A Phase II, Dose Finding Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)

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NS-065/NCNP-01 Study Chair

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Disclosure and Disclaimer

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

- Grant/Research Support: NIH, Department of Defense, MDA, Sanofi/Genzyme, Amicus, NS Pharma

- Advisory Boards/DSMB/Consultant: DSMB member for NIH and Pfizer. Pompe Disease Board of Advisors member for Sanofi/Genzyme. Consultant to Spark Therapeutics and UCB Biopharma
Introduction

- Approximately 8% of patients with DMD have an out-of-frame deletion amenable to exon 53 skipping
- In these patients, skipping exon 53 is predicted to restore the reading frame permitting translation of an internally deleted, partially functional dystrophin protein
- The goal of exon skipping is to convert the severe DMD phenotype to a milder Becker-like phenotype
NS-065/NCNP-01

- Antisense oligonucleotide with a novel morpholino backbone and molecule size
- Originated in Nippon Shinyaku jointly with National Center of Neurology and Psychiatry (NCNP)
- Exon 53 skipping agent (Mechanism of Action)
- IV infusion, once weekly
Mechanism of Action

Dystrophin mRNA

Normal

DMD

DMD + NS-065

Exon 53 skipping

Partly functional protein
Study 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
Screening

Randomization

NS-065/NCNP-01
40 or 80 mg/kg
N=6

Placebo
N=2

NS-065/NCNP-01
40 or 80 mg/kg
N=8

Patient Choice

Continue current dose in extension

30-day Follow-up

1st Muscle biopsy

2nd Muscle biopsy

Study Design

Day -21  Day 1  Period 1 (4 wks)  Week 5  Period 2 (20 wks)  Week 24  Extension (72 wks)

• All patients received NS-065/NCNP-01 40 or 80 mg/kg
• Muscle biopsy prior to treatment and after 24 weeks treatment
• First participant, first visit Dec 2016
• Last participant, last visit Mar 2018
Study Sites and Study Population

North American Study Sites

- Alberta Children's Hospital
  Calgary
- UC Davis
  Sacramento
- Lurie Children's Hospital
  Chicago
- Washington University
  St. Louis
- Children's Hospital
  Richmond
- Duke University
  Durham
- University of Florida
  Gainesville

Study Population

- Confirmed diagnosis of DMD with dystrophin mutation amenable to exon 53 skipping
- 4 - <10 years of age
- Ambulatory
- Stable treatment with glucocorticoid therapy

ClinicalTrials.gov Identifier: NCT02740972
## Demographics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>40 mg/kg n=8</th>
<th>80 mg/kg n=8</th>
<th>Total n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (range)</td>
<td>7.5 (4.3 – 9.8)</td>
<td>7.2 (4.8 – 9.8)</td>
<td>7.36 (4.3 – 9.8)</td>
</tr>
<tr>
<td>Mean weight, kg (range)</td>
<td>23.7 (14.9 – 30.4)</td>
<td>22.3 (15.5 – 35.4)</td>
<td>23.0 (14.9 – 35.4)</td>
</tr>
<tr>
<td>Steroid treatment, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deflazacort</td>
<td>6 (75%)</td>
<td>6 (75%)</td>
<td>12 (75%)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>2 (25%)</td>
<td>2 (25%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Deletion type, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-52</td>
<td>2 (25%)</td>
<td>5 (63%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>47-52</td>
<td>1 (13%)</td>
<td>0</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>48-52</td>
<td>1 (13%)</td>
<td>2 (25%)</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>49-52</td>
<td>2 (25%)</td>
<td>1 (13%)</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>50-52</td>
<td>2 (25%)</td>
<td>0</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>52</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Safety Data

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

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

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

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

<table>
<thead>
<tr>
<th>Conditions</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; (ng·hr/ml)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40mg/kg Week24</td>
<td>122,000</td>
<td>196,000</td>
<td>2.38</td>
</tr>
<tr>
<td>80mg/kg Week24</td>
<td>228,000</td>
<td>386,000</td>
<td>2.82</td>
</tr>
</tbody>
</table>
Sample of Western Blot Data

- Normalized to both alpha actinin and myosin heavy chain
- Standard curve: mixture of muscle extract from 5 healthy controls diluted with DMD muscle extract

### % dystrophin

<table>
<thead>
<tr>
<th></th>
<th>Pt 11 Week 24</th>
<th>Pt 11 Baseline</th>
<th>Pt 7 Week 24</th>
<th>Pt 7 Baseline</th>
<th>Pt 14 Baseline</th>
<th>Pt 14 Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10</td>
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<td></td>
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<tr>
<td>3</td>
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<tr>
<td>1</td>
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<tr>
<td>0</td>
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</tr>
</tbody>
</table>

- Dystrophin
- Alpha-Actinin
- Myosin heavy chain (Coomassie blue stained)
Sample of Dystrophin with Normalization

- Triplicate runs with stringent acceptance criteria
- Standard curve fit to first polynomial
- Blinded assays

\[
y = 2.4296x^2 + 3.4875x - 0.0222 \\
R^2 = 0.9981
\]

\[
y = 6.1013x^2 + 2.4371x + 0.0086 \\
R^2 = 0.9995
\]

\[
y = 4.2149x^2 + 3.0073x - 0.0143 \\
R^2 = 0.9993
\]
## Dystrophin protein: Western blot

<table>
<thead>
<tr>
<th>Dose</th>
<th>Baseline Mean % (range)</th>
<th>On-treatment Mean % (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mg/kg</td>
<td>0.3 (0.1 - 0.4)</td>
<td>5.7 (3.2 - 10.3)</td>
</tr>
<tr>
<td>80 mg/kg</td>
<td>0.6 (0.1 - 2.6)</td>
<td>5.9 (1.1 - 14.4)</td>
</tr>
</tbody>
</table>

### Graph

- **X-axis:** Dose (40 mg/kg, 80 mg/kg)
- **Y-axis:** Increase of dystrophin protein from baseline (% of normal)

- **40 mg/kg (n=8):**
  - Baseline: 0.3 (0.1 - 0.4)
  - On-treatment: 5.7 (3.2 - 10.3)

- **80 mg/kg (n=8):**
  - Baseline: 0.6 (0.1 - 2.6)
  - On-treatment: 5.9 (1.1 - 14.4)
Sample of RT-PCR Data
# Dystrophin Production Analysis

## RT-PCR and Western Blot

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

- 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

• Demonstration of exon skipping showing target engagement of the morpholino intervention with the dystrophin pre-mRNA
• Restoration of truncated dystrophin in patient muscle following 20-24 weeks of weekly infusions
• No safety signals to date
• Stable pharmacokinetics
• Analysis of clinical end-points planned for Fall 2018
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