163. Site-blocking oligos (SBO) upregulate utrophin

(University of Pennsylvania)

Asset Overview

Product Type	Gene therapy
Disease Area	Others
Indication	Duchenne Muscular Dystrophy (DMD)
Current Stage	Discovery
Target	Utrophin (a dystrophin related protein)
MoA	PMO-based SBOs complementary to the let-7c binding site in UTRN 3′UTR, with the goal of inhibiting let-7c interaction with UTRN mRNA and thus upregulating utrophin.
Brief Description	• The solution is a set of synthetic site-blocking oligonucleotides (SBO) which inhibits natural degradation of utrophin mRNA. These SBOs block downregulation of utrophin, increasing its expression in muscle cells which alleviates DMD symptoms. The invention works by binding to a site in utrophin mRNA which is usually targeted for degradation. Utrophin-upregulating SBOs may be used in combination with other upregulation strategies to further increase cellular expression of therapeutic utrophin. • To increase production of therapeutic utrophin in muscle cells, a set of five SBOs ranging from 24-29 base pairs were synthesized. All SBOs target the same utrophin mRNA 3′-UTR region. The SBOs increase utrophin expression by hybridizing to the Let-7c microRNA binding site. This prevents Let-7c microRNA from binding to utrophin mRNA and facilitating degradation through the RNA-induced silencing complex. Utrophin-upregulating SBOs are created using a nucleic acid mimic known as a phosphorodiamidate morpholino oligonucleotide (PMO) to decrease undesirable toxicity and increase tissue accumulation.
Intellectual Property	WO2022109432A2
Publication	PMO-based let-7c site blocking oligonucleotide (SBO) mediated utrophin upregulation in mdx mice, a therapeutic approach for Duchenne muscular dystrophy (DMD). Sci Rep, (2020)
Inventors	Tejvir S. Khurana

Highlights

- Higher binding affinity and tissue concentration compared to other oligo alternatives
- Endogenous utrophin signal increases by 1.5-fold for 48 hours in vitro
- Two-fold higher utrophin protein expression in skeletal muscles in DMD mouse model

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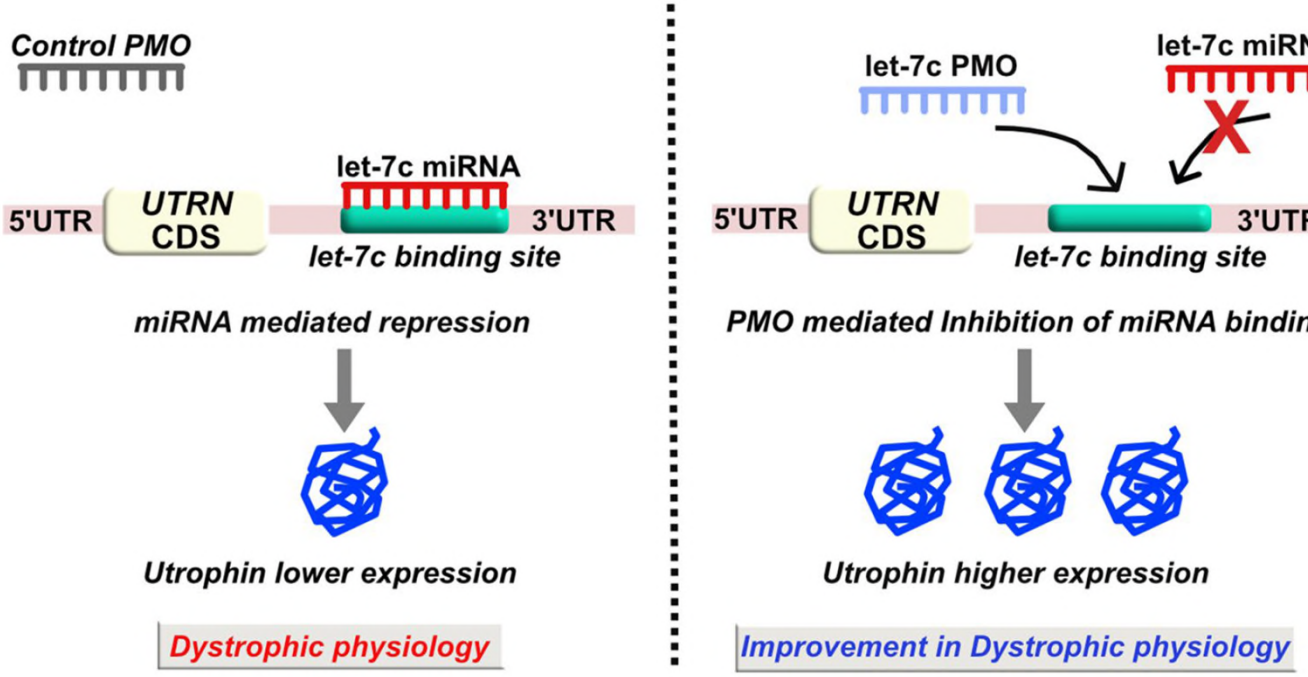
Key Data

Schematic representation of the PMO-based let-7c SBO strategy to alleviate let-7c miRNA repression of utrophin gene



The left panel shows, let-7c miRNA mediated post-transcriptional repression of utrophin in control PMO treated mice. Whereas the right panel shows let-7c PMO mediated blocking of miRNA binding to *UTRN* 3′UTR, resulting in higher utrophin expression and improvement in dystrophic pathophysiology.

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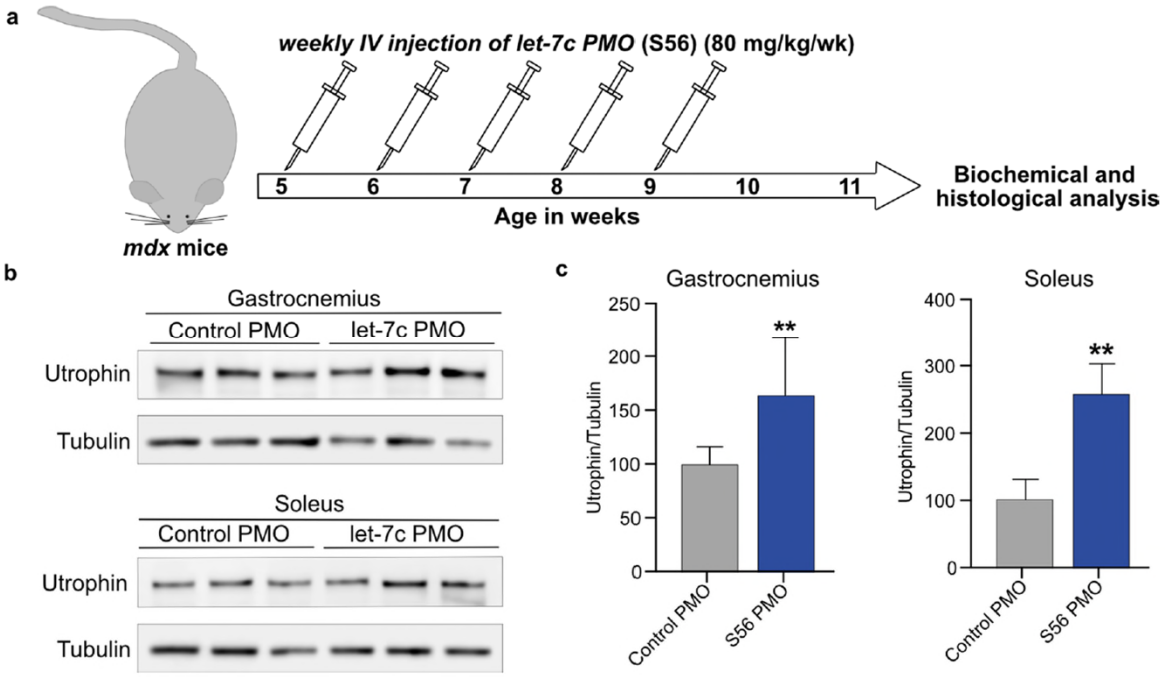
Higher expression of utrophin in mdx mice after five weeks of treatment with 80 mg/kg/wk S56 PMO



(**a**) Diagram of the experimental pipeline adopted for the study. (**b**) Western blot showed higher level of utrophin expression in gastrocnemius and soleus muscles of S56 PMO treated *mdx* mice. Samples from three different *mdx* mice treated with control or S56 PMO were shown. (**c**) Quantification of normalized utrophin level in control and S56 PMO treated *mdx* mice gastrocnemius and soleus muscle (n = 6 mice for both groups).

Data shown as percentage of normalized utrophin level compared to control PMO treated *mdx* mice. Each bar represents mean ± SEM. Statistical analysis performed by Mann–Whitney nonparametric test, $^{**}p < 0.01$.

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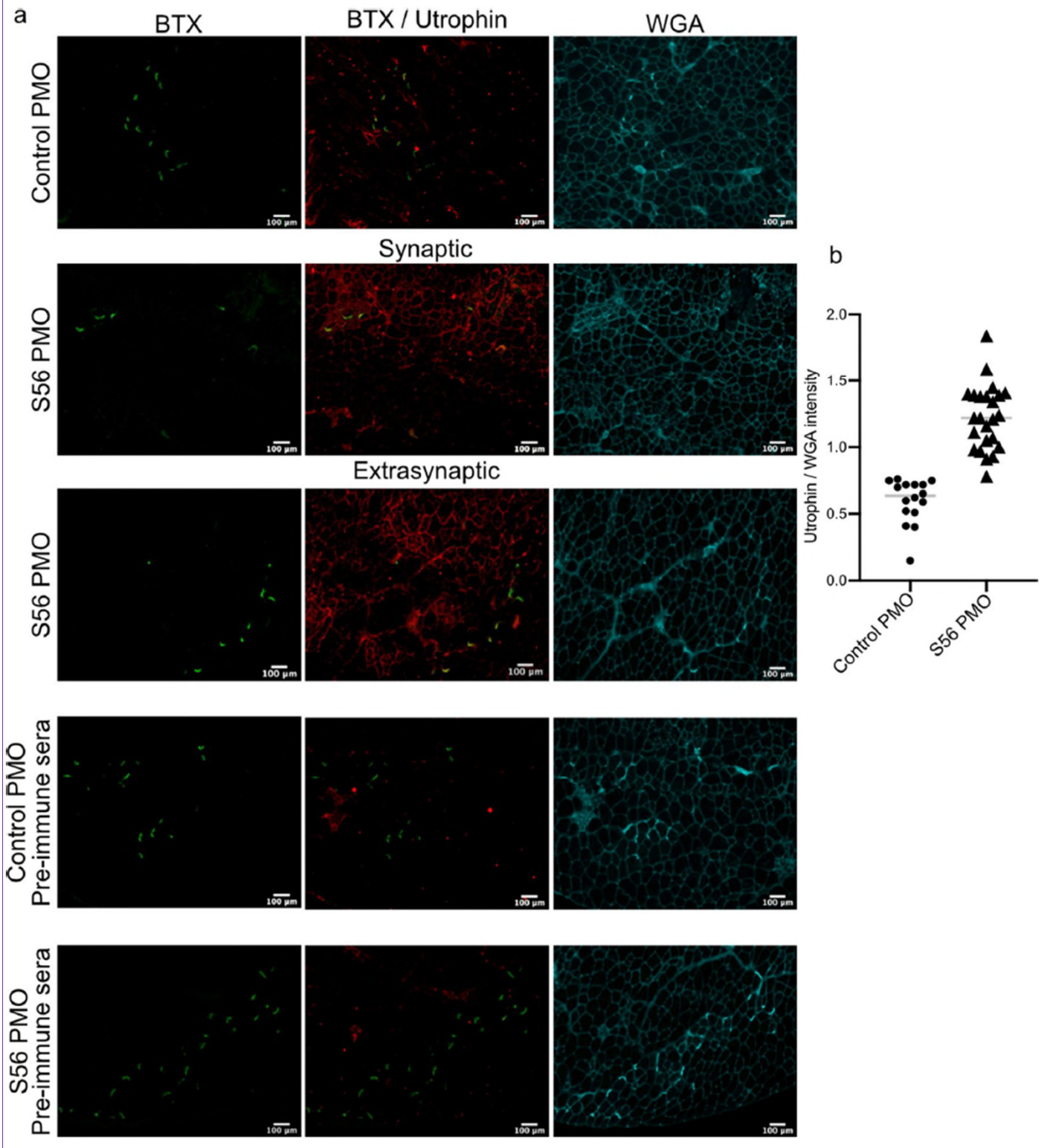
Immunohistochemistry of mdx TA muscles treated with S56 PMO showed higher sarcolemmal expression of utrophin



To be continued

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(**a**) TA muscles were stained with α-bungarotoxin (green), utrophin antibody (red) and wheat germ agglutinin (cyan). The control PMO and S56 PMO treated cryosections were also stained with utrophin pre-immune sera as control. Scale bar = 100 μm. The figures showed synaptic expression of utrophin in control PMO, and both synaptic and extra-synaptic sarcolemma associated expression of utrophin in S56 PMO treated mice. (**b**) Quantification of utrophin expression in sarcolemma normalized with WGA expression. S56 PMO treated muscles showed significantly higher expression of utrophin (****p = 0.0001, n = 4 mice for both groups). Statistical analysis performed by Mann–Whitney nonparametric test.

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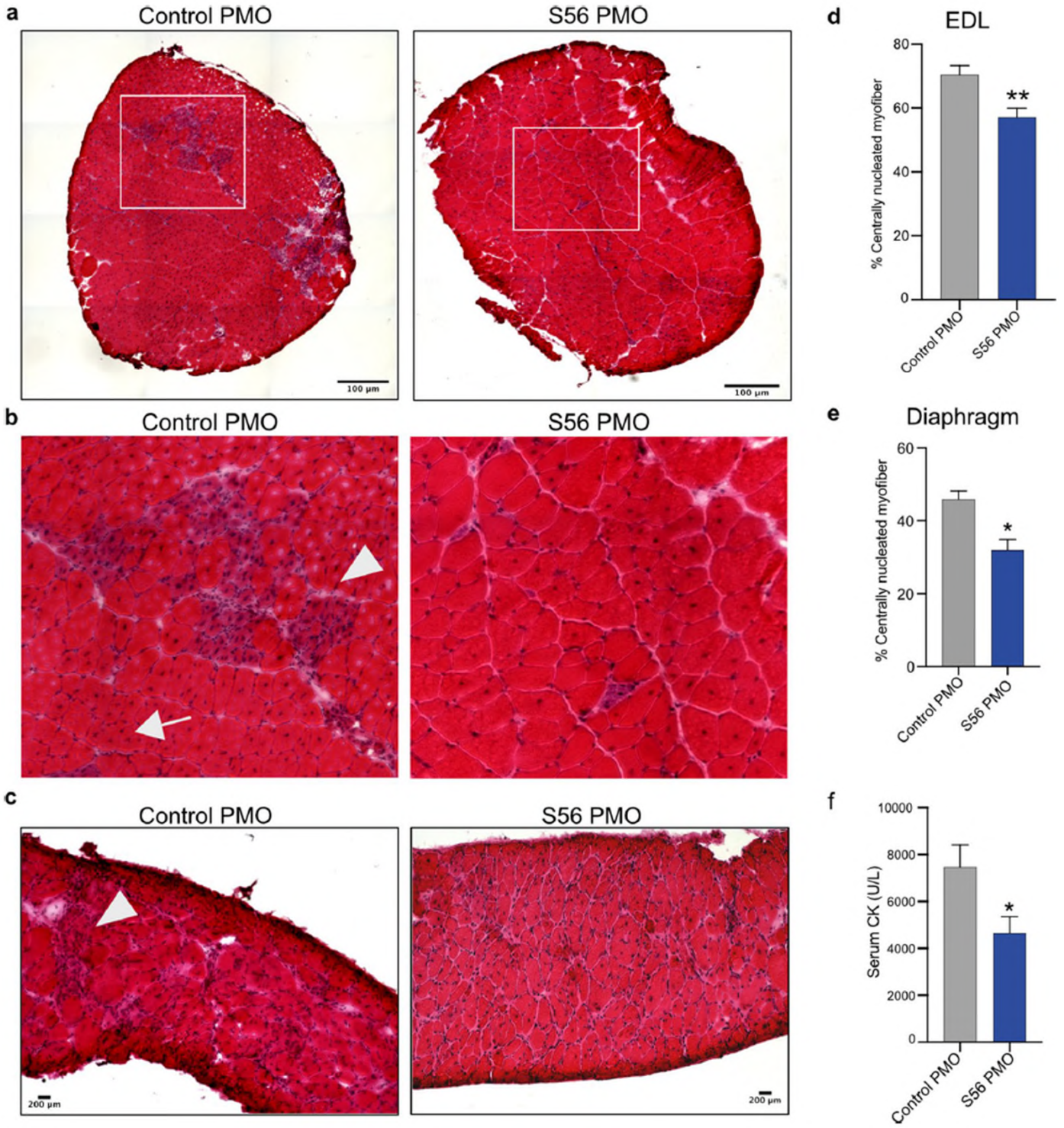
H&E staining of cryosections from control PMO or S56 PMO treated mdx mice EDL and diaphragm showed morphological improvement



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H&E staining of cryosections from control PMO or S56 PMO treated mdx mice EDL and diaphragm showed morphological improvement

(**a**) Representative H&E staining image of whole EDL cryosections (scale bar = 100 μm). (**b**) Regions highlighted by the white boxes were magnified (×3) (scale bar = 25 μm). (**c**) Representative H&E staining of diaphragm cryosections (scale bar = 200 μm). Control PMO treated EDL and diaphragm muscle cryosections show regenerated myofibers (arrow) and immune cell infiltration (arrowhead) and S56 PMO treatment appeared to alleviate symptoms. (**d, e**) Quantification of centrally nucleated EDL and diaphragm muscle fibers of *mdx* mice treated with control PMO or S56 PMO. The graphs show significant decrease in the percentage of CNFs of EDL and diaphragm muscles in S56 PMO treated *mdx* mice (***p* = 0.002, n = 10 mice for both groups of EDL muscles, **p* = 0.0140, n = 6 mice for both groups of diaphragm muscles). (**f**) The graph shows serum CK levels in S56 PMO treated mice were significantly lower than control PMO treated *mdx* mice (**p* = 0.02, n = 10 mice for both groups). Each bar represents mean ± SEM. Statistical analysis performed by Mann–Whitney nonparametric test.