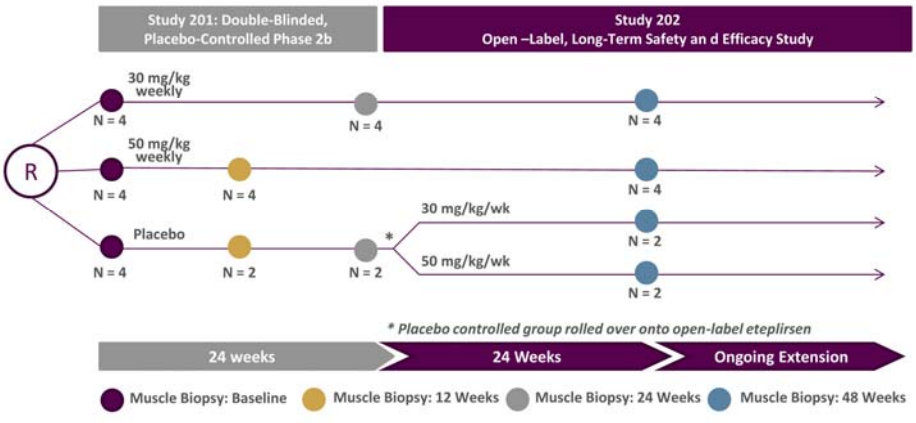
CXMD treated



dog trots

Yokota et al, Ann Neurol, 2009 65:667

Study leading to FDA application



n = 12, uncontrolled

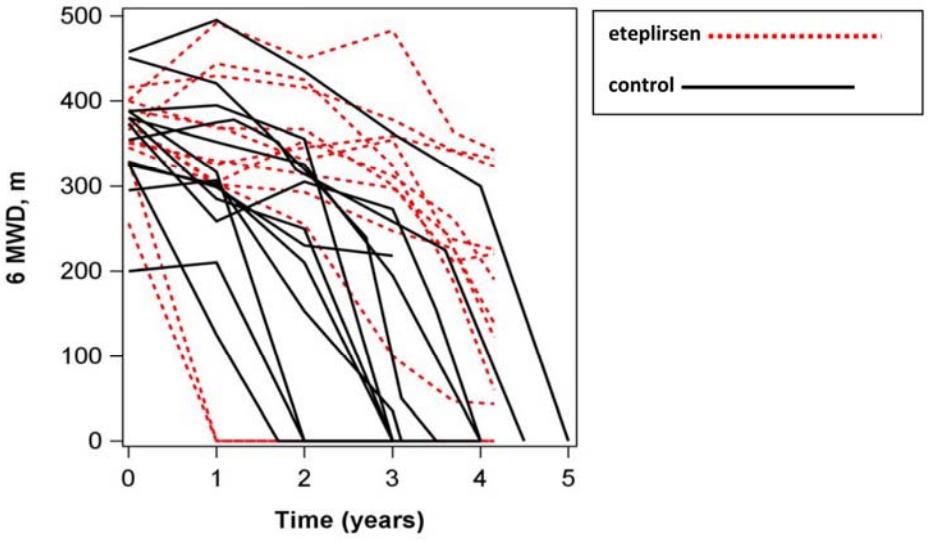
Patient	Western Blot % of normal
A	2.05
B	1.15
C	0.38
D	1.62
E	0.52
F	0.98
G	0
H	2.47
I	0.96
J	0
L	0.14

3.5 years on eteplirsen

Mean control: 0.08% of normal

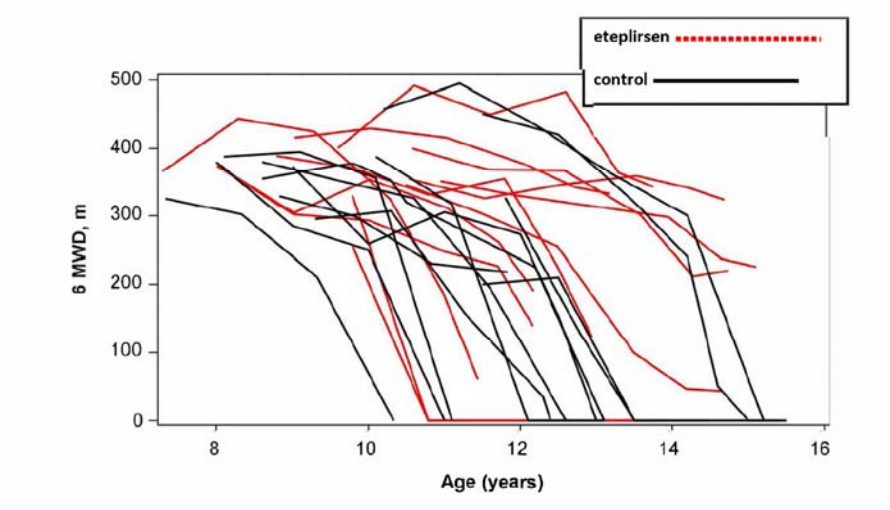
Mean 0.93% ± 0.84% for "11.6-fold increase"

Time on treatment



FDA briefing document from April 2016 Advisory Committee

by age



FDA briefing document from April 2016 Advisory Committee

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

TO: Janet Woodcock, M.D., Director, CDER
Ellis Unger, M.D., Director, Office of Drug Evaluation I, CDER
Luciana Borio, M.D., Chair, Agency Scientific Dispute Process Review Board

FROM: Robert M. Califf, M.D., Commissioner of Food and Drugs

RE: Scientific Dispute Regarding Accelerated Approval of Sarepta Therapeutics' Eteplirsen (NDA 206488) – **Commissioner's Decision**

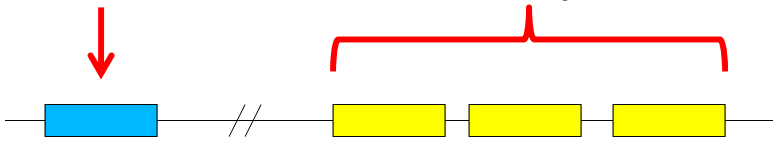
DATE: September 16, 2016

Splice shifting ASO for SMA

deletions on chr. 5 that remove *SMN1*

Deletions cause SMA

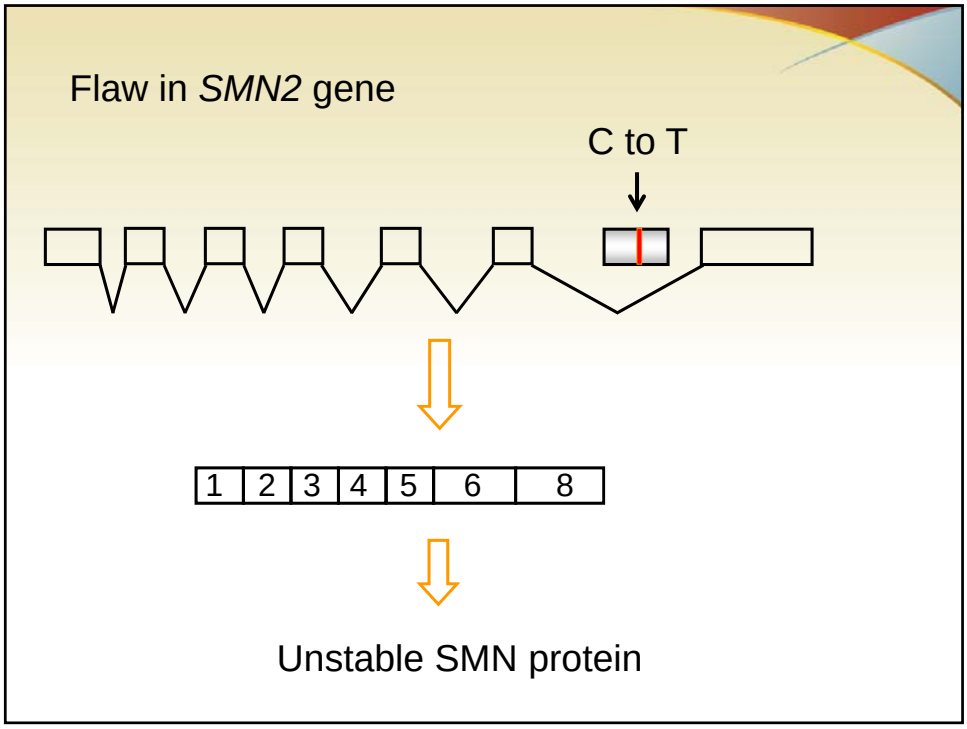
modify SMA

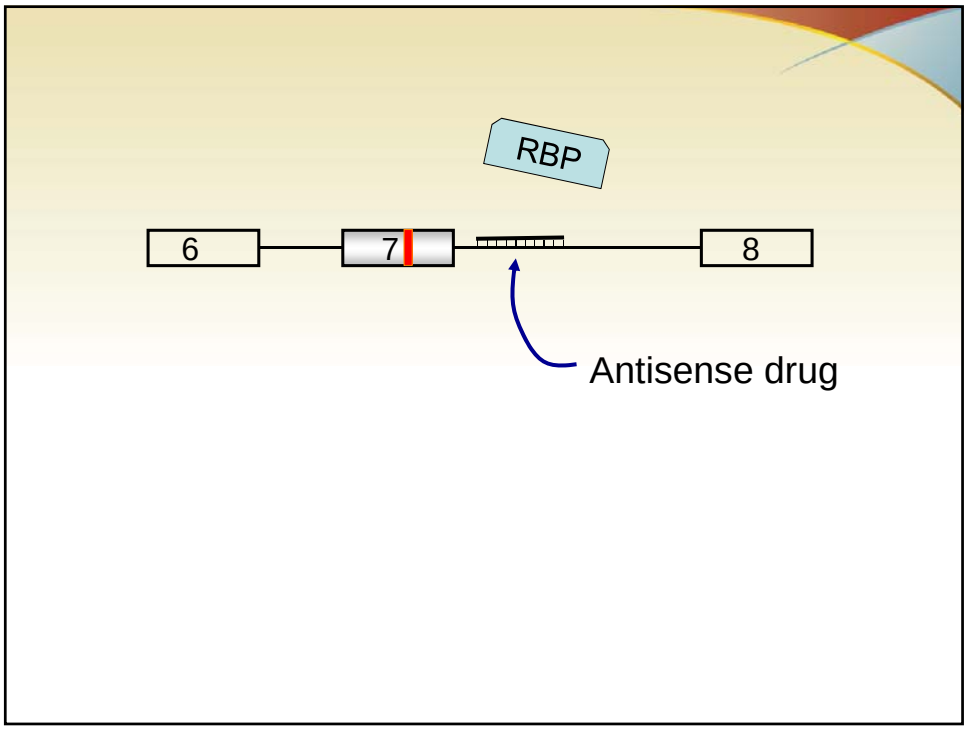


SMN1

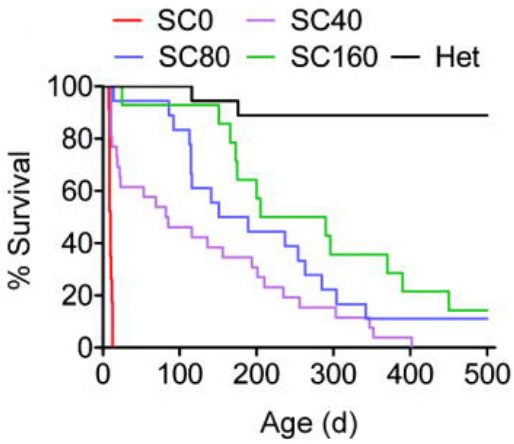
SMN2 (1 to 4 copies)

partial rescue from SMN defiency


Flaw in *SMN2* gene
C to T
1
2
3
4
5
6
8
Unstable SMN protein


RBP
6
7
8
Antisense drug

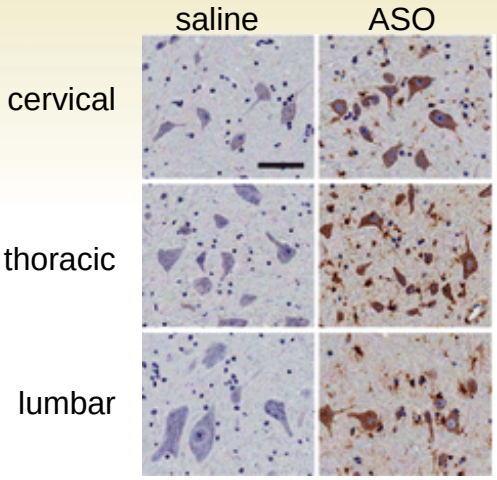
Restoration near-normal life span in mice with SMA



Hua et al, Nature, 2011

Intrathecal infusion in rhesus monkey

Intrathecal adminstration of ASO for SMA



Spinal cord penetration of ASOs

Passini et al, Science Trans Med, 2011

Phase 2 Study Interim Results
(as of January 26, 2016)

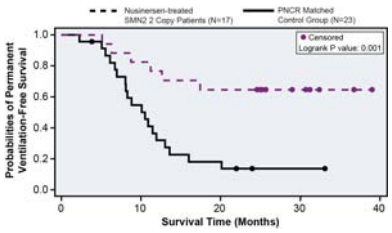
27

- **Safety/Tolerability**
  - **No safety or tolerability concerns identified**
  - **Intrathecal injections well tolerated in SMA infants**
- **Clinical Efficacy**
  - **Ventilation-free survival of nusinersen treated SMA infants is divergent compared to natural history**
    - 73% remain event-free and all are older than 24 months in the 12 mg cohort
  - **Increases in motor function scores**
    - Mean CHOP-INTEND score increase of 22 points in cohort 2 (12 mg dose)
    - Incremental achievement of HINE motor milestones
  - **Increase in ulnar and peroneal CMAP amplitude**

Increased Event-Free Survival & Muscle Function Scores Observed in Nusinersen-treated Infants with SMA
As of January 26, 2016, Compared to Natural History (PNCR)

28

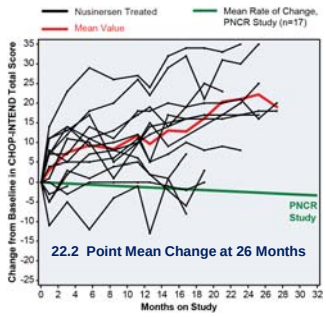
Increased Permanent Ventilation-free Survival (2 Copies of the *SMN2* Gene)



All infants continuing in the study are older than 2 years of age, some are older than 3 years of age

PNCR Natural History Study - Finkel et al. (2014) Neurology 83: 974-980

Increases in Motor Function Scores (Cohort 2, n=15)



- **No evidence of a therapeutic plateau**
- **Infants continue to demonstrate improvements in motor function**

Biogen

Published on *Biogen Media* (http://media.biogen.com) on 8/1/16 7:30 am EDT

Biogen and Ionis Pharmaceuticals Report Nusinersen Meets Primary Endpoint at Interim Analysis of Phase 3 ENDEAR Study in Infantile-Onset Spinal Muscular Atrophy

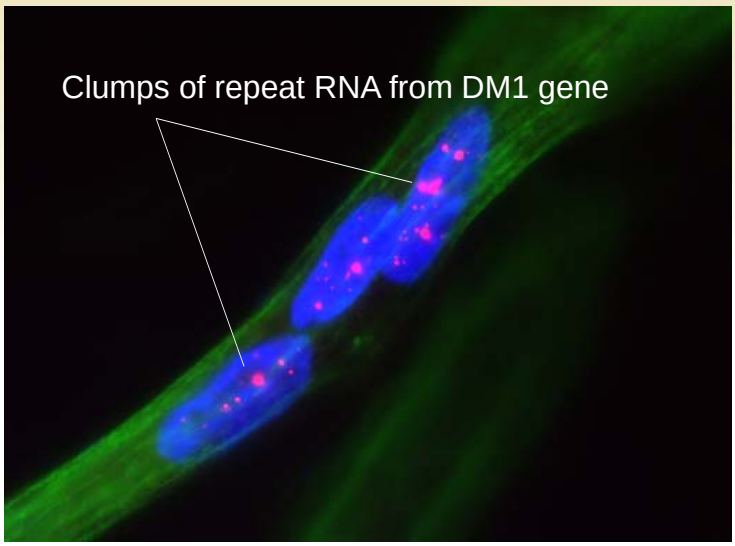
Release Date:
Monday, August 1, 2016 7:30 am EDT

- Prespecified interim analysis, infantile SMA, Phase 3
- Type 2 SMA trial ongoing

ASOs that act through target cleavage

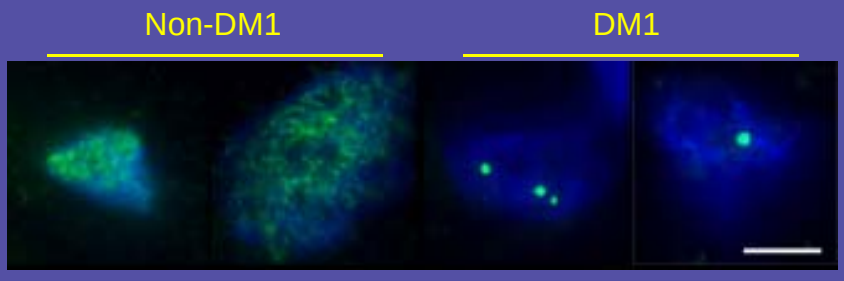
1. Familial amyloidotic polyneuropathy
2. Myotonic dystrophy type 1 (DM1)
3. Familial ALS (SOD1)
4. Huntington disease

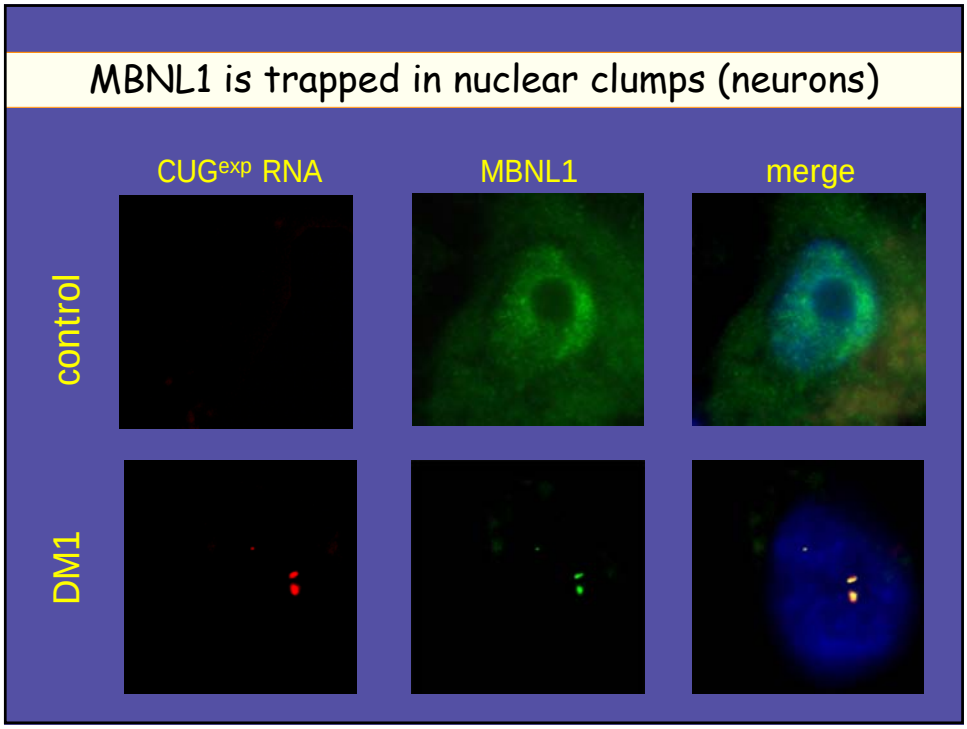
RNA Toxicity in myotonic dystrophy

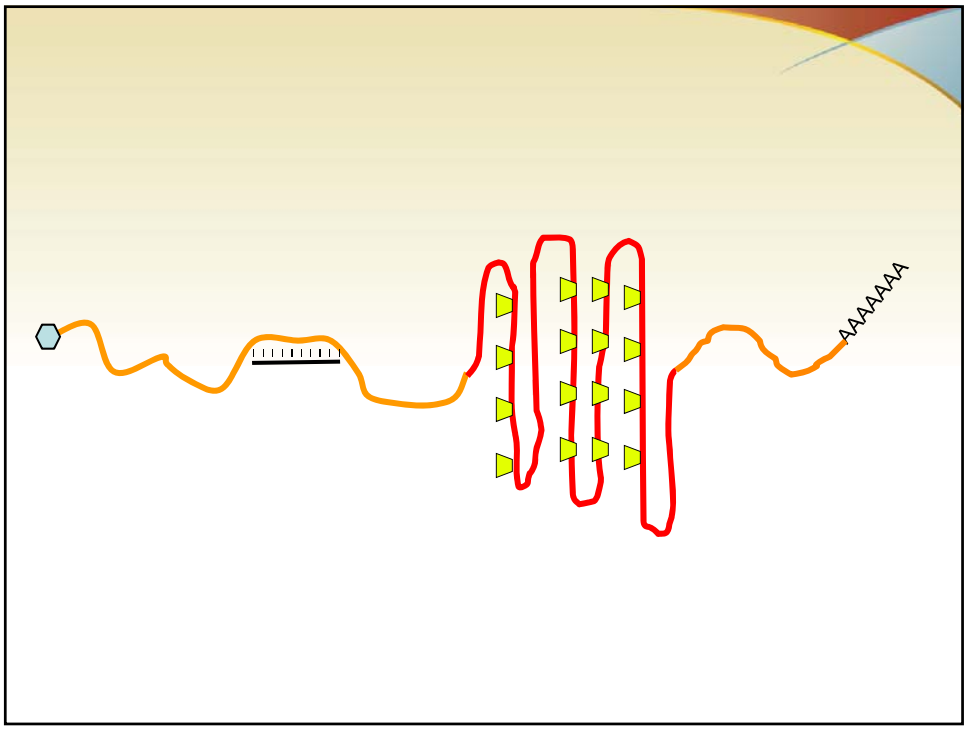


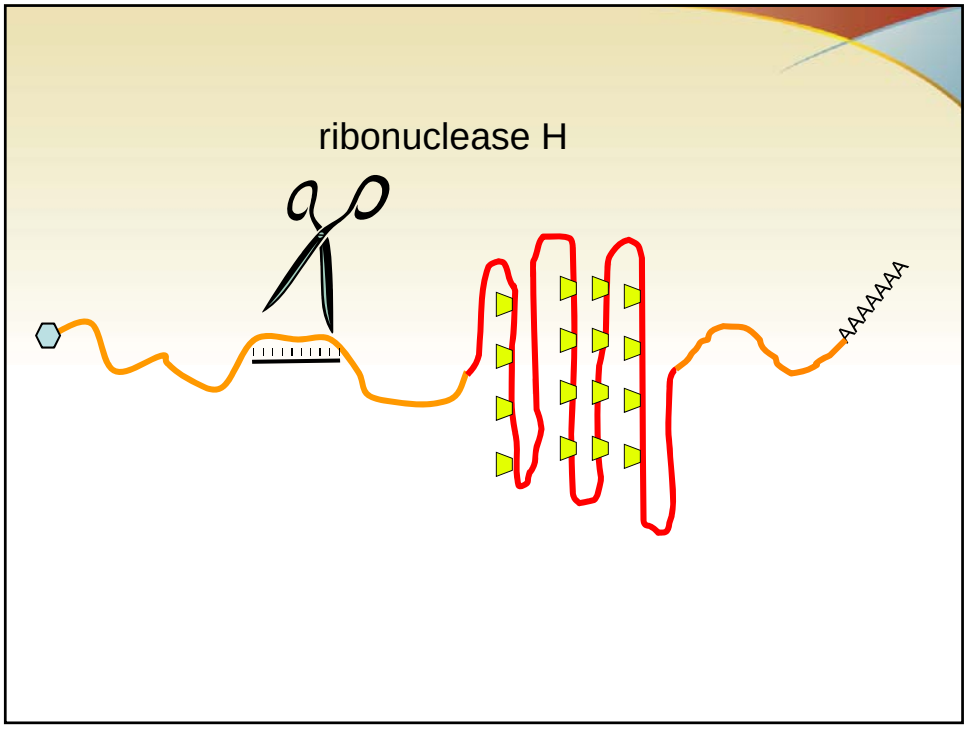
RNA with expanded repeat forms nuclear clumps

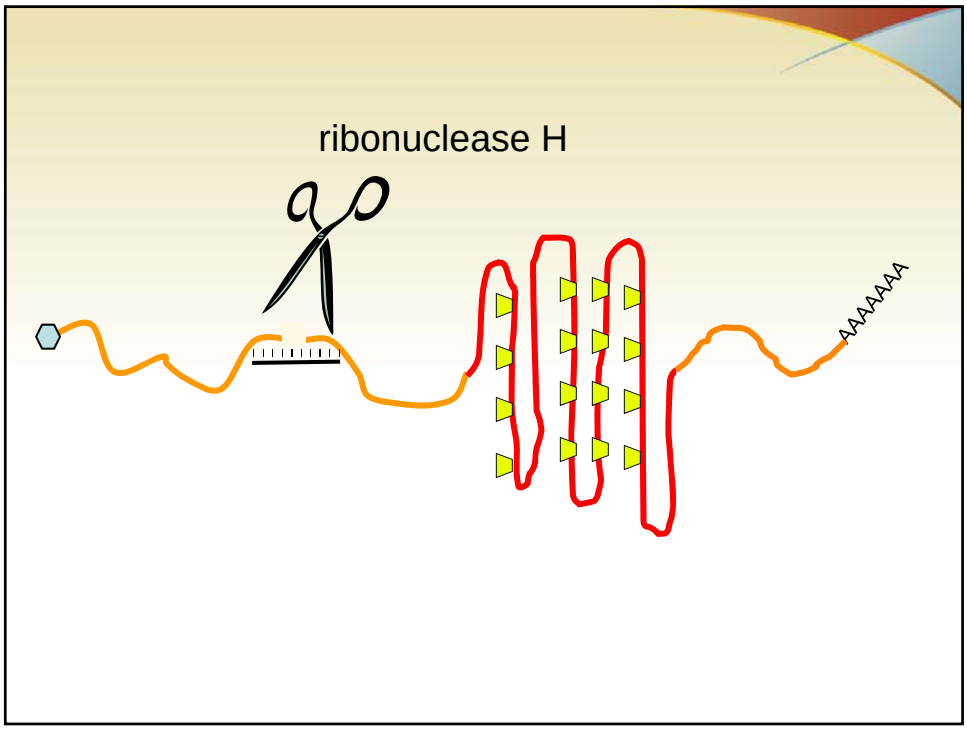
MBNL protein in cardiac cells

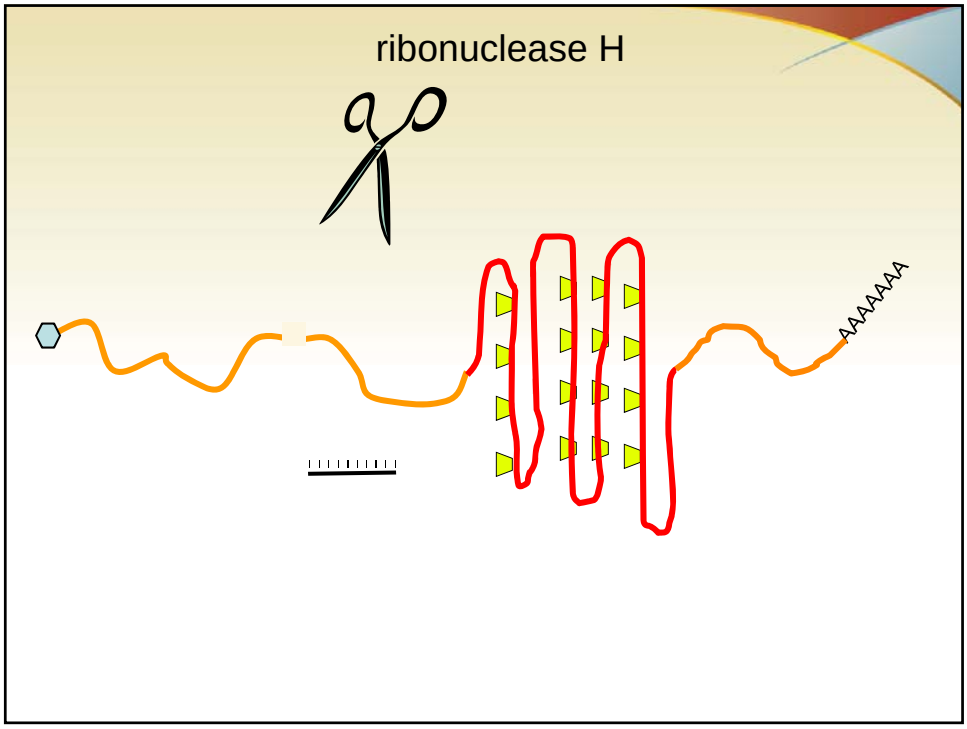


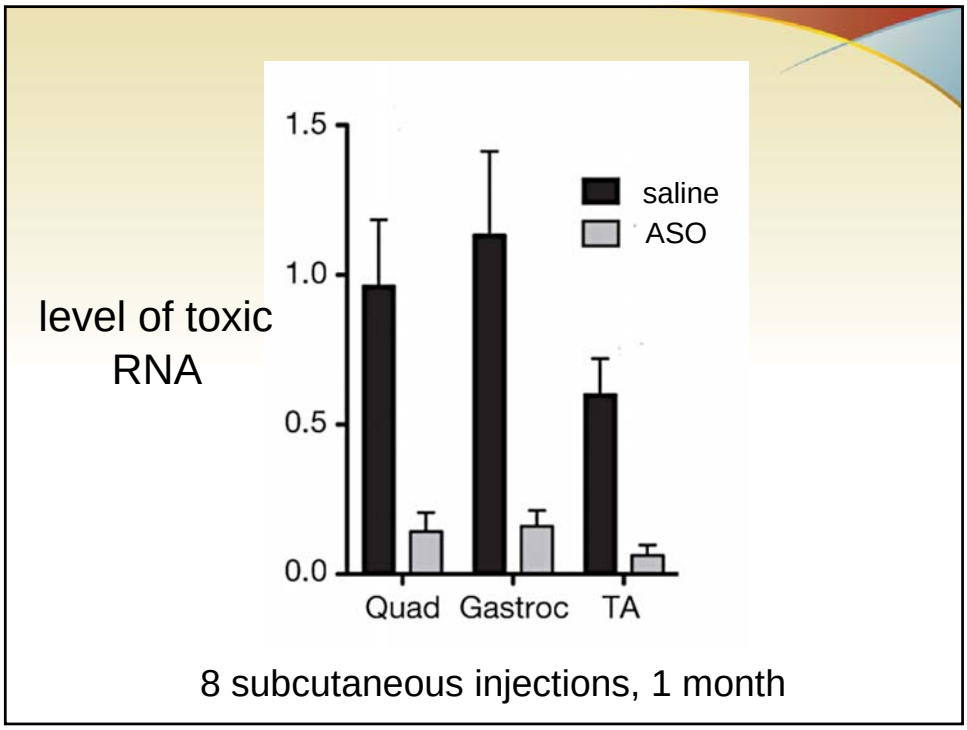

MBNL1 is trapped in nuclear clumps (neurons)
CUGexp RNA
MBNL1
merge
control
DM1


AAAAAAA

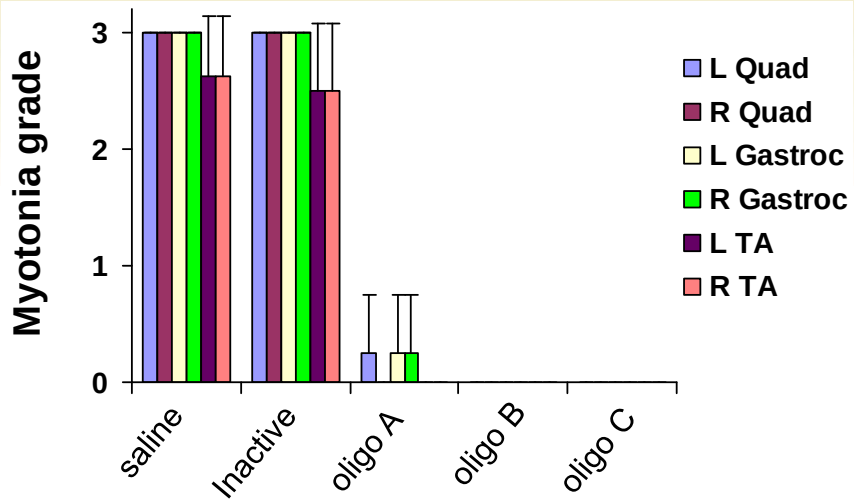

ribonuclease H
AAAAAAA


ribonuclease H
AAAAAAA


ribonuclease H
AAAAAAA


level of toxic RNA
1.5
1.0
0.5
0.0
saline
ASO
Quad
Gastroc
TA
8 subcutaneous injections, 1 month

Whole body correction of myotonia



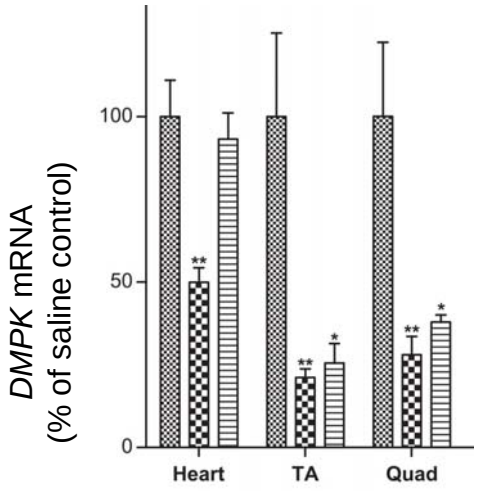
ASO targeting toxic RNA

- ✓ Reduces level of CUG-repeat RNA
- ✓ Eliminates myotonia
- ✓ Corrects splicing problems
- ✓ Improves histology
- ✓ Non-toxic and specific
- ✓ Durable

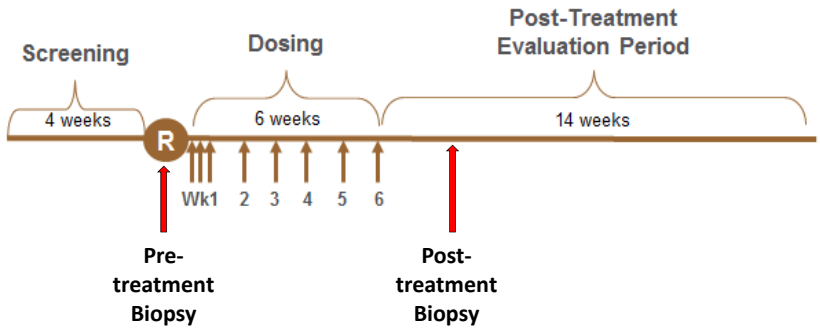
Increased sensitivity of nuclear-retained RNA to antisense

DMPK knockdown in monkeys
13 wks on, 13 wks off, SQ dosing

Saline
13 wks
26 wks
ISIS 486178



Pandey et al, JPET, 2015



Ionis-sponsored trial of ASO 598769 in DM1 (7 centers)
Phase 1/2a Safety and dose-finding study of around 50 patients

Summary

- Resurgence of interest in ASO drugs
- Tractable approach to modulate a specific gene
- remarkable specificity
- Biodistribution problems are not insurmountable
- Short development time for new targets
- Initial application for neurogenetic disease
- Neurologists may become heavy prescribers/injectors

Richard Moxley
Robert Griggs

Univ. Rochester
Eric Logigian
Thurman Wheeler
Masayuki Nakamori
Krzysztof Sobczak
Richard Moxley

Robert Dirksen Lab
John Lueck

Univ. Florida
Maurice Swanson

Isis Pharmaceuticals
Frank Bennett
Sanjay Pandey
Robert MacLeod

Genzyme
Andrew Leger
Bruce Wentworth
Seng Cheng

Biogen
Gersham Dent
John Carulli