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)
## Disclosure

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

- Speaker disclosures

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

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

Abnormal protein

Partly functional dystrophin protein

NS-065/NCNP-01
NS-065/NCNP-01 Clinical Program

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

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

**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

Timing of muscle biopsies
- All participants at baseline
- 4 participants each dose cohort at 12 weeks and at 24 weeks

Study Design
Phase I/II, Exploratory, Open label study

40 mg/kg week, N=8
N=16
80 mg/kg week, N=8

This study is registered as JapicCTI-163291
Safety Data

<table>
<thead>
<tr>
<th>Treatment-emergent adverse event</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>Nasopharyngitis</td>
<td>1 (12.5)</td>
<td>3 (37.5)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>1 (12.5)</td>
<td>3 (37.5)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>Contusion</td>
<td>3 (37.5)</td>
<td>0</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Beta-N-acetyl-D-glucosaminidase increased</td>
<td>1 (12.5)</td>
<td>2 (25.0)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>0</td>
<td>2 (25.0)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Influenza</td>
<td>2 (25.0)</td>
<td>0</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Brain natriuretic peptide increased</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Interleukin level increased</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Ejection fraction decreased</td>
<td>0</td>
<td>2 (25.0)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Pain in extremity</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Eczema</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Urticaria</td>
<td>0</td>
<td>2 (25.0)</td>
<td>2 (12.5)</td>
</tr>
</tbody>
</table>

- 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

Dystrophin

40 mg/kg
12 weeks
40 mg/kg
24 weeks
80 mg/kg
12 weeks
80 mg/kg
24 weeks

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

Statistically significant increase in mean dystrophin level from baseline was confirmed at 80 mg/kg cohort.
Exon skipping level by RT-PCR

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Biopsy (week)</th>
<th>Mean %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>40</td>
<td>15.6 (n=4)</td>
<td>28.0 (n=4)</td>
</tr>
<tr>
<td>80</td>
<td>35.1 (n=4)</td>
<td>49.7 (n=4)</td>
</tr>
<tr>
<td>Mean %</td>
<td>25.3 (n=8)</td>
<td>38.8 (n=8)</td>
</tr>
</tbody>
</table>

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

**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
- Long term Extension

- 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)

<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 drug-related AEs
- No AEs leading to discontinuation
- All AEs were mild or moderate
# Dystrophin protein: Western blot

<table>
<thead>
<tr>
<th>Dose</th>
<th>Baseline Mean % (range)</th>
<th>On treatment (24 weeks) 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:**

- **Y-axis:** Increase of dystrophin protein from baseline (% of normal)
- **X-axis:** Dose
- **Legend:**
  - Orange: 40 mg/kg, n=8
  - Green: 80 mg/kg, n=8
Sample of Western blot data

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

Dystrophin

Alpha-Actinin

Myosin heavy chain (Coomassie blue stained)

Dystrophin expression normalized to both alpha actinin and myosin heavy chain
# 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
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**
Eric Hoffman

**TRiNDS**
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**Sponsor: NS Pharma, Inc.**
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Youhei Satou, Taishi Yamashita

### Japan Phase I/II Study

**Site Investigators**
- Yasuhiro Takeshima, Hyogo College of Medicine
- Tsuyoshi Matsumura, Toneyama National Hospital
- Shiro Ozasa, Kumamoto University Hospital
- Michinori Funato, Nagara Medical Center

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