# Brogidirsen - The First Dual-Targeting Exon 44 Skipping Drug for Duchenne Muscular Dystrophy: Safety, Tolerability, and High Dystrophin Restoration in Long-Term Clinical Studies

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## 1. Background

- Duchenne muscular dystrophy (DMD) is a progressive genetic muscle disease linked to the X chromosome, caused by mutations in the DMD gene which encodes dystrophin, a protein crucial for muscle function.
- Brogidirsen, an investigational exon 44 skipping therapy which induces highly efficient exon 44 skipping and dystrophin protein expression in DMD patients with exon deletion(s) in the dystrophin gene amenable to exon 44 skipping, was developed by NCNP in collaboration with Nippon Shinyaku.
- Reported Phase 1/2 Study[1] and the Phase 2 Extension Study data up to Week 63[2] showed no safety or tolerability concerns, as well as dystrophin restoration and motor functional stabilization in DMD patient administered with Brogidirsen.

## 2. Objective

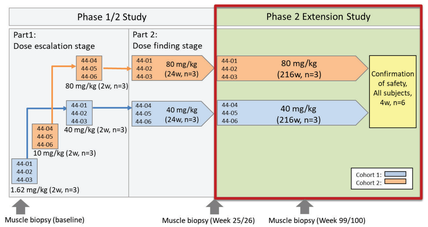
To comprehensively evaluate the safety and efficacy of Brogidirsen from the data of the Phase 1/2 Study and Phase 2 Extension Study up to Week 99/100.

## 3. Summary of Results

- The Phase 1/2 Study and the Phase 2 Extension Study (through Week 99/100) did not reveal any drug-related severe adverse events that interferes with the continuation of the study.
- Week 99/100 results showed trends of high dystrophin expression level and exon skipping efficiency in skeletal muscle.
- Plasma Biomarkers for DMD that could be useful for assessing therapeutic efficacy were discovered in plasma proteomics analysis.
- Motor function improvement or maintenance was observed up to Week 99/100.

## 4. Study Design

**Phase 1/2 Study** (NCNP/DMT02; NCT04129294).
**Phase 2 Extension Study** (NS-089/NCNP-02-P2OE; NCT05135663).




## 5. Participant Characteristics

| | | | 40 mg/kg (cohort 1, n=3) | 80 mg/kg (cohort 2, n=3) | Total (n=6) |
|---|---|---|---|---|---|
| Age at Phase 1/2 Study informed consent (years old) | Mean (SD) | | 9.0 (4.6) | 8.7 (0.6) | 8.8 (2.9) |
| | Range | | 4-13 (4, 10, 13) | 8-9 (8, 9, 9) | 4-13 |
| Age at Phase 1/2 Study Part 2 pre-dose (years old) | Mean (SD) | | 10.0 (4.6) | 9.7 (0.6) | 9.8 (2.9) |
| | Range | | 5-14 | 9-10 | 5-14 |
| Age at Phase 2 study W99/100 (years old) | Mean (SD) | | 12.0 (4.6) | 11.3 (0.6) | 11.6 (2.9) |
| | Range | | 7-16 | 11-12 | 7-16 |
| Mutation (deleted exons) | 45 | N | 3 (100) | 2 (66.7) | 5 (83.3) |
| | 45-54 | (%) | 0 (0) | 1 (33.3) | 1 (16.7) |
| Steroid treatment | Yes/No | | Yes* | Yes | Yes |

* 1 participant initiated steroid use during the post-treatment period of the Phase 1/2 Study

## 6. Safety Results

Adverse events related to Brogidirsen through Week 99/100 of the Phase 2 Extension Study were consistent with those previously observed in Phase 1/2 Study[2]. There was no severe adverse events related to Brogidirsen, or adverse events related to infusion-related reactions such as anaphylaxis, or immune-mediated myositis, or discontinuations up to Week 99/100.

**Drug-related Adverse Events** (MedDRA/J ver.25)

| Phase 2 Extension Study | 40 mg/kg (N=3) | 80 mg/kg (N=3) | Total (n=6) |
|---|---|---|---|
| Investigations | | | |
| Beta 2 microglobulin urine increased | 1 ( 33.3) | 0 ( 0.0) | 1 (16.7) |
| Prothrombin time prolonged | 1 ( 33.3) | 0 ( 0.0) | 1 (16.7) |
| Urine albumin/creatinine ratio increased | 2 ( 66.7) | 1 ( 33.3) | 3 (50.0) |
| Cystatin C increased | 1 ( 33.3) | 0 ( 0.0) | 1 (16.7) |
| Skin and subcutaneous tissue disorders | | | |
| Eczema | 0 ( 0.0) | 1 ( 33.3) | 1 (16.7) |

## 7. Efficacy Results (Dystrophin)

- DMD patients amenable to exon 44 skipping often show a certain amount of dystrophin expression even without treatment.[3] Week 99/100 results showed trends of high dystrophin expression level and exon skipping efficiency in skeletal muscle.
- Results of the in vitro assays using DMD participant-derived myoblast determination protein 1-transduced urine-derived cells (MYOD1-UDCs) **(A)** was consistent with W25/26 result **(B)**.

**Exon 44 Skipping Efficiency by RT-PCR**

**Phase 1/2 Study**

| Cohort | Cohort 1 (40 mg/kg, n=3) | Cohort 2 (80 mg/kg, n=3) | Total (n=6) |
|---|---|---|---|
| Baseline Mean (SD) Molarity (%) | 11.41 (7.00) | 18.42 (11.12) | 14.92 (9.16) |
| W25/26 Mean (SD) Molarity (%) | 40.97 (10.52) | 53.07 (15.94) | 47.02 (13.78) |

**Phase 2 Extension Study (Week 99/100)**

| Cohort | Cohort 1 (40 mg/kg, n=2) | Cohort 2 (80 mg/kg, n=3) | Total (n=5) |
|---|---|---|---|
| W99/100 Mean (SD) Molarity (%) | 47.84 (1.89) | 61.66 (16.55) | 56.14 (13.97) |

**Dystrophin Expression by Western blot***

**Phase 1/2 Study**

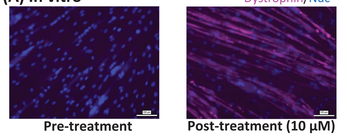
| Cohort | Cohort 1 (40 mg/kg, n=3) | Cohort 2 (80 mg/kg, n=3) | Total (n=6) |
|---|---|---|---|
| Baseline Mean (SD)(%) | 6.36 (3.45) | 8.68 (5.14) | 7.52 (4.12) |
| W25/26 Mean (SD)(%) | 16.63 (2.58) | 24.46 (2.08) | 20.55 (4.78) |

**Phase 2 Extension Study (Week 99/100)****

| Cohort | Cohort 1 (40 mg/kg, n=2) | Cohort 2 (80 mg/kg, n=3) | Total (n=5) |
|---|---|---|---|
| W99/100 Mean (SD)(%) | 13.18 (11.31) | 18.17 (4.10) | 16.17 (6.92) |

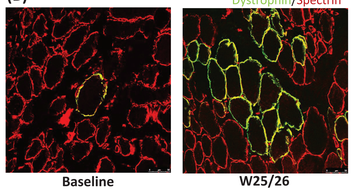
* Dystrophin expression normalized to α-Actinin2 (%)
** Quantitative comparison between Phase 1/2 Study and Phase 2 Extension Study is limited due to differences in measurement conditions.

**(A) In vitro**




**Participant 44-01 derived MYOD1-UDCs**

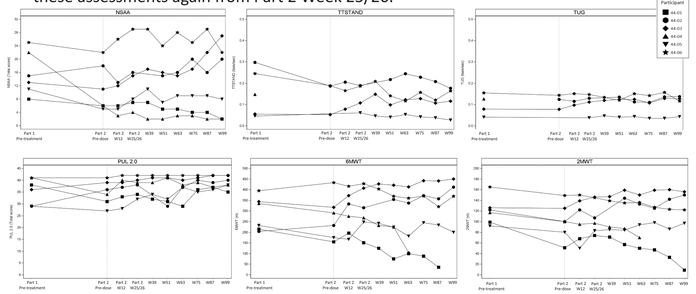
**(B)**




**DMD participant Muscle biopsy tissues from biceps brachii**

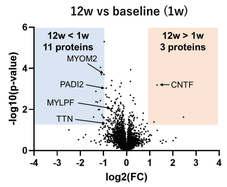
## 8. Efficacy Results (Motor function Assessment)

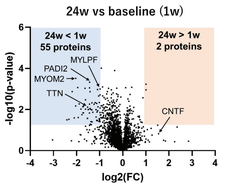
- Function assessment showed a trend of maintenance or improvement in motor function up to Week 99/100.
- More than 2 points changes in mean NSAA total scores was observed as early as Part 2 Week 25/26 in Cohort 2.
- Participant 44-05 could not perform TTSTAND and TUG assessment at Part 2 pre-dose and Part 2 Week 12 due to disease progression, however the participant was able to perform these assessments again from Part 2 Week 25/26.

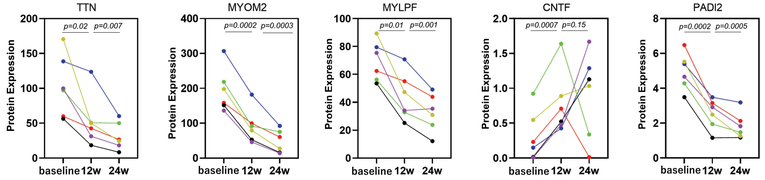



## 9. Plasma proteomics

- Plasma collected from six participants in Part 2 of Phase 1/2 Study was analyzed by the Olink Explore 3072 high-throughput proteomic analysis platform at three different time points: baseline and 12 and 24 weeks.
- Three proteins were upregulated and 11 proteins were downregulated at week 12 compared to baseline. Two proteins were upregulated and 55 were downregulated at week 24 compared to baseline.




**Plasma Proteomics**
- TTN:Titin
- MYOM2: Myomesin
- MYLPF: Myosin light chain Phosphorylatable fast skeletal muscle
- CNTF: Ciliary neurotrophic factor
- PADI2: Peptidyl arginine deiminase 2

## 10. Discussion

- Phase 1/2 study Part 1 initiation to Part 2 initiation took 8 to 13-months. During that period, participants only received a total of 4 doses with 3 to 5 months with no dosing[2]. To evaluate the motor function assessment results, the baseline was set as Part 2 pre-dose, right before initiating weekly Brogidirsen administration.
- The levels of TTN, MYOM2, and MYLPF, which were elevated in patients with DMD, decreased. These plasma markers are associated with muscle tissue necrosis and a decrease in their levels may indicate an improvement in muscle pathology.
- NSAA total scores of 2 to 3 points change in 48-week is considered clinically meaningful[4]. This trend was observed as early as after 24 weekly administration of Brogidirsen.
- The long-term results suggest the potential of Brogidirsen to slow the disease progression in patients with DMD who are amenable to exon 44 skipping.
- The limitations of this study include the small number of participants and lack of placebo control arm, therefore the effectiveness of Brogidirsen in maintaining motor function requires further verification.
- Further assessment of the safety and efficacy of Brogidirsen is being investigated in the ongoing Phase 2 study (Study ID: NS-089/NCNP-02-201; NCT05996003) sponsored by NS Pharma, Inc. (US subsidiary of Nippon Shinyaku).

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