

Muscle composition and muscle injury biomarkers improve with nivudirsen (BMN 351) treatment

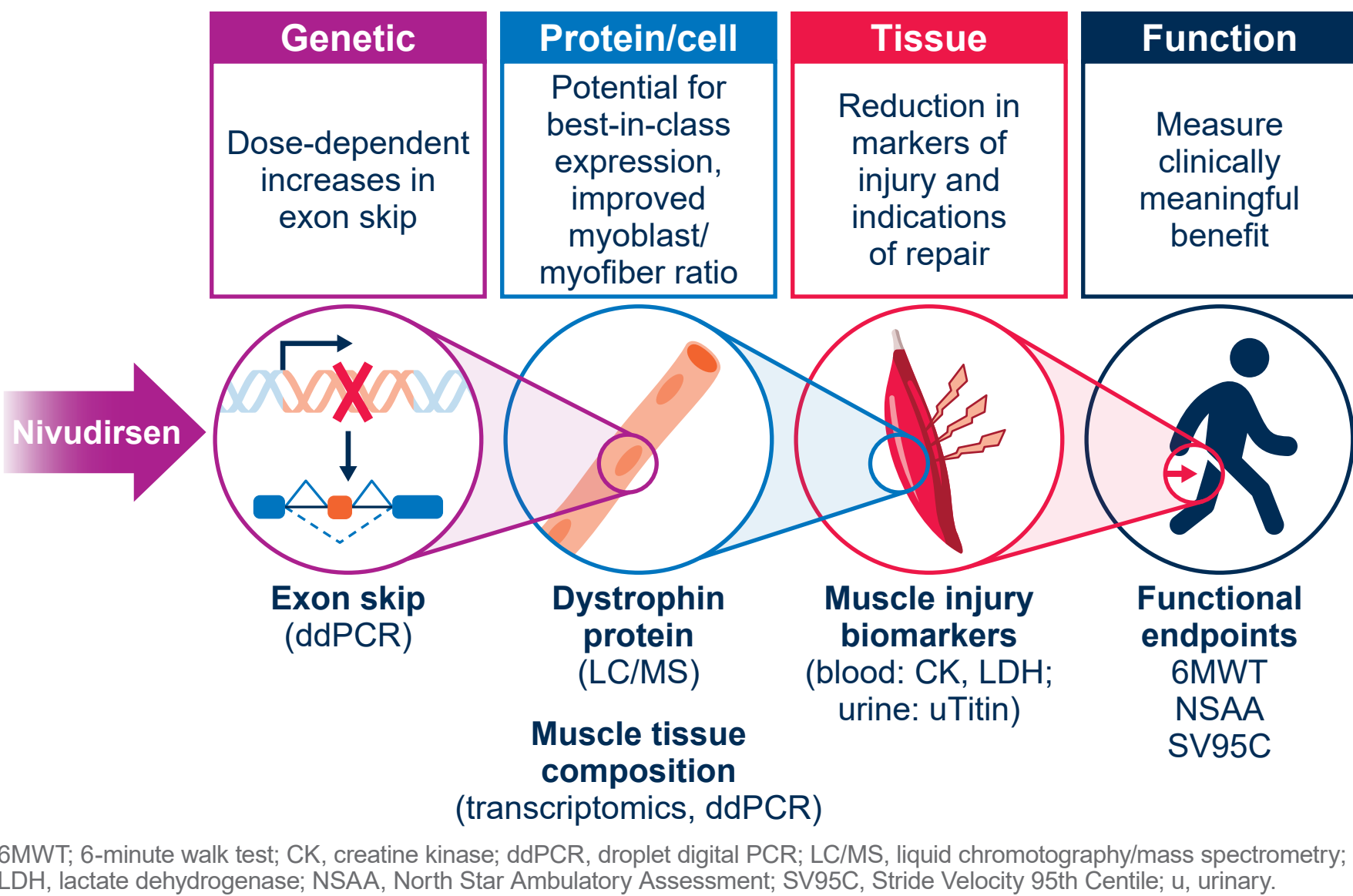
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Introduction

- Duchenne muscular dystrophy (DMD) is a neuromuscular disorder caused by variants in *dystrophin*, a protein required for preserving muscle function^{1,2}
- DMD is characterized by progressive muscle degeneration and changes to tissue composition (**Figure 1**)
 - Progressive muscle damage in DMD results in fibrosis, inflammatory cell infiltration, adipose accumulation, and muscle cell alterations.² Pathogenic changes in DMD muscle and responses to treatment can be evaluated through gene expression profiling
 - Muscle injury reduces muscle membrane integrity, releasing muscle-specific biomarkers into circulation³
 - Blood and urine muscle injury biomarkers offer minimally invasive indicators of disease progression and treatment response that can be evaluated repeatedly over time
- Nivudirsen (BMN 351), an antisense phosphorothioate-based oligonucleotide modified to enhance specificity and stability that targets a novel *dystrophin* exon 51 binding site,^{4,5} is being evaluated in the ongoing open-label, phase 1/2 dose-escalation study 351-201 (NCT06280209) in ambulatory participants with DMD amenable to exon 51 skipping

Figure 1. Transcriptomics and biomarkers measure the impact of nivudirsen on muscle quality



6MWT: 6-minute walk test; CK, creatine kinase; ddPCR, droplet digital PCR; LC/MS, liquid chromatography/mass spectrometry; LDH, lactate dehydrogenase; NSAA, North Star Ambulatory Assessment; SV95C, Stride Velocity 95th Centile; u, urinary.

Objective

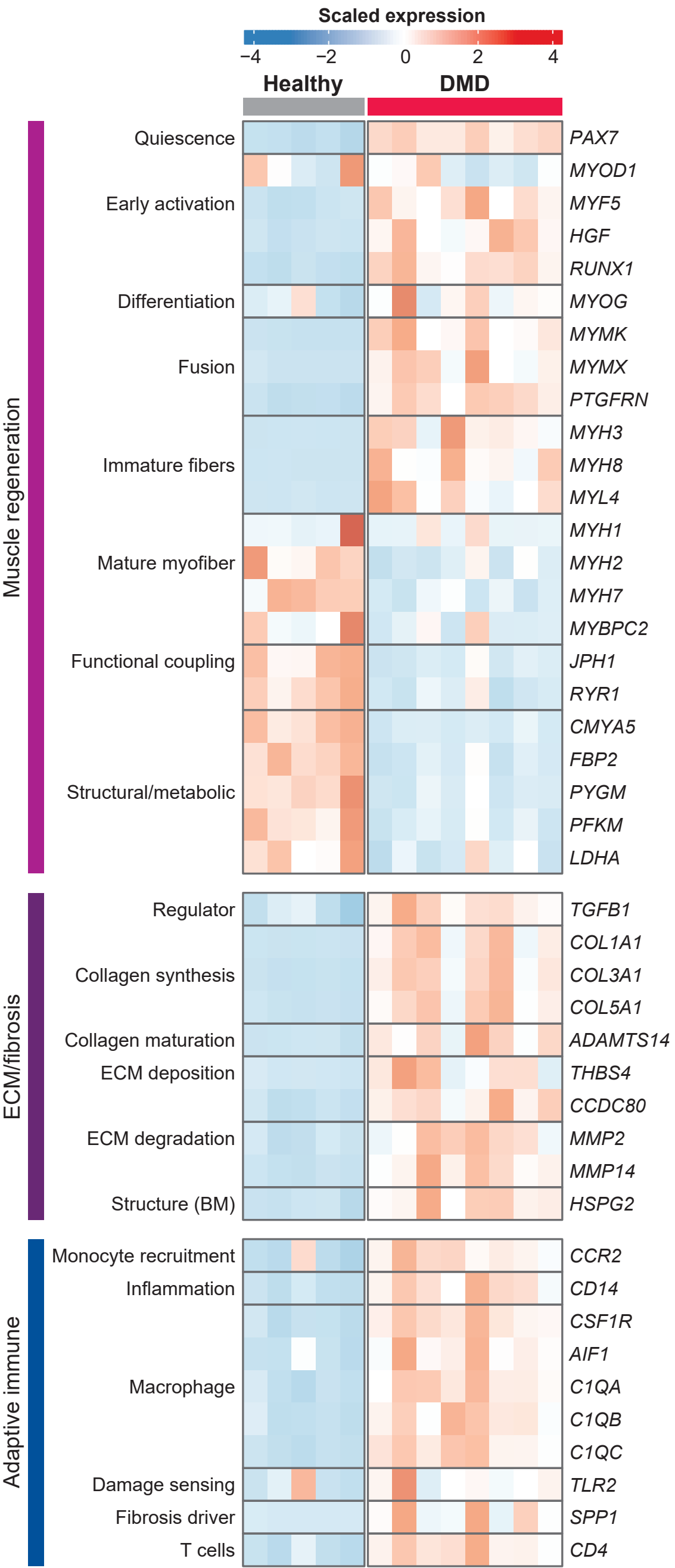
- To assess the ability of nivudirsen treatment to stabilize or improve muscle composition and quality by evaluating transcriptomic changes and serum and urinary biomarkers of muscle injury

Results

Transcriptome-wide signature of DMD in public datasets

- Transcriptome-wide expression changes in muscle biopsies of individuals with DMD compared to healthy skeletal muscle identified differential expression of biologically relevant pathways to disease, including genes associated with muscle regeneration, fibrosis and the extracellular matrix (ECM), and adaptive immune response (**Figure 2**)

Figure 2. Transcriptome-wide gene expression variance in healthy individuals vs individuals with DMD highlights key biologically relevant pathways

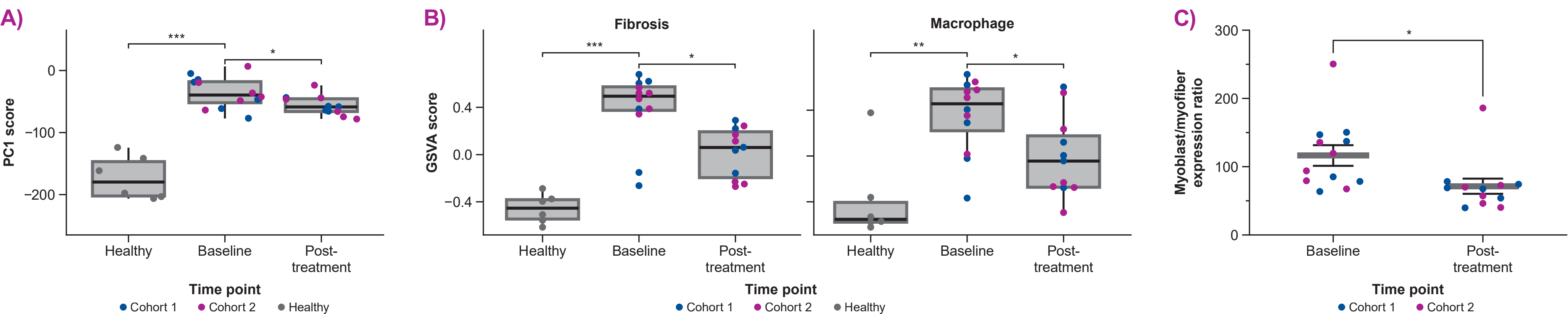


Heat maps were generated using variance-stabilized expression values from an unsupervised DESeq2 model. Data analyzed are from publicly available datasets containing DMD samples.¹ BM, bone marrow; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix.

Impact of nivudirsen on muscle transcriptomic profile and circulating or secreted muscle injury biomarkers in clinical samples

- RNAseq data from muscle biopsy samples were analyzed for genome-wide gene expression changes. The first principal component, PC1 score, captures the most variance in the data and scales with severity of muscular dystrophy phenotype
 - Following treatment with nivudirsen, the transcriptomics profile of participants from the BMN 351-201 clinical trial changed from baseline and trended toward healthy reference samples (**Figure 3A**)
- Gene expression profiles of fibrosis and immune response (**Figure 3B**) of participants with DMD were also significantly improved compared to baseline after nivudirsen treatment
 - No striking reduction in the gene expression profile for adipocytes was observed (data not shown), potentially due to the younger age of participants (mean age at enrollment: 6 mg/kg dose, 7.2 ± 1.3 years; 9 mg/kg dose, 7.3 ± 1.2 years) and their ambulatory status
- 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 3C**)

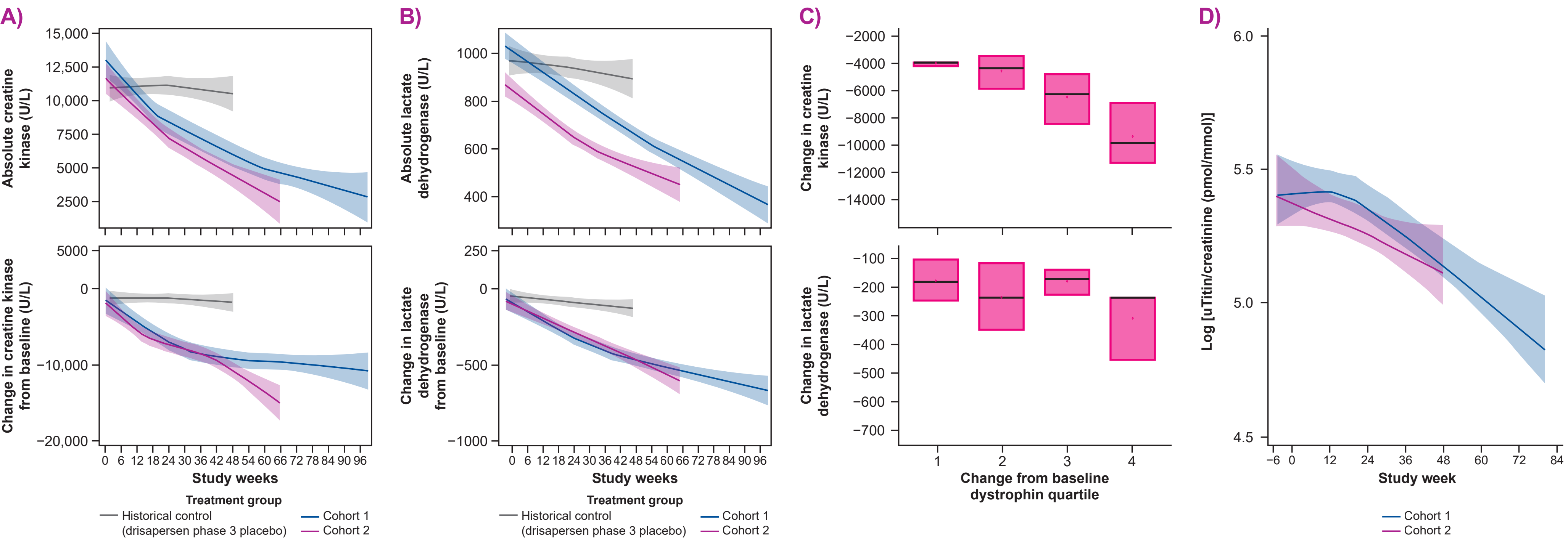
Figure 3. Skeletal muscle biopsy A) principal component scores, B) gene expression profiles of fibrosis and immune response, and C) myoblast-to-myofiber gene expression ratio of nivudirsen-treated participants with DMD



Participants were treated for 13 or 52 weeks with nivudirsen. In panel A, principal components were calculated with stats::prcomp() within datasets and were projected onto the PC analysis 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). In panel B, gene set variation analysis (GSEA) analyses were performed with the GSEA R package using 111 fibrosis and 44 macrophage genes that are differentially expressed between DMD and healthy individuals in public datasets and associated with gene ontology terms involving fibrosis and macrophage activity.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). Expression ratio is measured with ddPCR assays and composed of 3 genes that are expressed primarily in early, immature myoblasts or mature, functional myofibers, respectively, and is calculated with reference to healthy individual samples. Expression ratio statistical differences were determined using paired *t*-test (**P* < 0.05). Grey boxes represent the mean, and error bars represent the standard error. BL, baseline; ddPCR, droplet digital PCR; DMD, Duchenne muscular dystrophy; NA, not applicable; PC, principal component.

- Following nivudirsen treatment, participants in the 351-201 trial had reduced serum CK and LDH, enzymes released into circulation by muscle cells when injured,^{4,6} consistent with reduced muscle damage when assessed in the context of dystrophin expression (**Figure 4A–C**)
- Reductions in participant uTitin, a biomarker of muscle damage,⁷ were also observed, suggesting a decrease in muscular injury (**Figure 4D**)

Figure 4. Serum A) creatine kinase and B) lactate dehydrogenase levels over time and C) relationship with change in dystrophin, and D) urinary titin levels over time following treatment initiation with nivudirsen



Serum and urine samples were collected every 2 and 4 weeks, respectively. Data cutoff: March 2026. Lines represent locally estimated scatterplot smoothing (LOESS) estimates and shaded areas represent 95% point-wise confidence intervals. Historical controls plotted from participants in the placebo arm of the drisapersen phase 3 trial (NCT01254019; n = 61 participants; age range, 5–16 years of age). To calculate change from baseline for creatine kinase and lactate dehydrogenase, multiple measurements were averaged to reduce variability. Baseline values included all measurements from screening through day 14, while post-dose values included all measurements within 14 days before or after biopsy. For panel C, quartiles represent 4 groups of participants based on change from baseline dystrophin levels, with approximately 25% of participants in each group. u, urinary.

Conclusions

- Nivudirsen shifted muscle transcriptomic profiles toward healthy tissue, with improvements in inflammatory and fibrotic pathways
- The myoblast-to-myofiber transcript ratio decreased following treatment, consistent with muscle repair and improved tissue composition
- Muscle injury biomarkers (CK, LDH, and uTitin) declined with nivudirsen treatment, supporting improved muscle quality and reduced ongoing muscle damage
- Future analysis will evaluate associations between transcriptomic changes, circulating biomarkers, and functional outcomes in 351-201 trial participants

References

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Acknowledgments

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Disclosures

LAW, KOM, KL, KH, DG, HL, MA, LS, JI, EW, LL, CR, RP, and MAS 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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