ARCUS-mediated excision of the “hot spot” region of the human dystrophin gene results in functional improvement in a mouse model of Duchenne muscular dystrophy (DMD)

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INTRODUCTION

- Duchenne muscular dystrophy (DMD) is a genetic disorder associated with mutations in the dystrophin gene that prevent production of the dystrophin protein. Over time, children with DMD will develop problems walking and breathing, eventually leading to death in the second or third decade of life due to progressive cardiomyopathy and respiratory insufficiency.
- While there are many known pathogenic mutations that can cause DMD, ~50% of patients contain mutations within a “hot-spot” region of the gene between exons 45 and 55. DMD occurs in 1 in 3,500 to 5,000 male births, and currently there are limited approved therapies available for patients.
- Here we describe an ARCUS gene-editing approach for the treatment of DMD, which could allow for a single administration of a drug with possible life-long benefits and could be applicable to the broad population of patients with mutations in the dystrophin hot-spot region.

ARCUS THERAPEUTIC APPROACH

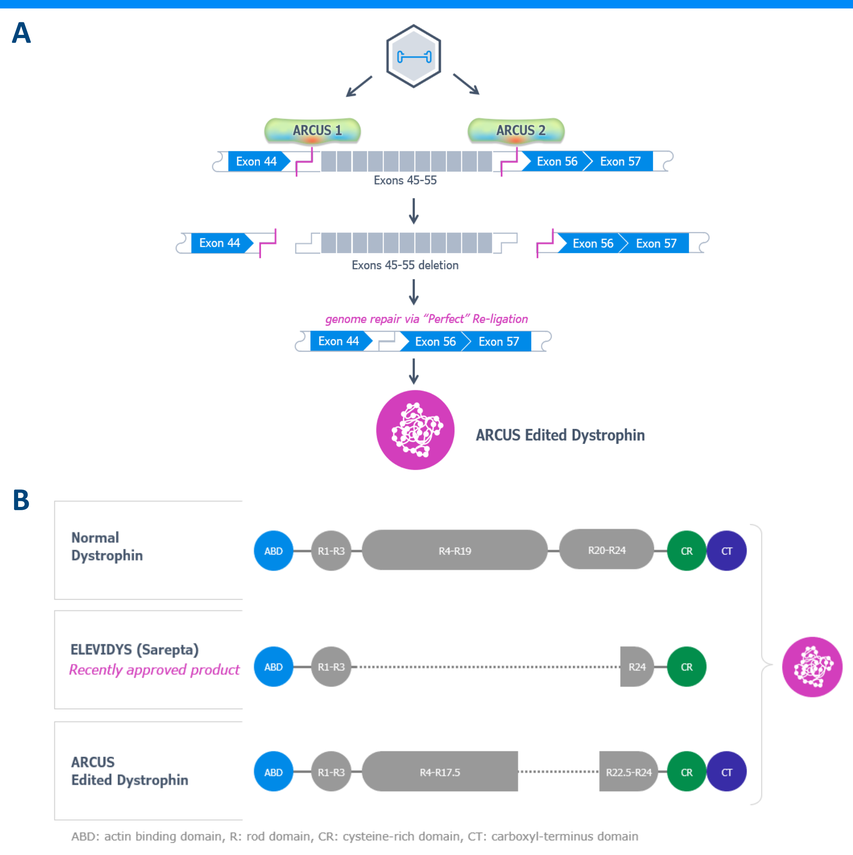

FIGURE 1. (A) Delivery of a single AAV encoding a pair of sequence-specific ARCUS nucleases that generate complementary 4 base pair, 3’ overhangs at their target sites to excise exons 45-55, restore the reading frame of the dystrophin protein following perfect re-ligation of the gene, and generate a functionally competent variant of the dystrophin protein. (B) ARCUS-edited dystrophin preserves majority of the natural protein domains with the goal of improving function.

METHODS

- To test the functional capacity of the ARCUS edited dystrophin, a study was conducted in a DMD mouse model. Mice were administered a single dose of AAV encoding the two DMD ARCUS nucleases with end point assessment at 8 weeks post dosing. Treated mice were compared to both untreated disease (*hDMD del52/mdx)* and healthy mice (*hDMD/mdx).*
- Excision of the hot spot region was measured using digital droplet PCR (ddPCR) utilizing primer pairs and probes that span the junction of the ARCUS 1 and ARCUS 2 target sites.
- Restoration of dystrophin was measured using WES, Protein Simple™. Dystrophin restoration was quantified comparing dystrophin protein extracted from target tissues to a protein standard curve generated from serial dilutions of total protein extracted from non-disease *hDMD/MDX* mouse.
- Muscle performance was measured *in vivo* with a 305C muscle lever system. A series of stimulations at increasing frequencies were performed on the plantar flexor muscle group. Force frequency was measured at 1, 20, 40, 50, 60, 80 &150Hz and maximal peak isometric force was recorded.
- Dystrophin protein restoration was characterized in the gastrocnemius with histological analysis using immunofluorescence. Muscle satellite cell editing was explored using BaseScope™ with probes specific for PAX7 and a probe specific for the exon 44/56 junction.

RESULTS

ARCUS Excision Results in “Perfect” Re-ligation

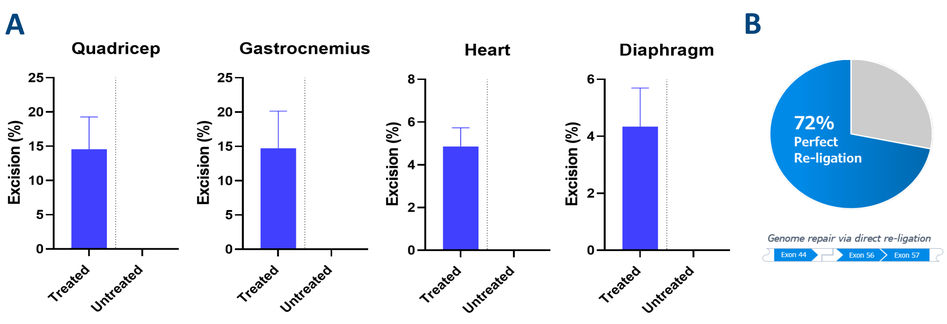

FIGURE 2. (A) Genomic excision of exons 45-55 was measured in all target tissues in a DMD mouse model. Excision was only detected in ARCUS-treated mice with 15% of genomes excised in skeletal muscle and >4% excision measured in the heart and diaphragm. (B) ARCUS nucleases cut their target sites leaving complementary 3’ overhangs. NGS analysis revealed that 72% of the excision events that occurred in the quadricep were repaired via perfect re-ligation.

RESULTS

ARCUS Editing Results in Dystrophin Protein Restoration

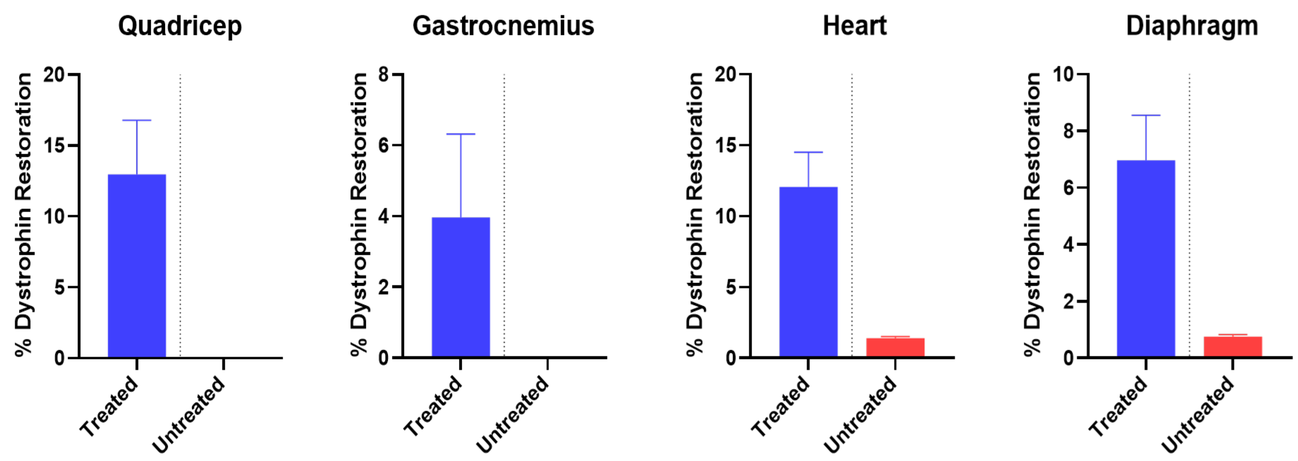

FIGURE 3. Dystrophin protein restoration was detected in skeletal, cardiac and diaphragm muscles of ARCUS-treated mice.

Maximum Force Output in the Gastrocnemius of ARCUS-treated Mice was Significantly Improved

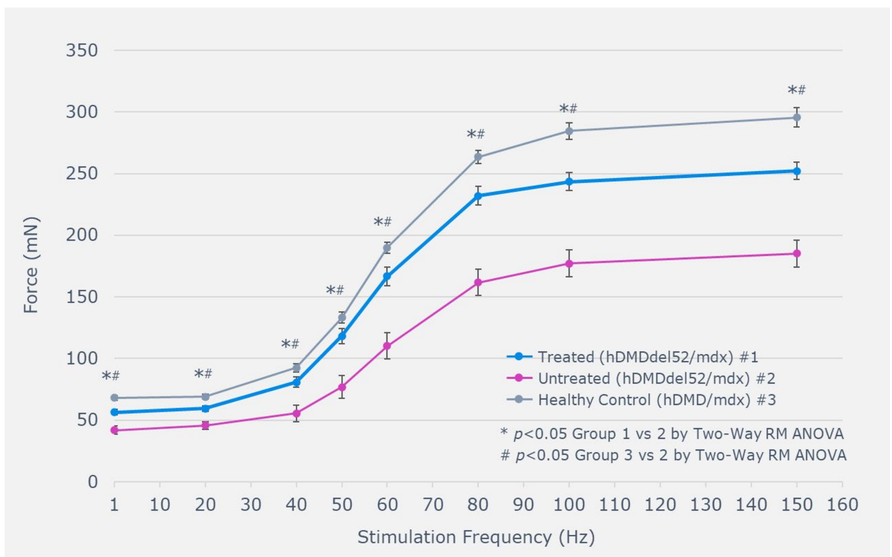

FIGURE 4. ARCUS treated mice (blue) had a significant improvement over untreated, diseased mice (pink), and had 86% force output of the non-diseased healthy mice (gray). These results demonstrate that the observed levels of an ARCUS-edited dystrophin protein produced meaningful therapeutic results.

Dystrophin Expression was Restored in ARCUS-treated Mice

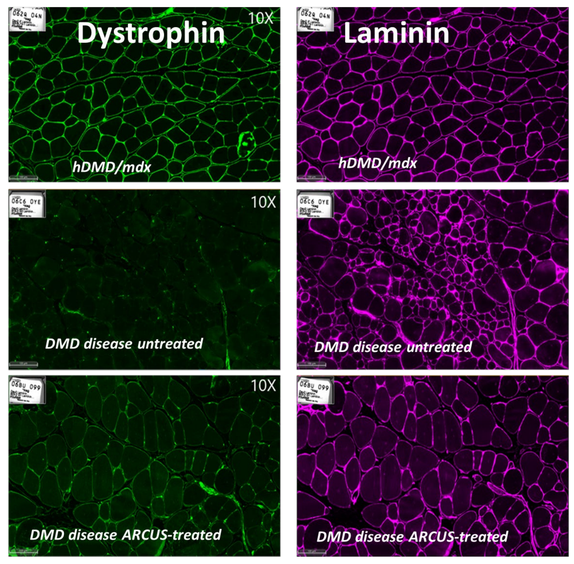

FIGURE 5. Histological tissue slides of ARCUS-treated gastrocnemius stained for human dystrophin and laminin as a marker for muscle fiber. Dystrophin protein is absent from non-treated diseased mice compared to the restoration seen in ARCUS-treated diseased mice.

ARCUS Edits Muscle Satellite Stem Cells

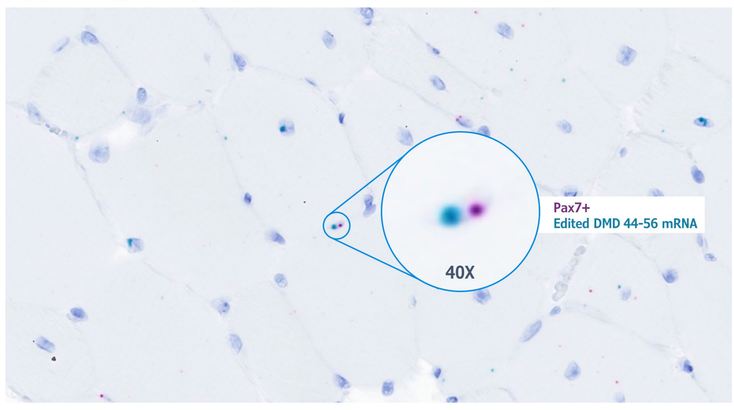

FIGURE 6. Histological tissue slides of ARCUS-treated gastrocnemius stained with BaseScope ™. Above example of muscle satellite cells identified by the purple PAX7 stain, also contain a blue signal, which indicates ARCUS editing (exon 44-56 splice edit) has occurred in this satellite cell type.

CONCLUSIONS

- **Following a single AAV delivery of two ARCUS nucleases, we observed the excision of exons 45-55 and the edited dystrophin protein in multiple tissue types including heart, diaphragm, and skeletal muscle.**
- **72% of the excision events in quadricep occurred by perfect re-ligation of the ARCUS target sites. This is likely attributable to the unique 3’ overhangs generated by ARCUS.**
- **The maximum force output (MFO) of the gastrocnemius muscle in ARCUS-treated animals was significantly improved, reaching 86% of the MFO levels observed in the non-disease, control mice.**
- **Dystrophin protein was restored to muscle fibers with evidence of the edited dystrophin transcript in PAX7+ cells, a marker for muscle satellite cells.**
- **This proof-of-concept study demonstrates the therapeutic potential of an ARCUS gene editing approach for the treatment of DMD and supports ongoing development toward clinical candidate nomination.**

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PRECISION BIOSCIENCES

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