Targeted Delivery of ASOs Demonstrates Potential to Treat Duchenne Muscular Dystrophy

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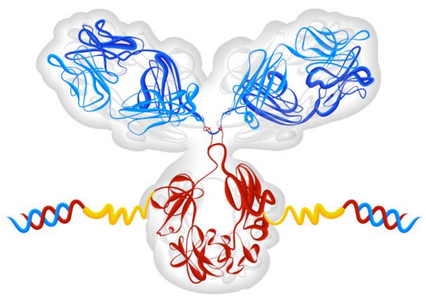
INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease caused by mutations in the dystrophin gene that lead to disruption in the mRNA reading frame, resulting in lack of dystrophin protein production. It is the most common pediatric-onset form of muscular dystrophy, characterized by progressive muscle degeneration and weakness that leads to early mortality as a result of respiratory and cardiac failure.

Figure 1. Duchenne Muscular Dystrophy (DMD) Overview.

Antisense oligonucleotides (ASOs) are promising therapeutics that can alter the mRNA reading frame through exon skipping to restore a more complete, functional dystrophin protein. However, the efficacy of current exon skipping ASO therapies has been limited by poor delivery and uptake to muscle cells.



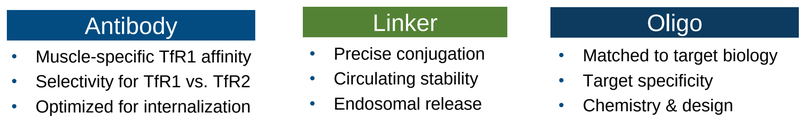


Figure 2. Dyne: Pioneering targeted therapies for muscle disease.

Rendering of Dyne therapeutic; not exact molecule

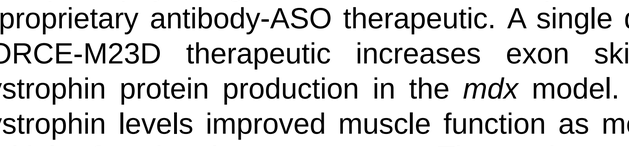
The FORCE platform, an antibody-mediated targeting technology developed by Dyne Therapeutics, improves delivery of therapeutic oligonucleotides to skeletal, cardiac and smooth muscles. We utilized the *mdx* mouse, a well-established animal model that mimics key aspects of human DMD pathophysiology, to evaluate the therapeutic potential of a proprietary antibody-ASO therapeutic. A single dose of the FORCE-M23D therapeutic increases exon skipping and dystrophin protein production in the *mdx* model. Increased dystrophin levels improved muscle function as measured by multiple functional assessments. These data support the therapeutic potential of Dyne's muscle-targeted oligonucleotides for the treatment of key muscle groups important in the pathobiology of DMD.

METHODS

Single-dose response study in the *mdx* mouse model

Group	Genotype	N	Treatment	Dose (mg/kg)	Endpoints
1	WT	4	Saline	0	• Exon skipping (RT-PCR) • Dystrophin Expression (Western blot)
2	*mdx*	4	Saline	0	
3	*mdx*	4	FORCE-M23D	10	
4	*mdx*	4	FORCE-M23D	20	
5	*mdx*	4	FORCE-M23D	30	

Single-dose functional study in *mdx* mouse model



Group	Genotype	N	Treatment	Dose (mg/kg)	Endpoints
1	WT	5	Saline	0	• Dystrophin Expression (IHC) • Creatine kinase • Functional outcomes (Hind Limb Fatigue, Running Wheel)
2	*mdx*	5	Saline	0	
3	*mdx*	5	M23D	30	
4	*mdx*	5	FORCE-M23D	30	

Mdx mouse contains nonsense mutation in exon 23 of dystrophin gene, dystrophin not expressed

Studies were conducted blinded at third-party CRO.

Key:
IV: intravenous dose
WT: Wild-type
RT-PCR: reverse transcription polymerase chain reaction
IHC: immunohistochemistry
ASO: antisense oligonucleotide
FORCE: Mouse Tfr1 targeting antibody ASO therapeutic
M23D: ASO targeting exon 23 mutation in *mdx* mouse. Utilized as "naked"

RESULTS

FORCE achieves effective exon skipping in *mdx* model

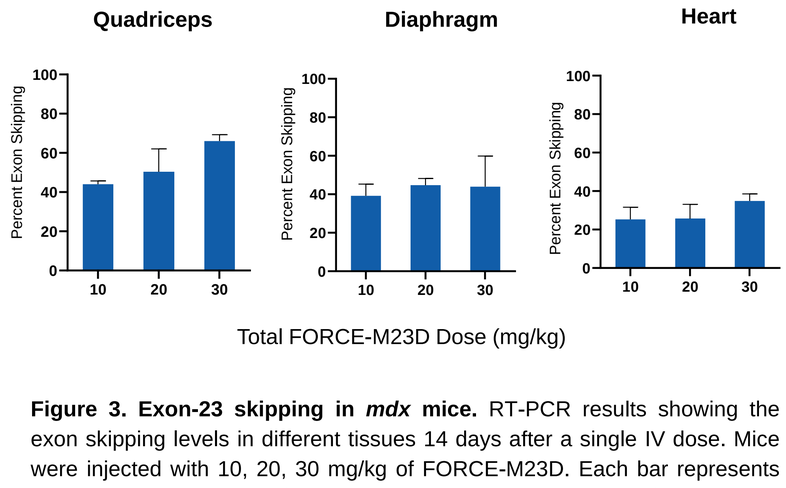


Figure 3. Exon-23 skipping in *mdx* mice. RT-PCR results showing the exon skipping levels in different tissues 14 days after a single IV dose. Mice were injected with 10, 20, 30 mg/kg of FORCE-M23D. Each bar represents an average of 4 mice. Wild-type and *mdx* mice treated with saline show no exon skipping in the tissues tested (not depicted in the graph).

FORCE-M23D results in dose-dependent dystrophin production

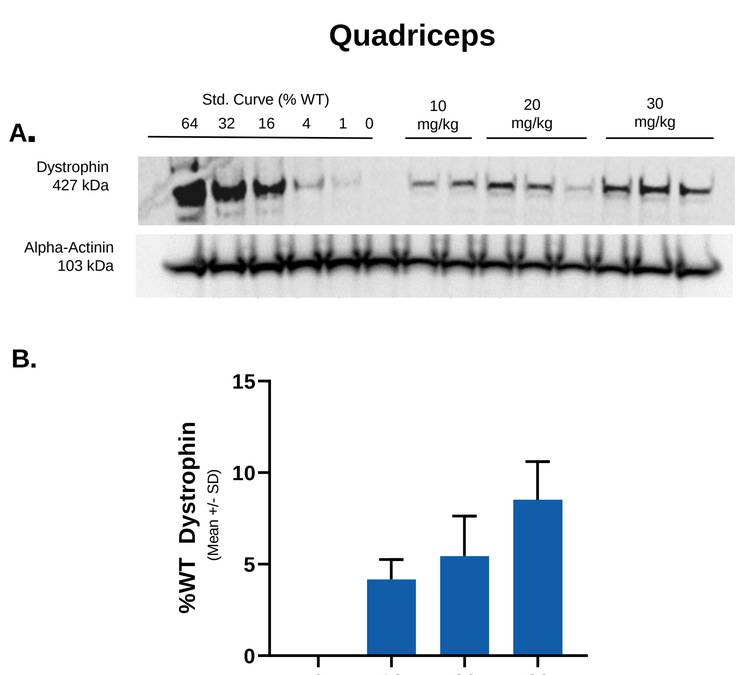


Figure 4. Dose dependent increase of dystrophin protein expression in *mdx* mice.
(A) Western blot results showing the dystrophin levels in quadriceps two weeks after a single IV dose. Mice were dosed with FORCE-M23D at 10, 20, 30 mg/kg. Dystrophin production was detected in the heart as well (western blot not depicted above).
(B) Bar graph representation of quadriceps western blot results. Each bar represents an average n=3 mice except at 10mg/kg where n=2.

Single dose of FORCE-M23D significantly decreases secreted creatine kinase in *mdx* model

Figure 5. Decrease in creatine kinase levels in *mdx* mice.
Results showing creatine kinase levels after 2 and 4-weeks treatment after a single 30mg/kg IV dose of M23D or FORCE-M23D. Control wild-type (WT) and *mdx* mice were dosed with saline. (****<0.0001).

Single dose of FORCE-M23D restores dystrophin expression to muscle membrane based on IHC

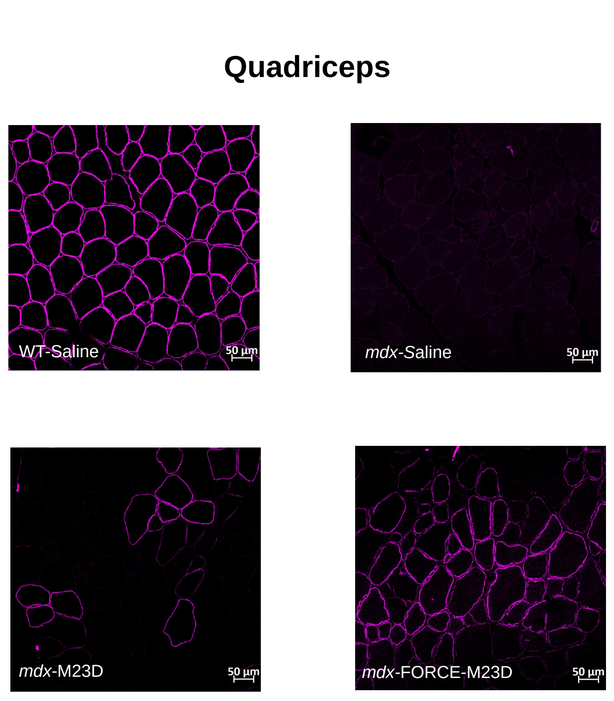
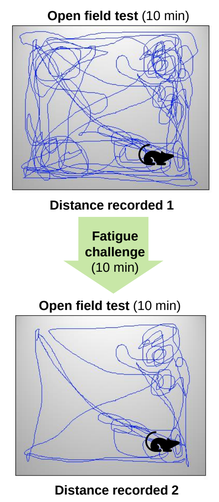


Figure 8. Immunofluorescent staining of dystrophin expression in *mdx* mice at 4 weeks post-dose.
Results showing dystrophin expression in quadriceps at four weeks after single IV dose at 30mg/kg. Control wild-type (WT) and *mdx* mice were dosed with saline.

Single-dose of FORCE-M23D demonstrates functional benefit in *mdx* model



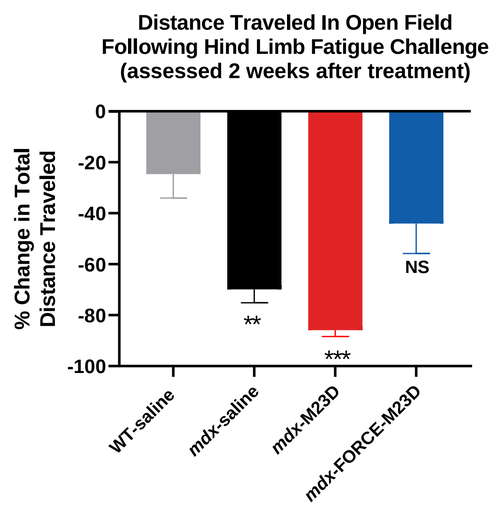
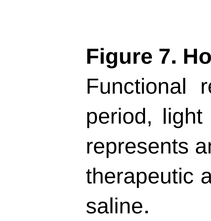


Figure 6. Hind Limb Fatigue Challenge in *mdx* mice.
Functional results showing the percent change in total distance travelled in an open-field after hind limb fatigue challenge at two weeks post-treatment. Each bar represents an average of 5 mice. Mice were dosed with M23D or FORCE-M23D therapeutic at 30mg/kg. Control wild-type (WT) and *mdx* mice were treated with saline. (**<0.01, ***<0.001, NS not significant).



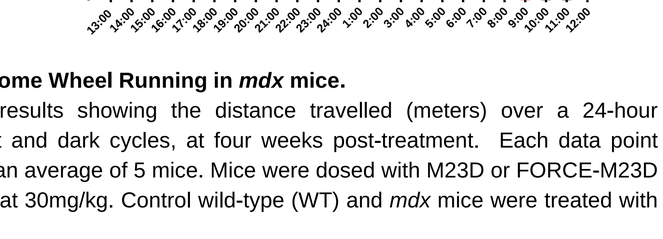


Figure 7. Home Wheel Running in *mdx* mice.
Functional results showing the distance travelled (meters) over a 24-hour period, light and dark cycles, at four weeks post-treatment. Each data point represents an average of 5 mice. Mice were dosed with M23D or FORCE-M23D therapeutic at 30mg/kg. Control wild-type (WT) and *mdx* mice were treated with saline.

CONCLUSIONS

Treatment in *mdx* mice with FORCE-M23D:

- Promotes exon skipping
- Significantly restores dystrophin protein expression in muscles including quadriceps, diaphragm and heart
- Significantly improves muscle function and health
  - Significant reduction in secreted creatine kinase
  - Improvement in multiple functional assessments
  - Restoration of dystrophin expression in muscle membrane
- Delivers sustained therapeutic effect for at least 4 weeks

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