A transcriptomics-based biomarker for muscle quality measures nivudirsen (BMN 351) treatment response in DMD patient biopsies

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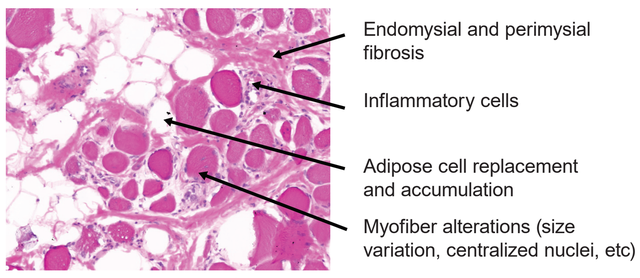
Introduction

- Duchenne muscular dystrophy (DMD) is caused by variants in the gene *dystrophin* that produce insufficient, truncated, or unstable protein[1]
  - Dystrophin protein is required for preserving muscle function, and insufficiency results in muscle degeneration[2]
- Muscle damage in DMD is progressive and associated with fibrotic tissue, inflammatory cell infiltration, adipose accumulation, and muscle cell alterations (**Figure 1**)[2]
  - Biomarkers related to muscle damage and composition could be indicators of disease progression or treatment response
- The ongoing, open-label, phase 1/2, dose-escalation study 351-201 (NCT06280209) is designed to evaluate nivudirsen (BMN 351), an antisense phosphorothioate-based oligonucleotide modified to enhance specificity and stability that targets a novel *dystrophin* exon 51 binding site[3,4] in ambulatory participants with DMD amenable to exon 51 skipping

Figure 1. Cross-section from a muscle biopsy of an individual with DMD stained with H&E

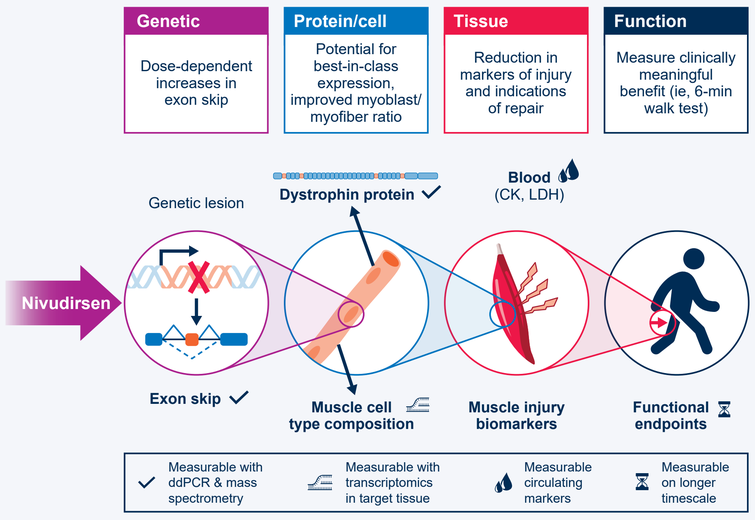


DMD, Duchenne muscular dystrophy; H&E, hematoxylin and eosin.

Objectives

- Here, we present exploratory transcriptomics biomarkers to supplement dystrophin expression as a measure of nivudirsen treatment response and assess the ability of nivudirsen to improve muscle composition and quality in a preclinical model and clinical trial participant samples (**Figure 2**)

Figure 2. Transcriptomics to measure impact of nivudirsen on muscle quality



ASO, antisense oligonucleotide; CK, creatine kinase; ddPCR, droplet digital polymerase chain reaction; LDH, lactate dehydrogenase.

Methods

Transcriptomics

- Bulk RNA sequencing data from publicly available datasets were analyzed from skeletal muscle biopsies from healthy individuals vs individuals with DMD[5] and from individuals with Becker muscular dystrophy (BMD) vs DMD[6]
- Bulk RNA sequencing data were generated (ribosomal RNA depletion) from muscle biopsies from participants of 351-201 who received 6 or 9 mg/kg of nivudirsen weekly for 13 or 25 weeks, and from nonclinical wild-type (WT) and DMD (hDMDdel52/*mdx*) mice treated for 13 weeks with vehicle or nivudirsen (18 mg/kg administered weekly), beginning at age 7 weeks when DMD mice were symptomatic[3]
- RNA sequencing was analyzed using a custom Snakemake pipeline; alignment and gene-level quantification were determined using STAR. Differential expression was assessed with DESeq2, and principal components analyses and gene set variation analyses were performed in R using stats and gene set variation analysis packages

RNA gene expression

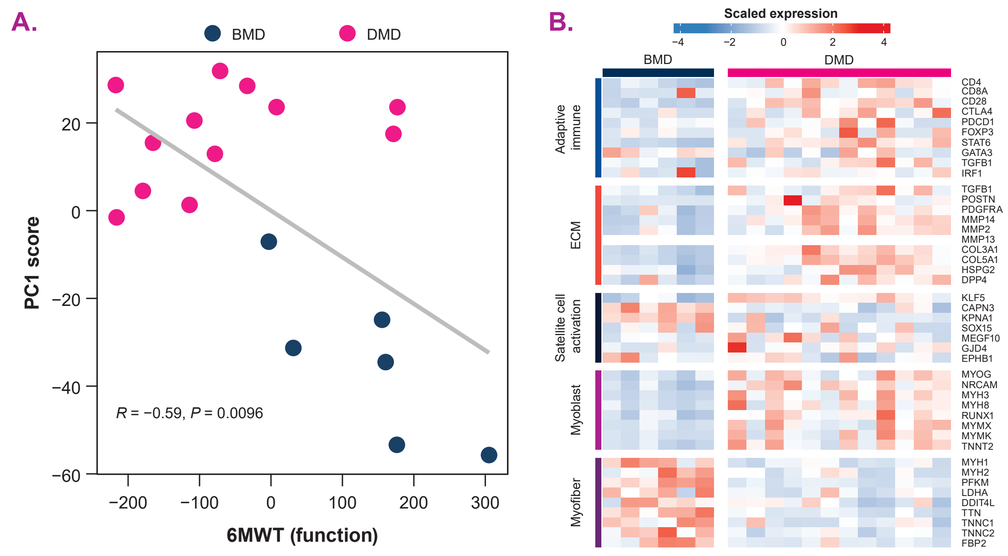
- Quantitative droplet digital polymerase chain reaction (ddPCR) assays were developed for murine and human myoblast- and myofiber-expressed genes
- ddPCR was performed on quadricep muscle from WT and hDMDdel52/*mdx* vehicle- or nivudirsen-treated mice and human skeletal muscle biopsies from healthy individuals (Reprocell, DX Biosamples, or AMS Bio) or individuals with DMD[7]
- A composition expression score was developed for the ratio of predominantly myoblast-expressed to myofiber-expressed genes, with reference to the corresponding gene expression in WT (mouse) or healthy (human) samples
- Skeletal muscle biopsies were analyzed from participants of 351-201, a phase 1/2 study evaluating nivudirsen in individuals with DMD

Results

Transcriptome-wide signature of DMD in public datasets and a murine model

- Transcriptome-wide expression changes in DMD and BMD skeletal muscle biopsies separate along the principal component 1 (PC1) axis and correlate with ambulatory function (**Figure 3A**)
- Five biologically relevant pathways were differentially expressed in DMD compared to healthy (data not shown) or BMD skeletal muscle biopsies (**Figure 3B**)

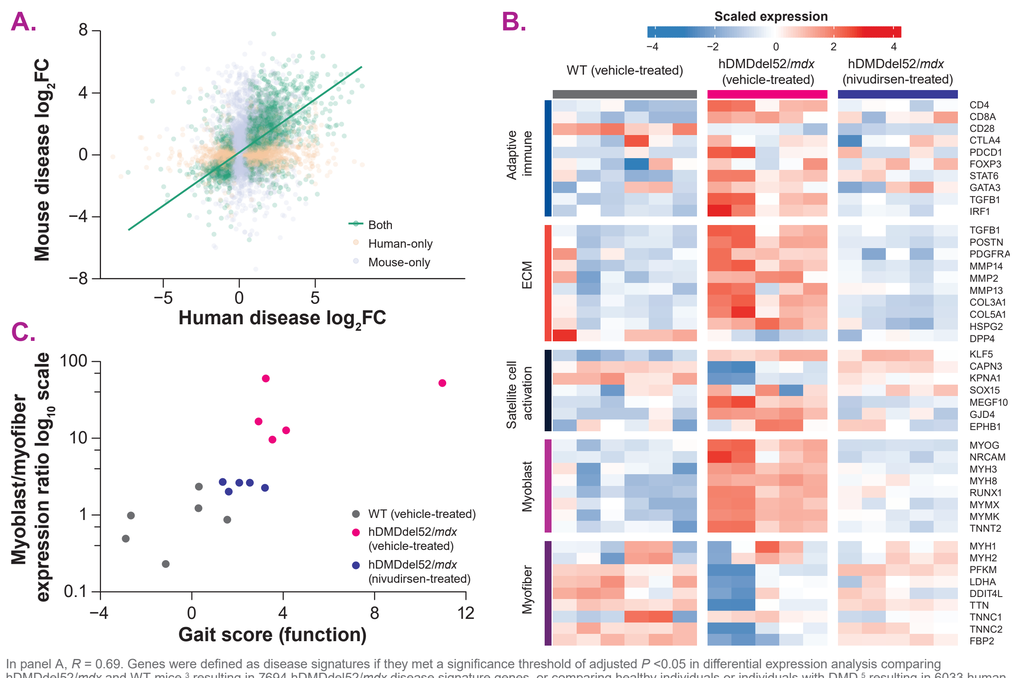
Figure 3. Transcriptome-wide gene expression variance in individuals with BMD vs DMD A) correlates with ambulatory function and B) highlights key biologically relevant pathways



Data analyzed in panel A are from the Rizzo 2025 dataset[6] and 6MWT values adjusted for age at evaluation and disease duration at biopsy. Heat maps were generated using variance-stabilized expression values from an unsupervised DESeq2 model.
BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; PC, principal component; 6MWT, 6-minute walk test.

- Observed DMD disease signatures were strongly concordant between individuals with DMD and the murine model of DMD (**Figure 4A**), and pathway changes in humans with DMD were also present in a murine model of DMD, with animals treated with nivudirsen shifting to a WT-like expression pattern (**Figure 4B**)
  - A total of 3684 genes were considered common DMD signatures and shared between mouse and human datasets, while 2349 genes were unique to the human dataset, and 4010 were unique to the mouse dataset
- Myoblast-associated gene expression was elevated and myofiber-associated gene expression was reduced in individuals with DMD vs healthy and in vehicle-treated hDMDdel52/*mdx* mice vs WT mice, respectively (data not shown)
- With nivudirsen treatment, the elevated myoblast-to-myofiber expression ratio in vehicle-treated hDMDdel52/*mdx* mice reduced to levels similar to WT mice and the ratio correlated with improved function (**Figure 4C**)

Figure 4. Gene expression differences associated with A) DMD were correlated between human and mouse disease signatures or B) muscle cell composition are conserved and reversible in WT and vehicle- or nivudirsen-treated hDMDdel52/*mdx* mice, and C) myoblast-to-myofiber expression ratio in WT and vehicle- or nivudirsen-treated hDMDdel52/*mdx* mice correlated with ambulatory function

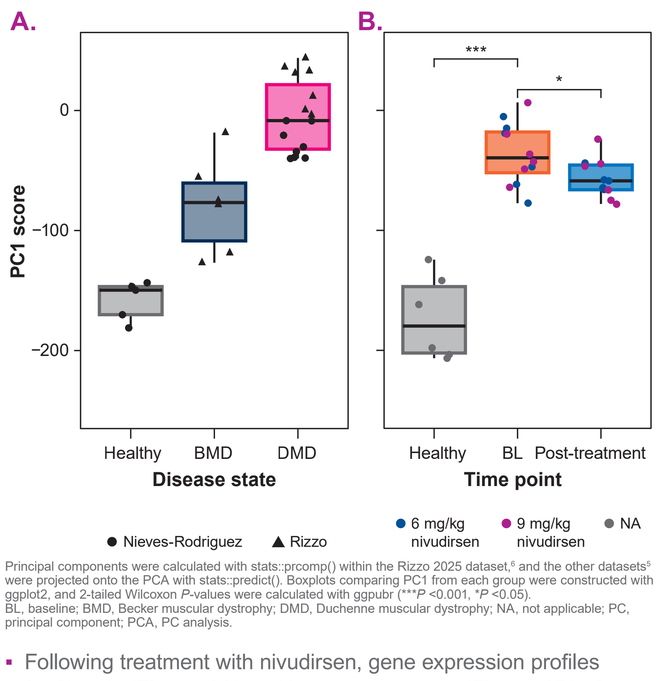


In panel A, $R = 0.69$. Genes were defined as disease signatures if they met a significance threshold of adjusted $P < 0.05$ in differential expression analysis comparing hDMDdel52/*mdx* and WT mice,[3] resulting in 7694 hDMDdel52/*mdx* disease signature genes, or comparing healthy individuals or individuals with DMD,[5] resulting in 6033 human DMD disease signature genes. The plot includes the union of disease signature genes identified in either species (correlation $P < 2.2e\text{-}16$). Heatmaps in panel B were generated using variance-stabilized expression values from an unsupervised DESeq2 model. In panel C, expression ratio is calculated from 3 genes expressed primarily in myoblasts and myofibers, respectively, and with reference to the WT group. Gait score was determined using fine motor kinetic testing; lower gait score indicates higher function.[3]
DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; FC, fold change; WT, wild-type.

Impact of nivudirsen on muscle transcriptomic profile, composition, and quality in clinical samples

- RNAseq data from muscle biopsy samples were analyzed for genome-wide expression changes. The first principal component, PC1 score, captured the most variance in the data as a single dimension
- Combined analysis from 2 published datasets identified that PC1 scores scaled with disease severity, as PC1 scores for individuals with BMD are intermediate to those of healthy and DMD (**Figure 5A**)[5,6]
- The range of transcriptomic profiles of participants from the BMN 351-201 clinical trial at baseline was consistent with individuals with DMD and reduced toward healthy following treatment with nivudirsen (**Figure 5B**)

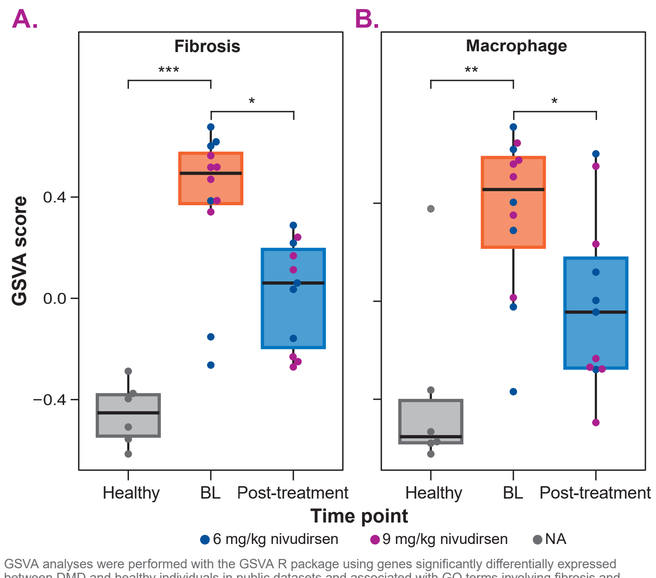
Figure 5. Skeletal muscle biopsy transcriptomics profile of A) healthy individuals and individuals with BMD or DMD and B) nivudirsen-treated participants with DMD vs postmortem healthy individuals



Principal components were calculated with stats::prcomp() within the Rizzo 2025 dataset,[6] and the other datasets[5] were projected onto the PCA with stats::predict(). Boxplots comparing PC1 from each group were constructed with ggplot2, and 2-tailed Wilcoxon *P*-values were calculated with ggpubr (***P <0.001, *P <0.05).
BL, baseline; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; NA, not applicable; PC, principal component; PCA, PC analysis.

- Following treatment with nivudirsen, gene expression profiles for fibrosis (**Figure 6A**) and immune response (**Figure 6B**) of participants with DMD were significantly improved compared to baseline

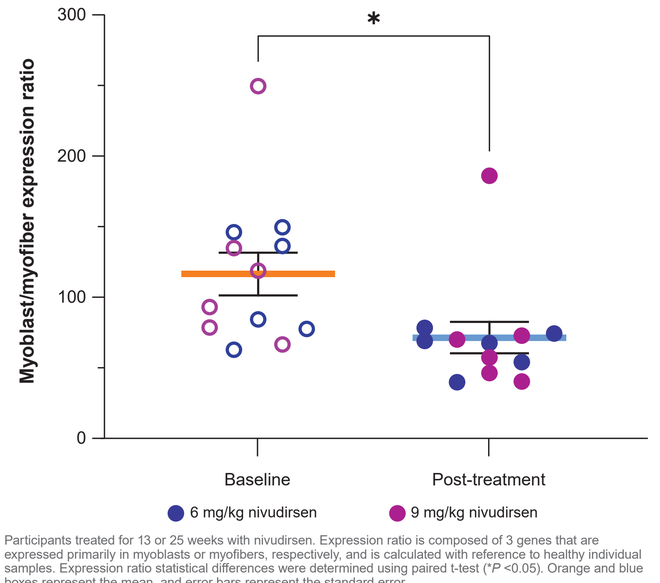
Figure 6. Gene expression profiles for A) fibrosis and B) macrophages of participants treated with nivudirsen in 351-201 compared to baseline and healthy individuals



GSVA analyses were performed with the GSVA R package using genes significantly differentially expressed between DMD and healthy individuals in public datasets and associated with GO terms involving fibrosis and macrophage activity.[5,6] Statistical differences for healthy vs BL and BL vs post-treatment groups were calculated using Wilcoxon signed-rank test and linear mixed effects models, respectively (***P <0.001, **P <0.01, *P <0.05).
BL, baseline; DMD, Duchenne muscular dystrophy; GO, gene ontology; GSVA, gene set variation analysis; NA, not available.

- In the 351-201 clinical trial, the myoblast-to-myofiber expression ratio decreased with nivudirsen treatment, suggesting improved muscle repair and maturation during tissue remodeling (**Figure 7**)

Figure 7. Effect of nivudirsen on the myoblast-to-myofiber gene expression ratio in participants in 351-201



Participants treated for 13 or 25 weeks with nivudirsen. Expression ratio is composed of 3 genes that are expressed primarily in myoblasts or myofibers, respectively, and is calculated with reference to healthy individual samples. Expression ratio statistical differences were determined using paired t-test (*P <0.05). Orange and blue boxes represent the mean, and error bars represent the standard error.

Conclusions

- Transcriptome-wide expression signature of DMD disease includes the same pathways in human and hDMDdel52/*mdx* mice
- Transcriptional changes with disease severity (individuals with BMD vs DMD) and with nivudirsen treatment (hDMDdel52/*mdx* mice) correlate with ambulatory function
- These data suggest that the near full-length dystrophin produced with nivudirsen alters the gene expression profile of muscle in both the hDMDdel52/*mdx* mice and clinical trial participants with DMD to align more closely with WT mice and healthy individuals, respectively
- Gene expression and pathway changes in clinical trial participant data suggest stability and reversal of DMD biology
- Future transcriptional analyses will evaluate the relationship between this exploratory biomarker and functional improvements in participants with DMD in nivudirsen clinical trials

References

1. Duan D, et al. *Nat Rev Dis Primers*. 2021;7(1):13. **2.** Cardone N, et al. *Acta Neuropathol Commun*. 2023;11(1):167. **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. **5.** Nieves-Rodriguez S, et al. *Front Genet*. 2023;14:1216066. **6.** Rizzo B, et al. *Int J Mol Sci*. 2025;26(14):6594. **7.** Goemans N, et al. *Neuromuscul Disord*. 2017;27(3):203–13.

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Disclosures

KOM, **EW**, **LL**, **MA**, **LS**, **DG**, **CR**, **JI**, **KL**, **KH**, and **RP** are employees and shareholders of BioMarin Pharmaceutical Inc. **IS** was an employee and shareholder of BioMarin Pharmaceutical Inc. at the time work was completed.

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