Published in final edited form as:

Curr Opin Neurol. 2009 October ; 22(5): 532–538. doi:10.1097/WCO.0b013e32832fd487.

Emerging genetic therapies to treat Duchenne muscular dystrophy

Stanley F. Nelson^{d,e}, Rachelle H. Crosbie^{a,e}, M. Carrie Miceli^{c,e}, and Melissa J. Spencer^{b,e}

^aDepartment of Physiological Science, UCLA, Los Angeles, California, USA

^bDepartment of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^cDepartment of Microbiology and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^dDepartments of Human Genetics and Psychiatry, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

^eCenter for Duchenne Muscular Dystrophy at UCLA, Los Angeles, California, USA

Abstract

Purpose of review—Duchenne muscular dystrophy is a progressive muscle degenerative disease caused by dystrophin mutations. The purpose of this review is to highlight two emerging therapies designed to repair the primary genetic defect, called 'exon skipping' and 'nonsense codon suppression'.

Recent findings—A drug, PTC124, was identified that suppresses nonsense codon translation termination. PTC124 can lead to restoration of some dystrophin expression in human Duchenne muscular dystrophy muscles with mutations resulting in premature stops. Two drugs developed for exon skipping, PRO051 and AVI-4658, result in the exclusion of exon 51 from mature mRNA. They can restore the translational reading frame to dystrophin transcripts from patients with a particular subset of dystrophin gene deletions and lead to some restoration of dystrophin expression in affected boys' muscle *in vivo*. Both approaches have concluded phase I trials with no serious adverse events.

Summary—These novel therapies that act to correct the primary genetic defect of dystrophin deficiency are among the first generation of therapies tailored to correct specific mutations in humans. Thus, they represent paradigm forming approaches to personalized medicine with the potential to lead to life changing treatment for those affected by Duchenne muscular dystrophy.

Keywords

antisense therapeutics; disease; dystrophin; dystrophy; exon skipping; mouse; muscle; nonsense codon suppression

Introduction

Duchenne muscular dystrophy (DMD) is a severe progressive muscle degenerative disease of childhood, occurring in about one of every 3500 live male births. DMD is caused by the absence of dystrophin due to mutation of *DMD* located on the X chromosome and, thus, primarily affects males [1–4]. The *mdx* mouse model has been a powerful tool for identifying potential therapeutic targets based on amelioration of the dystrophic phenotype. In general, approaches targeting the sarcolemmal defect that occurs due to lack of dystrophin and its associated proteins have proven most successful. Improvement in dystrophic features has been accomplished by upregulation of compensatory proteins (i.e. utrophin, integrin- α 7, or sarcospan) [5–7]; chemical repair of the weakened membrane (i.e. poloxamer) [8]; and increased glycosylation of α -dystroglycan to improve extracellular matrix attachment [9] (Fig. 1). Although these therapeutic approaches are promising, they have not entered the phase of clinical investigation. Recently, two novel therapies are being tested that target the primary genetic defect of abnormal dystrophin and are in phase I or phase II clinical trials. These agents generate a functional or partially functional dystrophin protein. In this review, we focus on these two emerging therapies, referred to as 'exon skipping' and 'nonsense codon suppression', which target the mRNA splicing machinery and ribosomal fidelity, respectively. Although viral-based gene therapy has the potential to restore dystrophin in DMD muscles and stem cell therapy has the potential to replace dystrophin-deficient muscle, these topics have been covered elsewhere [10–12], are more distant therapeutic options, and will not be discussed here.

Exon skipping

Dystrophin is a 427 kDa protein composed of four main domains, including an N-terminal actin-binding domain, a large central rod domain containing 24 spectrin repeats, a cysteine-rich region, and a carboxy-terminal domain. Although no true complete population-based systematic assessments have been performed, most mutation surveys indicate that approximately 70% of all DMD-causing dystrophin mutations are due to single or multiexon deletions with a higher mutational frequency observed within exons 44–55, which corresponds to the rod domain of dystrophin. Such deletions alter the reading frame of dystrophin and result in a prematurely truncated protein [13]. Given the architecture of the dystrophin protein, it is expected that for many of the DMD patients with rod deletions, restoration of dystrophin's reading frame by the targeted removal of an additional exon from the mature transcript will restore a partially functional dystrophin protein and thus provide clinical benefit. This expectation is based on studies showing that patients carrying large, in-frame deletions within the rod domain of the dystrophin protein frequently exhibit a milder clinical phenotype (referred to as Becker muscular dystrophy or BMD) [14,15]. A compelling example is that some individuals with large in-frame mutations, spanning exons 45–55, remained asymptomatic until 69 years of age [16]. These observations led to the hypothesis that the central rod domain of dystrophin was dispensable for dystrophin function. Elegant experiments from Jeff Chamberlain's group [17,18] defined the critical regions of dystrophin by testing the ability of mutant dystrophin, with internally truncated rod domain deletions, to rescue the *mdx* phenotype. Their investigations also demonstrated that larger deletions sometimes lead to a milder phenotype than smaller deletions [17]. These studies, along with the recognition that natural, *in vivo* 'exon skipping' occurred in the *mdx* mouse [19] and in humans with DMD [20,21], established the validity of targeting RNA splicing to restore the proper reading frame as a therapy.

Therapeutic exon skipping is now being tested in animal models of dystrophin deficiency and in human DMD trials. These studies utilize antisense oligonucleotides (AONs) to direct the lack of inclusion of targeted exons containing nonsense or frame-shift mutations into the

translated mRNA. Between 2001 and 2003, the feasibility of exon skipping was demonstrated with the successful administration of oligonucleotides and induction of exon skipping in *mdx* mice *in vivo* [22–24]. In 2005 and 2006, successful systemic administration was accomplished in the *mdx* mouse, although the efficiency was not yet to therapeutic levels [25,26]. Since that time, a variety of chemistries and delivery methods have been devised and tested in the *mdx* mouse and many researchers have continued to identify strategies to improve the efficiency of delivery, while keeping in mind toxicity and immunogenicity. For example, phosphorodiamidate morpholinos coupled to Arg-rich, cell-penetrating peptides effectively restored dystrophin in 96% of *mdx* skeletal muscle fibers, but were less effective in cardiac muscle (58%) [27,28]. Other studies using octaguanidine-coupled morpholinos demonstrated that the efficiency of delivery could be improved with this modification [26]. Studies have administered 2-*O*-methyl oligonucleotides to mice for as long as 8 months of treatment with continued phenotypic improvement apparent in the mice at 16 months of age [29], suggesting that this approach may be tolerated for extended periods of time, an important feature for a chronic disease such as DMD.

Whereas some researchers have focused upon improving the chemistries of the AONs to improve efficacy, others have coupled the oligonucleotides to various carriers to improve delivery. Agents such as nanopolymers of polyethylene glycol and polyethyleneimine [30] and polylactide-co-glycolic acid nanospheres [31] and cationic core shell nanoparticles [32] were used to deliver charged AONs (2-*O*-methyl) to *mdx* muscles. Although promising, all of these studies will require additional exploration of their potential toxicities.

An alternate approach to systemic antisense-based exon skipping has been proposed and tested in cell culture and the *mdx* mouse, in which the AON is cloned in tandem with a modified U7 small nuclear RNA sequence and expressed from an adeno-associated virus [33]. Although this requires a gene-therapy-like approach with its attendant problems, the possibility of a more permanent repair without the need for continued therapy is appealing. Improvements in this process have recently been published in which the AON is also linked to a short-binding sequence of heterogeneous ribonucleoprotein A1 [33].

It is not clear how small antisense sequences interfere with RNA splicing as the process is complex and is influenced by numerous RNA-binding proteins and splice enhancer sequences. Devising universal therapies targeted to specific exons is further complicated by the uniqueness of each DMD mutation and the associated deletion breakpoints. Another difficulty is that the optimal specific sequence to target is not always clear. Although some oligonucleotides are effective if targeted to splice donor and acceptor sites, these motifs are not always preferred targets [34••]. Furthermore, it will be imperative to optimize the oligonucleotides used for therapeutic intervention in the context of human cells *in vitro* [34••] or in the transgenic mouse expressing human dystrophin [35••], prior to initiating clinical trials.

The first human clinical trials for exon skipping are focused on exon 51, because AONs that efficiently induce exon 51 skipping were identified and because of the relatively large proportion of patients for whom exon 51 skipping would generate an in-frame dystrophin transcript. Patients with specific exon deletions (e.g. Δ 47–50, Δ 48–50, Δ 49–50, Δ 50, and – Δ 52) are in aggregate 13% of the DMD population and constitute the most common therapeutic targets in whom the skipping of a single exon is needed to restore reading frame. Trials are being conducted in Europe, targeting exon 51 using two different chemistries. In the Netherlands, researchers administered 2-*O*-methyl AONs that hybridize to an internal sequence of exon 51 (called PRO051) into the tibialis anterior muscle of four DMD patients bearing genetic deletions that were correctable by exon 51 skipping [36]. Biopsied, treated muscles from each patient exhibited detectable levels of dystrophin protein without adverse

effects, demonstrating successful exon skipping and establishing a key landmark for proof-of-principle studies in humans. Based on these promising results, phase I/II studies using systemic administration of PRO051 via subcutaneous delivery are underway and will test the safety and efficacy of a 5-week treatment regime and 13 weeks of follow-up. In parallel, local introduction of AVI-4658, which also targets the same region of exon 51 through an alternate backbone chemistry called morpholino, into the extensor digitorum brevis muscle was tested over a year ago and unpublished results indicate that some dystrophin expression was restored in the injected muscle. Similarly, a 12-week, phase I/IIa systemic delivery clinical trial of AVI-4658 has been initiated at Imperial College London by Drs Francesco Muntoni and Katherine Bushby (unpublished observation). Prior to the study, several different oligonucleotide chemistries were tested using cultured human muscle cells and using a mouse expressing a human dystrophin gene as a model system to identify optimal oligonucleotide conditions [37].

Mutational data indicate that following exon 51 skipping, the next six most common single exon skip targets are exons 45, 53, 44, 46, 52, and 50 (in that order based on frequency of the DMD mutations). Recently, two studies have demonstrated that exon skipping can also be used with complicated dystrophin mutations that lead to 'pseudoexons'. Thus, it is possible that more different types of mutations than it had previously been thought may benefit from this sort of therapeutic approach [38,39] and some point mutations will be amenable to this therapy. Given the large size and exon structure of DMD, there are a staggering 76 different single exons that could be therapeutically targeted in at least one observed mutation. Thus, even if exon 51-targeted therapy is successful, a tremendous amount of work is needed to develop a comprehensive approach to generate an armory of genetic mutation-targeted therapies, which will require an infrastructure to develop, design, and test each targeted therapeutic that may be used in only a single child. This challenge will make DMD a compelling experimental area for truly personalized medicine.

Notable reports of exceptionally mild (or asymptomatic) BMD in patients with large exon 45–55 deletions have led some investigators to explore the feasibility of developing a cocktail of AONs, which could be used as a single drug to treat as many as 63% of all patients with DMD [16,40]. Recent animal studies have led to encouragement that this approach may be feasible. Wilton and coworkers were able to use antisense oligomers to successfully restore dystrophin's reading frame in the *mdx4cv* mouse model, which is one that requires double skipping (of exons 52 and 53) to place the dystrophin transcript back in frame [41]. In addition, Hoffman and coworkers demonstrated the feasibility of multiple exon skipping using the canine muscular dystrophy dog model (CXMD), which has a mutation at the splice site of exon 7 of the dystrophin gene [42••]. To correct this mutation and to restore the reading frame requires skipping two exons (6 and 8) to create a fusion between exons 5 and 9. The authors used a cocktail of antisense morpholinos injected into the leg veins of the dogs and demonstrated some variable correction in all muscles tested of each dog, including the heart, but to a lesser extent. Thus, multi-exon skipping has now been successfully carried out *in vivo* in both small and large animal models. These studies are very encouraging for the development of an AON cocktail that could treat a large percentage of dystrophin mutations. Cocktails of 2-*O*-methyl oligonucleotides against all exons between 45 and 55 were tested in human cells *in vitro* [43]. Unfortunately, the researchers were not able to identify a cocktail that was effective for inducing such a large deletion; thus, additional studies will be necessary before therapeutic exon skipping between exons 45 and 55 becomes a reality.

These preliminary studies demonstrate that exon skipping is a viable strategy to induce the production of dystrophin in DMD boys. However, the success of this therapeutic approach rests on overcoming the inefficiencies of exon skipping, as it is unclear whether the levels of

skipped dystrophin currently being achieved will be sufficient to functionally reverse the disorder, particularly in DMD boys. Best estimates indicate that 30–60% of wild-type levels of skipped dystrophin will be required to functionally compensate for loss of dystrophin [17,44]. Early trial data, though promising, indicate that even high-dose local intramuscular delivery of AON falls short of inducing such levels, yielding only 3–35% of normal dystrophin. It is anticipated that systemic delivery of AON may be even more inefficient. Therefore, it will be imperative to increase the efficacy of exon skipping to replace dystrophin to functionally relevant levels, which is being pursued by altering the oligonucleotide backbone, altering the targeted sequence, modifying attachments to the oligonucleotide sequence, increasing delivery to muscle, and identifying small molecule facilitators of exon skipping. Nonetheless, the success of the early trials with PRO051 is encouraging.

Nonsense codon suppression

Approximately, 5–13% of all DMD causing mutations in dystrophin are nonsense mutations that lead to the creation of a nonsense codon. These stop codons halt translation of the mRNA by the ribosome and result in a truncated, nonfunctional protein. Ten years ago, Dr. Sweeney and coworkers [45] demonstrated that aminoglycoside antibiotics have the capacity to reduce ribosomal fidelity for recognizing these premature termination codons (PTCs) in the dystrophin transcript and, through this mechanism, induce ribosomal read-through of premature termination signals. Read-through of PTCs by the ribosome results in the generation of a full-length dystrophin protein with only one amino acid substitution, which corrects the primary genetic defect. Exposure of *mdx* cells or mice to the aminoglycoside gentamycin induced read-through of the PTC in exon 23 of the dystrophin transcript and production of dystrophin protein. This study was the first successful demonstration of pharmacological correction of a primary genetic defect *in vivo* and provided a proof-of-principle that such a therapy held promise. Unfortunately, these antibiotics were known to be too toxic for long-term therapy and were relatively inefficient. Subsequently, a screen of 800 000 compounds was conducted against a luciferase reporter that harbored a UGA premature stop codon. Through the screen and additional chemical modifications, a lead compound called PTC124 (ataluren) was identified [46]. This compound is a 284 Da, achiral, 1,2,4-oxadiazole linked to fluorobenzene and benzoic rings.

PTC124 has proven to be efficacious in *mdx* mice [46] and to some extent in clinical trials for both DMD [46] and cystic fibrosis [47]. Phase I and IIa clinical trials demonstrated good safety and tolerability in DMD boys and phase IIb clinical trials are fully enrolled with over 165 individuals. There is apparently little toxicity from the oral drug, though efficacy in protecting DMD has yet to be established. However, in the *mdx* mouse model, PTC administration does protect muscle from contraction-induced injury as measured by reduced force per cross-sectional area after five repeated eccentric contractions. Further, levels of dystrophin induced by PTC appear to be 35% and 40–60% of normal in mouse and human, respectively, within the range predicted to be necessary for functional improvement. Some caution that longer term exposure to nonsense codon suppression could permit reactivation of effectively silenced retrotransposons [48]. Although PTC124 targets a minority of DMD mutations, if successful it has the potential to be a substantial treatment for a subset of DMD patients. In addition, because PTC124 is not specific to the gene but rather to the type of mutation, it has the potential to be efficacious in many other recessive disorders that commonly include nonsense mutations.

Recently, some of the compounds successfully identified as read-through compounds in the cell-based assays used by Welch *et al.* [46], were shown to stabilize the luciferase protein and thus raised the possibility that PTC124 was identified not on the basis of truly inducing

read-through of nonsense codons [49]. Although Auld *et al.* [49] demonstrated that PTC124 can increase stability of firefly (Fluc) luciferase, alternate screening conditions to those used in the development of PTC were used. For instance, the initial high throughput screen used to identify PTC was carried out for only 2 h of drug incubation [46], whereas Auld *et al.* [49] incubated with the drug for 16–72 h. In addition, the reported stabilization occurred at high concentrations of drug compared with the concentrations in which read-through was observed (2 $\mu\text{mol/l}$ vs. 30 nmol/l). Further, PTC124 shows differential efficacy against different stop codons, a result that would not occur under the proposed mechanism of Auld *et al.* Most notably, Welch *et al.* [46] have validated the efficacy of PTC124 in the biological system they ultimately wish to treat, dystrophin-deficient muscle. Both in cell cultures made from human biopsies and in *mdx* mice, PTC124 restores at least some dystrophin protein production. Thus, from the published data, there appears to be read-through and dystrophin production induced by PTC124.

Although PTC124 is able to elicit read-through of stop codons, it does not act against normal stop codons. How is this drug able to differentiate between the same nucleic acid sequences in different parts of a transcript? One explanation relates to the secondary structure in the environment where the triplet resides. Another explanation is that it prevents nonsense-mediated decay, which is a mechanism used by the ribosome to identify nonsense codons. Additional studies will need to be carried out to identify the molecular target of PTC124.

Conclusion

Since the identification of the gene mutation in dystrophin leading to DMD in 1987, much has been learned about the function of dystrophin, its associated proteins, and downstream pathogenic mechanisms. Although much is known about these elements, a cure or even effective therapies for this disease have remained elusive. However, in the past 2 years, promising therapies have been developed and it is evident that in the next few years, treatments specific to DMD will likely be brought to market. Although these approaches are exciting, substantial work remains to make them highly effective therapies.

Acknowledgments

The present work was supported by the National Institutes of Health, the Muscular Dystrophy Association, the Department of Defense, the Foundation to Eradicate Duchenne, and the Parent Project for Muscular Dystrophy.

The authors thank their colleagues at the Center for Duchenne Muscular Dystrophy at UCLA for many helpful scientific discussions. Furthermore, the authors thank Terrence Partridge for sharing his insightful points of view with them and Guenter Scheuerbrandt for his informative website and reports (www.duchenne-information.eu).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Kunkel LM, Hejtmancik JF, Caskey CT, et al. Analysis of deletions in DNA from patients with Becker and Duchenne muscular dystrophy. *Nature* 1986;322:73–77. [PubMed: 3014348]

2. Monaco AP, Bertelson CJ, Middlesworth W, et al. Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment. *Nature* 1985;316:842–845. [PubMed: 2993910]
3. Monaco AP, Neve RL, Colletti-Feener C, et al. Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. *Nature* 1986;323:646–650. [PubMed: 3773991]
4. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;51:919–928. [PubMed: 3319190]
5. Peter AK, Marshall JL, Crosbie RH. Sarcospan reduces dystrophic pathology: stabilization of the utrophin-glycoprotein complex. *J Cell Biol* 2008;183:419–427. [PubMed: 18981229]
6. Tinsley J, Deconinck N, Fisher R, et al. Expression of full-length utrophin prevents muscular dystrophy in mdx mice. *Nat Med* 1998;4:1441–1444. [PubMed: 9846586]
7. Burkin DJ, Wallace GQ, Nicol KJ, et al. Enhanced expression of the alpha 7 beta 1 integrin reduces muscular dystrophy and restores viability in dystrophic mice. *J Cell Biol* 2001;152:1207–1218. [PubMed: 11257121]
8. Ng R, Metzger JM, Clafin DR, Faulkner JA. Poloxamer 188 reduces the contraction-induced force decline in lumbrical muscles from mdx mice. *Am J Physiol Cell Physiol* 2008;295:C146–C150. [PubMed: 18495816]
9. Nguyen HH, Jayasinha V, Xia B, et al. Overexpression of the cytotoxic T cell GalNAc transferase in skeletal muscle inhibits muscular dystrophy in mdx mice. *Proc Natl Acad Sci U S A* 2002;99:5616–5621. [PubMed: 11960016]
10. Peault B, Rudnicki M, Torrente Y, et al. Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. *Mol Ther* 2007;15:867–877. [PubMed: 17387336]
11. Darabi R, Santos FN, Perlingeiro RC. The therapeutic potential of embryonic and adult stem cells for skeletal muscle regeneration. *Stem Cell Rev* 2008;4:217–225. [PubMed: 18607783]
12. Thirion C, Lochmuller H. Current status of gene therapy for muscle diseases. *Drug News Perspect* 2007;20:357–363. [PubMed: 17925889]
13. Aartsma-Rus A, Van Deutekom JC, Fokkema IF, et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve* 2006;34:135–144. [PubMed: 16770791]
14. England SB, Nicholson LV, Johnson MA, et al. Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. *Nature* 1990;343:180–182. [PubMed: 2404210]
15. Love DR, Flint TJ, Genet SA, et al. Becker muscular dystrophy patient with a large intragenic dystrophin deletion: implications for functional minigenes and gene therapy. *J Med Genet* 1991;28:860–864. [PubMed: 1757963]
16. Ferreira V, Giliberto F, Muniz GM, et al. Asymptomatic Becker muscular dystrophy in a family with a multiexon deletion. *Muscle Nerve* 2009;39:239–243. [PubMed: 19012301]
17. Harper SQ, Hauser MA, DelloRusso C, et al. Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy. *Nat Med* 2002;8:253–261. [PubMed: 11875496]
18. Phelps SF, Hauser MA, Cole NM, et al. Expression of full-length and truncated dystrophin minigenes in transgenic mdx mice. *Hum Mol Genet* 1995;4:1251–1258. [PubMed: 7581361]
19. Lu QL, Morris GE, Wilton SD, et al. Massive idiosyncratic exon skipping corrects the nonsense mutation in dystrophic mouse muscle and produces functional revertant fibers by clonal expansion. *J Cell Biol* 2000;148:985–996. [PubMed: 10704448]
20. Nicholson LV, Bushby KM, Johnson MA, et al. Predicted and observed sizes of dystrophin in some patients with gene deletions that disrupt the open reading frame. *J Med Genet* 1992;29:892–896. [PubMed: 1479604]
21. Gualandi F, Neri M, Bovolenta M, et al. Transcriptional behavior of DMD gene duplications in DMD/BMD males. *Hum Mutat* 2009;30:E310–E319. [PubMed: 18853462]
22. Mann CJ, Honeyman K, Cheng AJ, et al. Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse. *Proc Natl Acad Sci U S A* 2001;98:42–47. [PubMed: 11120883]
23. Lu QL, Mann CJ, Lou F, et al. Functional amounts of dystrophin produced by skipping the mutated exon in the mdx dystrophic mouse. *Nat Med* 2003;9:1009–1014. [PubMed: 12847521]

24. Mann CJ, Honeyman K, McClorey G, et al. Improved antisense oligonucleotide induced exon skipping in the mdx mouse model of muscular dystrophy. *J Gene Med* 2002;4:644–654. [PubMed: 12439856]
25. Alter J, Lou F, Rabinowitz A, et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. *Nat Med* 2006;12:175–177. [PubMed: 16444267]
26. Lu QL, Rabinowitz A, Chen YC, et al. Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles. *Proc Natl Acad Sci U S A* 2005;102:198–203. [PubMed: 15608067]
27. Wu B, Moulton HM, Iversen PL, et al. Effective rescue of dystrophin improves cardiac function in dystrophin-deficient mice by a modified morpholino oligomer. *Proc Natl Acad Sci U S A* 2008;105:14814–14819. [PubMed: 18806224]
28. Wu B, Li Y, Morcos PA, et al. Octa-guanidine morpholino restores dystrophin expression in cardiac and skeletal muscles and ameliorates pathology in dystrophic mdx mice. *Mol Ther* 2009;17:864–871. [PubMed: 19277018]
29. Laws N, Cornford-Nairn RA, Irwin N, et al. Long-term administration of antisense oligonucleotides into the paraspinal muscles of mdx mice reduces kyphosis. *J Appl Physiol* 2008;105:662–668. [PubMed: 18499783]
30. Williams JH, Schray RC, Sirsi SR, Lutz GJ. Nanopolymers improve delivery of exon skipping oligonucleotides and concomitant dystrophin expression in skeletal muscle of mdx mice. *BMC Biotechnol* 2008;8:35. [PubMed: 18384691]
31. Sirsi SR, Schray RC, Wheatley MA, Lutz GJ. Formulation of polylactide-coglycolic acid nanospheres for encapsulation and sustained release of poly (ethylene imine)-poly(ethylene glycol) copolymers complexed to oligonucleotides. *J Nanobiotechnol* 2009;7:1.
32. Rimessi P, Sabatelli P, Fabris M, et al. Cationic PMMA nanoparticles bind and deliver antisense oligoribonucleotides allowing restoration of dystrophin expression in the mdx mouse. *Mol Ther* 2009;17:820–827. [PubMed: 19240694]
33. Goyenvallé A, Babbs A, van Ommen GJ, et al. Enhanced exon-skipping induced by U7 snRNA carrying a splicing silencer sequence: promising tool for DMD therapy. *Mol Ther* 2009;17:1234–1240. [PubMed: 19455105]
- 34•. Mitrpant C, Adams AM, Meloni PL, et al. Rational design of antisense oligomers to induce dystrophin exon skipping. *Mol Ther*. 2009 [Epub ahead of print]. Researchers used human muscle cell cultures to systematically assess the efficacy of different, potential oligonucleotides that might be used in therapeutic exon skipping. The authors found that oligonucleotides sometimes behaved differently in mice vs. human cells, emphasizing the importance of testing these agents in the appropriate model system.
- 35•. t Hoen PA, de Meijer EJ, Boer JM, et al. Generation and characterization of transgenic mice with the full-length human DMD gene. *J Biol Chem* 2008;283:5899–5907. [PubMed: 18083704] The investigators created a valuable transgenic mouse model in which the entire human dystrophin gene (2.3 Mb) was integrated to mouse chromosome 5. This model will allow for the testing of the impact of different oligonucleotides on exon skipping of the dystrophin gene. The authors also demonstrated for the first time that this human dystrophin gene could functionally compensate for the absence of mouse dystrophin in the *mdx* mouse.
36. van Deutekom JC, Janson AA, Ginjaar IB, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. *N Engl J Med* 2007;357:2677–2686. [PubMed: 18160687]
37. Arechavala-Gomez V, Graham IR, Popplewell LJ, et al. Comparative analysis of antisense oligonucleotide sequences for targeted skipping of exon 51 during dystrophin premRNA splicing in human muscle. *Hum Gene Ther* 2007;18:798–810. [PubMed: 17767400]
38. Madden HR, Fletcher S, Davis MR, Wilton SD. Characterization of a complex Duchenne muscular dystrophy-causing dystrophin gene inversion and restoration of the reading frame by induced exon skipping. *Hum Mutat* 2009;30:22–28. [PubMed: 18570328]
39. Gurvich OL, Tuohy TM, Howard MT, et al. DMD pseudoexon mutations: splicing efficiency, phenotype, and potential therapy. *Ann Neurol* 2008;63:81–89. [PubMed: 18059005]

40. Beroud C, Tuffery-Giraud S, Matsuo M, et al. Multiexon skipping leading to an artificial DMD protein lacking amino acids from exons 45 through 55 could rescue up to 63% of patients with Duchenne muscular dystrophy. *Hum Mutat* 2007;28:196–202. [PubMed: 17041910]
41. Mitropant C, Fletcher S, Iversen PL, Wilton SD. By-passing the nonsense mutation in the 4 CV mouse model of muscular dystrophy by induced exon skipping. *J Gene Med* 2009;11:46–56. [PubMed: 19006096]
42. Yokota T, Lu QL, Partridge T, et al. Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Ann Neurol* 2009;65:667–676. [PubMed: 19288467] The first demonstration of successful exon skipping by systemic administration of AONs to a large animal model. The study was also the first to demonstrate successful, multiexon skipping in a large animal model following systemic administration of oligonucleotides.
43. van Vliet L, de Winter CL, van Deutekom JC, et al. Assessment of the feasibility of exon 45–55 multiexon skipping for Duchenne muscular dystrophy. *BMC Med Genet* 2008;9:105. [PubMed: 19046429]
44. Neri M, Torelli S, Brown S, et al. Dystrophin levels as low as 30% are sufficient to avoid muscular dystrophy in the human. *Neuromuscul Disord* 2007;17(11–12):913–918. [PubMed: 17826093]
45. Barton-Davis ER, Cordier L, Shoturma DI, et al. Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. *J Clin Invest* 1999;104:375–381. [PubMed: 10449429]
46. Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007;447:87–91. [PubMed: 17450125]
47. Kerem E, Hirawat S, Armoni S, et al. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. *Lancet* 2008;372:719–727. [PubMed: 18722008]
48. Goodier JL, Mayer J. PTC124 for cystic fibrosis. *Lancet* 2009;373:1426. author reply 1427. [PubMed: 19394530]
49. Auld DS, Thorne N, Maguire WF, Inglese J. Mechanism of PTC124 activity in cell-based luciferase assays of nonsense codon suppression. *Proc Natl Acad Sci U S A* 2009;106:3585–3590. [PubMed: 19208811]

Figure 1. Therapeutic approaches to treat the primary defect in Duchenne muscular dystrophy
 (a) Schematic representation of the dystrophin–glycoprotein and utrophin–glycoprotein complexes (DGC and UGC, respectively) composed of dystrophin or utrophin, sarcoglycans (α , β , γ , δ -subunits; yellow), dystroglycans (α -subunit and β -subunit; red), and sarcospan (SSPN, green). In DMD, mutations in dystrophin result in loss of the entire DGC and sarcolemmal damage. Improvements in dystrophic pathology can be accomplished by several mechanisms, including upregulation of compensatory proteins, treatment of muscle with poloxamer compounds, and enhanced α -dystroglycan glycosylation, which improves muscle cell attachment to the extracellular matrix through mechanisms involving the UGC. Many compensatory proteins have been identified and only a subset of these is illustrated.

(b) Suppression of premature termination is an emerging therapy that attempts to bypass mutations in dystrophin that give rise to premature stop codons. Treatment of muscle with PTC124 results in the generation of full-length dystrophin protein with only one amino acid substitution at the site of the PTC (indicated in blue). (c) Therapeutic exon skipping utilizes antisense oligonucleotides that direct removal of exons containing nonsense or frame-shift mutations. In the example provided, a deletion mutation (exon 50; orange) alters the reading frame in the mRNA so that exon 49 (green) is spliced to exon 51 (purple). These splicing events result in a premature stop codon (TGA) within exon 51 and produce a truncated dystrophin protein that is nonfunctional and rapidly degraded. Oligonucleotides (PRO051) have been engineered to induce the spliceosome to skip this exon during RNA processing so that exon 49 is spliced directly to exon 52 (blue). The resultant mRNA encodes a truncated, but functional dystrophin protein lacking a small portion of the rod domain while maintaining the N-terminal and C-terminal regions important for protein interactions with actin (N-terminal dystrophin) and β -dystroglycan (C-terminal dystrophin).