Rescue of Full-Length Dystrophin Protein in a Mouse Model of Duchenne Muscular Dystrophy Using an AAV-tRNA Therapeutic

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DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is a fatal X-linked disorder caused by the absence of functional dystrophin, a large structural protein critical to the integrity of skeletal and cardiac muscle cells. DMD is characterized by progressive loss of muscle, respiratory insufficiency, and dilated cardiomyopathy. Existing therapeutic strategies are unable to restore full-length dystrophin due to the large size of the protein (427 kDa) and the diversity of unique mutations.

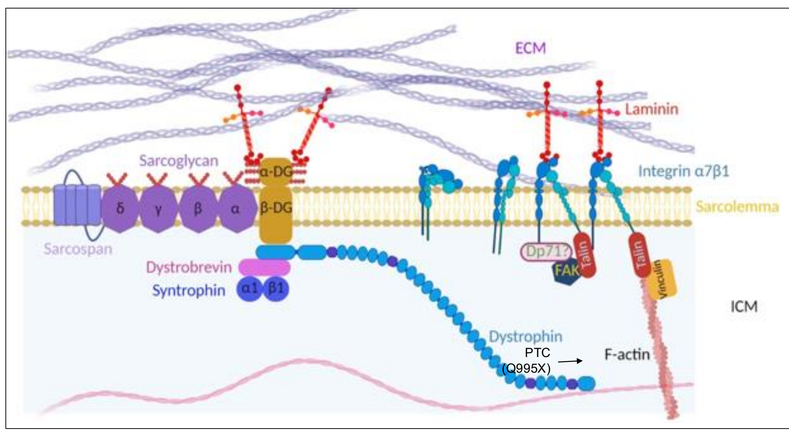

Figure 1. Dystrophin is the central protein of the dystrophin-glycoprotein complex (DGC) in muscle cells. Dystrophin connects the actin cytoskeleton to the extracellular matrix (ECM) to direct biomechanical forces during muscle contraction. Severing the link between the ECM and the intracellular cytoskeleton has a devastating impact on the homeostasis of skeletal muscle cells, leading to a range of muscular dystrophies. (*Wilson et al. Communications biology, 2022*)

SUPPRESSOR tRNA USED TO RESTORE DYSTROPHIN EXPRESSION

Nonsense mutations, in which a premature termination codon prevents translation of full-length dystrophin, are found in approximately 15% of patients with muscular dystrophy and are associated with more severe forms of the disorder. Suppressor tRNAs (SuptRNAs) are tRNA molecules in which the anticodon has been altered to enable ribosomes to read through nonsense mutations and produce full-length, functional protein. D2-*mdx* mouse model, which contains a TAA nonsense mutation and recapitulates key aspects of DMD pathology in humans, was used to test whether a transgene encoding a suppressor tRNA (Gln>TAA, tr0904) delivered systemically using a myotropic Adeno-Associated (MyoAAV2A) vector can restore full-length dystrophin protein expression and improve motor function.

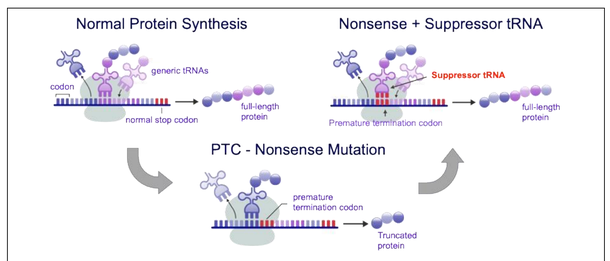

Figure 2. SuptRNAs read through Premature Termination Codons. Tevard Biosciences develops tRNA-based therapies with the potential to provide durable cures for DMD patients with nonsense mutations (TAA, TAG, TGA)

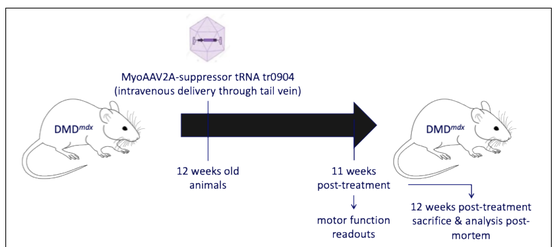

Figure 3. Gln>TAA suptRNA tr0904 was delivered with a myotropic capsid in D2-*mdx* mice through systemic delivery. SuptRNA safety and efficacy to restore dystrophin expression was determined 12 weeks post-injection

FULL-LENGTH DYSTROPHIN EXPRESSION IS RESTORED AFTER SUPtRNA tr0904 TREATMENT

- ❑ SuptRNA tr0904 is expressed in D2-*mdx* heart and skeletal muscles after systemic delivery using MyoAAV2A myotropic capsid.
- ❑ SuptRNA tr0904 restores full-length dystrophin protein in heart and skeletal muscles in a dose-dependent manner (up to 27% in heart, and 34% in skeletal muscle).

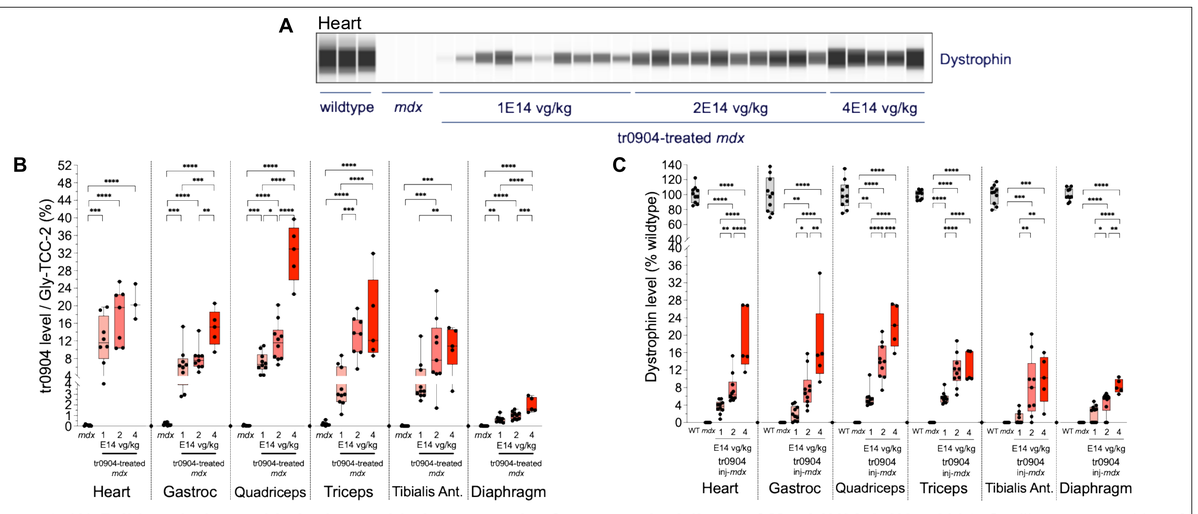

Figure 4. (A) Full-length dystrophin is detected in heart muscle after systemic delivery of MyoAAV2A-3xU6_tr0904. Capillary western blot of dystrophin expression 12 weeks after treatment with tr0904. **(B) SuptRNA tr0904 expression shows a dose-dependent increase in cardiac and skeletal muscles**. tr0904 expression was quantified by ddPCR and normalized to endogenous Gly-TCC-2 tRNA (%). **(C) SuptRNA tr0904 restores dystrophin protein expression in cardiac and skeletal muscles in a dose-dependent manner**. Dystrophin levels were measured by normalizing dystrophin to total protein signal intensity. (B)-(C) Plots show individual data and mean ± SEM. One-Way Anova test *<0.05, **<0.005, ***<0.0005 ****<0.00005.

AMELIORATION OF MORPHOLOGICAL PATHOLOGY IN CARDIAC AND SKELETAL MUSCLES FOLLOWING tr0904 TREATMENT

- ❑ Dystrophin is correctly organized at the muscle membrane in tr0904-treated D2-*mdx* mice.
- ❑ Up to 100% of muscle fibers are positive for dystrophin expression in tr0904-treated D2-*mdx* cardiac muscle.
- ❑ Muscle fiber size is reversed to wildtype levels in tr0904-treated D2-*mdx* cardiac muscle.

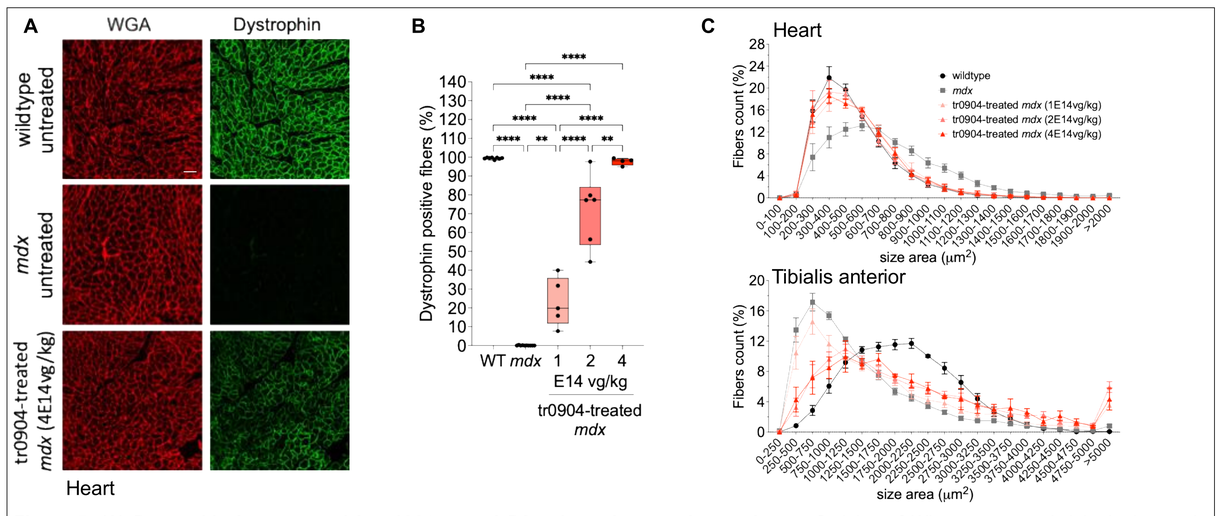

Figure 5. (A) Dystrophin is expressed in tr0904-treated D2-*mdx* at the muscle membrane. Staining of Wheat germ agglutinin (red, muscle membrane marker) and dystrophin (green) in cardiac muscles from wildtype, D2-*mdx*, and tr0904-treated mice (4E14vg/kg). **(B) Up to 100% muscle fibers express dystrophin in cardiac muscle following tr0904 treatment.** Quantification of dystrophin positive fibers normalized to total number of muscle fibers (%). Plots show individual data and mean ± SEM. One-Way Anova test *<0.05, ****<0.00005. **(C) Muscle fiber size distribution reverses to wild type following tr0904 treatment.** Classification of muscle fiber according to their size area range (µm²). Plots show mean ± SEM.

MOTOR FUNCTION IS IMPROVED AFTER tr0904 TREATMENT

- ❑ SuptRNA tr0904 leads to a dose-dependent restoration of motor function.
- ❑ SuptRNA tr0904 treatment of D2-*mdx* mice restores proteomic regulation (i.e. membrane repair, muscle injury) in a dose-dependent manner.
- ❑ IV delivery of SuptRNA tr0904 does not induce histopathologic changes in D2-*mdx* mice and significant off-target activity read through of normal TAA stop codons (data not shown).

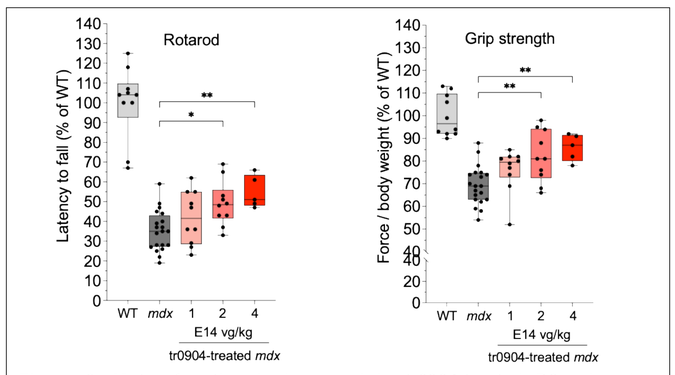

Figure 6. Motor function is improved in tr0904-treated D2-*mdx* mice. Untreated wildtype, D2-*mdx*, and tr0904-treated D2-*mdx* mice were compared for their grip strength (left) and their latency to fall from a rotating rotarod (right) 11 weeks post-treatment. Plots show individual data normalized to wildtype average and mean ± SEM. One-Way Anova test *<0.05, **<0.005.

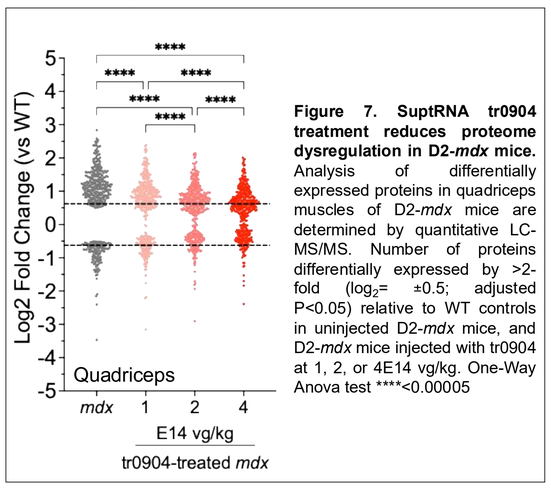

Figure 7. SuptRNA tr0904 treatment reduces proteome dysregulation in D2-*mdx* mice. Analysis of differentially expressed proteins in quadriceps muscles of D2-*mdx* mice are determined by quantitative LC-MS/MS. Number of proteins differentially expressed by >2-fold (log_2= ±0.5; adjusted P<0.05) relative to WT controls in uninjected D2-*mdx* mice, and D2-*mdx* mice injected with tr0904 at 1, 2, or 4E14 vg/kg. One-Way Anova test ****<0.00005

A NEW GENERATION OF SUPtRNAS OUTPERFORMS TR0904

- ❑ The third generation SuptRNA tr1622 can restore Dystrophin expression to 46% of wildtype levels after local delivery to tibialis anterior muscle.
- ❑ Tight correlation was observed between Gln>TAA, Gln>TGA, and Gln>TAG suptRNAs activity making them suitable to read through the whole spectrum of nonsense mutations.

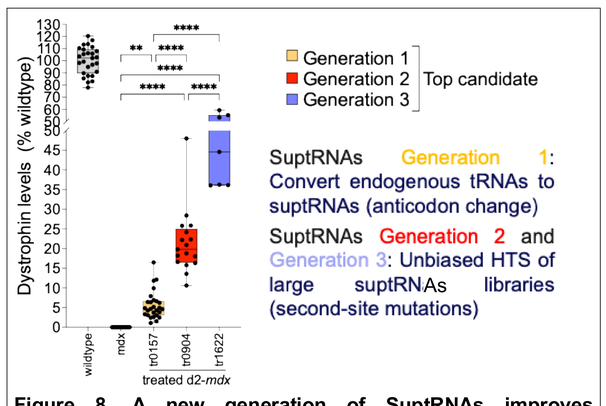

Figure 8. A new generation of SuptRNAs improves Dystrophin protein expression. Dystrophin levels were measured by normalizing dystrophin to total protein signal intensity. Plots show individual data and mean ± SEM. One-Way Anova test **<0.005, ****<0.00005.

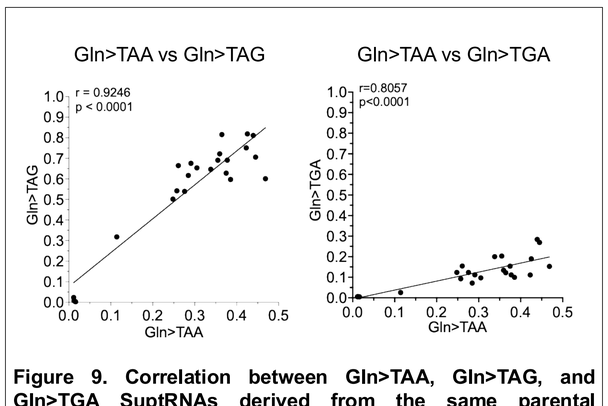

Figure 9. Correlation between Gln>TAA, Gln>TAG, and Gln>TGA SuptRNAs derived from the same parental tRNAs. SuptRNAs activity were determined by measuring the readthrough of the dual-luciferase PTC reporter and normalized to the WT reporter in HEK cells 24 hours after transient transfection. Pearson correlation ****<0.0001.

CONCLUSIONS

- ❑ Significant rescue of full-length dystrophin protein was detected following systemic delivery of SuptRNA tr0904 to D2-*mdx* mice.
- ❑ Systemic AAV delivery of SuptRNA tr0904 is efficacious to dramatically improve impaired motor function.
- ❑ Treatment with SuptRNA tr0904 successfully restores protein dysregulation in D2-*mdx* mice to wildtype levels.

PERSPECTIVES

- ❑ New generation of SuptRNA tr1622 will be evaluated for its safety and efficacy to restore dystrophin through systemic delivery in D2-*mdx* mice.
- ❑ Tevard's unique screening platform has revealed novel suptRNAs with enhanced expression and potency.
- ❑ Other classes of SuptRNAs (Gln>TGA / Gln>TAG) will be tested in DMD mouse models carrying TGA, and TAG PTCs.
- ❑ SuptRNA tr0904 is currently under investigation for other muscle and cardiology indications outside of DMD.

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