Ultrasound-mediated delivery of non-viral full-length dystrophin vector to skeletal, heart and diaphragm muscle tissues in DMD mice and non-human primates.

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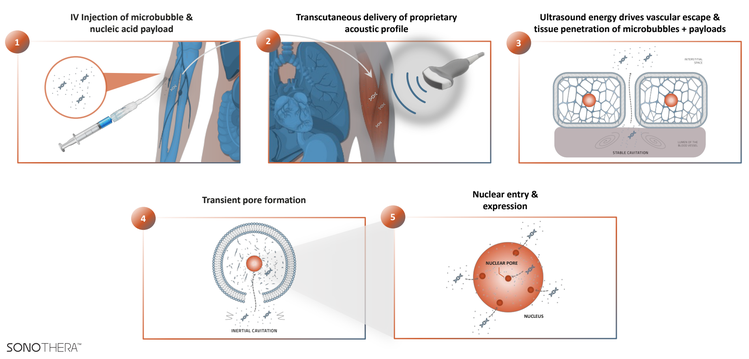
Duchenne muscular dystrophy (DMD) is a severe, progressive neuromuscular disorder caused by mutations in the dystrophin (DMD) gene, leading to a complete absence of functional dystrophin protein. Effective therapeutic strategies must restore dystrophin expression in skeletal, cardiac, and diaphragm muscles - the primary tissues affected by the disease. While current gene therapy approaches rely on truncated micro-dystrophin constructs delivered via viral vectors, their therapeutic efficacy is constrained by several limitations, including immunogenicity, inability to re-dose, restricted packaging capacity, and suboptimal tissue biodistribution. Notably, epidemiological data suggest that even low-level expression of full-length dystrophin (>0.5% of wild-type levels) may yield significant clinical benefit, highlighting the potential potency of the full-length DMD gene. Transcutaneous ultrasound-mediated gene delivery (UMGD) offers a noninvasive, non-viral and spatially targeted approach to address these challenges allowing efficient delivery of large genetic payloads. SonoThera has developed a proprietary UMGD-platform that enables highly efficient, selective, and durable delivery of genetic medicines to muscle tissues, offering a safe, redosable therapeutic solution for DMD and other muscle disorders.

To assess the safety and efficacy of UMGD for targeting muscle tissues, a luciferase reporter gene driven by a muscle-specific promoter was delivered to skeletal muscle, heart, and diaphragm in mice. This approach resulted in robust, tissue-selective luciferase expression with durable transgene activity, which was further enhanced following repeated dosing. Building on these findings, a DNA construct encoding the full-length human DMD transgene under the control of a muscle-specific promoter was developed. UMGD enabled efficient delivery of the full-length dystrophin transgene to skeletal, cardiac, and diaphragm muscles in DMD disease model mdx mice. Significant transgene expression and widespread biodistribution were confirmed by western blot, RNAscope and/or IHC assays.

To evaluate the feasibility of ultrasound-mediated gene delivery (UMGD) for delivering therapeutic payloads in large preclinical models, a non-viral DNA construct encoding the full-length DMD gene was delivered to skeletal muscle, heart, and diaphragm tissues in non-human primates (NHPs). Tissue analyses demonstrated high transgene delivery efficiency across all targeted muscle groups, with transgenic DNA detectable and stably maintained in tissues post-administration.

These results establish UMGD as an efficient and scalable platform for targeted delivery of genetic medicines to critical muscle groups affected in DMD. The approach enables robust expression of functional full-length dystrophin protein and provides a foundation for advancing next-generation therapeutic strategies with broad applicability across multiple forms of muscular dystrophy.

SonoThera delivery platform.



Durable luciferase expression >9 months in mouse skeletal muscle

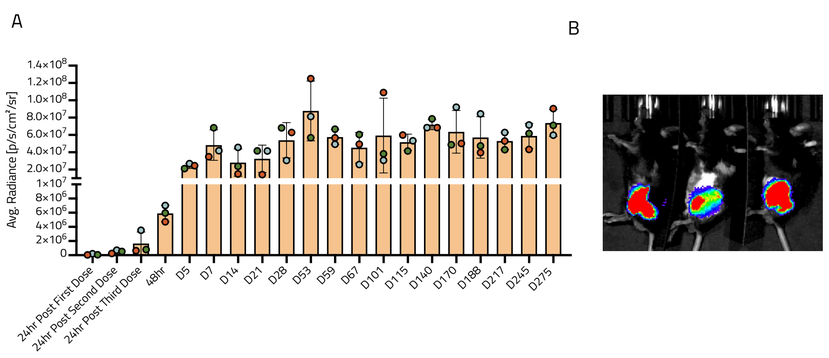


Figure 1: Expression of transgene in mouse skeletal muscle upon multiple UMGD-mediated delivery of DNA vector expressing firefly luciferase under control of muscle-specific promoter. (A) IVIS measurements over 275 days of observation. Error Bars SEM. (B) representative IVIS images.

Broad biodistribution and robust transgene expression in mouse skeletal muscle

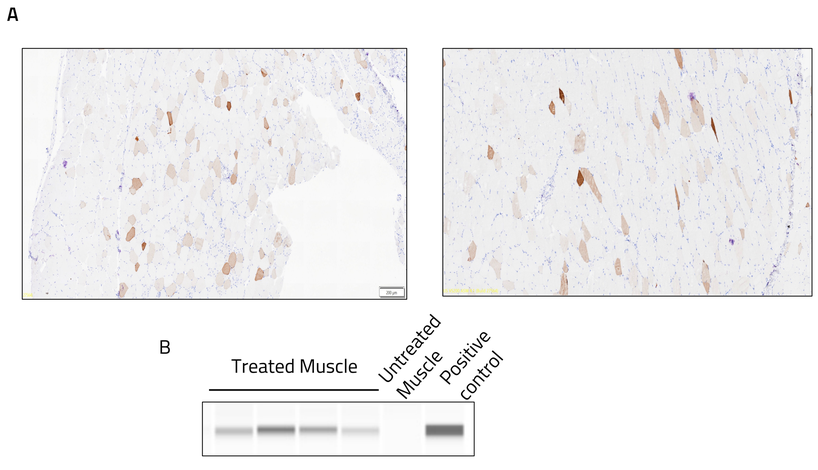


Figure 2: Expression of EGFP in mouse skeletal muscle one week post single treatment. (A) Representative images of IHC stain of treated tissues utilizing anti-EGFP antibody. (B) Capillary western blot analysis of treated and untreated tissues with anti-EGFP antibody. C2C12 cells transfected with EGFP expressing plasmid were used as positive control

Full-length human DMD expression in human DMD myocytes

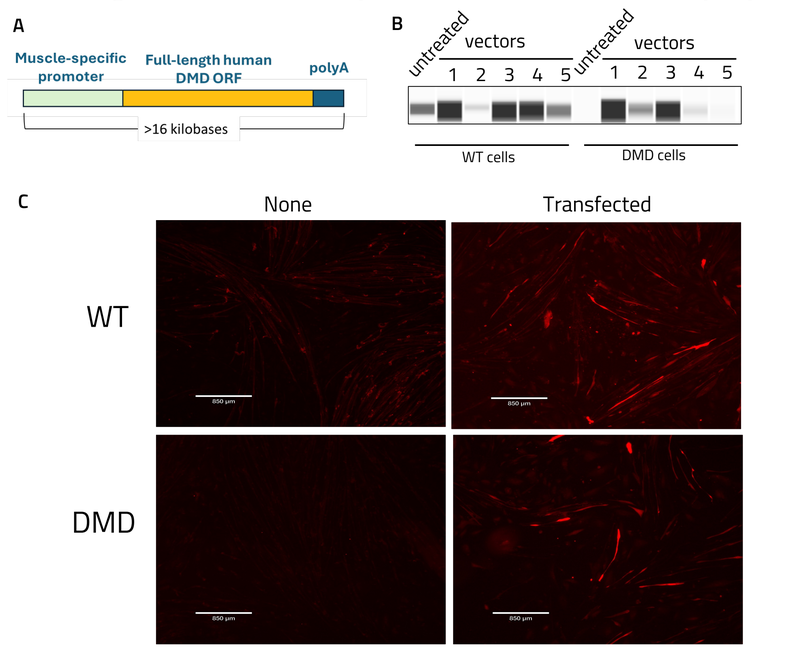


Figure 3: Expression of full-length human DMD in iPSC-derived DMD myocytes. (A) Schematic representation DNA vectors expressing full length human DMD under control of human-specific promoter. (B) Capillary western blot analysis of isogenic wild type control and DMD cells transfected with full-length DMD expressing vectors utilizing anti-DMD antibody. (C) Representative images of ICC stain of isogenic wild type control and DMD cells transfected with full-length DMD expressing vectors utilizing anti-DMD antibody. Untransfected cells were used as negative control

Robust full-length human DMD expression in mouse DMD disease model skeletal muscle

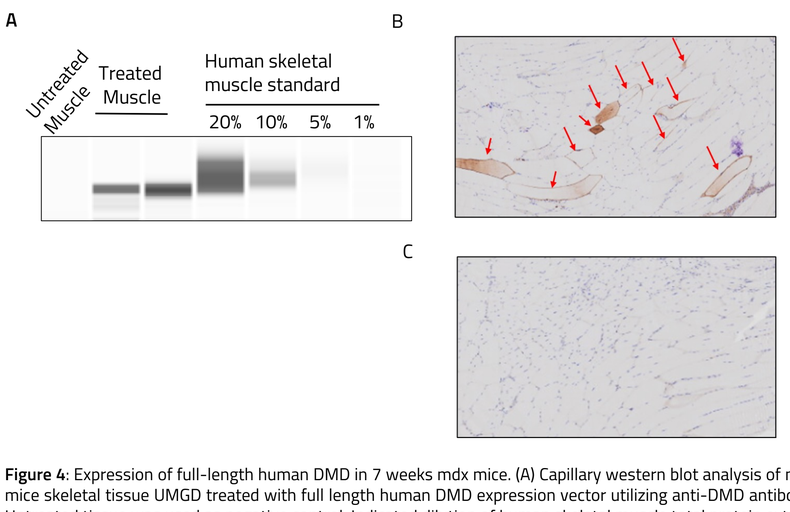


Figure 4: Expression of full-length human DMD in 7 weeks mdx mice. (A) Capillary western blot analysis of mdx mice skeletal tissue UMGD treated with full length human DMD expression vector utilizing anti-DMD antibody. Untreated tissue was used as negative control. Indicated dilution of human skeletal muscle total protein extract was used for expression level standard. (B) IHC analysis of treated mdx mouse skeletal muscle utilizing anti-DMD antibody. Red arrows mark fibers expressing full-length human DMD. (C) IHC analysis of age matched untreated mdx mouse skeletal muscle utilizing anti-DMD antibody. Of note, no revertant fibers were detected

Robust full length human DMD expression in NHP skeletal muscle

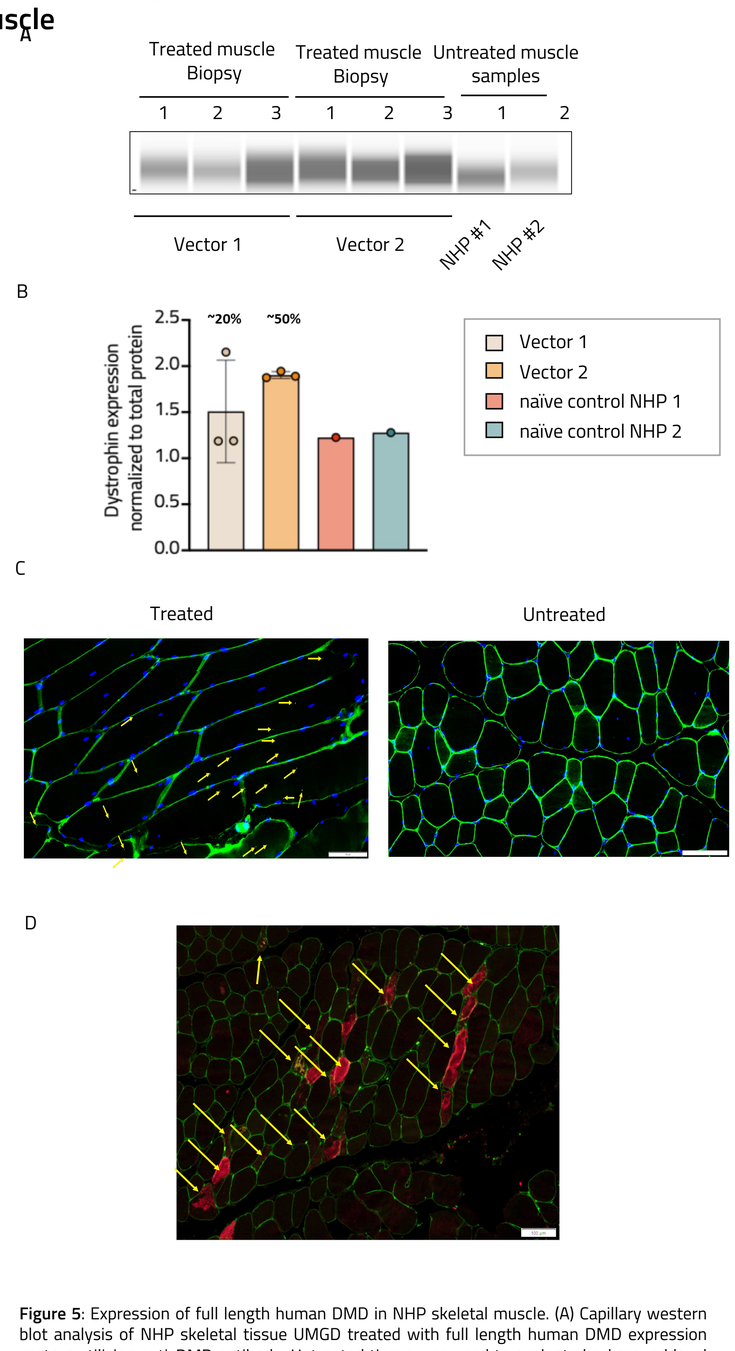


Figure 5: Expression of full length human DMD in NHP skeletal muscle. (A) Capillary western blot analysis of NHP skeletal tissue UMGD treated with full length human DMD expression vectors utilizing anti-DMD antibody. Untreated tissue was used to evaluate background level of endogenous NHP DMD protein expression. Of note, the anti-DMD antibody used cannot discriminate transgenic human DMD protein from endogenous monkey DMD. (B) Western blot signal quantification of the total DMD protein abundance. DMD signal normalized to the total protein. (C) BaseScope analysis of the treated NHP muscle tissues utilizing probe detecting transgenic DMD mRNA (yellow – transgenic DMD mRNA; green – WGA). Untreated NHP muscle tissue was stained as negative control. Yellow arrows mark transgenic DMD mRNA signal. (D) IHC analysis of NHP skeletal muscle tissue UMGD treated with DNA vector expressing HA-tagged full-length human DMD. The signal was detected by anti-HA antibody (red – HA; green – WGA). Yellow arrows mark skeletal fibers expressing full-length human DMD protein.

Transgene expression in mouse heart

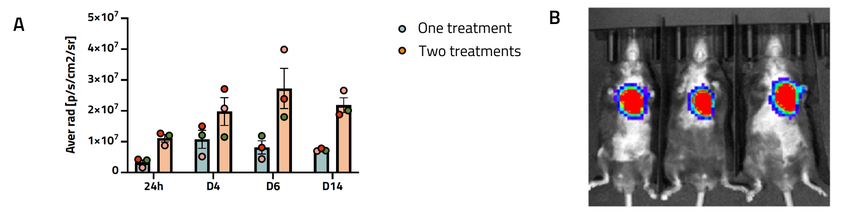


Figure 6: Mouse heart was single or double UMGD-treated with transgene expressing firefly luciferase (A) IVIS measurement of luciferase expression during 2 weeks post treatment. (B) Representative image of IVIS signal in treated mice

Full length human DMD expression in DMD disease mouse model heart

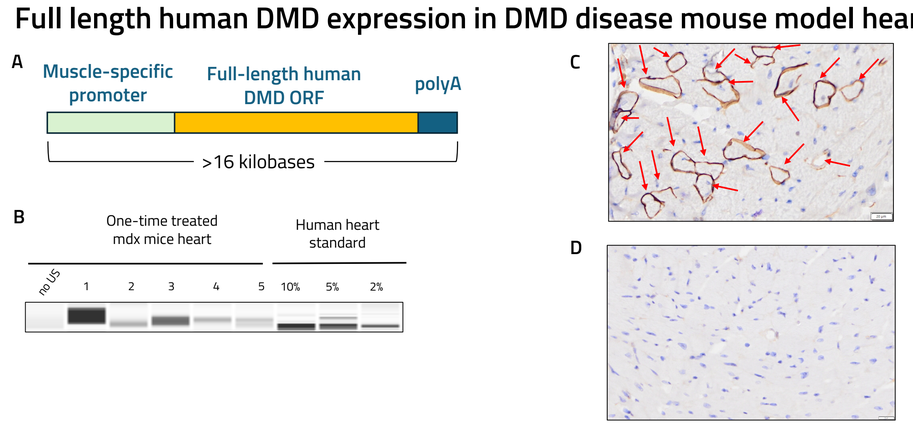


Figure 7. Full length human dystrophin is expressed in 7 weeks old mdx mouse heart upon UMGD treatment (A) Schematic representation DNA vectors expressing full length human DMD under control of human-specific promoter. (B) Capillary western blot analysis of mdx mice heart tissue UMGD treated with full length human DMD expression vector utilizing anti-DMD antibody. Untreated tissue was used as negative control. Indicated dilution of human heart muscle total protein extract was used for expression level standard. (C) IHC analysis of treated mdx mouse heart muscle utilizing anti-DMD antibody. Red arrows mark cells expressing full-length human DMD. (D) IHC analysis of age matched untreated mdx mouse heart muscle utilizing anti-DMD antibody. Of note, no revertant cells were detected

Transgene expression in mouse diaphragm

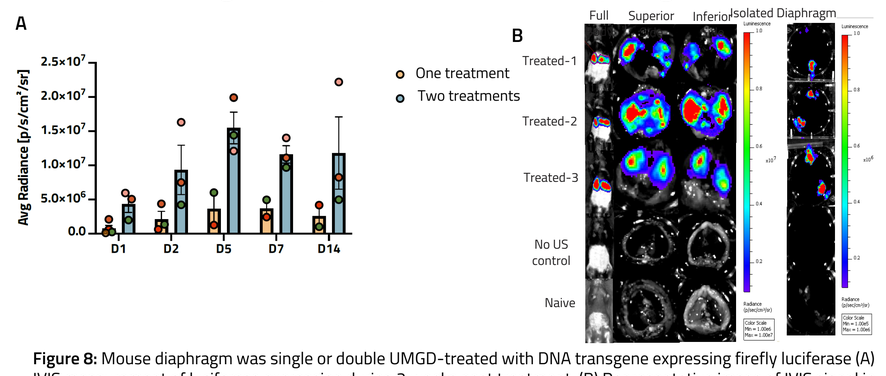


Figure 8: Mouse diaphragm was single or double UMGD-treated with DNA transgene expressing firefly luciferase (A) IVIS measurement of luciferase expression during 2 weeks post treatment. (B) Representative image of IVIS signal in treated mice .

Full length human DMD expression in DMD disease mouse model heart

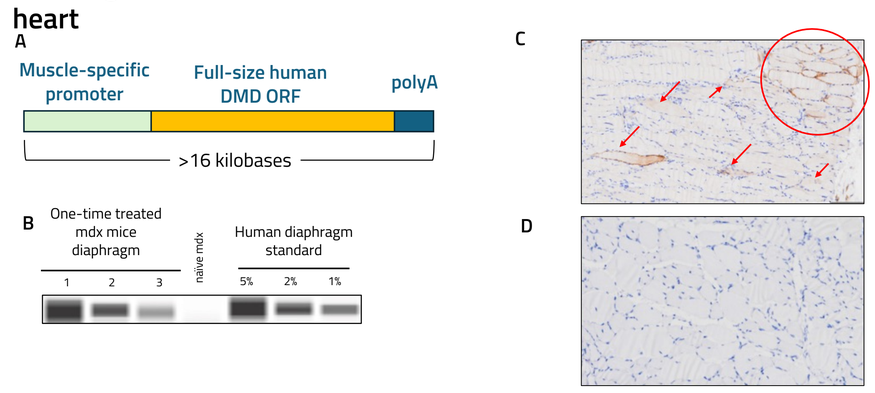


Figure 9: Full length human dystrophin is expressed in 7 weeks old mdx mouse diaphragm upon UMGD treatment (A) Schematic representation DNA vectors expressing full length human DMD under control of human-specific promoter. (B) Capillary western blot analysis of mdx mice diaphragm tissue UMGD treated with full length human DMD expression vector utilizing anti-DMD antibody. Untreated tissue was used as negative control. Indicated dilution of human diaphragm muscle total protein extract was used for expression level standard. (C) IHC analysis of treated mdx mouse diaphragm muscle utilizing anti-DMD antibody. Red arrows and circle mark cells expressing full-length human DMD. (D) IHC analysis of age matched untreated diaphragm mouse skeletal muscle utilizing anti-DMD antibody. Of note, no revertant cells were detected

Efficient transgenic DNA delivery to NHP heart

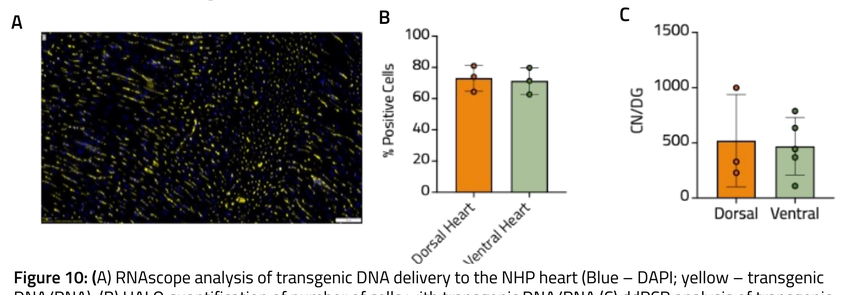


Figure 10: (A) RNAscope analysis of transgenic DNA delivery to the NHP heart (Blue – DAPI; yellow – transgenic DNA/RNA), (B) HALO quantification of number of cells with transgenic DNA/RNA (C) ddPCR analysis of transgenic DNA copy number per diploid genome

Efficient transgenic DNA delivery to NHP heart

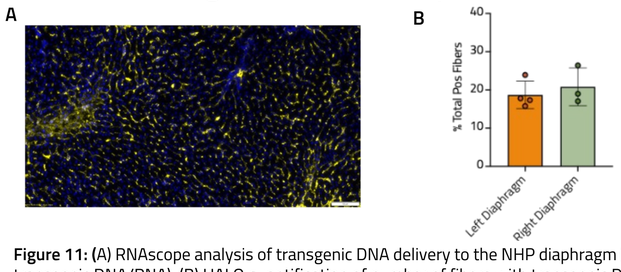


Figure 11: (A) RNAscope analysis of transgenic DNA delivery to the NHP diaphragm (Blue – DAPI; yellow – transgenic DNA/RNA), (B) HALO quantification of number of fibers with transgenic DNA/RNA

Efficient transgenic DNA delivery to major muscle groups in one UMGD treatment

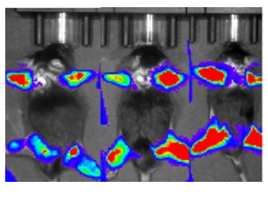


Figure 12: IVIS analysis of the firefly luciferase expression from DNA vector delivered to major mouse skeletal muscle groups upon single UMGD treatment

Summary

- established ultrasound-mediated gene delivery conditions for efficient and durable transgenic DNA delivery to mouse and NHP skeletal, hear and diaphragm muscle tissues
- developed potent full-length human DMD expression vector allowing full restoration of DMD expression in human DMD myocytes
- in DMD disease mouse model, achieved robust full length human DMD expression in major organs affected in DMD patients
- up to 50% of normal full length DMD expression is achieved in NHP skeletal tissue