

Non-Viral Genetic Medicine for Targeted Delivery of Full-Length Dystrophin to Skeletal, Cardiac, and Diaphragm Muscles in DMD Mouse Models and Non-Human Primates.

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Duchenne muscular dystrophy (DMD) is a life-threatening genetic condition caused by mutations in the DMD gene, which result in the complete loss of functional dystrophin protein. To effectively treat DMD, therapies must aim to restore dystrophin production in the skeletal, cardiac, and diaphragm muscles, as these are the primary tissues affected by the disorder. Current gene therapy strategies utilizing truncated micro-dystrophin transgenes have faced challenges in achieving effective outcomes. These challenges stem in part from the limitations of viral delivery systems, including immune responses, inability to re-dose, restricted payload capacity, and limited organ biodistribution. Epidemiological studies of DMD patients indicate that even minimal expression levels of full-length dystrophin gene (>0.5% of normal levels) may significantly alleviate the disease burden. This underscores the necessity to develop advanced non-viral genetic medicines capable of delivering full-length dystrophin (DMD) transgenes to achieve robust therapeutic efficacy. Transcutaneous ultrasound-mediated gene delivery (UMGD) represents a noninvasive and precision-targeted approach to address current challenges in genetic medicine delivery. SonoThera is advancing a proprietary UMGD-based non-viral genetic medicine platform designed to enable highly selective and efficient targeting of skeletal, cardiac, and diaphragm muscle tissues, offering a safe, extremely durable and redosable therapeutic solution.

To evaluate the potential of UMGD for safe and effective gene delivery to these muscle tissues, a nonviral DNA construct incorporating full-length human DMD transgene driven by a muscle-specific promoter was developed. Under optimized UMGD conditions, efficient delivery of the full-length DMD transgene was achieved in the skeletal, cardiac, and diaphragm muscles of the DMD disease (mdx) mouse model. Further analysis confirmed robust transgene expression across the targeted muscle tissues in the mdx mice.

To assess the feasibility of UMGD for delivering therapeutic payloads in large preclinical models, the full-length DMD nonviral DNA construct was delivered to muscle tissues in non-human primates (NHPs). Molecular analyses confirmed efficient transgene delivery to the NHP target tissues, resulting in high levels of transgenic DMD protein expression in the treated tissues.

These findings position UMGD as an efficient and scalable platform for delivering genetic medicines to key muscle groups impacted in DMD patients, facilitating robust expression of functional full-length dystrophin protein. This innovative approach lays a foundation for the development of next-generation therapeutic strategies with broad potential applications across various muscular dystrophies.

Robust transgene expression in skeletal muscle following repeat treatment

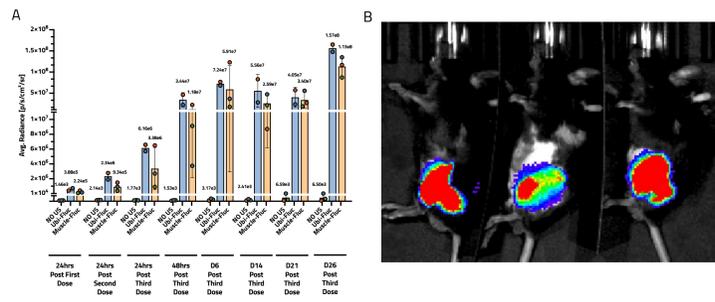


Figure 1: Efficient transgene delivery to mouse skeletal muscle. A) IVIS signal from treated skeletal muscle after one, two or three UMGD treatments. B) Representative bioluminescent images of mice treated with skeletal targeted UMGD.

Robust transgene expression in heart muscle following repeat treatment

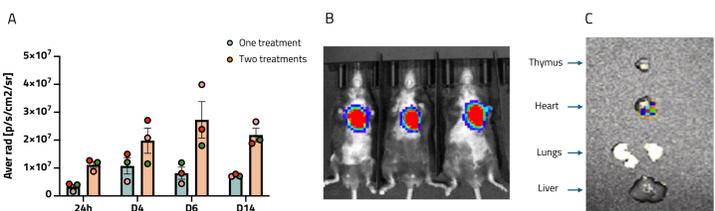


Figure 2: Efficient transgene delivery to mouse heart muscle. A) IVIS signal from treated heart muscle after one or two UMGD treatments. B) Representative bioluminescent images of mice treated with heart targeted UMGD. C) Representative IVIS images of isolated thymus, heart, lung and liver from UMGD treated mouse

Robust transgene expression in diaphragm muscle following repeat treatment

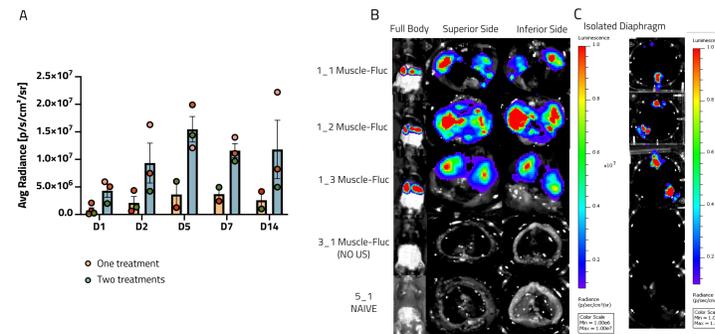


Figure 3: Efficient transgene delivery to mouse diaphragm muscle. A) IVIS signal from treated diaphragm muscle after one or two UMGD treatments. B) Representative bioluminescent images of mice treated with diaphragm targeted UMGD. C) Representative IVIS images of isolated diaphragm from UMGD treated and control mice

Summary:

- » Established UMGD conditions for targeted, redosable, and durable payload delivery to mouse and NHP skeletal, heart, and diaphragm muscle tissues.
- » Demonstrated full-length dystrophin transgene can be efficiently delivered to the skeletal, heart, and diaphragm muscles of a DMD mouse model (mdx) to provide 5% of normal human dystrophin protein expression following a single dose, as determined by Western Blot assay.
- » Demonstrated efficient delivery of large transgenes, including expression of full-length dystrophin, to NHP skeletal, heart, and diaphragm muscles.

Robust transgene expression in mdx mice skeletal muscle

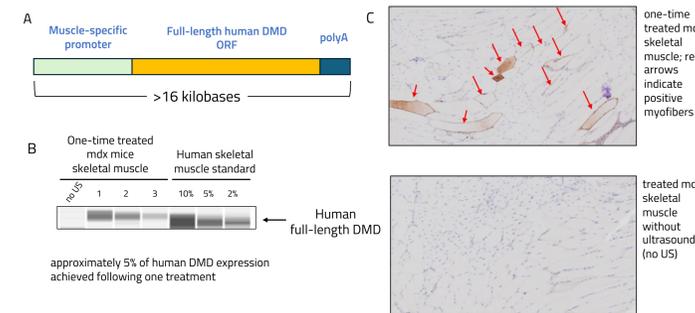


Figure 4: Efficient human full-length DMD transgene delivery to mdx mouse skeletal muscle. A) Vector schematics. B) Capillary western blot analysis of transgenic DMD expression in skeletal muscle. C) IHC analysis of skeletal tissue with anti-DMD antibody.

Robust transgene expression in mdx mice heart muscle

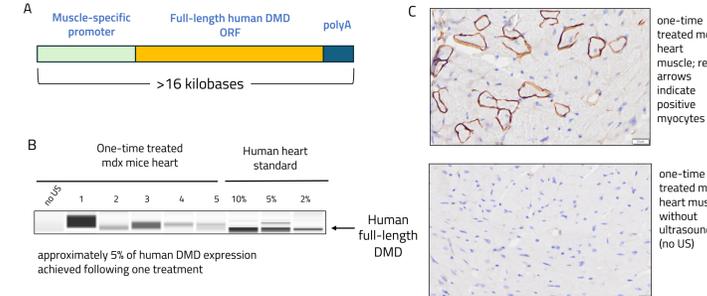


Figure 5: Efficient human full-length DMD transgene delivery to mdx mouse heart muscle. A) Vector schematics. B) Capillary western blot analysis of transgenic DMD expression in heart muscle. C) IHC analysis of heart tissue with anti-DMD antibody.

Robust transgene expression in mdx mice diaphragm muscle

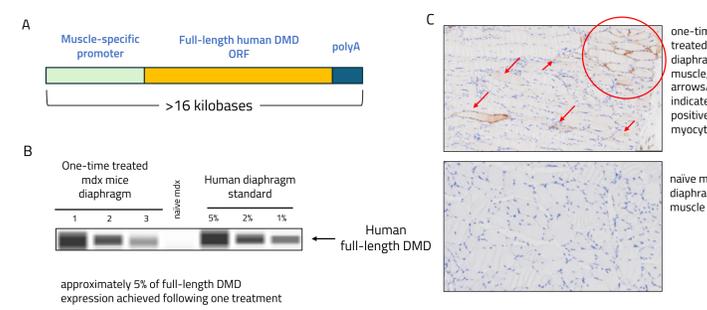


Figure 6: Efficient human full-length DMD transgene delivery to mdx mouse diaphragm muscle. A) Vector schematics. B) Capillary western blot analysis of transgenic DMD expression in diaphragm muscle. C) IHC analysis of diaphragm tissue with anti-DMD antibody.

Robust delivery of full-length human dystrophin to NHP skeletal muscle

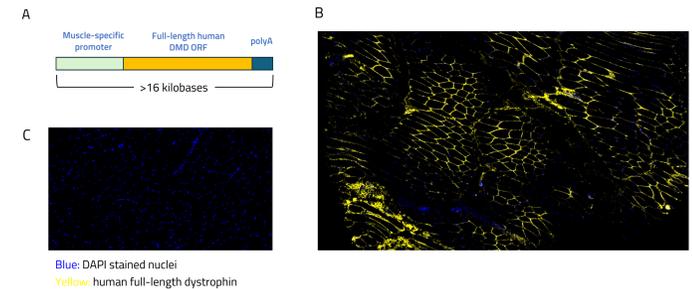


Figure 7: Efficient human full-length DMD transgene delivery to NHP skeletal muscle. A) Vector schematics. B) RNAscope analysis of UMGD treated NHP skeletal tissue. C) Naive tissue RNAscope staining.

Robust delivery of full-length human dystrophin to NHP heart muscle

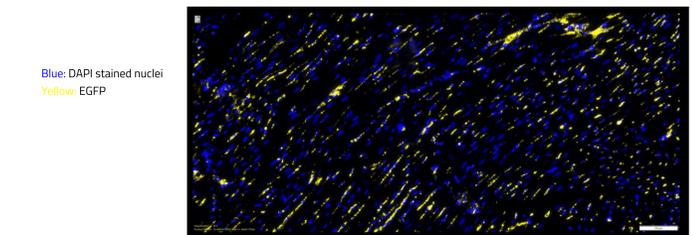


Figure 8: Efficient transgene delivery to NHP heart muscle. RNAscope analysis of UMGD treated NHP heart tissue.

Robust delivery of full-length human dystrophin to NHP diaphragm muscle

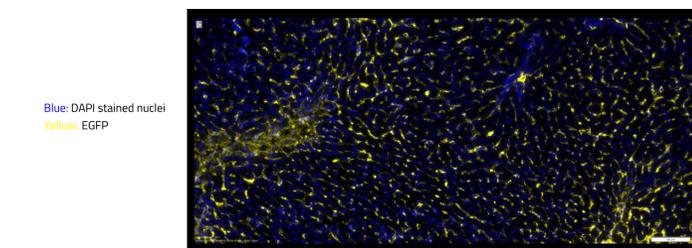


Figure 9: Efficient transgene delivery to NHP diaphragm muscle. RNAscope analysis of UMGD treated NHP diaphragm tissue.

SonoThera delivery platform

1. Ultrasound enhancing agents (a.k.a. microbubbles) co-infused intravenously with nucleic acid payload (e.g. DNA/RNA)
2. Ultrasound energy is externally applied to target organ
3. Expression of the therapeutic payload occurs in target cells

