Orthogonal methods demonstrate pharmacodynamic responses in Duchenne muscular dystrophy patients treated with nivudirsen (BMN 351)

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Introduction

- Duchenne muscular dystrophy (DMD) is a neuromuscular disorder characterized by progressive muscle weakness that can be caused by frameshift variants that produce truncated and non-functional dystrophin protein[1,2]
- Nivudirsen (BMN 351) is an antisense oligonucleotide (ASO) designed to exclude exon 51 from the dystrophin pre-mRNA to restore the open reading frame and enable synthesis of functional, near full-length dystrophin protein in exon 51 skip-amenable individuals[3,4]
- Study 351-201 (NCT06280209) is an ongoing, open-label, phase 1/2 clinical trial of nivudirsen in participants with DMD amenable to exon 51 skipping
- The measurement of exon 51 skipping and dystrophin protein can provide early pharmacodynamic assessments of ASOs under clinical investigation
  - Immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry (IA-UPLC-MS/MS) offers an improved quantitative measure of dystrophin protein compared with semiquantitative western blot methods

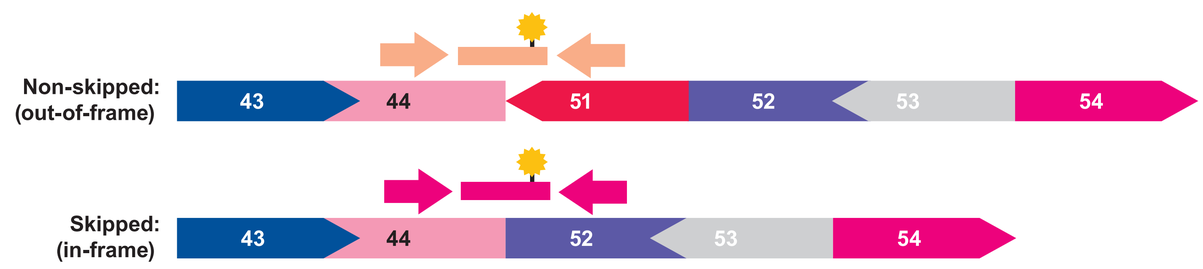
Objectives

- Three key pharmacodynamic biomarkers were measured in participant muscle biopsy tissue from the 351-201 clinical trial to evaluate early participant responses from the first 2 of 3 total nivudirsen doses

Methods

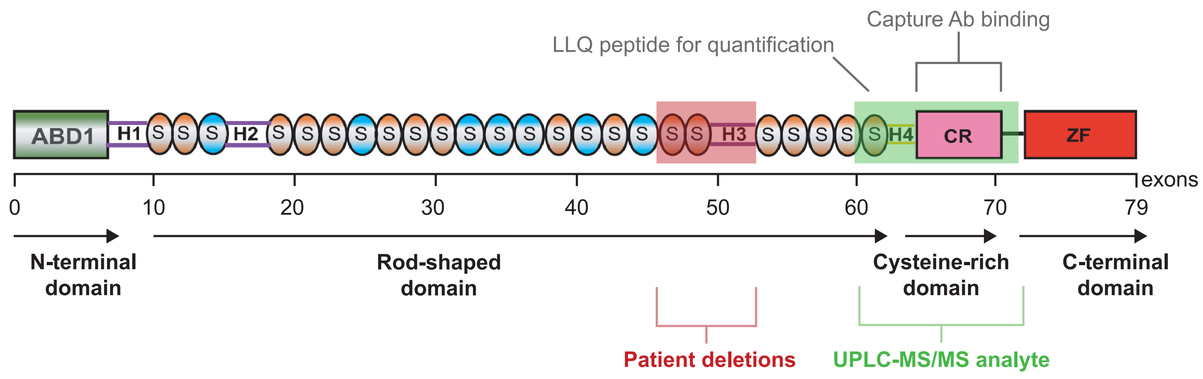
- Tissue homogenate aliquots were used to measure specific mRNA targets by droplet digital PCR (ddPCR), dystrophin, and α-actinin-2 proteins by IA-UPLC-MS/MS, and total protein by the bicinchoninic acid (BCA) assay
- A QX ONE ddPCR instrument from BioRad was used to quantify in-frame and out-of-frame dystrophin mRNA (**Figure 1**) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The percent of exon skipping was calculated as in-frame / (in-frame + out-of-frame) dystrophin mRNA
- Dystrophin protein was measured by semiquantitative western blot and quantitative IA-UPLC-MS/MS method (**Figure 2**)
  - The western blot method used mouse monoclonal anti-dystrophin antibody MANDYS106 with chemiluminescent detection and myosin heavy chain (MYH) or ACTN2 for normalization
  - The IA-UPLC-MS/MS method used a Sciex Triple Quad 6500+ system and ACTN2 or total protein measurement for normalization

Figure 1. Schematic of ddPCR dystrophin mRNA analytes



The illustration shows a representative genotype of exon 45 to 50 deletion; a skipped ddPCR transcript is only produced when exon 51 is skipped.
Arrows represent primers, and bars represents fluorescent labeled probes.
ddPCR, droplet digital PCR

Figure 2. Schematic of IA-UPLC-MS/MS analyte

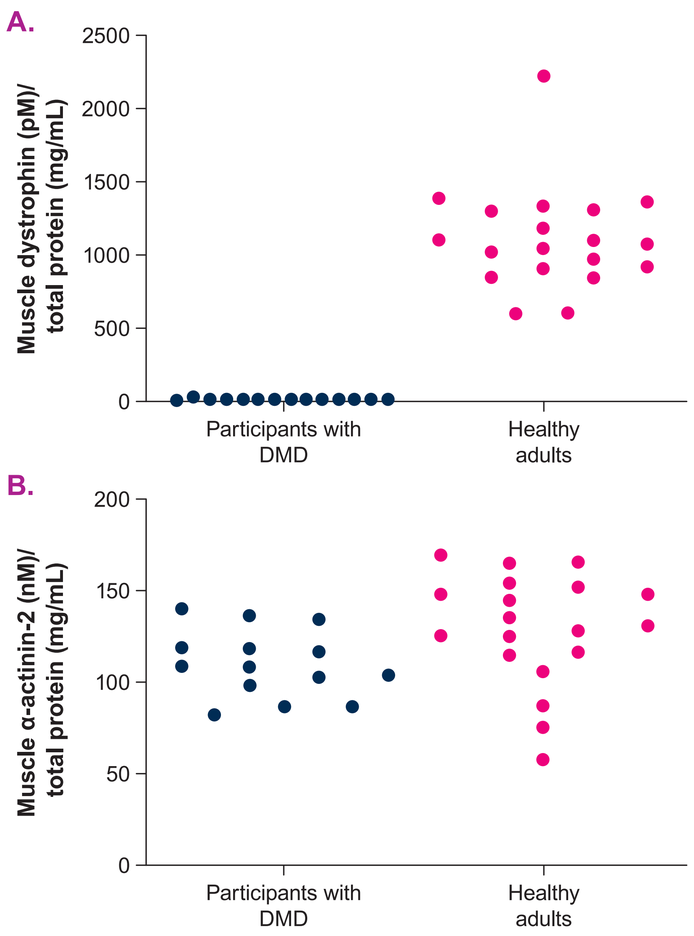


Ab, antibody; ABD, actin-binding domain; CR, cysteine-rich domain; H, hinge region; LLQ, LLQVAVEDR; S, spectrin; IA-UPLC-MS/MS, immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry; ZF, zinc-finger domain.
Figure 2 reproduced with minor changes from Wang Y, et al. Prenatal diagnosis of Duchenne muscular dystrophy revealed a novel mosaic mutation in dystrophin gene: a case report. *BMC Med Genet.* 2020;11;21(1):222. Licensed under CC BY 4.0 and cropped from original.

Results

- The dystrophin and α-actinin-2 protein concentrations measured by IA-UPLC-MS/MS in 19 healthy adult muscle samples were used as a reference for reporting the percentage of normal dystrophin concentrations in muscle samples from 14 participants with DMD, 1 sample per participant, in study 351-201 (**Figure 3**)
  - α-actinin-2/total protein content was correlated with biopsy myofiber content in participants with DMD as measured by hematoxylin and eosin or Masson's trichrome histology (Spearman rank correlation, $P = 0.002$, $R = 0.75$ and $P = 0.02$, $R = 0.067$, respectively)

Figure 3. Establishing a healthy adult reference range for the percentage of A) dystrophin and B) α-actinin-2 protein concentrations by IA-UPLC-MS/MS



DMD, Duchenne muscular dystrophy; IA-UPLC-MS/MS, immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry.

- From the healthy adult muscle samples, 6 were selected for further evaluation to compare the precision of dystrophin detection by western blot vs IA-UPLC-MS/MS (**Table 1**)

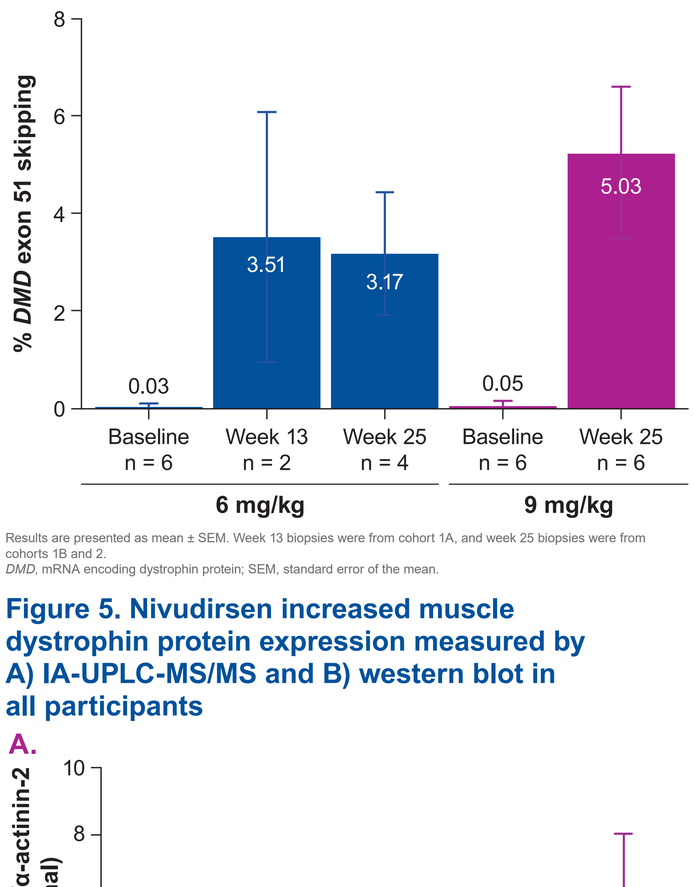
Table 1. Head-to-head comparison of western blot vs IA-UPLC-MS/MS human muscle dystrophin assay precision

Detection method	CV type	Precision (%CV)
Western blot[a]	Intra-assay	26.7 (4.2–86.8)
	Inter-assay	19.6 (9.7–26.6)
IA-UPLC-MS/MS[a]	Intra-assay	2.6 (0.0–9.5)
	Inter-assay	11.2 (3.8–15.6)

[a]For both detection methods, dystrophin was normalized to α-actinin-2 and the IA-UPLC-MS/MS method was validated to M10. For the comparison, 3 aliquots of each healthy adult muscle sample (n = 6) were measured in triplicate (western blot) or duplicate (IA-UPLC-MS/MS) across 3 independent runs.
CV, coefficient of variation; IA-UPLC-MS/MS, immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry.

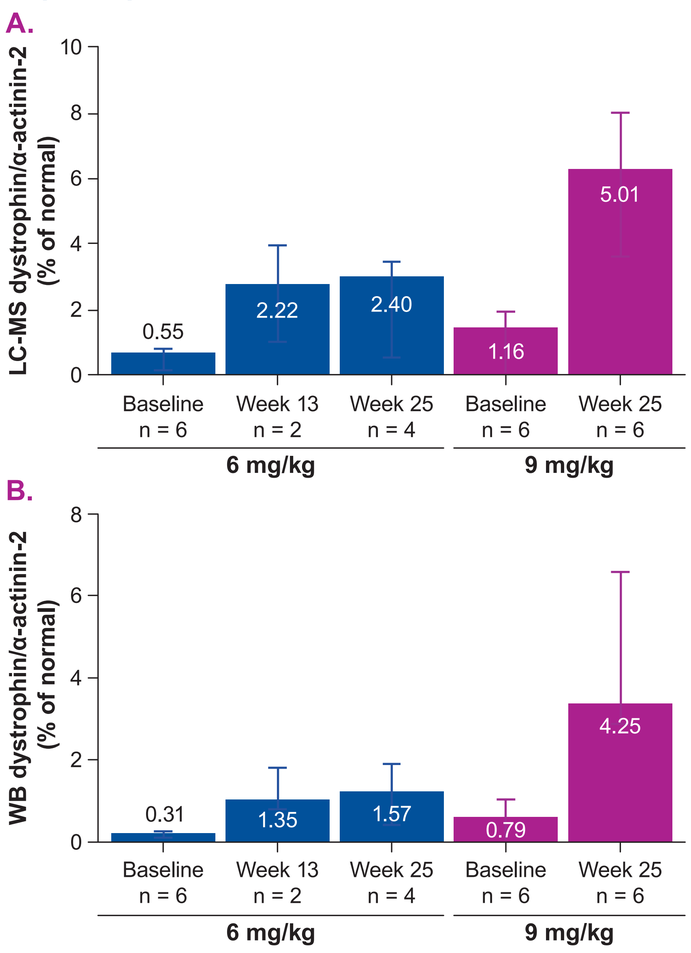
- In study 351-201, nivudirsen demonstrated dose-dependent increases in exon skipping (**Figure 4**) and dystrophin protein (**Figure 5**) in muscle samples from 12 participants with DMD at the 6- and 9-mg/kg doses
  - These results demonstrate early on-target activity of nivudirsen for participants with DMD

Figure 4. Nivudirsen demonstrated dose-dependent exon 51 skipping in all participants



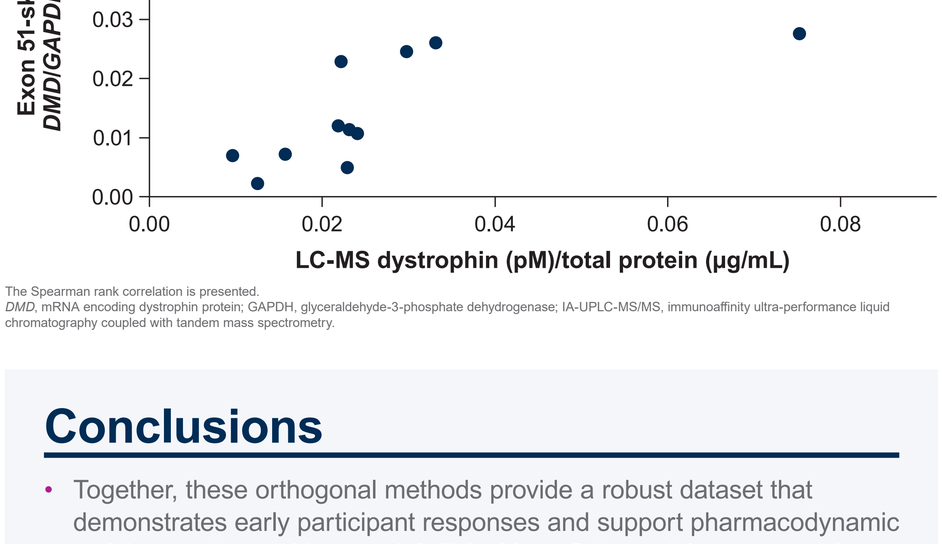
Results are presented as mean ± SEM. Week 13 biopsies were from cohort 1A, and week 25 biopsies were from cohorts 1B and 2.
DMD, mRNA encoding dystrophin protein; SEM, standard error of the mean.

Figure 5. Nivudirsen increased muscle dystrophin protein expression measured by A) IA-UPLC-MS/MS and B) western blot in all participants



Results are presented as mean ± SEM. Week 13 biopsies were from cohort 1A, and week 25 biopsies were from cohorts 1B and 2.
IA-UPLC-MS/MS, immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry; SEM, standard error of the mean; WB, western blot.

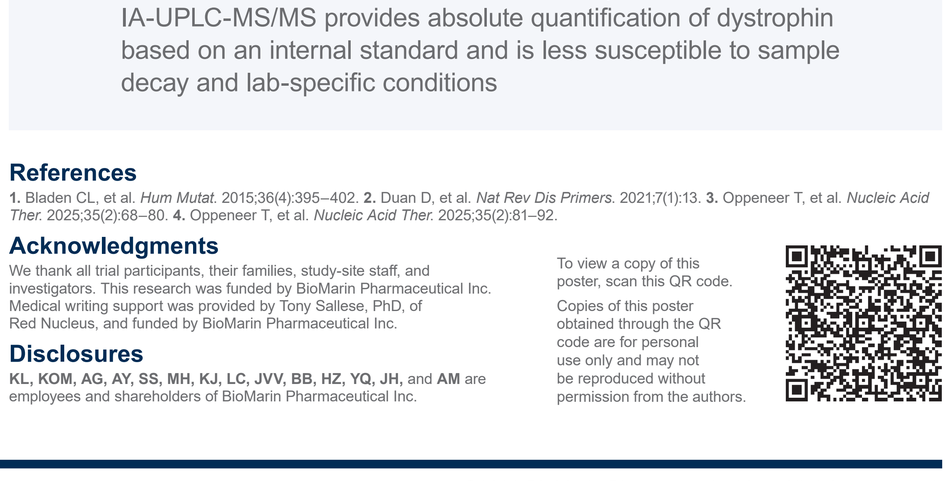
Figure 6. Semiquantitative western blot method provides useful qualitative information about dystrophin protein size and isoform heterogeneity in each sample



For panel B, the western blot image revealed multiple dystrophin isoforms with unknown functional capacity at baseline and a higher molecular weight dystrophin isoform after nivudirsen treatment
DMD, Duchenne muscular dystrophy; QC, quality control; W, week.

- After nivudirsen treatment, exon 51 skipping correlated with detection of near full-length dystrophin protein (**Figure 7**)

Figure 7. Exon-skipped dystrophin mRNA correlated with dystrophin protein measured by IA-UPLC-MS/MS after nivudirsen treatment



The Spearman rank correlation is presented.
DMD, mRNA encoding dystrophin protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IA-UPLC-MS/MS, immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry.

Conclusions

- Together, these orthogonal methods provide a robust dataset that demonstrates early participant responses and support pharmacodynamic activity consisent with potential clinical benefit in participants treated with nivudirsen
- IA-UPLC-MS/MS and western blot are complimentary methods of protein measurement that offer quantitative and qualitative assessments, respectively
  - Whereas western blot is considered semi-quantitative, IA-UPLC-MS/MS provides absolute quantification of dystrophin based on an internal standard and is less susceptible to sample decay and lab-specific conditions

References

1. Bladen CL, et al. *Hum Mutat.* 2015;36(4):395–402. **2.** Duan D, et al. *Nat Rev Dis Primers.* 2021;7(1):13. **3.** Oppeneer T, et al. *Nucleic Acid Ther.* 2025;35(2):68–80. **4.** Oppeneer T, et al. *Nucleic Acid Ther.* 2025;35(2):81–92.

Acknowledgments

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Disclosures

KL, KOM, AG, AY, SS, MH, KJ, LC, JVV, BB, HZ, YQ, JH, and **AM** are employees and shareholders of BioMarin Pharmaceutical Inc.

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