Clinical trial development of NS-065/NCNP-01 for exon 53 skipping in DMD

- **Japanese Phase I/II trial**

  Presented by Hirofumi Komaki, National Center of Neurology and Psychiatry (Tokyo, Japan)

- **North American Phase II trial**

  Presented by Paula Clemens, University of Pittsburgh (Pittsburgh, USA)

PPMD 2018 Conference
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Disclosure

- National Center of Neurology and Psychiatry and Nippon Shinyaku Co., Ltd are co-inventors of NS-065/NCNP-01
- Speaker disclosures

	Dr. Komaki	Dr. Clemens
Grant funding	Sanofi, Chugai, PTC Therapeutics, Nippon Shinyaku, Taiho, Pfizer, Sumitomo Dainippon, Daiichi Sankyo, Bristol-Myers Squibb	NIH, Department of Defense, MDA, Sanofi, Amicus, NS Pharma
Consulting/lectures/ advisory boards/DSMB	Biogen, PTC Therapeutics	NIH, Pfizer, Sanofi, Spark, UCB Biopharma

Introduction

National Center of Neurology and Psychiatry (Tokyo, Japan)

- Leading medical center of research and care for muscular diseases in Japan
- Sponsor of Phase I study in Japan

Nippon Shinyaku Co., Ltd (Kyoto, Japan)

- Japan-based pharmaceutical company
- Sponsor of Phase I/II study in Japan

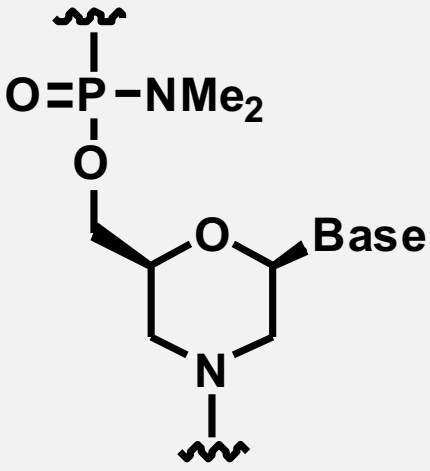
NS Pharma, Inc. (Paramus, NJ, USA)

- Wholly-owned, US subsidiary of Nippon Shinyaku Co., Ltd.
- Sponsor of Phase II study in North America

NS-065/NCNP-01

Origin : Nippon Shinyaku jointly with National Center of Neurology and Psychiatry (NCNP)

Mechanism : Exon 53 skipping



PMO
Phosphorodiamidate Morpholine Oligomer

Characteristics

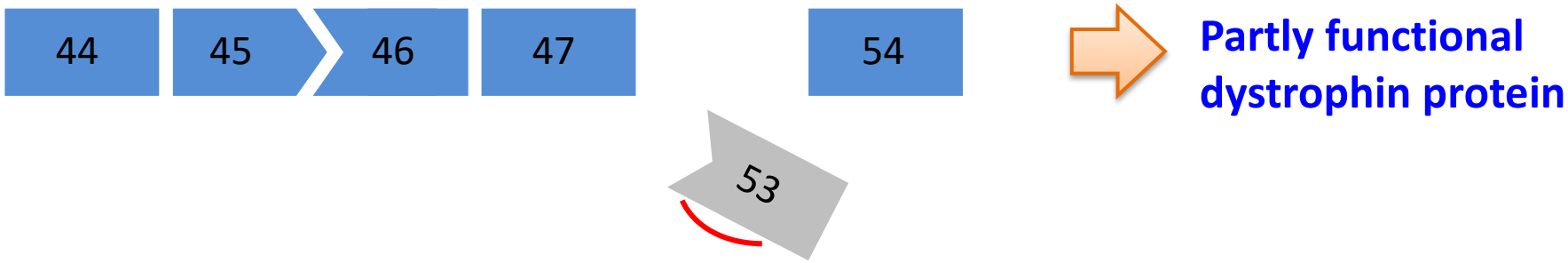
- High potential of exon 53 skipping activity
- PMO : Charge neutral
- I.V. administration, once weekly
- Excreted through the kidney

Exon skipping strategy

Example of a deletion that disrupts the dystrophin mRNA reading frame that is restored to in-frame by exon 53 skipping

Exon 48-52 deletion: disrupts reading frame

Exon 53 skipping by NS-065/NCNP-01: restores reading frame



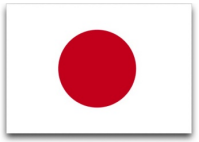
NS-065/NCNP-01 Clinical Program

NCNP : National center of Neurology and Psychiatry

Phase I Investigator Initiated study (JAPAN)
1.25, 5, 20 mg/kg for 12 weeks

Nippon Shinyaku

Phase I/II Dose finding study (JAPAN)
40, 80 mg/kg for 24 weeks



NS Pharma

Phase II Dose finding study (NORTH AMERICA)
40, 80mg/kg for 24 weeks

Phase II Extension study (NORTH AMERICA)
40, 80mg/kg, ongoing

Phase I/II: Dose finding study in Japan

Primary Objectives

- Dystrophin expression (WB, IHC)
- Exon skipping level (RT-PCR)

Secondary Objectives

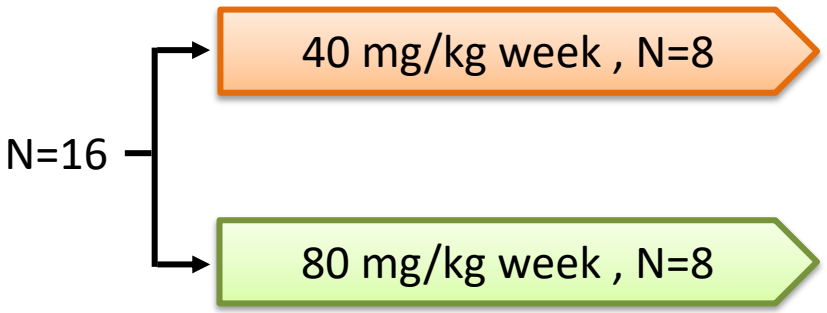
- Physical function
- CK level
- Safety
- Pharmacokinetics

Key inclusion Criteria

- Age : 5-17 years
- Amenable to exon 53 skipping
- Ambulant and non-ambulant

Study Design

Phase I/II, Exploratory, Open label study



This study is registered as JapicCTI-163291

Timing of muscle biopsies

- All participants at baseline
- 4 participants each dose cohort at 12 weeks and at 24 weeks

Safety Data

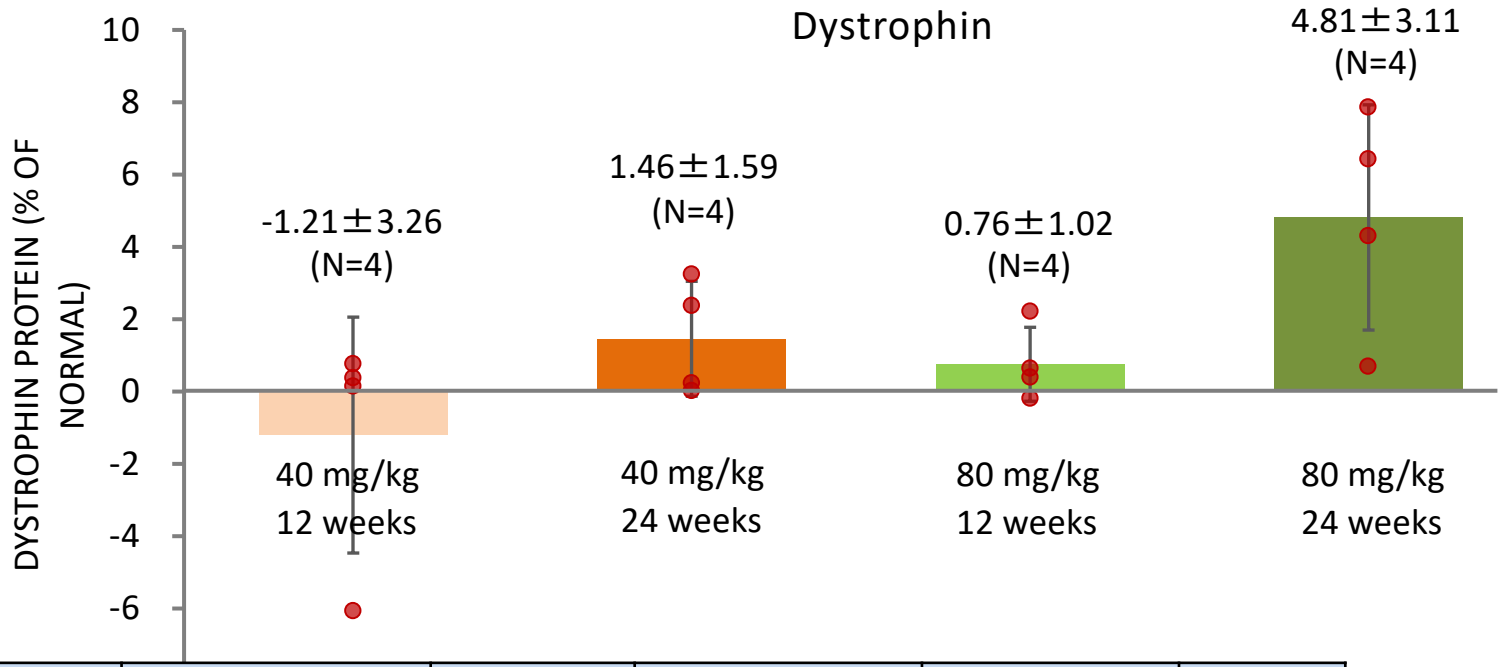
Treatment-Emergent Adverse Events by Preferred Term (occurring in ≥2)

Treatment-emergent adverse event	40 mg/kg n=8 (%)	80 mg/kg n=8 (%)	Total n=16 (%)
Nasopharyngitis	1 (12.5)	3 (37.5)	4 (25.0)
Upper respiratory tract infection	1 (12.5)	3 (37.5)	4 (25.0)
Contusion	3 (37.5)	0	3 (18.8)
Beta-N-acetyl-D-glucosaminidase increased	1 (12.5)	2 (25.0)	3 (18.8)
Pyrexia	0	2 (25.0)	2 (12.5)
Influenza	2 (25.0)	0	2 (12.5)
Brain natriuretic peptide increased	1 (12.5)	1 (12.5)	2 (12.5)
Interleukin level increased	1 (12.5)	1 (12.5)	2 (12.5)
Ejection fraction decreased	0	2 (25.0)	2 (12.5)
Pain in extremity	1 (12.5)	1 (12.5)	2 (12.5)
Eczema	1 (12.5)	1 (12.5)	2 (12.5)
Rash	1 (12.5)	1 (12.5)	2 (12.5)
Urticaria	0	2 (25.0)	2 (12.5)

- There were no AEs leading to discontinuation
- All AEs were mild or moderate
- One serious adverse event (Upper respiratory tract infection) was not treatment-related

Western Blot

Dystrophin Expression (12, 24 weeks) by dose group



Dose	Biopsy	Baseline Mean (SD)	After Treatment Mean(SD)	Mean change from baseline	P-value
40 mg/kg	12, 24 weeks, n=8	1.13 (2.13)	1.26 (1.34)	0.13 (2.77)	0.901
80 mg/kg	12, 24 weeks, n=8	0.41 (0.12)	3.19 (3.04)	2.78 (3.05)	< 0.05

Statistically significant increase in mean dystrophin level from baseline was confirmed at 80 mg/kg cohort.

Exon skipping level by RT-PCR

		Biopsy (week)		Mean %
		12	24	
Dose (mg/kg)	40	15.6 (n=4)	28.0 (n=4)	**21.8 (n=8)**
	80	35.1 (n=4)	49.7 (n=4)	**42.4 (n=8)**
Mean %		**25.3 (n=8)**	**38.8 (n=8)**	**32.1 (n=16)**

Mean exon skipping level of each group

- All 16 patients demonstrated an increase in exon 53 skipping level at 12 or 24 weeks
- Level of exon 53 skipping was dose dependent
- Level of exon 53 skipping was higher at 24 weeks than 12 weeks at both dose levels

Phase II: Dose finding study in US and Canada
Objectives

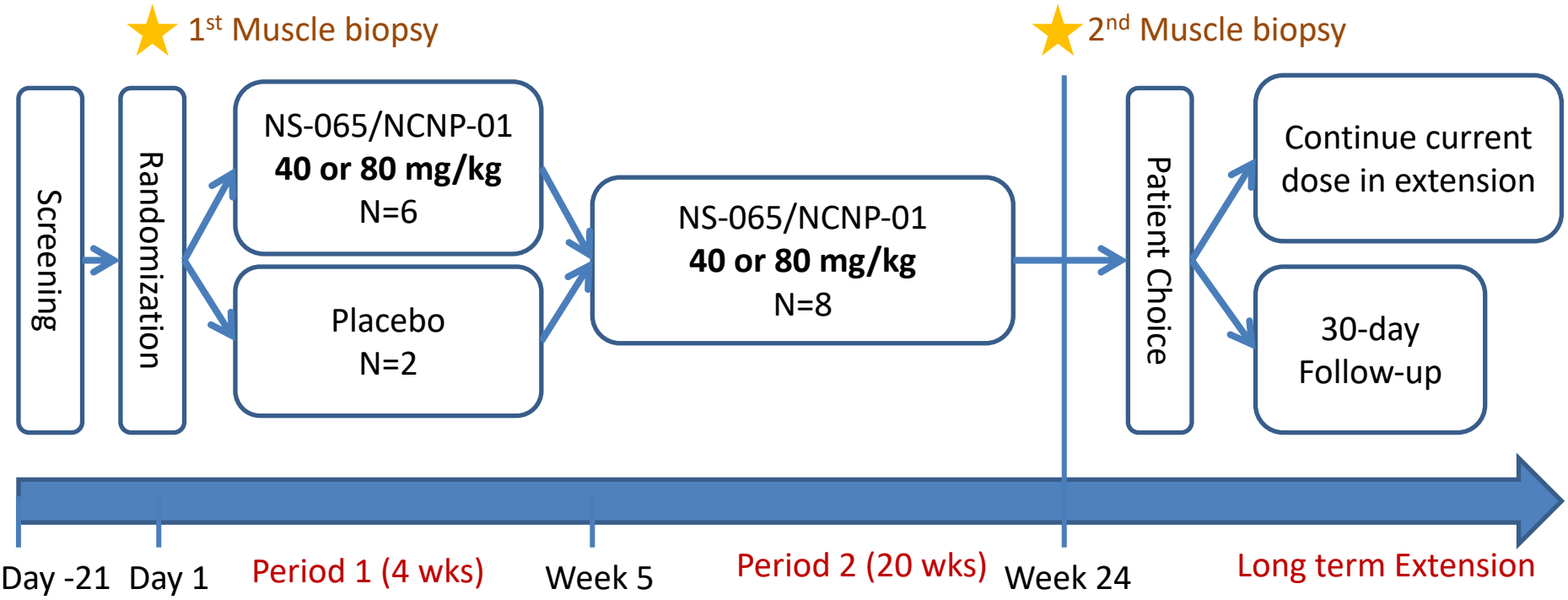
Primary

- Safety and tolerability of NS-065/NCNP-01
- Pharmacokinetics
- Muscle dystrophin expression by Western blot

Secondary

- Exon skipping by RT-PCR
- Muscle dystrophin expression by immunohistochemistry
- Muscle strength and function

Study Design



- **4 - <10 years of age**; **ambulant** with DMD amenable to exon 53 skipping
- **16 participants** enrolled at **6 CINRG network sites** in US and Canada
- Enrollment began **Dec 2016**; 24 week study complete **Mar 2018**
- ClinicalTrials.gov Identifier: NCT02740972

Safety Data

Treatment-Emergent Adverse Events by Preferred Term (occurring in ≥2)

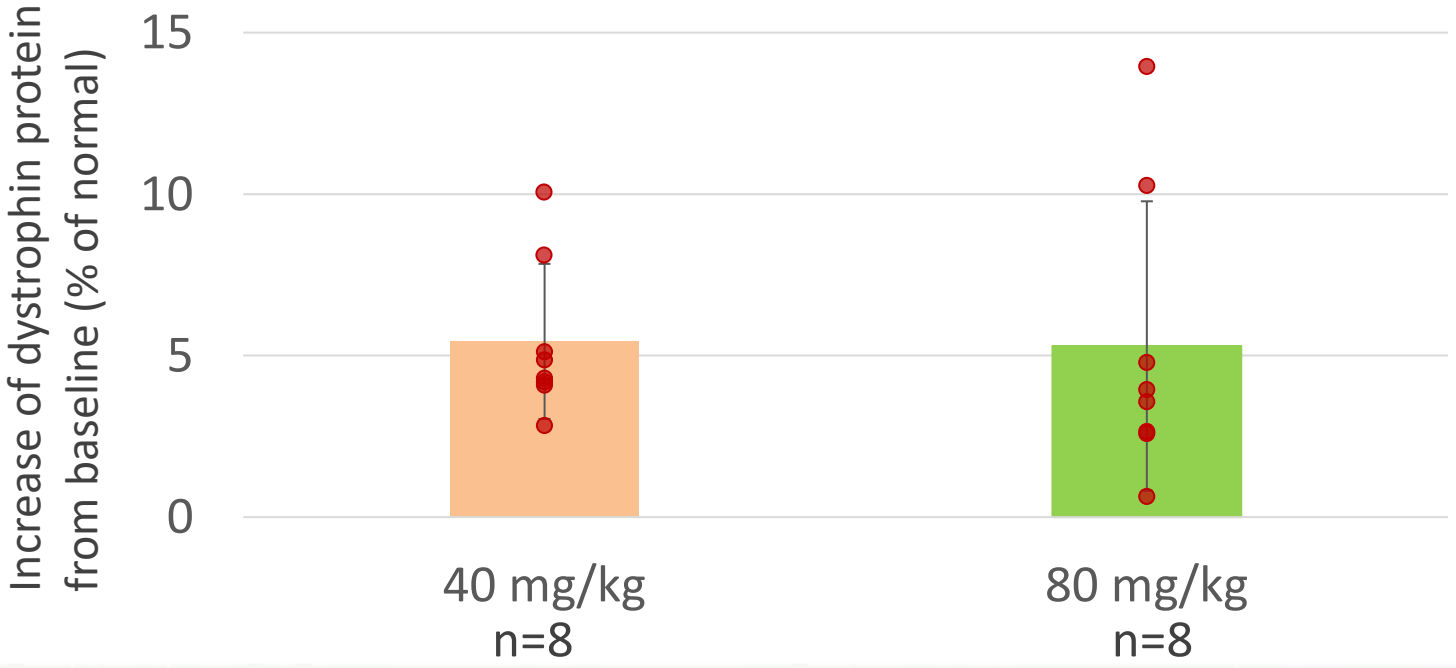
Adverse events (MedDRA/J ver.20.1)	40 mg/kg N=8 (%)	80 mg/kg N=8 (%)	Total N=16 (%)
Cough	4 (50.0)	5 (62.5)	9 (56.3)
Nasopharyngitis	2 (25.0)	4 (50.0)	6 (37.5)
Vomiting	2 (25.0)	2 (25.0)	4 (25.0)
Diarrhea	2 (25.0)	1 (12.5)	3 (18.8)
Pyrexia	2 (25.0)	1 (12.5)	3 (18.8)
Rash	2 (25.0)	1 (12.5)	3 (18.8)
Arthropod bite	2 (25.0)	0	2 (12.5)
Contusion	0	2 (25.0)	2 (12.5)
Nasal congestion	2 (25.0)	0	2 (12.5)
Respiratory tract congestion	1 (12.5)	1 (12.5)	2 (12.5)

Number (%) of patients, as of Apr 25th 2018

- One serious adverse event (Left tibia/fibular fracture) observed was not treatment-related.
- No drug-related AEs
- No AEs leading to discontinuation
- All AEs were mild or moderate

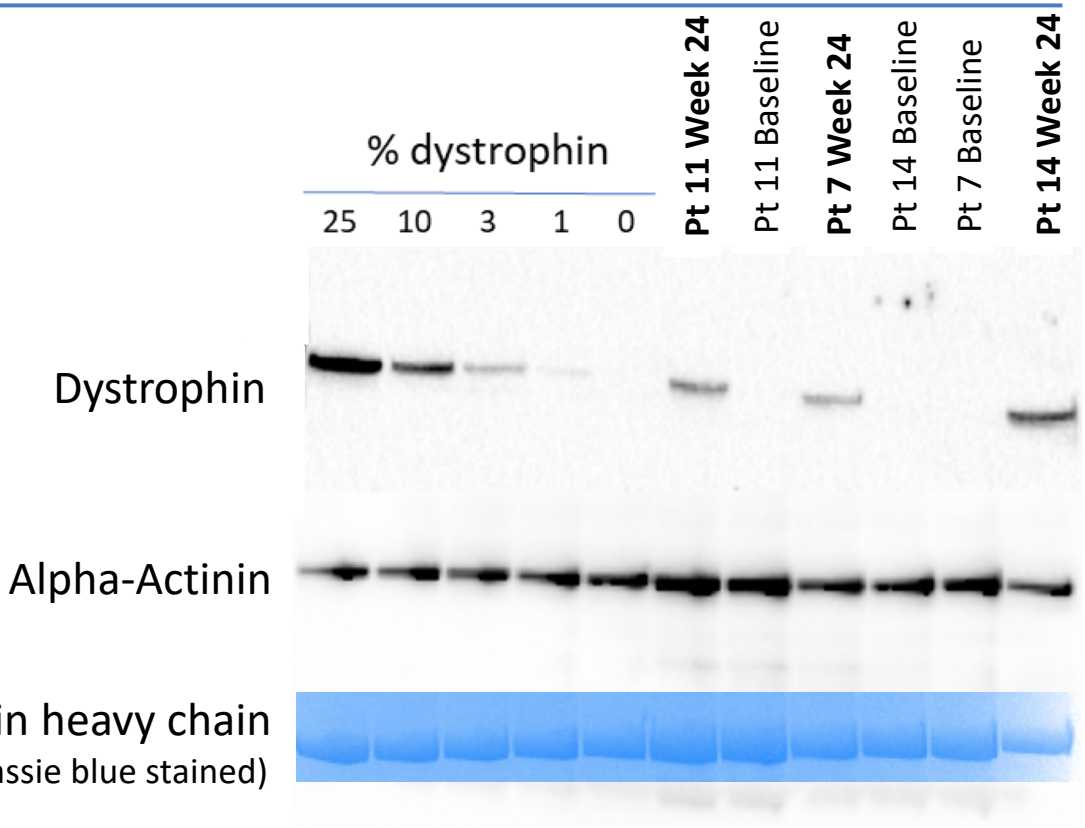
Dystrophin protein: Western blot

Dose	Baseline Mean % (range)	On treatment (24 weeks) Mean % (range)
40 mg/kg	0.3 (0.1 - 0.4)	**5.7 (3.2 - 10.3)**
80 mg/kg	0.6 (0.1 - 2.6)	**5.9 (1.1 - 14.4)**



Sample of Western blot data

- Triplicate, blinded assays
- Standard curve: mixture of muscle extract from 5 healthy controls diluted with DMD muscle extract



Dystrophin expression normalized to both alpha actinin and myosin heavy chain

Dystrophin Production Analysis
RT-PCR and Western Blot

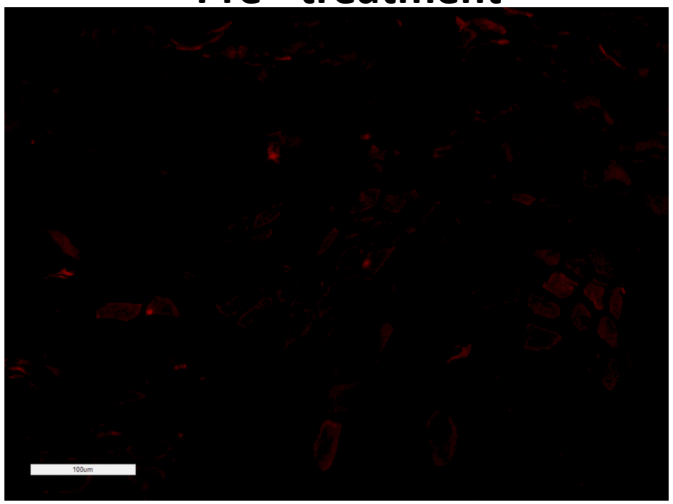
Method	Dose	Baseline Mean % (SD)	On-treatment Mean % (SD)
% exon skipped molality (RT-PCR)	40 mg/kg	0.0 (0.0)	**17.4 (7.2)**
	80 mg/kg	0.0 (0.0)	**43.9 (16.7)**
% dystrophin (Western blot)	40 mg/kg	0.3 (0.1)	5.7 (2.4)
	80 mg/kg	0.6 (0.8)	5.9 (4.5)

- RT-PCR shows clear dose-response
- 2-fold increase in drug = 2-fold increase in skipped mRNA

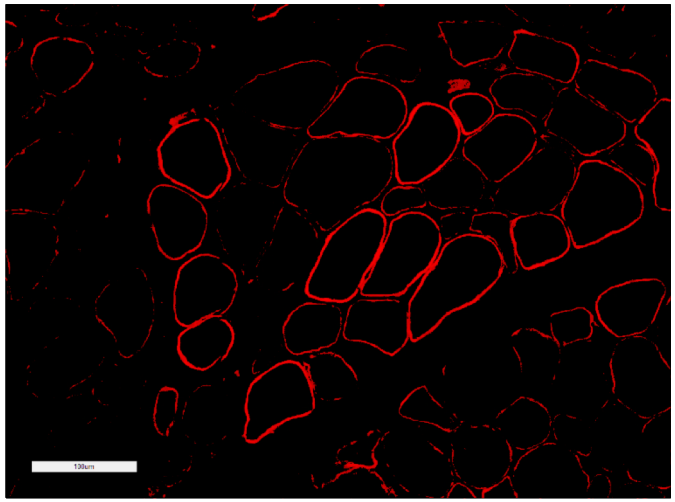
Immunohistochemistry

Representative images

Pre - treatment

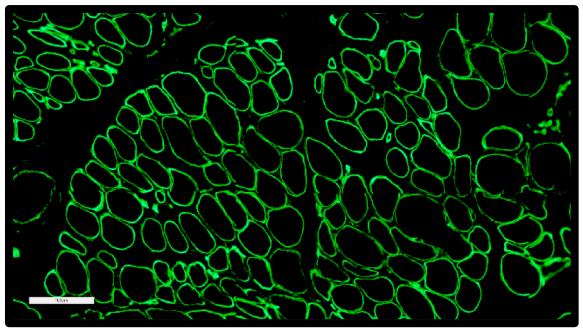


Post - treatment

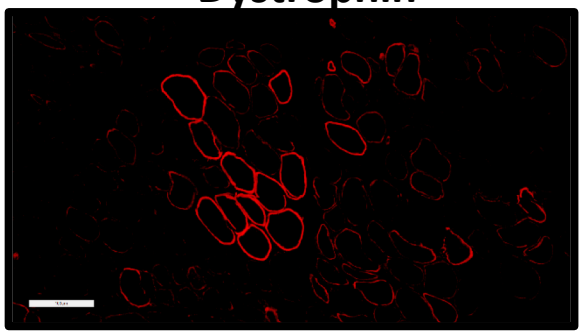


Scale = 100 µm

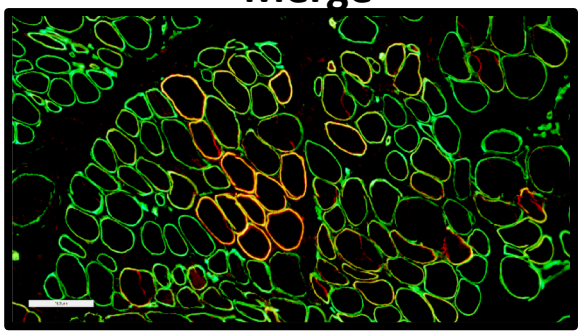
Laminin-α2



Dystrophin



Merge



Summary

Japan Phase I/II and North American Phase II Studies

- Demonstration of exon skipping showing target engagement of the morpholino intervention with the dystrophin pre-mRNA
  - At 24 weeks: JP – Mean% 21.8/42.4; NA – Mean% 17.4/43.9
- Restoration of truncated dystrophin in patient muscle following 20-24 weekly infusions
  - At 24 weeks: JP - % increase 1.5/4.8; NA - % increase 5.4/5.3
- No safety signals to date
- Stable pharmacokinetics
- Analysis of clinical end-points planned for Fall 2018

Acknowledgements

North American Phase II study

Site Investigators

- Vamshi Rao, Lurie Children's Hospital, Chicago, IL
- Anne Connolly, Washington University, St. Louis, MO
- Amy Harper, Children's Hospital of Richmond, Richmond, VA
- Jean Mah, Alberta Children's Hospital, Calgary, Alberta, Canada
- Edward Smith, Duke Children's Hospital, Durham, NC
- Craig McDonald, University of California, Davis, Sacramento, CA
- Barry Byrne, University of Florida, Gainesville, FL

AGADA Biosciences

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TRiNDS

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Japan Phase I/II Study

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