Golodirsen Induces Exon Skipping Leading to Sarcolemmal Dystrophin Expression in Patients With Genetic Mutations Amenable to Exon 53 Skipping

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Perry B. Shieh, MD, PhD
UCLA Medical Center

On Behalf of:

Francesco Muntoni, Diane E. Frank, Jennifer Morgan, Volker Straub, Eugenio Mercuri, Laurent Servais

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Duchenne Muscular Dystrophy (DMD)

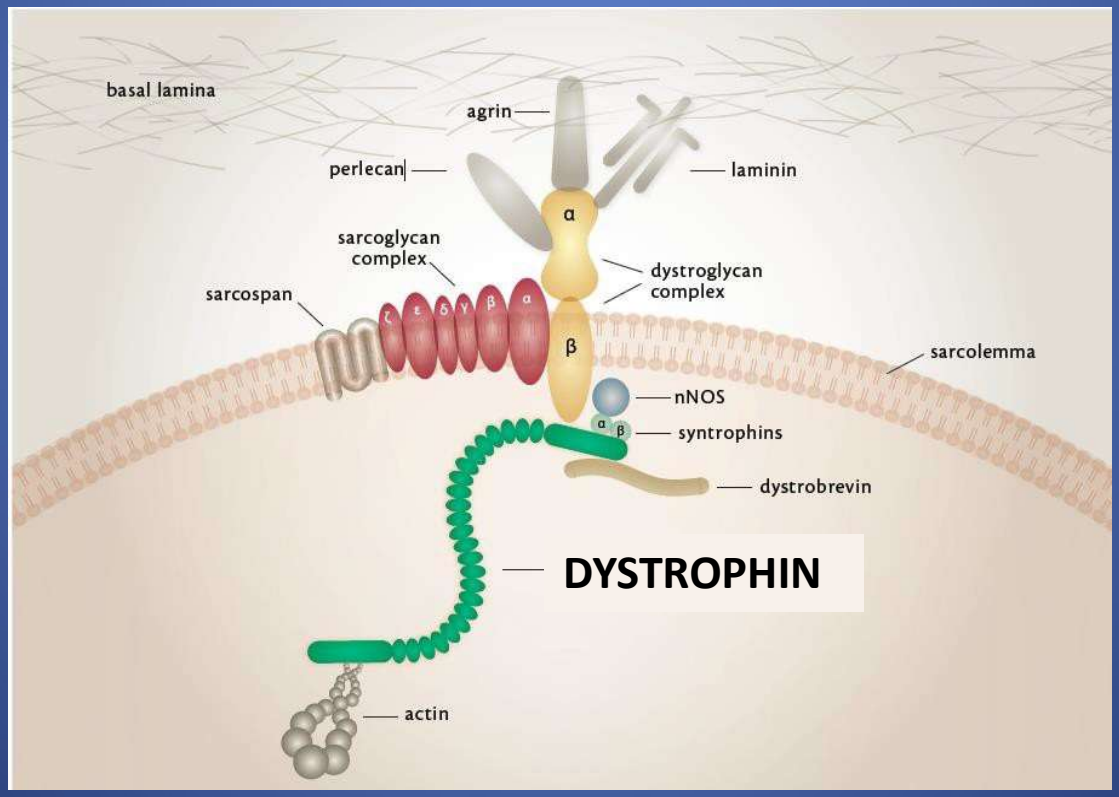
- DMD is a rare, fatal, degenerative neuromuscular disease with X-linked recessive inheritance[1,2]
- Due to mutations in the *DMD* gene, and most of these mutations disrupt the dystrophin mRNA reading frame and prevent production of functional dystrophin[3]

mRNA, messenger ribonucleic acid.

1. Zaharieva IT, et al. *PLoS One* 2013;8:e80263. **2.** Moat SJ, et al. *Eur J Hum Genet* 2013;21:1049-53. **3.** Kobayashi YM, Campbell KP. In: Hill JA, Olson EN, eds. *Muscle: Fundamental Biology and Mechanisms of Disease Volume 2*. New York, NY: Academic Press; 2012:935-42.

Dystrophin Protein

- Dystrophin is a critical protein that functions to prevent muscle damage during eccentric contraction[1-5]
- Clinical effect of dystrophin loss is progressive muscle wasting and weakness, loss of function, and premature death[3,6,7]



1. Kobayashi YM, Campbell KP. In: Hill JA, Olson EN, eds. *Muscle: Fundamental Biology and Mechanisms of Disease Volume 2*. New York, NY: Academic Press; 2012:935-42. **2.** Kole R, Krieg AM. *Adv Drug Deliv Rev* 2015;87:104-7. **3.** Allen DG, et al. *Physiol Rev* 2016;96:253-305. **4.** Aartsma-Rus A, et al. *J Med Genet* 2016;53:145-51. **5.** Cirak S, et al. *Mol Ther* 2012;20:462-7. **6.** Mah JK. *Neuropsychiatr Dis Treat* 2016;12:1795-807. **7.** Falzarano MS, et al. *Molecules* 2015;20:18168-84

Disease Progression in DMD[1-3]

5 TO 7 YEARS

- Motor delay
- Enlarged calves
- Toe walking
- Standing from supine, climbing stairs more difficult

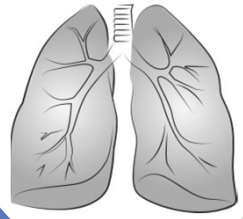
8 TO 11 YEARS

- Increasing loss of walking ability
- Part-time wheelchair use

EARLY TEENS

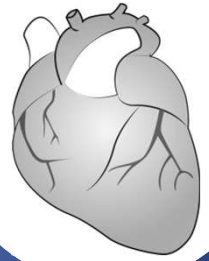
- Loss of ambulation
- Full-time wheelchair use
- Increasing loss of upper limb function

TEENS



- Increasing respiratory impairment
- Ventilatory support often required
- Unable to perform activities of daily living

TEENS TO TWENTIES



- Increasing cardiac dysfunction
- Heart failure
- Death

EARLY AMBULATORY → LATE AMBULATORY → EARLY NON-AMBULATORY → LATE NON-AMBULATORY

1. Bushby K, Finkel R, Birnkrant DJ, et al. *Lancet Neurol.* 2010;9:77-93.
2. Emery AEH. *Lancet*. 2002;359:687-695.
3. Landfeldt E, Lindgren P, Bell CF, et al. *Neurology*. 2014;83(6):529-536.

PMOs Eteplirsen and Golodirsen

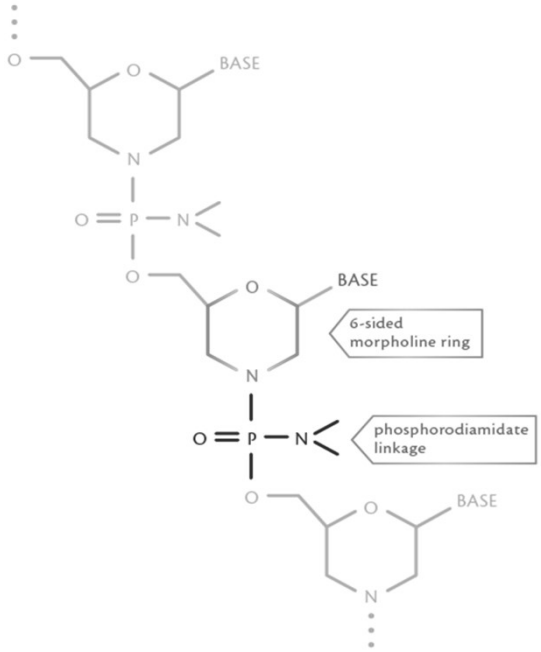
- Eteplirsen is approved by the US FDA for the treatment of DMD patients with confirmed mutations amenable to exon 51 skipping[1–6]
  - Approximately 13% of DMD patients carry out-of-frame deletion mutations amenable to exon 51 skipping
- Golodirsen (formerly SRP-4053) binds and excludes exon 53 during dystrophin mRNA processing, allowing production of internally shortened dystrophin protein
  - Approximately 8% of DMD patients have mutations amenable to exon 53 skipping[1]
- Additional exon targeting may address an unmet need for other patients

1. Aartsma-Rus A, et al. *Hum Mutat* 2009;30:293-9. **2.** Bladen CL, et al. *Hum Mutat* 2015;36:395-402. **3.** Emery AE. *Neuromuscul Disord* 1991;1:19-29. **4.** Exondys 51 [package insert]. Cambridge, MA: Sarepta Therapeutics, Inc.; 2016. **5.** Cirak S, et al. *Lancet* 2011;378:595-605. **6.** Mendell JR, et al. *Ann Neurol* 2016;79:257-71.

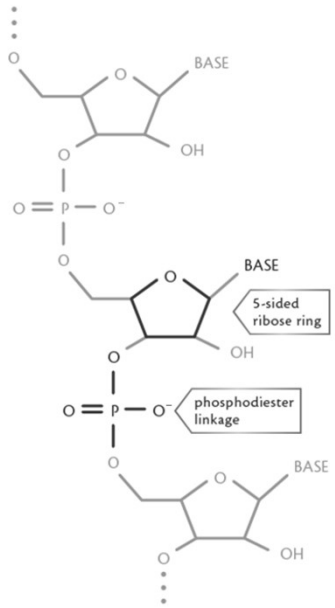
Phosphorodiamidate Morpholino Oligomers (PMOs)

- PMOs are a class of unique RNA therapeutics with an uncharged backbone that target endogenous nucleic acids through Watson-Crick base pairing[1–4]
  - Sequences are designed complementary to the desired target

PMO Structure



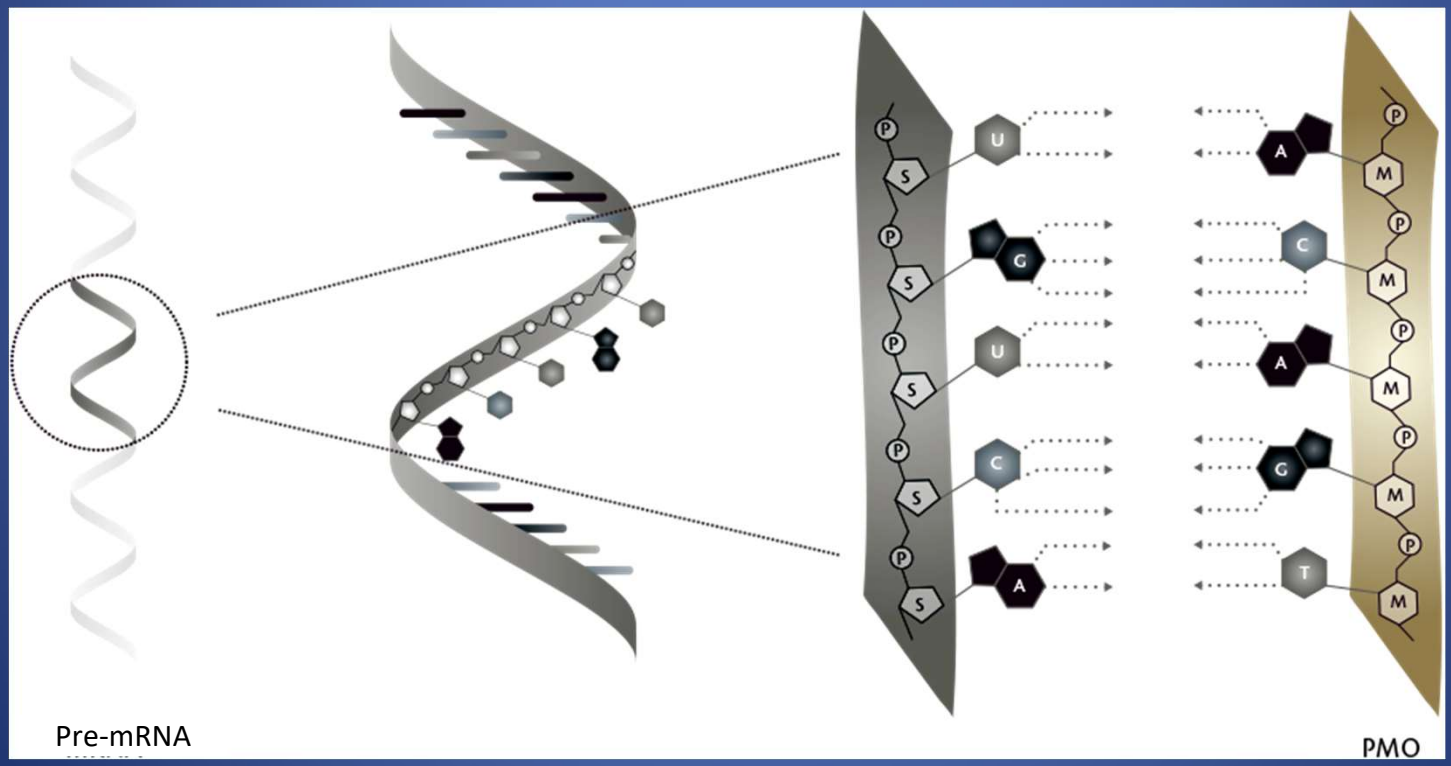
RNA Structure



1. Wilton SD, et al. *Mol Ther* 2007;15:1288-96. **2.** Wilton SD, Fletcher S. *Curr Gene Ther* 2011;11:259-75. **3.** Wilton SD, et al. *Neuromuscul Disord* 1999; 9:330-8. **4.** Popplewell LJ, et al. *Mol Ther* 2009;17:554-61.; 5. Kole R, Leppert BJ. *Discov Med.* 2012;14(74):59-69.

Sequence Specific Binding of PMOs to pre-mRNA

- In DMD, PMOs have been designed with a goal of skipping targeted exons to restore the dystrophin mRNA reading frame and allow production of internally shortened dystrophin protein



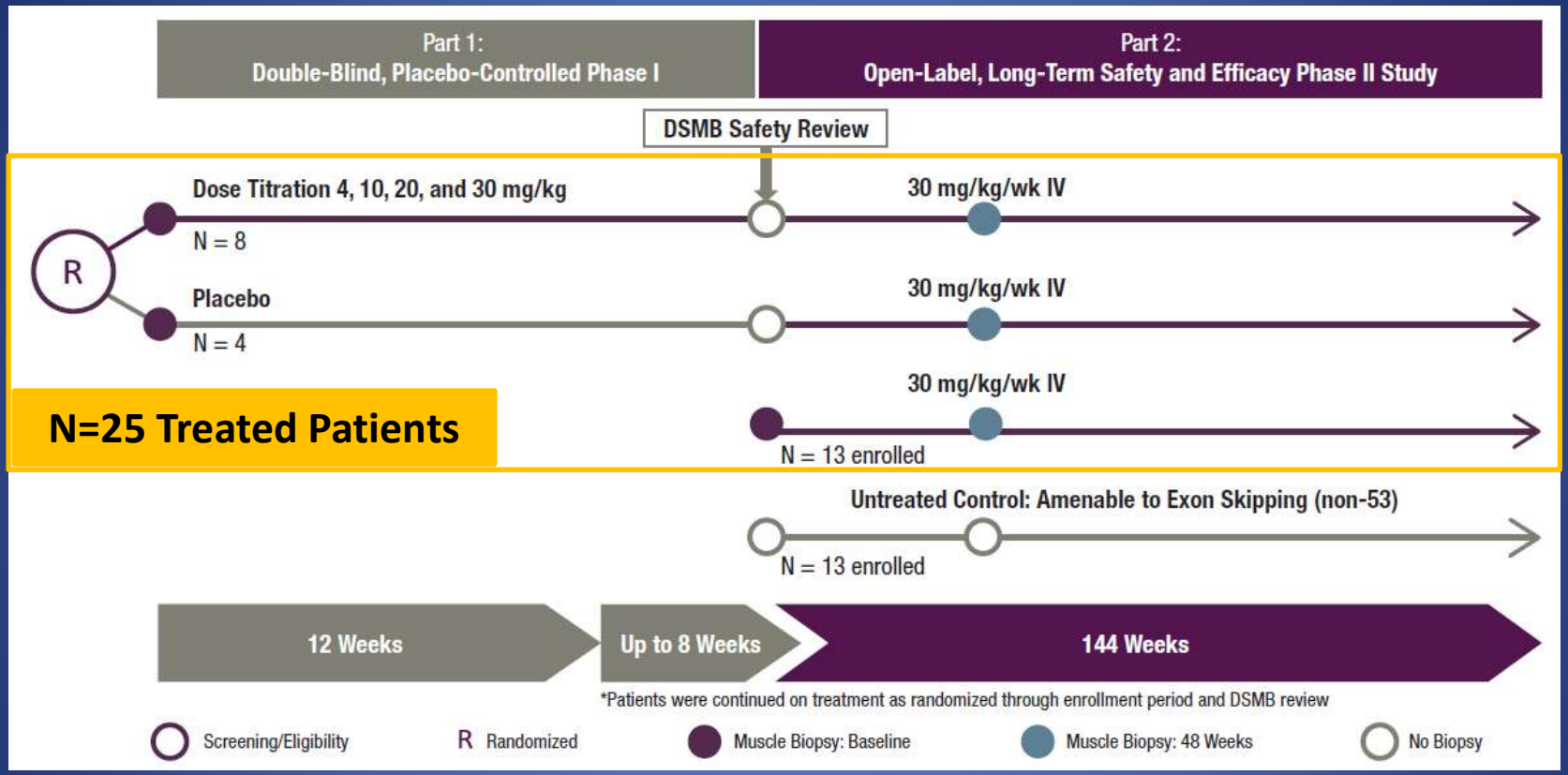
1. Kole R, Leppert B. *Discov Med*. 2012;14(74):59-69.
2. Sarepta Therapeutics Data on File

SKIP-NMD Consortium

- The consortium is a broad group encompassing advocacy, research, healthcare providers and industry
- Francesco Muntoni is project lead (UCL, GOSH). Individual site leads are as follows:
  - Institut de Myologie (Laurent Servais)
  - University of Newcastle upon Tyne (Volker Straub)
  - Università Cattolica del Sacro Cuore (Eugenio Mercuri)
  - Royal Holloway & Bedford New College (George Dickson)- in vitro sequence selection & steering
- All stakeholders had a chance to contribute to trial design

Study 4053-101: SKIP-NMD
An International Collaborative Study

SKIP-NMD
Skipping exon 53 of the dystrophin gene



Population:
- Age: 6-15 yrs
- 6MWT ≥250 m
- NSAA Total >17
  or Rise Time <7 sec

Key Endpoints (Part 2):
- 1º: 6MWT (Week 144), Western Blot (Week 48)
- PFTs
- Dystrophin Intensity, PDPF
- Exon Skipping

6MWT, 6-minute walk test; NSAA, North Star Ambulatory Assessment, PDPF, percent dystrophin-positive fibers; PFT, pulmonary function test.

Methods: Western Blot and RT-PCR

- RT-PCR completed to evaluate dystrophin exon 53 skipping
  - Semi-quantitative, end point method
  - Sanger DNA sequencing to confirm correct skipping

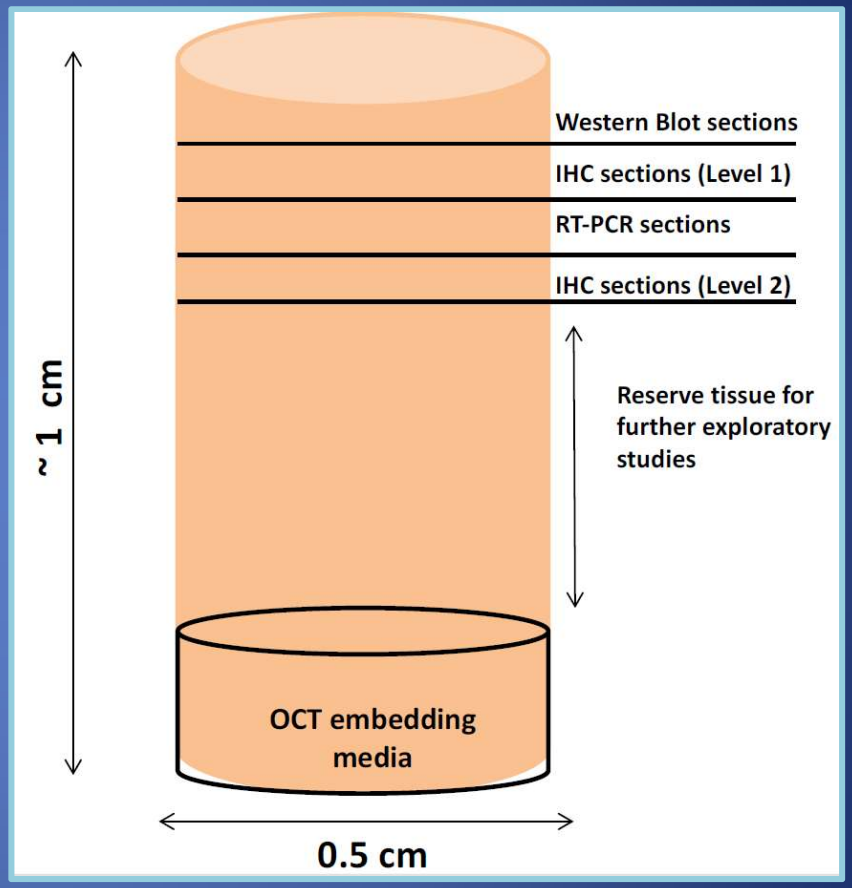
- Western blot: A validated quantitative assay assessed dystrophin protein levels

cDNA, complementary deoxyribonucleic acid; GCLP, Good Clinical Laboratory Practice; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

Muscle Biopsies and Tissue Allocation

- Muscle biopsies:
  - Baseline & Week 48 (Part 2)
  - Contralateral biceps brachii
  - Standardized surgical procedure

- 2 sections of muscle (A + B) were excised during each surgery
  - Allocated and analyzed separately
  - Western blot: 4 replicates A+B, duplicate gels
  - Exon skipping: 8 replicates A+B, quadruplicate reactions
  - IHC: 4 replicates A+B, level 1 + 2 slides



IHC, immunohistochemistry; OCT, optimal cutting temperature; RT-PCR, reverse transcription polymerase chain reaction.

Results: Patient Demographics and Baseline Disease Characteristics

Baseline Characteristic	Mean (SD) N=25 Patients
Age, years	8.2 (2.2)
Height, cm	120.1 (10.4)
Weight, kg	28.2 (9.1)
BMI, kg/m^2	19.1 (3.7)
6MWT distance, m	403.7 (56.7)
Time since DMD diagnosis, months	55.2 (24.9)
Duration of corticosteroid use, months	34.6 (24.7)

Note: Values shown are from the N=25 patients who were treated and received a muscle biopsy

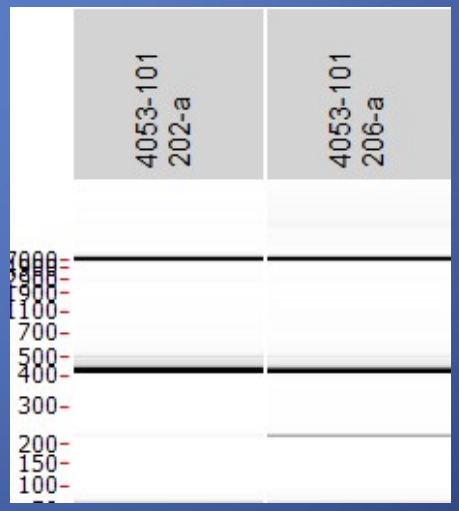
BMI, body mass index.

Results: RT-PCR for Exon 53 Skipping

100% exon skipping response rate

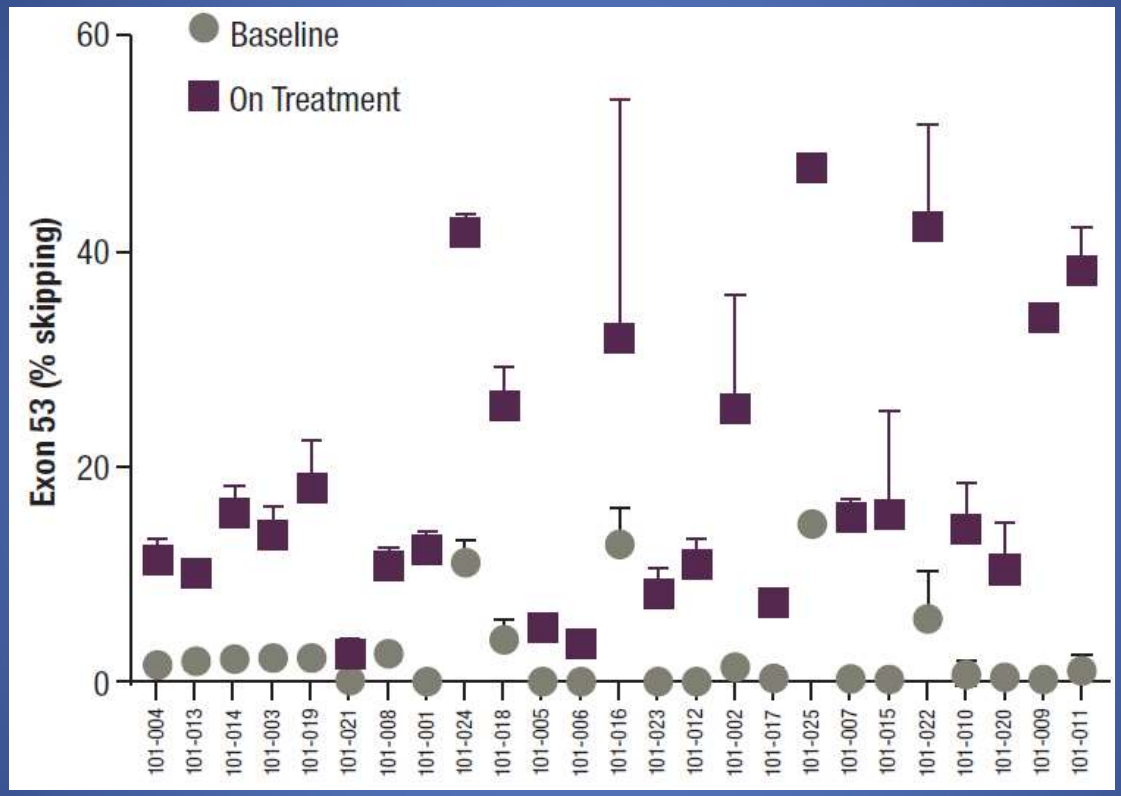
- All 25 patients displayed an increase in the exon 53 skipped band (*P*<0.001) over baseline levels at Week 48

Mean % Exon Skipping				
BL	Wk48	Δ	P	Fold↑
2.6	19.0	+16.4	<0.001	+7.3



Sample Result
Del 49-52
15.42% skip

Results: RT-PCR for Exon 53 Skipping

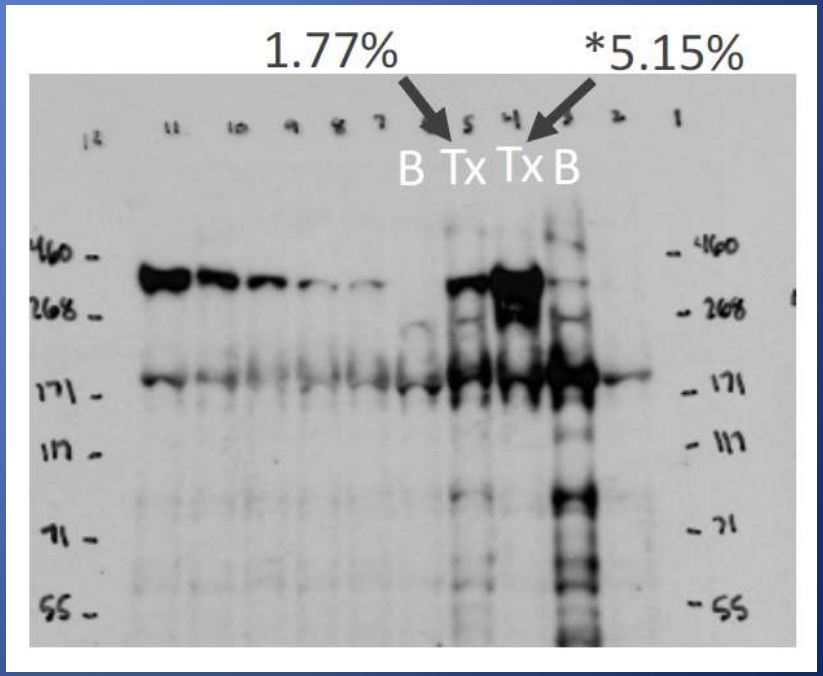


- Range of individual mean percent increase from baseline: 2.50% to 37.32%

Results: Western Blot for Dystrophin

Significant increase in dystrophin protein observed from baseline to Week 48 in golodirsen treated patients

Mean % Normal Dystrophin				
BL	Wk48	Δ	P	Fold↑
0.1	1.0	+0.9	<0.001	10.7

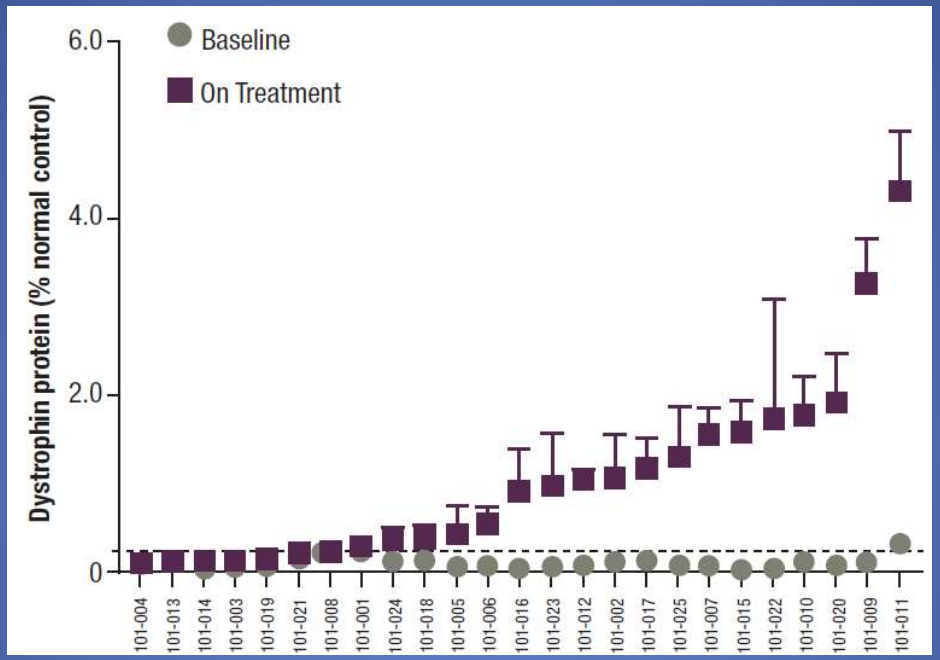


*above 4% upper limit of quantitation

SD, standard deviation.

Results: Western Blot for Dystrophin Protein

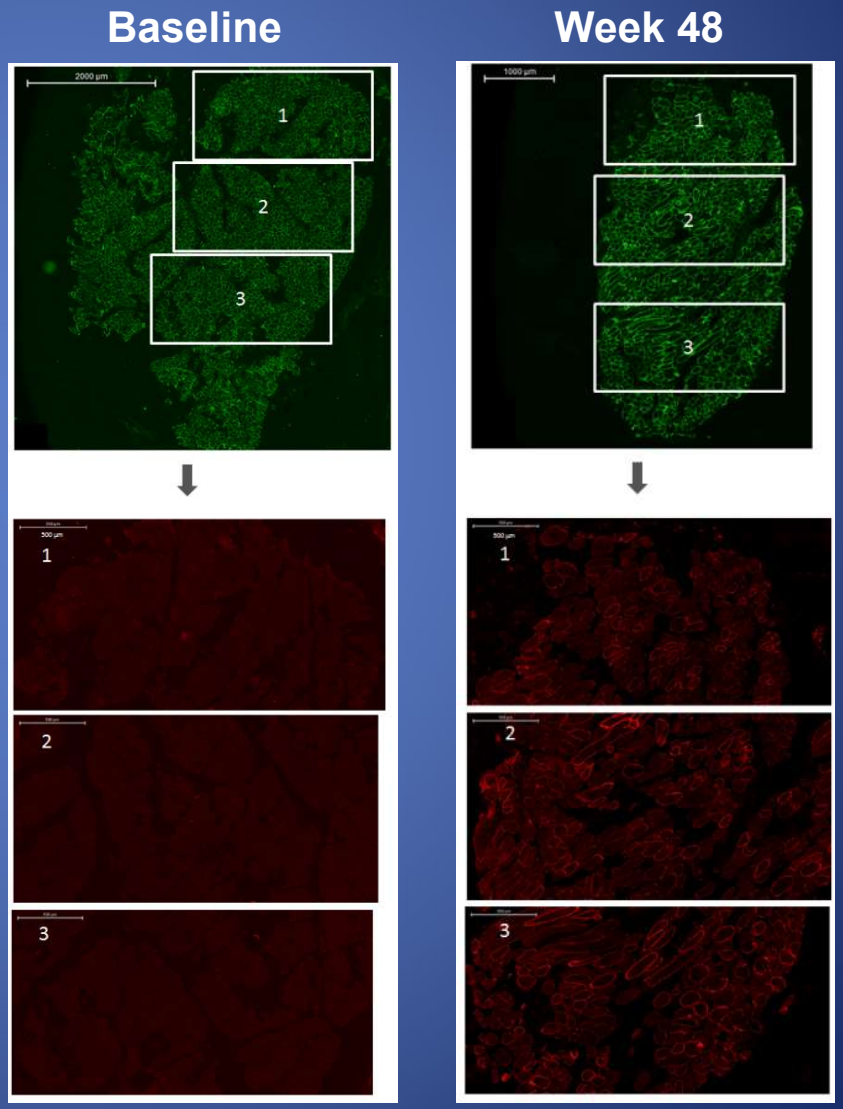
Western Blot, Mean Dystrophin % Normal



- Range of dystrophin in individual patient biopsies: 0.09%–4.30% of normal
- Baseline biopsies (N=24) uniformly expressed very low levels of dystrophin
  - Only one had dystrophin above BLOQ (0.31%)
- Significant positive correlation between exon skipping and de novo dystrophin protein expression observed
  - Spearman-r correlation coefficient, 0.500; *P*=0.011

Results: IHC for Dystrophin Localization

- Dystrophin localization to the sarcolemma clearly demonstrated
  - Evidence that the dystrophin is functional and present throughout the muscle fiber
  - Whole-slide scan images at baseline and Week 48 from 1 patient is shown as an example



Note: Indirect immunofluorescence staining of tissue cryosections was performed using MANDYS106 and anti-laminin 2 alpha antibodies

Conclusions

- Treatment with golodirsen resulted in increases in dystrophin
  - 1º biological endpoint achieved: Statistically significant mean increase in dystrophin observed ($p<0.001$)
  - ↑ exon 53 skipping observed in all patients
  - Dystrophin correctly localized to sarcolemma
  - Exon skipping significantly correlated to dystrophin expression

- Golodirsen is the second PMO to demonstrate increased dystrophin expression and sarcolemmal membrane localization through exon skipping
  - These findings further validate the potential of the PMO platform in DMD

- Using rigorous methods to measure dystrophin expression should facilitate the evaluation of dystrophin restorative therapies

Acknowledgments

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