

SIMPLE WESTERN ADVANCES CUTTING-EDGE DUCHENNE MUSCULAR DYSTROPHY RESEARCH



INTRODUCTION TO DUCHENNE MUSCULAR DYSTROPHY

Duchenne Muscular Dystrophy (DMD) is a severe genetic disease characterized by the progressive breakdown of skeletal and cardiac muscles, resulting in weakness, loss of cardiac function and premature death. The cause of DMD is frameshift mutations to dystrophin, a gene responsible for linking the actin network to transmembrane components of the dystrophin-associated glycoprotein complex (DGC) (FIGURE 1). These mutations result in strong reduction or complete absence of functional dystrophin. While recent advancements in gene editing therapies are offering hope of restoring dystrophin function in human cells, reliable detection and quantification of dystrophin remains a challenge. As one of the largest proteins in the human body (427 kDa), its extreme molecular weight makes dystrophin very difficult to reliably detect, let alone quantify, with traditional techniques like [Western blot](#).

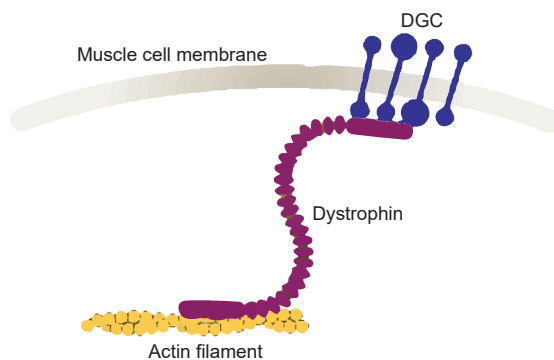


FIGURE 1. Schematic of dystrophin connecting the actin network to the transmembrane components of the DGC. In individuals with DMD, dystrophin is missing or made in very small amounts, which damages muscle tissue and ultimately leads to death by early adulthood.

SIMPLE WESTERN SIZES UP DYSTROPHIN

Dystrophin's large molecular weight is no problem for [Simple Western](#), a capillary-based immunoassay platform from ProteinSimple. With a range of up to 440 kDa, Simple Western can easily detect and quantify full-length dystrophin¹. This major advantage over traditional Western blot is accompanied by automation, high throughput, high sensitivity, and low-sample size requirements. As a result, Simple Western's contributions to DMD research is rapidly increasing²⁻¹², including in the world's premier journals like *Science*² and *Nature Medicine*³.

SIMPLE WESTERN VALIDATES PROMISING NEW DMD TREATMENT STRATEGIES

Among the recent breakthrough gene therapy studies with the potential to treat DMD, Simple Western is emerging as the method of choice to monitor dystrophin expression. Thus, Simple Western is quickly becoming an integral part in developing a cure to this tragic disease. We highlight two notable examples in this spotlight and provide references for additional reading.

SOMATIC GENE EDITING AMELIORATES SKELETAL AND CARDIAC MUSCLE FAILURE IN PIG AND HUMAN MODELS OF DUCHENNE MUSCULAR DYSTROPHY

Following genetic reprogramming to correct DMD, how do you know if dystrophin protein expression is restored? Simple Western is the solution, as was shown in this 2019 study in *Nature Medicine*³. Using AAVs as delivery vectors, Moretti et al. injected Cas9 and gRNA targeting dystrophin exon 51 in patient-derived induced pluripotent stem cells lacking dystrophin exon 52 (DMD Δ 52), an out-of-frame and non-functional isoform of dystrophin. The nuclease activity of Cas9 restored the DMD reading frame, creating a slightly shortened, but largely functional isoform of dystrophin (DMD Δ 51-52). The transduced cells were analyzed by Wes, a Simple Western instrument, for dystrophin expression. The quantification capability of Simple Western showed significantly increased dystrophin expression in treated skeletal muscle cells and cardiomyocytes (FIGURE 2).

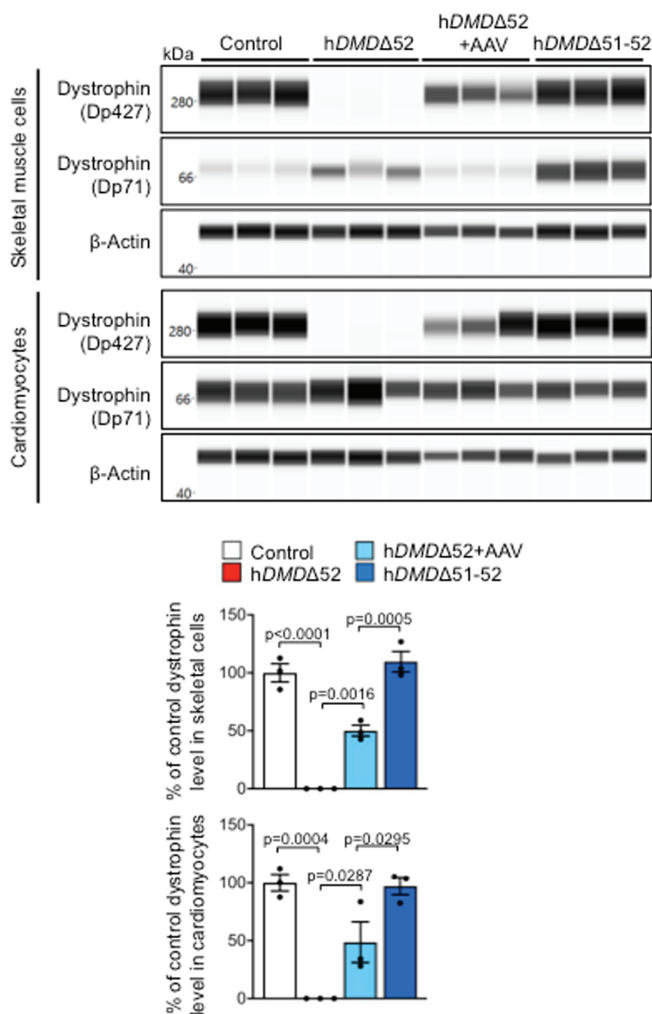


FIGURE 2. (Top) Dystrophin detection by Wes after myotube induction of control, untreated or AAV6-Cas9/gE51-transduced hDMD Δ 52 and hDMD Δ 51-52 skeletal myoblasts and in control, untreated or AAV6-Cas9/gE51-transduced hDMD Δ 52 and hDMD Δ 51-52 cardiomyocytes from 3 independent differentiations. Bands represent the main (Dp427) and a shorter dystrophin isoform (Dp71). β -actin, loading control. (Bottom) Dystrophin (Dp427) levels normalized to β -actin expressed as percentage of mean level in control cells are depicted for skeletal muscle cells and cardiomyocytes as mean \pm SEM (p values from one-way ANOVA with Bonferroni's multiple comparison test; Skeletal cells F=63.46, df=8, Cardiomyocytes F=21.59, df=8). Figure adapted from Moretti et al.³.

EXTRACELLULAR NANOVESICLES FOR PACKAGING OF CRISPR-CAS9 PROTEIN AND SGRNA TO INDUCE THERAPEUTIC EXON SKIPPING

Prolonged expression of the CRISPR-Cas9 nuclease and gRNA from viral vectors like AAV may cause off-target mutagenesis and immunogenicity. Thus, in this 2020 study in *Nature Communications*, Gee et al. used extracellular nanovesicles as transient delivery vectors to reduce these risks while restoring dystrophin function⁵. They used Cas9 and sgRNA targeting exon 45 SA (DMD1) and exon 45 SD (DMD23) in Δ exon 44 DMD iPSC-differentiated skeletal muscle cells. Targeting Cas9 to these sites can induce exon 45 skipping and restore dystrophin protein expression. Then, they used Wes to monitor dystrophin expression in response to treatment. Dystrophin protein expression in the iPSC-differentiated skeletal muscle cells correlated with exon skipping data and were highest when NanoMEDIC targeting SA and SD were multiplexed (FIGURE 3).

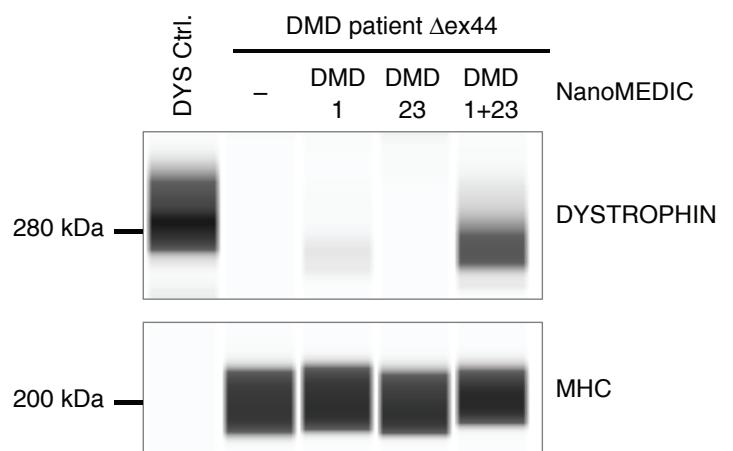


FIGURE 3. Dystrophin protein expression was analyzed by Wes and compared with HEK293T cells overexpressing dystrophin cDNA. Myosin heavy chain protein was also analyzed as a loading control. Results are representative of two independent Wes runs from a single experiment. Figure adapted from Gee et al.⁵.

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