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Ed Kaye, Abstract No. 37, Nucleic Acid Therapeutics, New Rochelle, doi:10.1089/nat.2012.1501, (2012-12-01), page A13, Abstracts 8th Annual Meeting of the Oligonucleotide Therapeutics Society Boston, Massachusetts October 28-31, 2012
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(57) Abstract: Improved compositions and methods for treating muscular dystrophy by administering an antisense molecule such as eteplirsen, capable of binding to a selected target site in the human dystrophin gene to induce exon skipping, are described.

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IMPROVED COMPOSITIONS FOR TREATING MUSCULAR DYSTROPHY

RELATED APPLICATIONS

This patent application claims the benefit of U.S. Provisional Patent Application Serial No. 61/793,463, filed March 15, 2013. The entire contents of the above-referenced provisional patent application are incorporate herein by reference.

FIELD OF THE INVENTION

The present invention relates to improved methods for treating muscular dystrophy in a patient. It also provides compositions suitable for facilitating exon skipping in the human dystrophin gene.

BACKGROUND OF THE INVENTION

Antisense technologies are being developed using a range of chemistries to affect gene expression at a variety of different levels (transcription, splicing, stability, translation). Much of that research has focused on the use of antisense compounds to correct or compensate for abnormal or disease-associated genes in a wide range of indications. Antisense molecules are able to inhibit gene expression with specificity, and because of this, many research efforts concerning oligonucleotides as modulators of gene expression have focused on inhibiting the expression of targeted genes or the function of cis-acting elements. The antisense oligonucleotides are typically directed against RNA, either the sense strand (e.g., mRNA), or minus-strand in the case of some viral RNA targets. To achieve a desired effect of specific gene down-regulation, the oligonucleotides generally either promote the decay of the targeted mRNA, block translation of the mRNA or block the function of cis-acting RNA elements, thereby effectively preventing either *de novo* synthesis of the target protein or replication of the viral RNA.

However, such techniques are not useful where the object is to up-regulate production of the native protein or compensate for mutations that induce premature termination of translation, such as nonsense or frame-shifting mutations. In these cases, the defective gene transcript should not be subjected to targeted degradation or steric inhibition, so the antisense oligonucleotide chemistry should not promote target mRNA decay or block translation.

In a variety of genetic diseases, the effects of mutations on the eventual expression of a gene can be modulated through a process of targeted exon skipping during the splicing process. The splicing process is directed by complex multi-component machinery that brings adjacent exon-intron junctions in pre-mRNA into close proximity and performs cleavage of phosphodiester bonds at the ends of the introns with their subsequent reformation between exons that are to be spliced together. This complex and highly precise process is mediated by sequence motifs in the pre-mRNA that are relatively short, semi-conserved RNA segments to which various nuclear splicing factors that are then involved in the splicing reactions bind. By changing the way the splicing machinery reads or recognizes the motifs involved in pre-mRNA processing, it is possible to create differentially spliced mRNA molecules. It has now been recognized that the majority of human genes are alternatively spliced during normal gene expression, although the mechanisms involved have not been identified. Bennett *et al.* (U.S. Patent No. 6,210,892) describe antisense modulation of wild-type cellular mRNA processing using antisense oligonucleotide analogs that do not induce RNAse H-mediated cleavage of the target RNA. This finds utility in being able to generate alternatively spliced mRNAs that lack specific exons (e.g., as described by (Sazani, Kole, et al. 2007) for the generation of soluble TNF superfamily receptors that lack exons encoding membrane spanning domains.

In cases where a normally functional protein is prematurely terminated because of mutations therein, a means for restoring some functional protein production through antisense technology has been shown to be possible through intervention during the splicing processes, and that if exons associated with disease-causing mutations can be specifically deleted from some genes, a shortened protein product can sometimes be produced that has similar biological properties of the native protein or has sufficient biological activity to ameliorate the disease caused by mutations associated with the exon (see *e.g.,* Sierakowska, Sambade et al. 1996; Wilton, Lloyd et al. 1999; van Deutekom, Bremmer-Bout et al. 2001; Lu, Mann et al. 2003; Aartsma-Rus, Janson et al. 2004). Kole *et al.* (U.S. Patent Nos. 5,627,274; 5,916,808; 5,976,879; and 5,665,593) disclose methods of combating aberrant splicing using modified antisense oligonucleotide analogs that do not promote decay of the targeted pre-mRNA. Bennett et al. (U.S. Patent No. 6,210,892) describe antisense modulation of wild-type cellular mRNA processing also using antisense oligonucleotide analogs that do not induce RNAse H-mediated cleavage of the target RNA.

The process of targeted exon skipping is likely to be particularly useful in long genes where there are many exons and introns, where there is redundancy in the genetic constitution of the exons or where a protein is able to function without one or more particular exons. Efforts to redirect gene processing for the treatment of genetic diseases associated with truncations caused by mutations in various genes have focused on the use of antisense oligonucleotides that either: (1) fully or partially overlap with the elements involved in the splicing process; or (2) bind to the pre-mRNA at a position sufficiently close to the element to disrupt the binding and function of the splicing factors that would normally mediate a particular splicing reaction which occurs at that element.

Duchenne muscular dystrophy (DMD) is caused by a defect in the expression of the protein dystrophin. The gene encoding the protein contains 79 exons spread out over more than 2 million nucleotides of DNA. Any exonic mutation that changes the reading frame of the exon, or introduces a stop codon, or is characterized by removal of an entire out of frame exon or exons, or duplications of one or more exons, has the potential to disrupt production of functional dystrophin, resulting in DMD.

Disease onset can be documented at birth with elevated creatine kinase levels, and significant motor deficits may be present in the first year of life. By the age of seven or eight, most patients with DMD have an increasingly labored gait and are losing the ability to rise from the floor and climb stairs; by ages 10 to 14, most are wheelchair-dependent. DMD is uniformly fatal; affected individuals typically die of respiratory and/or cardiac failure in their late teens or early 20s. The continuous progression of DMD allows for therapeutic intervention at all stages of the disease; however, treatment is currently limited to glucocorticoids, which are associated with numerous side effects including weight gain, behavioral changes, pubertal changes, osteoporosis, Cushingoid facies, growth inhibition, and cataracts. Consequently, developing better therapies to treat the underlying cause of this disease is imperative.

A less severe form of muscular dystrophy, Becker muscular dystrophy (BMD) has been found to arise where a mutation, typically a deletion of one or more exons, results in a correct reading frame along the entire dystrophin transcript, such that translation of mRNA into protein is not prematurely terminated. If the joining of the upstream and downstream exons in the processing of a mutated dystrophin pre-mRNA maintains the correct reading frame of the gene,

the result is an mRNA coding for a protein with a short internal deletion that retains some activity, resulting in a Becker phenotype.

For many years it has been known that deletions of an exon or exons which do not alter the reading frame of a dystrophin protein would give rise to a BMD phenotype, whereas an exon deletion that causes a frame-shift will give rise to DMD (Monaco, Bertelson et al. 1988). In general, dystrophin mutations including point mutations and exon deletions that change the reading frame and thus interrupt proper protein translation result in DMD. It should also be noted that some BMD and DMD patients have exon deletions covering multiple exons.

Modulation of mutant dystrophin pre-mRNA splicing with antisense oligoribonucleotides has been reported both *in vitro* and *in vivo* (see *e.g.,* Matsuo, Masumura et al. 1991; Takeshima, Nishio et al. 1995; Pramono, Takeshima et al. 1996; Dunckley, Eperon et al. 1997; Dunckley, Manoharan et al. 1998; Errington, Mann et al. 2003).

The first example of specific and reproducible exon skipping in the *mdx* mouse model was reported by Wilton *et al.* (Wilton, Lloyd et al. 1999). By directing an antisense molecule to the donor splice site, consistent and efficient exon 23 skipping was induced in the dystrophin mRNA within 6 hours of treatment of the cultured cells. Wilton *et al.* also describe targeting the acceptor region of the mouse dystrophin pre-mRNA with longer antisense oligonucleotides. While the first antisense oligonucleotide directed at the intron 23 donor splice site induced consistent exon skipping in primary cultured myoblasts, this compound was found to be much less efficient in immortalized cell cultures expressing higher levels of dystrophin. However, with refined targeting and antisense oligonucleotide design, the efficiency of specific exon removal was increased by almost an order of magnitude (Mann, Honeyman et al. 2002).

Recent studies have begun to address the challenge of achieving sustained dystrophin expression accompanied by minimal adverse effects in tissues affected by the absence of dystrophin. Intramuscular injection of an antisense oligonucleotide targeted to exon 51 (PRO051) into the tibialis anterior muscle in four patients with DMD resulted in specific skipping of exon 51 without any clinically apparent adverse effects (Mann, Honeyman et al. 2002; van Deutekom, Janson et al. 2007). Studies looking at systemic delivery of an antisense phosphorodiamidate morpholino oligomer conjugated to a cell-penetrating peptide (PPMO) targeted to exon 23 in *mdx* mice produced high and sustained dystrophin protein production in

skeletal and cardiac muscles without detectable toxicity (Jearawiriyapaisarn, Moulton et al. 2008; Wu, Moulton et al. 2008; Yin, Moulton et al. 2008).

Recent clinical trials testing the safety and efficacy of splice switching oligonucleotides (SSOs) for the treatment of DMD are based on SSO technology to induce alternative splicing of pre-mRNAs by steric blockade of the spliceosome (Cirak *et al.*, 2011; Goemans *et al.*, 2011; Kinali *et al.*, 2009; van Deutekom *et al.*, 2007). However, despite these successes, the pharmacological options available for treating DMD are limited. Notably, an antisense oligonucleotide (drisapersen), which utilizes a negatively charged phosphorothioate backbone, has been associated in clinical trials with proteinuria, increased urinary α1-microglobulin, thrombocytopenia and injection site reactions, such as erythema and inflammation.

Eteplirsen, which is being developed by the assignee of this application, has been the subject of clinical studies to test its safety and efficacy and clinical development is ongoing. Eteplirsen is an oligonucleotide structurally distinct from drisapersen. Specifically, the chemical backbone of eteplirsen is phosphorodiamidate mopholino (PMO), whereas the chemical backbone of drisapersen is 2'O-methyl phosphorothioate (2'-OMe). These structural differences and their potential impact on clinical outcomes were recently described. See *Molecular Therapy Nucleic Acids* (2014) **3**, e152; doi:10.1038/mtna.2014.6 (Published online 11 March 2014).

The sequence of eteplirsen has previously been described. See, for example, U.S. Patent No. 7,807,816, which is exclusively licensed to Applicants. U.S. Patent No. 7,807,816, however, does not explicitly discuss optimum dosing schedules and routes of administration for eteplirsen.

Thus, there remains a need for improved compositions and methods for treating muscular dystrophy, such as DMD and BMD in patients.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on compelling evidence of a therapeutic effect of an exon skipping antisense oligonucleotide, eteplirsen, which represents a major advance in the treatment of DMD by addressing the underlying cause of the disease. The novel finding that treatment with an exon 51 skipping antisense oligonucleotide, eteplirsen, produced reliable increases in novel dystrophin and stabilized walking ability (e.g., stabilization of ambulation), as measured by the 6 Minute Walk Test (6MWT), underscores the potential to alter the course of the disease. Significantly, no drug-related adverse events were seen in 576

infusions administered over one year. When applied to other exons, the use of exon skipping antisense oligonucleotides could treat an estimated 70% to 80% of patients who have DMD due to a deletion in the dystrophin gene.

Accordingly, in one aspect the present invention relates to a method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering to the patient a dose of eteplirsen of about 30 mg/kg once a week over 120 weeks, thereby delaying the progression of the disease over 120 weeks as determined by one or both of the following:

(i) the patient maintaining stable walking ability over 120 weeks, as measured by a decline of less than 5 percent, or less than 13.9 meters, in walking ability, relative to baseline in the 6 Minute Walk Test (6MWT); and

(ii) the patient showing an increase in respiratory muscle function over 120 weeks relative to baseline, as measured by a 14.6 percent increase in maximum inspiratory pressure (MIP) and a 15 percent increase in maximum expiratory pressure (MEP). In some embodiments, eteplirsen is administered in a single dose. In some embodiments eteplirsen is administered intravenously. In some embodiments, the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene. In some embodiments, the patient is a pediatric patient.

In another aspect, the present invention relates to a method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering to the patient a dose of eteplirsen of about 30 mg/kg once a week, wherein the patient is administered an oral corticosteroid for at least 24 weeks prior to the first dose of eteplirsen.

In yet another aspect, the present invention provides a method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering intravenously to the patient a single dose of eteplirsen of about 30 mg/kg once a week, wherein the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene.

Other embodiments of the invention relate to a method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering to the patient a dose of eteplirsen of about 50 mg/kg once a week over 120 weeks, thereby delaying the progression of the disease over 120 weeks as determined by one or both of the following:

(i) the patient maintaining stable walking ability over 120 weeks, as measured by a decline of less than 5 percent, or less than 13.9 meters, in walking ability, relative to baseline in the 6 Minute Walk Test (6MWT); and

(ii) the patient showing an increase in respiratory muscle function over 120 weeks relative to baseline, as measured by a 14.6 percent increase in maximum inspiratory pressure (MIP) and a 15 percent increase in maximum expiratory pressure (MEP). In some embodiments, eteplirsen is administered in a single dose. In some embodiments, eteplirsen is administered intravenously. In some embodiments the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene. In some embodiments, the patient is a pediatric patient. In yet other embodiments, the patient is administered an oral corticosteroid for at least 24 weeks prior to the first dose of eteplirsen.

In another aspect, the invention relates to a method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering intravenously to the patient a single dose of eteplirsen of about 50 mg/kg once a week, wherein the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene.

In yet another aspect, the invention provides a method for increasing dystrophin production in a patient having Duchenne muscular dystrophy comprising administering to the

patient a dose of eteplirsen of about 30 mg/kg once a week. In some embodiments, a dose of eteplirsen of about 50 mg/kg once a week is administered. In some embodiments, eteplirsen is administered in a single dose. In some embodiments, eteplirsen is administered intravenously. In other embodiments, the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene. In some embodiments the patient is a pediatric patient. In other embodiments, the patient is administered an oral corticosteroid for at least 24 weeks prior to the first dose of eteplirsen.

Accordingly, the present invention relates to methods of treating Duchenne muscular dystrophy (DMD) or Becker muscular dystrophy (BMD) in patients by administering an effective amount of a composition comprising an antisense oligonucleotide of 20 to 50 nucleotides in length comprising at least 10 consecutive nucleotides complementary to a target region in an exon of the human dystrophin gene to specifically hybridize to the target region, induce exon skipping, and thereby treat the disease. In one embodiment, an effective amount is at least 20 mg/kg for a period of time sufficient to increase the number of dystrophin-positive fibers in a subject to at least 20% of normal, and stabilize, maintain, or improve walking distance from a 20% deficit, for example in a 6 MWT, in the patient, relative to a healthy peer. In another embodiment, an effective amount is at least 20 mg/kg to about 30 mg/kg, about 25 mg/kg to about 30 mg/kg, or about 30 mg/kg to about 50 mg/kg. In yet another embodiment, an effective amount is about 30 mg/kg or about 50 mg/kg.

In another aspect, an effective amount is at least 20 mg/kg, about 25 mg/kg, about 30mg/kg, or about 30 mg/kg to about 50 mg/kg, for at least 24 weeks, at least 36 weeks, or at least 48 weeks, to thereby increase the number of dystrophin-positive fibers in a subject to at least 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% of normal, and stabilize or improve walking distance from a 20% deficit, for example in a 6 MWT, in the patient relative to a healthy peer. In one embodiment, treatment increases the number of dystrophin-positive fibers to 20-60%, or 30-50% of normal in the patient. In some embodiments, treatment is by systemic administration, such as once weekly by infusion. In other embodiments, treatment includes administering another therapeutic agent, such as a steroid to the subject.

In another aspect, the present invention provides a method of treating DMD or BMD in a patient by administering about 30 mg/kg to about 50 mg/kg of a composition comprising an

antisense oligonucleotide of 20 to 50 nucleotides in length comprising at least 10 consecutive nucleotides complementary to a target region in an exon of the human dystrophin gene, wherein the antisense oligonucleotide specifically hybridizes to the target region inducing exon skipping, thereby treating the subject. In one embodiment, the antisense oligonucleotide is substantially uncharged. In another embodiment, the antisense oligonucleotide comprises morpholino subunits linked by phosphorus-containing intersubunit linkages joining a morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent subunit. In yet another embodiment, the antisense oligonucleotide comprises morpholino subunits linked by substantially uncharged phosphorus-containing intersubunit linkages joining a morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent subunit. In other aspects, the antisense oligonucleotide comprises morpholino subunits and phosphorodiamidate intersubunit linkages.

In some embodiments, the antisense oligonucleotide is 20 to 50, 30 to 50, or 20 to 30 nucleotides in length comprising at least 10, 12, 15, 17, or 20 consecutive nucleotides complementary to a target region in an exon of the human dystrophin gene selected from the group consisting of exon 51, exon 50, exon 53, exon 45, exon 46, exon 44, exon 52, exon 55 and exon 8. In one embodiment, the antisense is 20 to 50, 30 to 50, or 20 to 30 nucleotides in length and includes at least 20 consecutive nucleotides eteplirsen (SEQ ID NO: 1). In another embodiment, the antisense oligonucleotide is 20 to 50, 30 to 50, or 20 to 30 nucleotides in length and includes at least 10, 12, 15, 17, or 20 consecutive nucleotides of the antisense oligonucleotide set forth as SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9. In yet another embodiment, the antisense oligonucleotide is 20 to 50, 30 to 50, or 20 to 30 nucleotides in length and includes at least 10, 12, 15, 17, or 20 consecutive nucleotides of a nucleotide sequences set forth in Tables 3 and 4, wherein uracil bases in the antisense oligonucleotide are optionally thymine bases.

In one embodiment, the composition includes eteplirsen (SEQ ID NO: 1), and, optionally, a pharmaceutically acceptable carrier. In another embodiment, the composition includes an antisense oligonucleotide selected from the group consisting of SEQ ID NOS: 1-9, such as SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9. In yet another embodiment, the antisense oligonucleotide is any one or a combination of the nucleotide sequences set forth in Tables 3 and 4, wherein uracil bases in the antisense oligonucleotide are optionally thymine bases. In some

aspects, the antisense oligonucleotide is chemically linked to one or more moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide, such as an arginine-rich peptide.

In another aspect, the present invention provides a method of treating DMD or BMD in a patient by administering at least 20 mg/kg of a composition comprising eteplirsen (SEQ ID NO: 1) for a period of time sufficient to increase the number of dystrophin-positive fibers in a subject to at least about 20% of normal, and stabilize or improve walking distance from a 20% deficit, for example in a 6 MWT, in the patient, relative to a healthy peer. In another embodiment, an effective amount is at least 20 mg/kg to about 30 mg/kg, about 25 mg/kg to about 30 mg/kg, or about 30 mg/kg to about 50 mg/kg of a composition comprising eteplirsen (SEQ ID NO: 1), and, optionally, a pharmaceutically acceptable carrier, such as phosphate-buffered saline.

In another aspect, an effective amount of a composition comprising eteplirsen (SEQ ID NO: 1) is at least 20 mg/kg, about 25 mg/kg, about 30mg/kg, or about 30 mg/kg to about 50 mg/kg, for at least 24 weeks, at least 36 weeks, or at least 48 weeks, to thereby increase the number of dystrophin-positive fibers in a subject to at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% of normal, and stabilize or improve walking distance from a 20% deficit, for example in a 6 MWT, in the patient relative to a healthy peer. In some embodiments, treatment with antisense oligonucleotides of the present invention slows or reduces the loss of ambulation that would be expected without treatment. In some embodiments, treatment with the antisense oligonucleotides of the present invention stabilizes, maintains, or increases a stable walking distance in a patient. For example, treatment may increase the stable walking distance in the patient from baseline to greater than 3, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 or 50 meters (including all integers in between).

Other aspects of the invention relate to treatment with an antisense oligonucleotide of the invention, such as eteplirsen, which slows or reduces the progressive respiratory muscle dysfunction and/or failure in patients with DMD that would be expected without treatment. In one embodiment, treatment with an antisense oligonucleotide of the invention may reduce or eliminate the need for ventilation assistance that would be expected without treatment. In one embodiment, measurements of respiratory function for tracking the course of the disease, as well as the evaluation of potential therapeutic interventions include Maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) and forced vital capacity (FVC).

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A shows an exemplary morpholino oligomer structure with a phosphorodiamidate linkage.

FIG. 1B shows a conjugate of an arginine-rich peptide and an antisense oligomer, in accordance with an embodiment of the invention.

FIG. 1C shows a conjugate as in FIG. 1B, wherein the backbone linkages contain one or more positively charged groups.

FIGs. 1D-G show the repeating subunit segment of exemplary morpholino oligonucleotides, designated D through G.

FIG. 2 is a schematic representation of the study design for treating DMD patients. Twelve DMD patients were randomized to one of three cohorts in the double-blind, placebo-controlled study, 201: Cohort 1, eteplirsen 30 mg/kg/wk; Cohort 2, eteplirsen 50 mg/kg/wk; and Cohort 3, placebo/delayed eteplirsen. At week 25, placebo-treated patients in Cohort 3 switched to open-label treatment with 30 or 50 mg/kg/week eteplirsen. Patients were maintained on their same dose of eteplirsen under the open-label extension study, 202. **Muscle Biopsies.** Patients underwent biceps biopsies at baseline and deltoid biopsies at week 48 for analysis of dystrophin. Additional biceps biopsies were obtained at week 12 (from patients in Cohort 2 and two patients in Cohort 3) or week 24 (from patients in Cohort 1 and two patients in Cohort 3). **Efficacy Evaluations.** The 6MWT was used as a functional outcome measure and was performed pre-treatment and every 12 weeks post treatment through week 48.

FIG. 3 depicts dystrophin-positive muscle fibers after 12, 24, and 48 weeks of eteplirsen. Panels **A** and **B** show the mean absolute change from baseline in the percentage of dystrophin-positive fibers at weeks 12, 24, and week 48 by treatment group. In Panel A: *P-value is for comparison between eteplirsen and placebo using the pooled results from weeks 12 and 24, and is based on an analysis of covariance model for ranked data with treatment as a fixed effect and baseline value and time since DMD diagnosis as covariates. Mean changes shown are based on descriptive statistics. † P-value is from a paired t-test comparing the week 48 value to baseline. ‡ Results from the placebo-treated patients biopsied at weeks 12 and 24 are pooled.
§ Placebo/delayed eteplirsen patients began receiving eteplirsen at week 25 and had received a

total of 24 doses at week 48. Abbreviations: BL=baseline; NA=not applicable; ND=not done; NS=not significant; SE=standard error.

FIG. 4 shows the effects of eteplirsen on the dystrophin-associated glycoprotein complex. **(A)** Representative examples of time-dependent increases in dystrophin-positive fibers in relation to treatment for all participating study patients. **(B)** nNOS μ staining in muscle from DMD (a) and normal (c) control patients (not in study), and from patient 6 at baseline (b) and week 48 (d), demonstrates restoration of nNOSμ binding with eteplirsen. β– sarcoglycan (e) and γ– sarcoglycan (f) staining in patient 6 at week 48 demonstrate restoration of the sarcoglycan complex with eteplirsen. **(C)** RT-PCR shows skipped product (289 bp) post-treatment in the muscle of patient 12.

FIG. 5 graphically depicts the functional efficacy of eteplirsen. The dark purple line shows the change from baseline in distance walked on the 6MWT over time for the 6 evaluable patients who received eteplirsen from the start of 201 (two boys were unable to at or beyond week 24 were excluded from this analysis). The gray line shows change from baseline in distance walked on the 6MWT for the 4 patients who received placebo for the first 24 weeks and eteplirsen for the last 24 weeks.

DETAILED DESCRIPTION

Embodiments of the present invention relate to improved methods for treating muscular dystrophy, such as DMD and BMD, by administering antisense compounds that are specifically designed to induce exon skipping in the human dystrophin gene. Dystrophin plays a vital role in muscle function, and various muscle-related diseases are characterized by mutated forms of this gene. Hence, in certain embodiments, the improved methods described herein may be used for inducing exon skipping in mutated forms of the human dystrophin gene, such as the mutated dystrophin genes found in DMD and BMD.

Due to aberrant mRNA splicing events caused by mutations, these mutated human dystrophin genes either express defective dystrophin protein or express no measurable dystrophin at all, a condition that leads to various forms of muscular dystrophy. To remedy this condition, the antisense compounds of the present invention hybridize to selected regions of a pre-processed RNA of a mutated human dystrophin gene, induce exon skipping and differential splicing in that otherwise aberrantly spliced dystrophin mRNA, and thereby allow muscle cells to

produce an mRNA transcript that encodes a functional dystrophin protein. In certain embodiments, the resulting dystrophin protein is not necessarily the "wild-type" form of dystrophin, but is rather a truncated, yet functional or semi-functional, form of dystrophin.

By increasing the levels of functional dystrophin protein in muscle cells, these and related embodiments are useful in the prophylaxis and treatment of muscular dystrophy, especially those forms of muscular dystrophy, such as DMD and BMD, that are characterized by the expression of defective dystrophin proteins due to aberrant mRNA splicing. The methods described herein further provide improved treatment options for patients with muscular dystrophy and offer significant and practical advantages over alternate methods of treating relevant forms of muscular dystrophy. For example, in some embodiments, the improved methods relate to the administration of an antisense compound for inducing exon skipping in the human dystrophin gene at a higher dose and/or for a longer duration than prior approaches.

Thus, the invention relates to improved methods for treating muscular dystrophy such as DMD and BMD, by inducing exon skipping in a patient. In some embodiments, exon skipping is induced by administering an effective amount of a composition which includes a charge-neutral, phosphorodiamidate morpholino oligomer (PMO), such as eteplirsen, which selectively binds to a target sequence in an exon of dystrophin pre-mRNA. In some embodiments, the invention relates to methods of treating DMD or BMD in which an effective amount of a composition e.g., at least 20 mg/kg, about 25 mg/kg, about 30 mg/kg or about 30 mg/kg to about 50 mg/kg, which includes an antisense as described herein, such as eteplirsen, over a period of time sufficient to treat the disease.

Some embodiments of the present invention relate to the use of eteplirsen as a disease-modifying therapy for treating DMD. Without being bound by theory, the clinical efficacy seen to date with eteplirsen may be derived from its safety profile due to its unique chemical composition, which is characterized by nucleotides bound to morpholine rings linked through charge-neutral phosphorodiamidate moieties.

In DMD patients treated with eteplirsen for one year, the mean percentage of dystrophin-positive fibers was increased to 47% of normal, relative to baseline. The magnitude of the increase was dependent upon treatment duration. Significant increases in dystrophin levels were observed in the 24-week biopsies taken from patients in Cohort 1 (30 mg/kg) and in the 48-week biopsies from patients in Cohort 3 (who started eteplirsen at week 25).

Eteplirsen's clinical benefit mirrored its ability to induce exon skipping and restore functional dystrophin production. Clinical effect was assessed with the 6MWT, a measure of endurance and muscular capacity that goes beyond the assessment of strength in individual muscle groups. Patients who received 30 or 50 mg/kg eteplirsen from the beginning maintained a stable walking distance over 48 weeks, consistent with eteplirsen-induced increases in novel dystrophin expression between weeks 12 and 24. In contrast, patients in the placebo/delayed eteplirsen cohort lost 70 meters by week 36, but appeared to stabilize by week 48 (24 weeks after initiating eteplirsen). This is the same timeframe in which a clinical impact was seen in patients who received 30 or 50 mg/kg eteplirsen once a week from the start of the study. Both cohorts have maintained stable 6MWT results over 120 weeks as described below.

At 120 weeks, patients in the 30 mg/kg and 50 mg/kg eteplirsen cohorts who were able to perform the 6MWT (modified Intent-to-Treat or mITT population; n=6) experienced a general stability with a slight decline of 13.9 meters, or less than 5 percent, from baseline in walking ability. A statistically significant treatment benefit of 64.9 meters ($p \leq 0.006$) was observed for the mITT population compared with the placebo/delayed-treatment cohort (n=4), which initiated treatment at Week 25 following 24 weeks of placebo. After experiencing a substantial decline earlier in the study (prior to treatment with etiplersen), the placebo/delayed-treatment cohort also demonstrated stabilization in walking ability for more than 1.5 years, from Week 36 through 120, the period from which meaningful levels of dystrophin were likely produced, with a decline of 9.5 meters over this timeframe. These analyses were based on the maximum 6MWT score when the test was performed on two consecutive days.

Respiratory muscle function from baseline through Week 120 in both dosing cohorts, as measured by maximum inspiratory and expiratory pressure (MIP and MEP), showed a 14.6 percent mean increase in MIP and a 15.0 percent mean increase in MEP. Analyses of MIP percent predicted (MIP adjusted for weight) and MEP percent predicted (MEP adjusted for age) demonstrated a mean increase from 90.2 percent at baseline to 95.2 percent at Week 120 in MIP percent predicted, and a slight mean increase from 79.3 percent at baseline to 79.6 percent at Week 120 in MEP percent predicted. In addition, there was a mean increase in forced vital capacity (FVC), a measure of lung volume, of 8.7 percent from baseline to Week 120, and FVC percent predicted (FVC adjusted for age and height) was maintained above a mean of 90 percent through Week 120, with 101 percent at baseline and 93 percent at Week 120.

The present invention is based, at least in part, on the evidence of a therapeutic effect of eteplirsen, which represents a major advance in the treatment of DMD by addressing the underlying cause of the disease. Accordingly, the invention relates to methods of treating DMD or BMD in patients by administering an effective amount of a composition which includes an antisense oligonucleotide, such as eteplirsen, which is complementary to a target region in an exon of the human dystrophin gene to specifically hybridize to the target region, induce exon skipping, and treat the disease. In one embodiment, treatment is by administering one or more antisense oligonucleotides of the present invention (e.g., a nucleotide sequence shown in Tables 3 and 4), optionally as part of a pharmaceutical formulation or dosage form, to a subject in need thereof. Treatment includes inducing exon-skipping in a subject by administering an effective amount of one or more antisense oligonucleotides, in which the exon is any one or more of exons 1-79 from the dystrophin gene. Preferably, the exon is exon 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 8 from the human dystrophin gene.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

I. Definitions

By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

The terms "complementary" and "complementarity" refer to polynucleotides (i.e., a sequence of nucleotides) related by base-pairing rules. For example, the sequence "T-G-A (5'-3')," is complementary to the sequence "T-C-A (5'-3')." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and

strength of hybridization between nucleic acid strands. While perfect complementarity is often desired, some embodiments can include one or more but preferably 6, 5, 4, 3, 2, or 1 mismatches with respect to the target RNA. Variations at any location within the oligomer are included. In certain embodiments, variations in sequence near the termini of an oligomer are generally preferable to variations in the interior, and if present are typically within about 6, 5, 4, 3, 2, or 1 nucleotides of the 5' and/or 3' terminus.

The terms "cell penetrating peptide" and "CPP" are used interchangeably and refer to cationic cell penetrating peptides, also called transport peptides, carrier peptides, or peptide transduction domains. The peptides, as shown herein, have the capability of inducing cell penetration within 100% of cells of a given cell culture population and allow macromolecular translocation within multiple tissues *in vivo* upon systemic administration. A preferred CPP embodiment is an arginine-rich peptide as described further below.

The terms "antisense oligomer" and "antisense compound" and "antisense oligonucleotide" are used interchangeably and refer to a sequence of cyclic subunits, each bearing a base-pairing moiety, linked by intersubunit linkages that allow the base-pairing moieties to hybridize to a target sequence in a nucleic acid (typically an RNA) by Watson-Crick base pairing, to form a nucleic acid:oligomer heteroduplex within the target sequence. The cyclic subunits are based on ribose or another pentose sugar or, in a preferred embodiment, a morpholino group (see description of morpholino oligomers below). The oligomer may have exact or near sequence complementarity to the target sequence; variations in sequence near the termini of an oligomer are generally preferable to variations in the interior.

Such an antisense oligomer can be designed to block or inhibit translation of mRNA or to inhibit natural pre-mRNA splice processing, and may be said to be "directed to" or "targeted against" a target sequence with which it hybridizes. The target sequence is typically a region including an AUG start codon of an mRNA, a Translation Suppressing Oligomer, or splice site of a pre-processed mRNA, a Splice Suppressing Oligomer (SSO). The target sequence for a splice site may include an mRNA sequence having its 5' end 1 to about 25 base pairs downstream of a normal splice acceptor junction in a preprocessed mRNA. A preferred target sequence is any region of a preprocessed mRNA that includes a splice site or is contained entirely within an exon coding sequence or spans a splice acceptor or donor site. An oligomer is more generally said to be "targeted against" a biologically relevant target, such as a protein,

virus, or bacteria, when it is targeted against the nucleic acid of the target in the manner described above.

The terms "morpholino oligomer" or "PMO" (phosphoramidate- or phosphorodiamidate morpholino oligomer) refer to an oligonucleotide analog composed of morpholino subunit structures, where (i) the structures are linked together by phosphorus-containing linkages, one to three atoms long, preferably two atoms long, and preferably uncharged or cationic, joining the morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent subunit, and (ii) each morpholino ring bears a purine or pyrimidine base-pairing moiety effective to bind, by base specific hydrogen bonding, to a base in a polynucleotide. See, for example, the structure in Figure 1A, which shows a preferred phosphorodiamidate linkage type. Variations can be made to this linkage as long as they do not interfere with binding or activity. For example, the oxygen attached to phosphorus may be substituted with sulfur (thiophosphorodiamidate). The 5' oxygen may be substituted with amino or lower alkyl substituted amino. The pendant nitrogen attached to phosphorus may be unsubstituted, monosubstituted, or disubstituted with (optionally substituted) lower alkyl. The purine or pyrimidine base pairing moiety is typically adenine, cytosine, guanine, uracil, thymine or inosine. The synthesis, structures, and binding characteristics of morpholino oligomers are detailed in U.S. Patent Nos. 5,698,685, 5,217,866, 5,142,047, 5,034,506, 5,166,315, 5,521,063, 5,506,337, 8,076,476, 8,299,206 and 7,943,762 (cationic linkages), all of which are incorporated herein by reference. Modified intersubunit linkages and terminal groups are detailed in PCT application US2011/038459 and publication WO/2011/150408 which are incorporated herein by reference in their entirety.

"Eteplirsen", also known as "AVN-4658" is a PMO having the base sequence 5'-CTCCAACATCAAGGAAGATGGCATTTCTAG-3' (SEQ ID NO:1). Eteplirsen is registered under CAS Registry Number 1173755-55-9. Chemical names include:

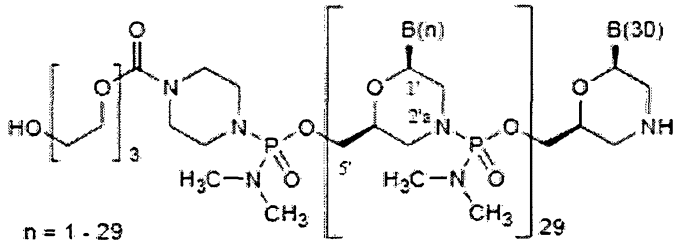
RNA, [*P*-deoxy-*P*-(dimethylamino)](2',3'-dideoxy-2',3'-imino-2',3'-seco)(2'a→5')(C-m5U-C-C-A-A-C-A-m5U-C-A-A-G-G-A-A-G-A-m5U-G-G-C-A-m5U-m5U-m5U-C-m5U-A-G), 5'-[*P*-[4-[[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]carbonyl]-1-piperazinyl]-*N*,*N*-dimethylphosphonamidate]

and

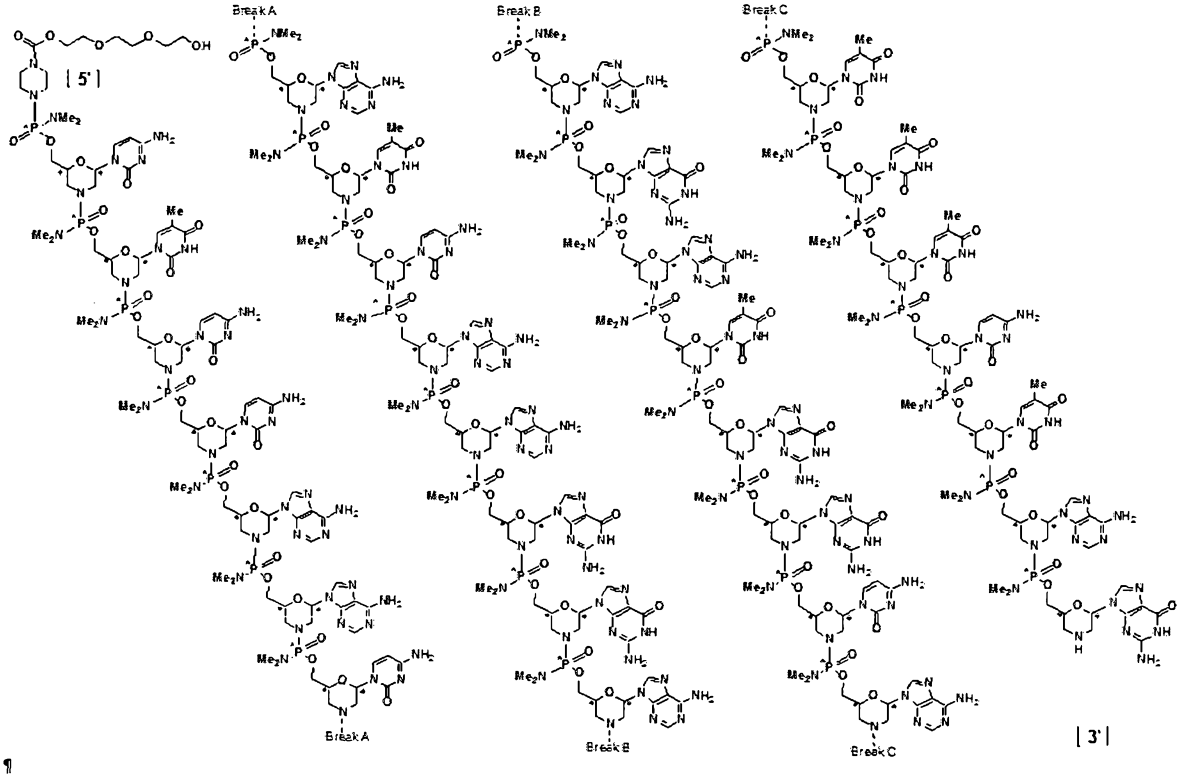
P,2',3'-trideoxy-*P*-(dimethylamino)-5'-*O*-{*P*-[4-(10-hydroxy-2,5,8-trioxadecanoyl)piperazin-1-yl]-*N*,*N*-dimethylphosphonamidoyl}-2',3'-imino-2',3'-secocytidylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secocytidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secocytidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secocytidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secocytidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoguanylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoguanylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoguanylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoguanylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoguanylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-*imino*-2',3'-secocytidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secocytidylyl-(2'a→5')-*P*,3'-dideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secothymidylyl-(2'a→5')-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-secoadenylyl-(2'a→5')-2',3'-dideoxy-2',3'-imino-2',3'-secoguanosine

Eteplirsen has the following structure:

STRUCTURAL FORMULA



B(1-30):
C-T-C-C-A-A-C-A-T-C-A-A-G-G-A-A-G-A-T-G-G-C-A-T-T-T-C-T-A-G


Break A
Break B
Break C
[5']
[3']

¶

An "amino acid subunit" or "amino acid residue" can refer to an α-amino acid residue (-CO-CHR-NH-) or a β- or other amino acid residue (*e.g.*–CO-$(CH_2)_n$CHR-NH-), where R is a side chain (which may include hydrogen) and n is 1 to 6, preferably 1 to 4.

The term "naturally occurring amino acid" refers to an amino acid present in proteins found in nature. The term "non-natural amino acids" refers to those amino acids not present in proteins found in nature, examples include beta-alanine (β-Ala), 6-aminohexanoic acid (Ahx) and 6-aminopentanoic acid.

An "exon" refers to a defined section of nucleic acid that encodes for a protein, or a nucleic acid sequence that is represented in the mature form of an RNA molecule after either portions of a pre-processed (or precursor) RNA have been removed by splicing. The mature RNA molecule can be a messenger RNA (mRNA) or a functional form of a non-coding RNA, such as rRNA or tRNA. The human dystrophin gene has about 79 exons.

An "intron" refers to a nucleic acid region (within a gene) that is not translated into a protein. An intron is a non-coding section that is transcribed into a precursor mRNA (pre-mRNA), and subsequently removed by splicing during formation of the mature RNA.

An "effective amount" or "therapeutically effective amount" refers to an amount of therapeutic compound, such as an antisense oligonucleotide, administered to a human subject, either as a single dose or as part of a series of doses, which is effective to produce a desired therapeutic effect. For an antisense oligonucleotide, this effect is typically brought about by inhibiting translation or natural splice-processing of a selected target sequence. In some embodiments, an effective amount is at least 20 mg/kg of a composition including an antisense oligonucleotide for a period of time to treat the subject. In one embodiment, an effective amount is at least 20 mg/kg of a composition including an antisense oligonucleotide to increase the number of dystrophin-positive fibers in a subject to at least 20% of normal. In another embodiment, an effective amount is at least 20 mg/kg of a composition including an antisense oligonucleotide to stabilize, maintain, or improve walking distance from a 20% deficit, for example in a 6 MWT, in a patient, relative to a healthy peer. In another embodiment, an effective amount is at least 20 mg/kg to about 30 mg/kg, about 25 mg/kg to about 30 mg/kg, or about 30 mg/kg to about 50 mg/kg. In yet another embodiment, an effective amount is about 30 mg/kg or about 50 mg/kg. In another aspect, an effective amount is at least 20 mg/kg, about 25 mg/kg, about 30mg/kg, or about 30 mg/kg to about 50 mg/kg, for at least 24 weeks, at least 36

weeks, or at least 48 weeks, to thereby increase the number of dystrophin-positive fibers in a subject to at least 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% of normal, and stabilize or improve walking distance from a 20% deficit, for example in a 6 MWT, in the patient relative to a healthy peer. In one embodiment, treatment increases the number of dystrophin-positive fibers to 20-60%, or 30-50% of normal in the patient.

"Exon skipping" refers generally to the process by which an entire exon, or a portion thereof, is removed from a given pre-processed RNA, and is thereby excluded from being present in the mature RNA, such as the mature mRNA that is translated into a protein. Hence, the portion of the protein that is otherwise encoded by the skipped exon is not present in the expressed form of the protein, typically creating an altered, though still functional, form of the protein. In certain embodiments, the exon being skipped is an aberrant exon from the human dystrophin gene, which may contain a mutation or other alteration in its sequence that otherwise causes aberrant splicing. In certain embodiments, the exon being skipped is any one or more of exons 1-79 of the human dystrophin gene, such as 3-8, 10-16, 19-40, 42-47, and 50-55, though exons 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 8 of the human dystrophin gene are preferred.

"Dystrophin" is a rod-shaped cytoplasmic protein, and a vital part of the protein complex that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix through the cell membrane. Dystrophin contains multiple functional domains. For instance, dystrophin contains an actin binding domain at about amino acids 14-240 and a central rod domain at about amino acids 253-3040. This large central domain is formed by 24 spectrin-like triple-helical elements of about 109 amino acids, which have homology to alpha-actinin and spectrin. The repeats are typically interrupted by four proline-rich non-repeat segments, also referred to as hinge regions. Repeats 15 and 16 are separated by an 18 amino acid stretch that appears to provide a major site for proteolytic cleavage of dystrophin. The sequence identity between most repeats ranges from 10-25%. One repeat contains three alpha-helices: 1, 2 and 3. Alpha-helices 1 and 3 are each formed by 7 helix turns, probably interacting as a coiled-coil through a hydrophobic interface. Alpha-helix 2 has a more complex structure and is formed by segments of four and three helix turns, separated by a Glycine or Proline residue. Each repeat is encoded by two exons, typically interrupted by an intron between amino acids 47 and 48 in the first part of

alpha-helix 2. The other intron is found at different positions in the repeat, usually scattered over helix-3. Dystrophin also contains a cysteine-rich domain at about amino acids 3080-3360), including a cysteine-rich segment (i.e., 15 Cysteines in 280 amino acids) showing homology to the C-terminal domain of the slime mold (Dictyostelium discoideum) alpha-actinin. The carboxy-terminal domain is at about amino acids 3361-3685.

The amino-terminus of dystrophin binds to F-actin and the carboxy-terminus binds to the dystrophin-associated protein complex (DAPC) at the sarcolemma. The DAPC includes the dystroglycans, sarcoglycans, integrins and caveolin, and mutations in any of these components cause autosomally inherited muscular dystrophies. The DAPC is destabilized when dystrophin is absent, which results in diminished levels of the member proteins, and in turn leads to progressive fibre damage and membrane leakage. In various forms of muscular dystrophy, such as Duchenne's muscular dystrophy (DMD) and Becker's muscular dystrophy (BMD), muscle cells produce an altered and functionally defective form of dystrophin, or no dystrophin at all, mainly due to mutations in the gene sequence that lead to incorrect splicing. The predominant expression of the defective dystrophin protein, or the complete lack of dystrophin or a dystrophin-like protein, leads to rapid progression of muscle degeneration, as noted above. In this regard, a "defective" dystrophin protein may be characterized by the forms of dystrophin that are produced in certain subjects with DMD or BMD, as known in the art, or by the absence of detectable dystrophin.

As used herein, the terms "function" and "functional" and the like refer to a biological, enzymatic, or therapeutic function.

A "functional" dystrophin protein refers generally to a dystrophin protein having sufficient biological activity to reduce the progressive degradation of muscle tissue that is otherwise characteristic of muscular dystrophy, typically as compared to the altered or "defective" form of dystrophin protein that is present in certain subjects with DMD or BMD. In certain embodiments, a functional dystrophin protein may have about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (including all integers in between) of the in vitro or in vivo biological activity of wild-type dystrophin, as measured according to routine techniques in the art. As one example, dystrophin-related activity in muscle cultures in vitro can be measured according to myotube size, myofibril organization (or disorganization), contractile activity, and spontaneous clustering of acetylcholine receptors (see, e.g., Brown et al., Journal of Cell Science.

112:209-216, 1999). Animal models are also valuable resources for studying the pathogenesis of disease, and provide a means to test dystrophin-related activity. Two of the most widely used animal models for DMD research are the mdx mouse and the golden retriever muscular dystrophy (GRMD) dog, both of which are dystrophin negative (see, e.g., Collins & Morgan, Int J Exp Pathol 84: 165-172, 2003). These and other animal models can be used to measure the functional activity of various dystrophin proteins. Included are truncated forms of dystrophin, such as those forms that are produced by certain of the exon-skipping antisense compounds of the present invention.

The term "restoration" of dystrophin synthesis or production refers generally to the production of a dystrophin protein including truncated forms of dystrophin in a patient with muscular dystrophy following treatment with an antisense oligonucleotide as described herein. In some embodiments, treatment results in an increase in novel dystrophin production in a patient by 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% (including all integers in between). In some embodiments, treatment increases the number of dystrophin-positive fibers to at least 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90 % or about 95% to 100% of normal in the subject. In other embodiments, treatment increases the number of dystrophin-positive fibers to about 20% to about 60%, or about 30% to about 50% of normal in the subject. The percent of dystrophin-positive fibers in a patient following treatment can be determined by a muscle biopsy using known techniques. For example, a muscle biopsy may be taken from a suitable muscle, such as the biceps brachii muscle in a patient.

Analysis of the percentage of positive dystrophin fibers may be performed pre-treatment and/or post-treatment or at time points throughout the course of treatment. In some embodiments, a post-treatment biopsy is taken from the contralateral muscle from the pre-treatment biopsy. Pre- and post-treatment dystrophin expression studies may be performed using any suitable assay for dystrophin. In one embodiment, immunohistochemical detection is performed on tissue sections from the muscle biopsy using an antibody that is a marker for dystrophin, such as a monoclonal or a polyclonal antibody. For example, the MANDYS106 antibody can be used which is a highly sensitive marker for dystrophin. Any suitable secondary antibody may be used.

In some embodiments, the percent dystrophin-positive fibers are calculated by dividing

the number of positive fibers by the total fibers counted. Normal muscle samples have 100% dystrophin-positive fibers. Therefore, the percent dystrophin-positive fibers can be expressed as a percentage of normal. To control for the presence of trace levels of dystrophin in the pretreatment muscle as well as revertant fibers a baseline can be set using sections of pre-treatment muscles from each patient when counting dystrophin-positive fibers in post-treatment muscles. This may be used as a threshold for counting dystrophin-positive fibers in sections of post-treatment muscle in that patient. In other embodiments, antibody-stained tissue sections can also be used for dystrophin quantification using Bioquant image analysis software (Bioquant Image Analysis Corporation, Nashville, TN). The total dystrophin fluorescence signal intensity can be reported as a percentage of normal. In addition, Western blot analysis with monoclonal or polyclonal anti-dystrophin antibodies can be used to determine the percentage of dystrophin positive fibers. For example, the anti-dystrophin antibody NCL-Dys1 from Novacastra may be used. The percentage of dystrophin-positive fibers can also be analyzed by determining the expression of the components of the sarcoglycan complex (β,γ) and/or neuronal NOS.

In some embodiments, treatment with an antisense oligonucleotide of the invention, such as eteplirsen, slows or reduces the progressive respiratory muscle dysfunction and/or failure in patients with DMD that would be expected without treatment. In one embodiment, treatment with an antisense oligonucleotide of the invention may reduce or eliminate the need for ventilation assistance that would be expected without treatment. In one embodiment, measurements of respiratory function for tracking the course of the disease, as well as the evaluation of potential therapeutic interventions include Maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) and forced vital capacity (FVC). MIP and MEP measure the level of pressure a person can generate during inhalation and exhalation, respectively, and are sensitive measures of respiratory muscle strength. MIP is a measure of diaphragm muscle weakness.

In one embodiment, MEP may decline before changes in other pulmonary function tests, including MIP and FVC. In another embodiment, MEP may be an early indicator of respiratory dysfunction. In another embodiment, FVC may be used to measure the total volume of air expelled during forced exhalation after maximum inspiration. In patients with DMD, FVC increases concomitantly with physical growth until the early teens. However, as growth slows or is stunted by disease progression, and muscle weakness progresses, the vital capacity enters a

descending phase and declines at an average rate of about 8 to 8.5 percent per year after 10 to 12 years of age. In another embodiment, MIP percent predicted (MIP adjusted for weight), MEP percent predicted (MEP adjusted for age) and FVC percent predicted (FVC adjusted for age and height) are supportive analyses.

By "isolated" is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated polynucleotide," as used herein, may refer to a polynucleotide that has been purified or removed from the sequences that flank it in a naturally-occurring state, e.g., a DNA fragment that has been removed from the sequences that are normally adjacent to the fragment.

As used herein, "sufficient length" refers to an antisense oligonucleotide that is complementary to at least 8, more typically 8-30, contiguous nucleobases in a target dystrophin pre-mRNA. In some embodiments, an antisense of sufficient length includes at least 8, 9, 10, 11, 12, 13, 14, or 15 contiguous nucleobases in the target dystrophin pre-mRNA. In other embodiments an antisense of sufficient length includes at least 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleobases in the target dystrophin pre-mRNA. An antisense oligonucleotide of sufficient length has at least a minimal number of nucleotides to be capable of specifically hybridizing to any one or more of exons 1-79 of the dystrophin gene. Preferably, the antisense oligonucleotide of the invention has a minimal number of nucleotides to be capable of specifically hybridizing to any one or more of exons 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 8 of the human dystrophin gene. Preferably an oligonucleotide of sufficient length is from about 10 to about 50 nucleotides in length, including oligonucleotides of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 and 40 or more nucleotides. In one embodiment, an oligonucleotide of sufficient length is from 10 to about 30 nucleotides in length. In another embodiment, an oligonucleotide of sufficient length is from 15 to about 25 nucleotides in length. In yet another embodiment, an oligonucleotide of sufficient length is from 20 to 30, or 20 to 50, nucleotides in length. In yet another embodiment, an oligonucleotide of sufficient length is from 25 to 28 nucleotides in length.

By "enhance" or "enhancing," or "increase" or "increasing," or "stimulate" or "stimulating," refers generally to the ability of one or antisense compounds or compositions to produce or cause a greater physiological response (i.e., downstream effects) in a cell or a subject, as compared to the response caused by either no antisense compound or a control compound. A

measurable physiological response may include increased expression of a functional form of a dystrophin protein, or increased dystrophin-related biological activity in muscle tissue, among other responses apparent from the understanding in the art and the description herein. Increased muscle function can also be measured, including increases or improvements in muscle function by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. The percentage of muscle fibers that express a functional dystrophin can also be measured, including increased dystrophin expression in about 1%, 2%, %, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of muscle fibers. For instance, it has been shown that around 40% of muscle function improvement can occur if 25-30% of fibers express dystrophin (see, e.g., DelloRusso et al, Proc Natl Acad Sci USA 99: 12979-12984, 2002). An "increased" or "enhanced" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1), e.g., 1.5, 1.6, 1.7, 1.8, etc.) the amount produced by no antisense compound (the absence of an agent) or a control compound.

The term "reduce" or "inhibit" may relate generally to the ability of one or more antisense compounds of the invention to "decrease" a relevant physiological or cellular response, such as a symptom of a disease or condition described herein, as measured according to routine techniques in the diagnostic art. Relevant physiological or cellular responses (*in vivo* or *in vitro*) will be apparent to persons skilled in the art, and may include reductions in the symptoms or pathology of muscular dystrophy, or reductions in the expression of defective forms of dystrophin, such as the altered forms of dystrophin that are expressed in individuals with DMD or BMD. A "decrease" in a response may be statistically significant as compared to the response produced by no antisense compound or a control composition, and may include a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% decrease, including all integers in between.

Also included are vector delivery systems that are capable of expressing the oligomeric, dystrophin-targeting sequences of the present invention, such as vectors that express a

polynucleotide sequence comprising any one or more of the sequences shown in Tables 3 and 4, and variants thereof, as described herein. By "vector" or "nucleic acid construct" is meant a polynucleotide molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, yeast or virus, into which a polynucleotide can be inserted or cloned. A vector preferably contains one or more unique restriction sites and can be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrated with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating vector, i.e., a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g., a linear or closed circular plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial chromosome. The vector can contain any means for assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated.

"Treatment" of an individual (e.g. a mammal, such as a human) or a cell is any type of intervention used in an attempt to alter the natural course of the individual or cell. Treatment includes, but is not limited to, administration of a pharmaceutical composition, and may be performed either prophylactically or subsequent to the initiation of a pathologic event or contact with an etiologic agent. Treatment includes any desirable effect on the symptoms or pathology of a disease or condition associated with the dystrophin protein, as in certain forms of muscular dystrophy, and may include, for example, minimal changes or improvements in one or more measurable markers of the disease or condition being treated. Also included are "prophylactic" treatments, which can be directed to reducing the rate of progression of the disease or condition being treated, delaying the onset of that disease or condition, or reducing the severity of its onset. "Treatment" or "prophylaxis" does not necessarily indicate complete eradication, cure, or prevention of the disease or condition, or associated symptoms thereof.

In one embodiment, treatment with an antisense oligonucleotide of the invention increases novel dystrophin production and slows or reduces the loss of ambulation that would be expected without treatment. For example, treatment may stabilize, maintain, improve or increase walking ability (e.g., stabilization of ambulation) in the subject. In some embodiments, treatment maintains or increases a stable walking distance in a patient, as measured by, for

example, the 6 Minute Walk Test (6MWT), described by McDonald, et al. (Muscle Nerve, 2010; 42:966-74, herein incorporated by reference). A change in the 6 Minute Walk Distance (6MWD) may be expressed as an absolute value, a percentage change or a change in the %-predicted value. In some embodiments, treatment maintains or improves a stable walking distance in a 6MWT from a 20% deficit in the subject relative to a healthy peer. The performance of a DMD patient in the 6MWT relative to the typical performance of a healthy peer can be determined by calculating a %-predicted value. For example, the %-predicted 6MWD may be calculated using the following equation for males: 196.72 + (39.81 x age) – (1.36 x age^2) + (132.28 x height in meters). For females, the %-predicted 6MWD may be calculated using the following equation: 188.61 + (51.50 x age) – (1.86 x age^2) + (86.10 x height in meters) (Henricson et al. PLoS Curr., 2012, version 2, herein incorporated by reference). In some embodiments, treatment with an antisense oligonucleotide increases the stable walking distance in the patient from baseline to greater than 3, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 or 50 meters (including all integers in between).

Loss of muscle function in patients with DMD may occur against the background of normal childhood growth and development. Indeed, younger children with DMD may show an increase in distance walked during 6MWT over the course of about 1 year despite progressive muscular impairment. In some embodiments, the 6MWD from patients with DMD is compared to typically developing control subjects and to existing normative data from age and sex matched subjects. In some embodiments, normal growth and development can be accounted for using an age and height based equation fitted to normative data. Such an equation can be used to convert 6MWD to a percent-predicted (%-predicted) value in subjects with DMD. In certain embodiments, analysis of %-predicted 6MWD data represents a method to account for normal growth and development, and may show that gains in function at early ages (e.g., less than or equal to age 7) represent stable rather than improving abilities in patients with DMD (Henricson et al. PLoS Curr., 2012, version 2, herein incorporated by reference).

A "subject," as used herein, includes any animal that exhibits a symptom, or is at risk for exhibiting a symptom, which can be treated with an antisense compound of the invention, such as a subject that has or is at risk for having DMD or BMD, or any of the symptoms associated with these conditions (e.g., muscle fibre loss). Suitable subjects (patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets

(such as a cat or dog). Non-human primates and, preferably, human patients, are included.

A "pediatric patient" as used herein is a patient from age 1 to 21, inclusive.

"Alkyl" or "alkylene" both refer to a saturated straight or branched chain hydrocarbon radical containing from 1 to 18 carbons. Examples include without limitation methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tert-butyl, n-pentyl and n-hexyl. The term "lower alkyl" refers to an alkyl group, as defined herein, containing between 1 and 8 carbons.

"Alkenyl" refers to an unsaturated straight or branched chain hydrocarbon radical containing from 2 to 18 carbons and comprising at least one carbon to carbon double bond. Examples include without limitation ethenyl, propenyl, iso-propenyl, butenyl, iso-butenyl, tert-butenyl, n-pentenyl and n-hexenyl. The term "lower alkenyl" refers to an alkenyl group, as defined herein, containing between 2 and 8 carbons.

"Alkynyl" refers to an unsaturated straight or branched chain hydrocarbon radical containing from 2 to 18 carbons comprising at least one carbon to carbon triple bond. Examples include without limitation ethynyl, propynyl, iso-propynyl, butynyl, iso-butynyl, tert-butynyl, pentynyl and hexynyl. The term "lower alkynyl" refers to an alkynyl group, as defined herein, containing between 2 and 8 carbons.

"Cycloalkyl" refers to a mono- or poly-cyclic alkyl radical. Examples include without limitation cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

"Aryl" refers to a cyclic aromatic hydrocarbon moiety containing from to 18 carbons having one or more closed ring(s). Examples include without limitation phenyl, benzyl, naphthyl, anthracenyl, phenanthracenyl and biphenyl.

"Aralkyl" refers to a radical of the formula RaRb where Ra is an alkylene chain as defined above and Rb is one or more aryl radicals as defined above, for example, benzyl, diphenylmethyl and the like.

"Thioalkoxy" refers to a radical of the formula –SRc where Rc is an alkyl radical as defined herein. The term "lower thioalkoxy" refers to an alkoxy group, as defined herein, containing between 1 and 8 carbons.

"Alkoxy" refers to a radical of the formula –ORda where Rd is an alkyl radical as defined herein. The term "lower alkoxy" refers to an alkoxy group, as defined herein, containing between 1 and 8 carbons. Examples of alkoxy groups include, without limitation, methoxy and ethoxy.

"Alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group.

"Carbonyl" refers to the C(=O) – radical.

"Guanidynyl" refers to the $H_2N(C=NH_2)$ –NH– radical.

"Amidinyl" refers to the $H_2N(C=NH_2)CH$– radical.

"Amino" refers to the NH_2 radical.

"Alkylamino" refers to a radical of the formula –NHRd or –NRdRd where each Rd is, independently, an alkyl radical as defined herein. The term "lower alkylamino" refers to an alkylamino group, as defined herein, containing between 1 and 8 carbons.

"Heterocycle" means a 5- to 7-membered monocyclic, or 7- to 10-membered bicyclic, heterocyclic ring which is either saturated, unsaturated, or aromatic, and which contains from 1 to 4 heteroatoms independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen heteroatom may be optionally quaternized, including bicyclic rings in which any of the above heterocycles are fused to a benzene ring. The heterocycle may be attached via any heteroatom or carbon atom. Heterocycles include heteroaryls as defined below. Thus, in addition to the heteroaryls listed below, heterocycles also include morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperizinyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiopyranyl, and the like.

"Heteroaryl" means an aromatic heterocycle ring of 5- to 10 members and having at least one heteroatom selected from nitrogen, oxygen and sulfur, and containing at least 1 carbon atom, including both mono- and bicyclic ring systems. Representative heteroaryls are pyridyl, furyl, benzofuranyl, thiophenyl, benzothiophenyl, quinolinyl, pyrrolyl, indolyl, oxazolyl, benzoxazolyl, imidazolyl, benzimidazolyl, thiazolyl, benzothiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, cinnolinyl, phthalazinyl, and quinazolinyl.

The terms "optionally substituted alkyl", "optionally substituted alkenyl", "optionally substituted alkoxy", "optionally substituted thioalkoxy", "optionally substituted alkyl amino", "optionally substituted lower alkyl", "optionally substituted lower alkenyl", "optionally substituted lower alkoxy", "optionally substituted lower thioalkoxy", "optionally substituted lower alkyl amino" and "optionally substituted heterocyclyl" mean that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=O) two

hydrogen atoms are replaced. In this regard, substituents include: deuterium, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocycle, optionally substituted cycloalkyl, oxo, halogen, –CN, –ORx, NRxRy, NRxC(=O)Ry, NRxSO2Ry, –NRxC(=O)NRxRy, C(=O)Rx, C(=O)ORx, C(=O)NRxRy, –SOmRx and –SOmNRxRy, wherein m is 0, 1 or 2, Rx and Ry are the same or different and independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocycle or optionally substituted cycloalkyl and each of said optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocycle and optionally substituted cycloalkyl substituents may be further substituted with one or more of oxo, halogen, –CN, –ORx, NRxRy, NRxC(=O)Ry, NRxSO2Ry, –NRxC(=O)NRxRy, C(=O)Rx, C(=O)ORx, C(=O)NRxRy, –SOmRx and –SOmNRxRy.

An antisense molecule nomenclature system was proposed and published to distinguish between the different antisense molecules (see Mann et al., (2002) J Gen Med 4, 644-654). This nomenclature became especially relevant when testing several slightly different antisense molecules, all directed at the same target region, as shown below:

H#A/D(x:y).

The first letter designates the species (e.g. H: human, M: murine, C: canine). "#" designates target dystrophin exon number. "A/D" indicates acceptor or donor splice site at the beginning and end of the exon, respectively. (x y) represents the annealing coordinates where "-" or "+" indicate intronic or exonic sequences respectively. For example, A(-6+18) would indicate the last 6 bases of the intron preceding the target exon and the first 18 bases of the target exon. The closest splice site would be the acceptor so these coordinates would be preceded with an "A". Describing annealing coordinates at the donor splice site could be D(+2-18) where the last 2 exonic bases and the first 18 intronic bases correspond to the annealing site of the antisense molecule. Entirely exonic annealing coordinates that would be represented by A(+65+85), that is the site between the 65th and 85th nucleotide from the start of that exon.

II. Constructing the Antisense Oligonucleotide

Exemplary embodiments of the invention relate to morpholino oligonucleotides having phosphorus-containing backbone linkages are illustrated in Figs. 1A-1C. Preferred is a phosphorodiamidate-linked morpholino oligonucleotide such as shown in Fig. 1C, which is modified, in accordance with one aspect of the present invention, to contain positively charged groups at preferably 10%-50% of its backbone linkages. Morpholino oligonucleotides with uncharged backbone linkages, including antisense oligonucleotides, are detailed, for example, in (Summerton and Weller 1997) and in co-owned U.S. Patent Nos. 5,698,685, 5,217,866, 5,142,047, 5,034,506, 5,166,315, 5,185, 444, 5,521,063, 5,506,337, 8,076,476, 8,299,206 and 7,943,762 all of which are expressly incorporated by reference herein.

Important properties of the morpholino-based subunits include: 1) the ability to be linked in a oligomeric form by stable, uncharged or positively charged backbone linkages; 2) the ability to support a nucleotide base (*e.g.* adenine, cytosine, guanine, thymidine, uracil and inosine) such that the polymer formed can hybridize with a complementary-base target nucleic acid, including target RNA, Tm values above about 45°C in relatively short oligonucleotides (*e.g.*, 10-15 bases); 3) the ability of the oligonucleotide to be actively or passively transported into mammalian cells; and 4) the ability of the antisense oligonucleotide:RNA heteroduplex to resist RNAse and RNase H degradation, respectively.

Exemplary backbone structures for antisense oligonucleotides of the claimed subject matter include the morpholino subunit types shown in Figs. 1D-G, each linked by an uncharged or positively charged, phosphorus-containing subunit linkage. Fig. 1D shows a phosphorus-containing linkage which forms the five atom repeating-unit backbone, where the morpholino rings are linked by a 1-atom phosphoamide linkage. Fig. 1E shows a linkage which produces a 6-atom repeating-unit backbone. In this structure, the atom Y linking the 5' morpholino carbon to the phosphorus group may be sulfur, nitrogen, carbon or, preferably, oxygen. The X moiety pendant from the phosphorus may be fluorine, an alkyl or substituted alkyl, an alkoxy or substituted alkoxy, a thioalkoxy or substituted thioalkoxy, or unsubstituted, monosubstituted, or disubstituted nitrogen, including cyclic structures, such as morpholines or piperidines. Alkyl, alkoxy and thioalkoxy preferably include 1-6 carbon atoms. The Z moieties are sulfur or oxygen, and are preferably oxygen.

The linkages shown in Figs. 1F and 1G are designed for 7-atom unit-length backbones. In structure 1F, the X moiety is as in Structure 1E, and the Y moiety may be methylene, sulfur, or, preferably, oxygen. In Structure 1G, the X and Y moieties are as in Structure 1E. Particularly preferred morpholino oligonucleotides include those composed of morpholino subunit structures of the form shown in Fig. 1E, where X=NH_2, $N(CH_3)_2$, or 1-piperazine or other charged group, Y=O, and Z=O.

A substantially uncharged oligonucleotide may be modified, in accordance with an aspect of the invention, to include charged linkages, *e.g.,* up to about 1 per every 2-5 uncharged linkages, such as about 4-5 per every 10 uncharged linkages. In certain embodiments, optimal improvement in antisense activity may be seen when about 25% of the backbone linkages are cationic. In certain embodiments, enhancement may be seen with a small number *e.g.*, 10-20% cationic linkages, or where the number of cationic linkages are in the range 50-80%, such as about 60%.

Oligomers having any number of cationic linkages are provided, including fully cationic-linked oligomers. Preferably, however, the oligomers are partially charged, having, for example, 10%-80%. In preferred embodiments, about 10% to 60%, and preferably 20% to 50% of the linkages are cationic.

In one embodiment, the cationic linkages are interspersed along the backbone. The partially charged oligomers preferably contain at least two consecutive uncharged linkages; that is, the oligomer preferably does not have a strictly alternating pattern along its entire length.

Also considered are oligomers having blocks of cationic linkages and blocks of uncharged linkages; for example, a central block of uncharged linkages may be flanked by blocks of cationic linkages, or vice versa. In one embodiment, the oligomer has approximately equal-length 5', 3' and center regions, and the percentage of cationic linkages in the center region is greater than about 50%, preferably greater than about 70%.

In certain embodiments, the antisense compounds can be prepared by stepwise solid-phase synthesis, employing methods detailed in the references cited above, and below with respect to the synthesis of oligonucleotides having a mixture or uncharged and cationic backbone linkages. In some cases, it may be desirable to add additional chemical moieties to the antisense compound, *e.g.,* to enhance pharmacokinetics or to facilitate capture or detection of the compound. Such a moiety may be covalently attached, according to standard synthetic methods.

For example, addition of a polyethylene glycol moiety or other hydrophilic polymer, *e.g.*, one having 1-100 monomeric subunits, may be useful in enhancing solubility.

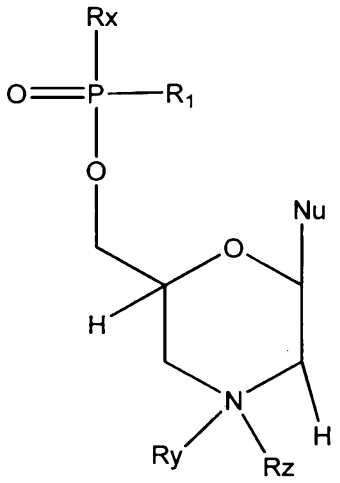
A reporter moiety, such as fluorescein or a radiolabeled group, may be attached for purposes of detection. Alternatively, the reporter label attached to the oligomer may be a ligand, such as an antigen or biotin, capable of binding a labeled antibody or streptavidin. In selecting a moiety for attachment or modification of an antisense compound, it is generally of course desirable to select chemical compounds of groups that are biocompatible and likely to be tolerated by a subject without undesirable side effects.

Oligomers for use in antisense applications generally range in length from about 10 to about 50 subunits, more preferably about 10 to 30 subunits, and typically 15-25 bases. For example, an oligomer of the invention having 19-20 subunits, a useful length for an antisense compound, may ideally have two to ten, *e.g.,* four to eight, cationic linkages, and the remainder uncharged linkages. An oligomer having 14-15 subunits may ideally have two to seven, *e.g.*, 3, 4, or 5, cationic linkages and the remainder uncharged linkages. In a preferred embodiment, the oligomers have 25 to 28 subunits.

Each morpholino ring structure supports a base pairing moiety, to form a sequence of base pairing moieties which is typically designed to hybridize to a selected antisense target in a cell or in a subject being treated. The base pairing moiety may be a purine or pyrimidine found in native DNA or RNA (*e.g.*, A, G, C, T or U) or an analog, such as hypoxanthine (the base component of the nucleoside inosine) or 5-methyl cytosine.

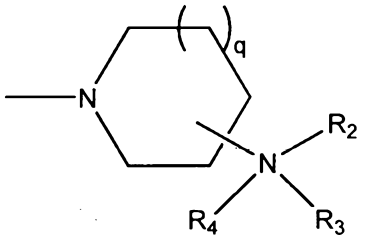
As noted above, certain embodiments are directed to oligomers comprising novel intersubunit linkages, including PMO-X oligomers and those having modified terminal groups. In some embodiments, these oligomers have higher affinity for DNA and RNA than do the corresponding unmodified oligomers and demonstrate improved cell delivery, potency, and/or tissue distribution properties compared to oligomers having other intersubunit linkages. The structural features and properties of the various linkage types and oligomers are described in more detail in the following discussion. The synthesis of these and related oligomers is described in co-owned U.S. Application No. 13/118,298, which is incorporated by reference in its entirety.

In certain embodiments, the invention provides for an oligonucleotide having a sequence complementary to the target sequence which is associated with a human disease, and comprises a sequence of nucleotides having a formula:



wherein Nu is a nucleobase;

R_1 has the formula



q is 0, 1, or 2;

R_2 is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_1-C_5 aralkyl, and a formamidinyl group, and

R_3 is selected from the group consisting of hydrogen, C_1-C_{10} acyl, C_1-C_{10} aminoacyl, acyl moiety of a natural or unnatural alpha or beta amino acid, C_1-C_{10} aralkyl, and C_1-C_{10} alkyl, or

R_2 and R_3 are joined to form a 5-7 membered ring where the ring may be optionally substituted with a substituent selected from the group consisting of C_1-C_{10} alkyl, phenyl, halogen, and C_1-C_{10} aralkyl;

R_4 is selected from the group consisting of an electron pair, hydrogen, a C_1-C_6 alkyl and C_1-C_6 aralkyl;

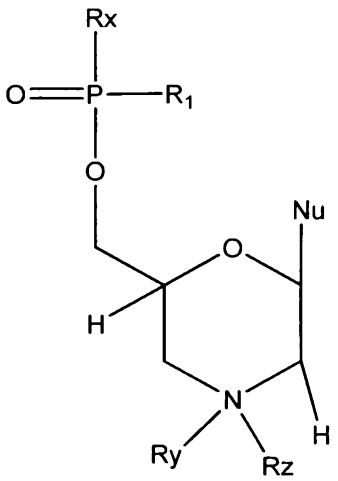
Rx is selected from the group consisting of sarcosinamide, hydroxyl , a nucleotide, a cell penetrating peptide moiety, and piperazinyl;

Ry is selected from the group consisting of hydrogen, a C_1-C_6 alkyl, a nucleotide a cell penetrating peptide moiety, an amino acid, a formamidinyl group, and C_1-C_6 acyl; and,

Rz is selected from the group consisting of an electron pair, hydrogen, a C_1-C_6 alkyl, and C_1-C_6 acyl pharmaceutically acceptable salts thereof.

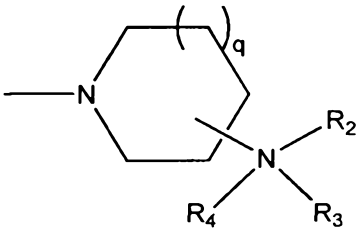
Nu may be selected from the group consisting of adenine, guanine, thymine, uracil, cytosine, and hypoxanthine. More preferably Nu is thymine or uracil.

In preferred embodiments, the invention provides an oligonucleotide having a sequence of nucleotides having a formula:



wherein Nu is a nucleobase;

R_1 is selected from the group consisting of R_1' and R_1'' wherein R_1' is dimethyl- amino and R_1'' has the formula



wherein at least one R_1 is R_1'';

q is 0, 1, or 2; with the proviso that at least one of R_1 is a piperidinyl moiety;

R_2 is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_1-C_5 aralkyl, and a formamidinyl group, and

R_3 is selected from the group consisting of hydrogen, C_1-C_{10} acyl, C_1-C_{10} aminoacyl, acyl moiety of a natural or unnatural alpha or beta amino acid, C_1-C_{10} aralkyl, and C_1-C_{10} alkyl, or

R_2 and R_3 are joined to form a 5-7 membered ring where the ring may be optionally substituted with a substituent selected from the group consisting of C_1-C_{10} alkyl, phenyl, halogen, and C_1-C_{10} aralkyl;

R_4 is selected from the group consisting of an electron pair, hydrogen, a C_1-C_6 alkyl and aralkyl;

Rx is selected from the group consisting of sarcosinamide, hydroxyl , a nucleotide, a cell penetrating peptide moiety, and piperazinyl;

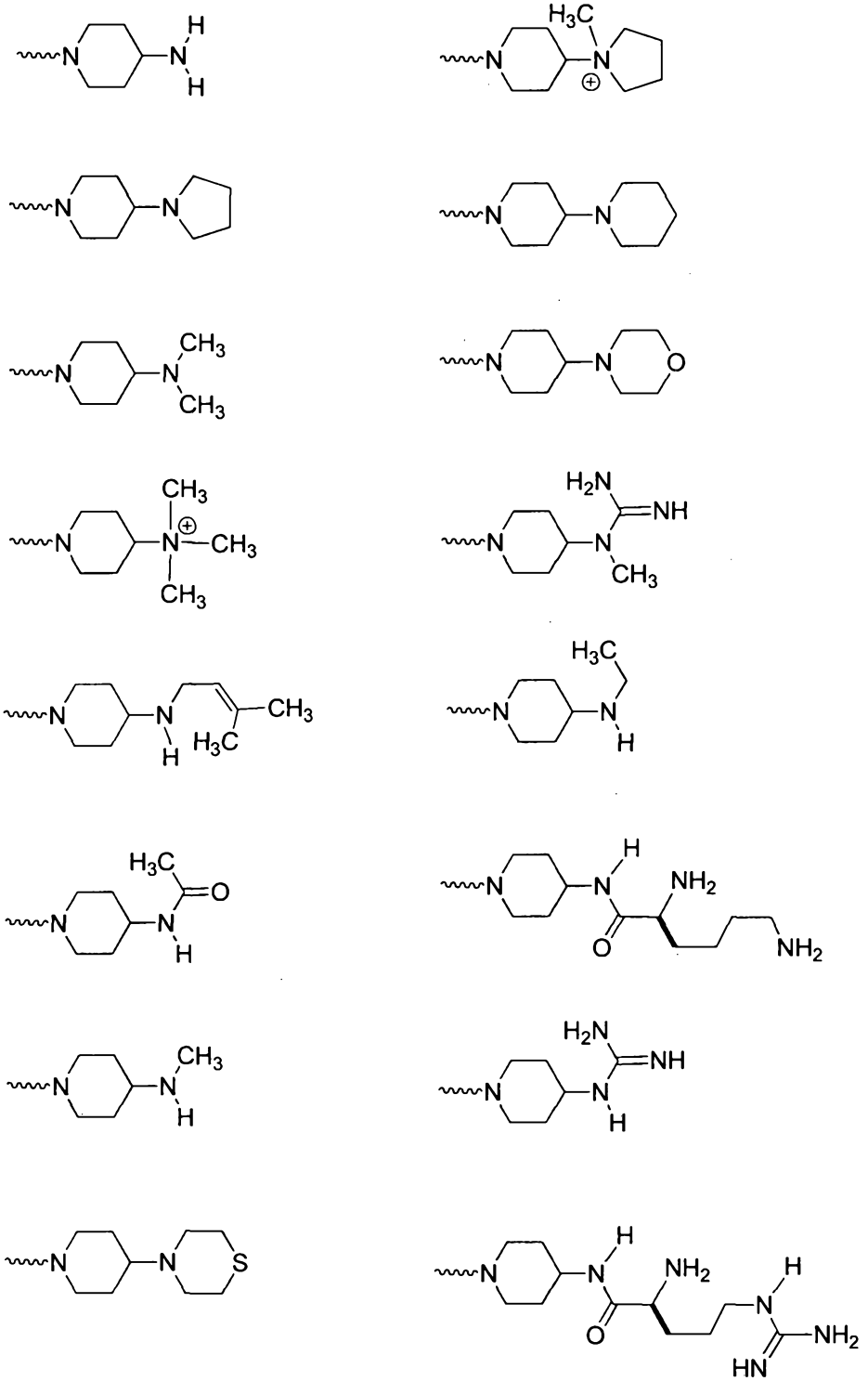
Ry is selected from the group consisting of hydrogen, a C_1-C_6 alkyl, a nucleotide a cell penetrating peptide moiety, an amino acid, a formamidinyl group, and C_1-C_6 acyl; and,

Rz is selected from the group consisting of an electron pair, hydrogen, a C_1-C_6 alkyl, and C_1-C_6 acyl pharmaceutically acceptable salts thereof.

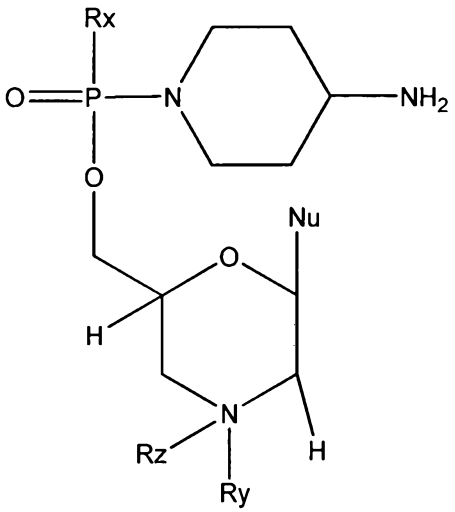
Nu may be selected from the group consisting of adenine, guanine, thymine, uracil, cytosine, and hypoxanthine. More preferably Nu is thymine or uracil.

About 90-50% of the R_1 groups are dimethylamino (i.e. R_1'). More, preferably, 90-50% of the R_1 groups are dimethylamino. Most, preferably about 66% of the R_1 groups are dimethylamino.

R_1" may be selected from the group consisting of



Preferably, at least one nucleotide of the oligonucleotide has the formula:



wherein Rx, Ry, Rz, and Nu are as stated above. Most preferably, Nu is thymine or uracil.

Although thymine (T) is the preferred base pairing moiety (Nu or Pi) containing the chemical modifications described above, any base subunit known to a person of skill in the art can be used as the base pairing moiety.

The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense molecule need not be 100% complementary to that of its target sequence to be specifically hybridizable. An antisense molecule is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is

desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

While the above method may be used to select antisense molecules capable of deleting any exon from within a protein that is capable of being shortened without affecting its biological function, the exon deletion should not lead to a reading frame shift in the shortened transcribed mRNA. Thus, if in a linear sequence of three exons the end of the first exon encodes two of three nucleotides in a codon and the next exon is deleted then the third exon in the linear sequence must start with a single nucleotide that is capable of completing the nucleotide triplet for a codon. If the third exon does not commence with a single nucleotide there will be a reading frame shift that would lead to the generation of truncated or a non-functional protein.

It will be appreciated that the codon arrangements at the end of exons in structural proteins may not always break at the end of a codon, consequently there may be a need to delete more than one exon from the pre-mRNA to ensure in-frame reading of the mRNA. In such circumstances, a plurality of antisense oligonucleotides may need to be selected by the method of the invention wherein each is directed to a different region responsible for inducing splicing in the exons that are to be deleted.

The length of an antisense molecule may vary so long as it is capable of binding selectively to the intended location within the pre-mRNA molecule. The length of such sequences can be determined in accordance with selection procedures described herein. Generally, the antisense molecule will be from about 10 nucleotides in length up to about 50 nucleotides in length. It will be appreciated however that any length of nucleotides within this range may be used in the method. Preferably, the length of the antisense molecule is between 10-30 nucleotides in length.

The most common method for producing antisense molecules is the methylation of the 2' hydroxyribose position and the incorporation of a phosphorothioate backbone produces molecules that superficially resemble RNA but that are much more resistant to nuclease degradation.

To avoid degradation of pre-mRNA during duplex formation with the antisense molecules, the antisense molecules used in the method may be adapted to minimize or prevent cleavage by endogenous RNase H. This property is highly preferred as the treatment of the RNA with the unmethylated oligonucleotides either intracellularly or in crude extracts that contain

RNase H leads to degradation of the pre-mRNA: antisense oligonucleotide duplexes. Any form of modified antisense molecules that is capable of by-passing or not inducing such degradation may be used in the present method. An example of antisense molecules which when duplexed with RNA are not cleaved by cellular RNase H is 2'-O-methyl derivatives. 2'-O-methyl-oligoribonucleotides are very stable in a cellular environment and in animal tissues, and their duplexes with RNA have higher Tm values than their ribo- or deoxyribo-counterparts.

Antisense molecules that do not activate RNase H can be made in accordance with known techniques (see, e.g., U.S. Pat. No. 5,149,797). Such antisense molecules, which may be deoxyribonucleotide or ribonucleotide sequences, simply contain any structural modification which sterically hinders or prevents binding of RNase H to a duplex molecule containing the oligonucleotide as one member thereof, which structural modification does not substantially hinder or disrupt duplex formation. Because the portions of the oligonucleotide involved in duplex formation are substantially different from those portions involved in RNase H binding thereto, numerous antisense molecules that do not activate RNase H are available. For example, such antisense molecules may be oligonucleotides wherein at least one, or all, of the inter-nucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphorothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues may be modified as described. In another non-limiting example, such antisense molecules are molecules wherein at least one, or all, of the nucleotides contain a 2' lower alkyl moiety (e.g., C_1-C_4, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl). For example, every other one of the nucleotides may be modified as described.

While antisense oligonucleotides are a preferred form of the antisense molecules, the present invention comprehends other oligomeric antisense molecules, including but not limited to oligonucleotide mimetics such as are described below.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural inter-nucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art,

modified oligonucleotides that do not have a phosphorus atom in their inter-nucleoside backbone can also be considered to be oligonucleosides.

In other preferred oligonucleotide mimetics, both the sugar and the inter-nucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleo-bases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. Certain nucleo-bases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds that are chimeric compounds. "Chimeric" antisense compounds

or "chimeras," in the context of this invention, are antisense molecules, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the increased resistance to nuclease degradation, increased cellular uptake, and an additional region for increased binding affinity for the target nucleic acid.

III. Peptide Transporters

The antisense compounds of the invention may include an oligonucleotide moiety conjugated to a CPP, preferably an arginine-rich peptide transport moiety effective to enhance transport of the compound into cells. The transport moiety is preferably attached to a terminus of the oligomer, as shown, for example, in **FIGs 1B** and **1C**. The peptides have the capability of inducing cell penetration within 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of cells of a given cell culture population, including all integers in between, and allow macromolecular translocation within multiple tissues *in vivo* upon systemic administration. In one embodiment, the cell-penetrating peptide may be an arginine-rich peptide transporter. In another embodiment, the cell-penetrating peptide may be Penetratin or the Tat peptide. These peptides are well known in the art and are disclosed, for example, in US Publication No. 2010-0016215 A1, incorporated by reference in its entirety. A particularly preferred approach to conjugation of peptides to antisense oligonucleotides can be found in PCT publication WO2012/150960, which is incorporated by reference in its entirety. A preferred embodiment of a peptide conjugated oligonucleotide of the present invention utilizes glycine as the linker between the CPP and the antisense oligonucleotide. For example, a preferred peptide conjugated PMO consists of R_6-G-PMO.

The transport moieties as described above have been shown to greatly enhance cell entry of attached oligomers, relative to uptake of the oligomer in the absence of the attached transport moiety. Uptake is preferably enhanced at least ten fold, and more preferably twenty fold, relative to the unconjugated compound.

The use of arginine-rich peptide transporters (i.e., cell-penetrating peptides) are particularly useful in practicing the present invention. Certain peptide transporters have been shown to be highly effective at delivery of antisense compounds into primary cells including

muscle cells (Marshall, Oda et al. 2007; Jearawiriyapaisarn, Moulton et al. 2008; Wu, Moulton et al. 2008). Furthermore, compared to other known peptide transporters such as Penetratin and the Tat peptide, the peptide transporters described herein, when conjugated to an antisense PMO, demonstrate an enhanced ability to alter splicing of several gene transcripts (Marshall, Oda et al. 2007).

Exemplary peptide transporters, excluding linkers are given below in Table 1.

Table 1. Exemplary peptide transporters

NAME (DESIGNATION)	SEQUENCE	SEQ ID NO[A]
rTAT	RRRQRRKKR	876
Tat	RKKRRQRRR	877
R_9F_2	RRRRRRRRRFF	878
$R_5F_2R_4$	RRRRRFFRRRR	879
R_4	RRRR	880
R_5	RRRRR	881
R_6	RRRRRR	882
R_7	RRRRRRR	883
R_8	RRRRRRRR	884
R_9	RRRRRRRRR	885
$(RX)_8$	RXRXRXRXRXRXRXRX	886
$(RAhxR)_4$; (P007)	RAhxRRAhxRRAhxRRAhxR	887
$(RAhxR)_5$; (CP04057)	RAhxRRAhxRRAhxRRAhxRRAhxR	888
$(RAhxRRBR)_2$; (CP06062)	RAhxRRBRRAhxRRBR	889
$(RAR)_4F_2$	RARRARRARRARFF	890
$(RGR)_4F_2$	RGRRGRRGRRGRFF	891

[A]Sequences assigned to SEQ ID NOs do not include the linkage portion (*e.g.*, C, G, P, Ahx, B, AhxB where Ahx and B refer to 6-aminohexanoic acid and beta-alanine, respectively).

IV. Formulations and Treatment

In certain embodiments, the present invention provides formulations or compositions suitable for the therapeutic delivery of antisense oligomers, as described herein. Hence, in certain embodiments, the present invention provides pharmaceutically acceptable compositions that comprise a therapeutically-effective amount of one or more of the oligomers described herein, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. While it is possible for an oligomer of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

The compositions of the present invention may be administered alone or in combination with another therapeutic. The additional therapeutic may be administered prior, concurrently or subsequently to the administration of the composition of the present invention. For example, the compositions may be administered in combination with a steroid and/or an antibiotic. The steroid may be a glucocorticoid or prednisone. Glucocorticoids such as cortisol control carbohydrate, fat and protein metabolism, and are anti-inflammatory by preventing phospholipid release, decreasing eosinophil action and a number of other mechanisms. Mineralocorticoids such as aldosterone control electrolyte and water levels, mainly by promoting sodium retention in the kidney. Corticosteroids are a class of chemicals that includes steroid hormones naturally produced in the adrenal cortex of vertebrates and analogues of these hormones that are synthesized in laboratories. Corticosteroids are involved in a wide range of physiological processes, including stress response, immune response, and regulation of inflammation, carbohydrate metabolism, protein catabolism, blood electrolyte levels, and behavior. Corticosteroids include Betamethasone, Budesonide, Cortisone, Dexamethasone, Hydrocortisone, Methylprednisolone, Prednisolone, and Prednisone.

Other agents which can be administered include an antagonist of the ryanodine receptor, such as dantrolene, which has been shown to enhance antisense-mediated exon skipping in patient cells and a mouse model of DMD (G. Kendall et al. Sci Tranl Med 4 164ra160 (2012), incorporated herein by reference).

Methods for the delivery of nucleic acid molecules are described, for example, in Akhtar et al., 1992, Trends Cell Bio., 2:139; and Delivery Strategies for Antisense Oligonucleotide Therapeutics, ed. Akhtar; Sullivan et al., PCT WO 94/02595. These and other protocols can be

utilized for the delivery of virtually any nucleic acid molecule, including the isolated oligomers of the present invention.

As detailed below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Some examples of materials that can serve as pharmaceutically-acceptable carriers include, without limitation: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12)

esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

Additional non-limiting examples of agents suitable for formulation with the antisense oligomers of the instant invention include: PEG conjugated nucleic acids, phospholipid conjugated nucleic acids, nucleic acids containing lipophilic moieties, phosphorothioates, P-glycoprotein inhibitors (such as Pluronic P85) which can enhance entry of drugs into various tissues; biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after implantation (Emerich, D F et al., 1999, Cell Transplant, 8, 47-58) Alkermes, Inc. Cambridge, Mass.; and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (Prog Neuropsychopharmacol Biol Psychiatry, 23, 941-949, 1999).

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, branched and unbranched or combinations thereof, or long-circulating liposomes or stealth liposomes). Oligomers of the invention can also comprise covalently attached PEG molecules of various molecular weights. These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic et al. Chem. Rev. 1995, 95, 2601-2627; Ishiwata et al., Chem. Pharm. Bull. 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic et al., Science 1995, 267, 1275-1276; Oku et al., 1995, Biochim. Biophys. Acta, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu et al., J. Biol. Chem. 1995, 42, 24864-24870; Choi et al., International PCT Publication No. WO 96/10391; Ansell et al., International PCT Publication No. WO 96/10390; Holland et al., International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease

degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

In a further embodiment, the present invention includes oligomer compositions prepared for delivery as described in U.S. Pat. Nos. 6,692,911, 7,163,695 and 7,070,807. In this regard, in one embodiment, the present invention provides an oligomer of the present invention in a composition comprising copolymers of lysine and histidine (HK) (as described in U.S. Pat. Nos. 7,163,695, 7,070,807, and 6,692,911) either alone or in combination with PEG (e.g., branched or unbranched PEG or a mixture of both), in combination with PEG and a targeting moiety or any of the foregoing in combination with a crosslinking agent. In certain embodiments, the present invention provides antisense oligomers in compositions comprising gluconic-acid-modified polyhistidine or gluconylated-polyhistidine/transferrin-polylysine. One skilled in the art will also recognize that amino acids with properties similar to His and Lys may be substituted within the composition.

Certain embodiments of the oligomers described herein may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared in situ in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, e.g., Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19).

The pharmaceutically acceptable salts of the subject oligomers include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic,

glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In certain embodiments, the oligomers of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared in situ in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, e.g., Berge et al., *supra*).

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that

amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

In certain embodiments, a formulation of the present invention comprises an excipient selected from cyclodextrins, celluloses, liposomes, micelle forming agents, e.g., bile acids, and polymeric carriers, e.g., polyesters and polyanhydrides; and an oligomer of the present invention. In certain embodiments, an aforementioned formulation renders orally bioavailable an oligomer of the present invention.

Methods of preparing these formulations or compositions include the step of bringing into association an oligomer of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. An oligomer of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules, trouches and the like), the active ingredient may be mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds and surfactants, such as

poloxamer and sodium lauryl sulfate; (7) wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, zinc stearate, sodium stearate, stearic acid, and mixtures thereof; (10) coloring agents; and (11) controlled release agents such as crospovidone or ethyl cellulose. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (e.g., gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g., freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations or dosage forms for the topical or transdermal administration of an oligomer as provided herein include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active oligomers may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required. The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an oligomer of the present invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and

polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of an oligomer of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the oligomer in the proper medium. Absorption enhancers can also be used to increase the flux of the agent across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the agent in a polymer matrix or gel, among other methods known in the art.

Pharmaceutical compositions suitable for parenteral administration may comprise one or more oligomers of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject oligomers may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished

by the use of a liquid suspension of crystalline or amorphous material having poor water solubility, among other methods known in the art. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms may be made by forming microencapsule matrices of the subject oligomers in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of oligomer to polymer, and the nature of the particular polymer employed, the rate of oligomer release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations may also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissues.

When the oligomers of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99% (more preferably, 10 to 30%) of active ingredient in combination with a pharmaceutically acceptable carrier.

As noted above, the formulations or preparations of the present invention may be given orally, parenterally, systemically, topically, rectally or intramuscular administartion. They are typically given in forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it

enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

Regardless of the route of administration selected, the oligomers of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, may be formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art. Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unacceptably toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular oligomer of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular oligomer being employed, the rate and extent of absorption, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular oligomer employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, oral, intravenous, intracerebroventricular, intramuscular and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated effects, will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

Preferred doses of the oligomers of the present invention (*e.g.,* phosphorodiamidate morpholino oligomers; eteplirsen) are administered generally from about 20-100 mg/kg. In some cases, doses of greater than 100 mg/kg may be necessary. For i.v. administration, preferred

doses are from about 0.5 mg to 100 mg/kg. In some embodiments, the oligomers are administered at doses of about 20mg/kg, 21mg/kg, 25mg/kg, 26 mg/kg, 27mg/kg, 28 mg/kg, 29 mg/kg, 30mg/kg, 31mg/kg, 32mg/kg, 33mg/kg, 34mg/kg, 35mg/kg, 36mg/kg, 37mg/kg, 38mg/kg, 39mg/kg, 40mg/kg, 41mg/kg, 42mg/kg, 43mg/kg, 44mg/kg, 45mg/kg, 46mg/kg, 47mg/kg, 48mg/kg, 49mg/kg 50mg/kg, 51mg/kg, 52mg/kg, 53mg/kg, 54mg/kg, 55mg/kg, 56mg/kg, 57mg/kg, 58mg/kg, 59mg/kg, 60mg/kg, 65mg/kg, 70mg/kg, 75mg/kg, 80mg/kg, 85mg/kg, 90mg/kg, 95mg/kg, 100mg/kg, including all integers in between. In a preferred embodiment, the oligomer is administered at 30 mg/kg. In another preferred embodiment, the oligomer is administered at 50mg/kg.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain situations, dosing is one administration per day. In certain embodiments, dosing is one or more administration per every 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, as needed, to maintain the desired expression of a functional dystrophin protein.

In some embodiments, the oligomers of the present invention (*e.g.,* phosphorodiamidate morpholino oligomers; eteplirsen) are administered, generally at regular intervals (e.g., daily, weekly, biweekly, monthly, bimonthly). The oligomers may be administered at regular intervals, e.g., daily; once every two days; once every three days; once every 3 to 7 days; once every 3 to 10 days; once every 7 to 10 days; once every week; once every two weeks; once monthly. For example, the oligomers may be administered once weekly by intravenous infusion. The oligomers may be administered intermittently over a longer period of time, e.g., for several weeks, months or years. For example, the oligomers may be administered once every one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve months. In addition, the oligomers may be administered once every one, two, three, four or five years. Administration may be followed by, or concurrent with, administration of an antibiotic, steroid or other therapeutic agent. The treatment regimen may be adjusted (dose, frequency, route, etc.) as indicated, based on the results of immunoassays, other biochemical tests and physiological examination of the subject under treatment.

Nucleic acid molecules can be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, as described herein and known in the art. In certain embodiments, microemulsification technology may be utilized to improve bioavailability of lipophilic (water insoluble) pharmaceutical agents. Examples include Trimetrine (Dordunoo, S. K., et al., Drug Development and Industrial Pharmacy, 17(12), 1685-1713, 1991 and REV 5901 (Sheen, P. C., et al., J Pharm Sci 80(7), 712-714, 1991). Among other benefits, microemulsification provides enhanced bioavailability by preferentially directing absorption to the lymphatic system instead of the circulatory system, which thereby bypasses the liver, and prevents destruction of the compounds in the hepatobiliary circulation.

In one aspect of invention, the formulations contain micelles formed from an oligomer as provided herein and at least one amphiphilic carrier, in which the micelles have an average diameter of less than about 100 nm. More preferred embodiments provide micelles having an average diameter less than about 50 nm, and even more preferred embodiments provide micelles having an average diameter less than about 30 nm, or even less than about 20 nm.

While all suitable amphiphilic carriers are contemplated, the presently preferred carriers are generally those that have Generally-Recognized-as-Safe (GRAS) status, and that can both solubilize the compound of the present invention and microemulsify it at a later stage when the solution comes into a contact with a complex water phase (such as one found in human gastro-intestinal tract). Usually, amphiphilic ingredients that satisfy these requirements have HLB (hydrophilic to lipophilic balance) values of 2-20, and their structures contain straight chain aliphatic radicals in the range of C-6 to C-20. Examples are polyethylene-glycolized fatty glycerides and polyethylene glycols.

Examples of amphiphilic carriers include saturated and monounsaturated polyethyleneglycolyzed fatty acid glycerides, such as those obtained from fully or partially hydrogenated various vegetable oils. Such oils may advantageously consist of tri-, di-, and mono-fatty acid glycerides and di- and mono-polyethyleneglycol esters of the corresponding fatty acids, with a particularly preferred fatty acid composition including capric acid 4-10, capric acid 3-9, lauric acid 40-50, myristic acid 14-24, palmitic acid 4-14 and stearic acid 5-15%. Another useful class of amphiphilic carriers includes partially esterified sorbitan and/or sorbitol,

with saturated or mono-unsaturated fatty acids (SPAN-series) or corresponding ethoxylated analogs (TWEEN-series).

Commercially available amphiphilic carriers may be particularly useful, including Gelucire-series, Labrafil, Labrasol, or Lauroglycol (all manufactured and distributed by Gattefosse Corporation, Saint Priest, France), PEG-mono-oleate, PEG-di-oleate, PEG-mono-laurate and di-laurate, Lecithin, Polysorbate 80, etc (produced and distributed by a number of companies in USA and worldwide).

In certain embodiments, the delivery may occur by use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, a nanoparticle or the like. The formulation and use of such delivery vehicles can be carried out using known and conventional techniques.

Hydrophilic polymers suitable for use in the present invention are those which are readily water-soluble, can be covalently attached to a vesicle-forming lipid, and which are tolerated in vivo without toxic effects (i.e., are biocompatible). Suitable polymers include polyethylene glycol (PEG), polylactic (also termed polylactide), polyglycolic acid (also termed polyglycolide), a polylactic-polyglycolic acid copolymer, and polyvinyl alcohol. In certain embodiments, polymers have a molecular weight of from about 100 or 120 daltons up to about 5,000 or 10,000 daltons, or from about 300 daltons to about 5,000 daltons. In other embodiments, the polymer is polyethyleneglycol having a molecular weight of from about 100 to about 5,000 daltons, or having a molecular weight of from about 300 to about 5,000 daltons. In certain embodiments, the polymer is polyethyleneglycol of 750 daltons (PEG(750)). Polymers may also be defined by the number of monomers therein; a preferred embodiment of the present invention utilizes polymers of at least about three monomers, such PEG polymers consisting of three monomers (approximately 150 daltons).

Other hydrophilic polymers which may be suitable for use in the present invention include polyvinylpyrrolidone, polymethoxazoline, polyethyloxazoline, polyhydroxypropyl methacrylamide, polymethacrylamide, polydimethylacrylamide, and derivatized celluloses such as hydroxymethylcellulose or hydroxyethylcellulose.

In certain embodiments, a formulation of the present invention comprises a biocompatible polymer selected from the group consisting of polyamides, polycarbonates, polyalkylenes, polymers of acrylic and methacrylic esters, polyvinyl polymers, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses, polypropylene, polyethylenes, polystyrene, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), poly(lactide-co-caprolactone), polysaccharides, proteins, polyhyaluronic acids, polycyanoacrylates, and blends, mixtures, or copolymers thereof.

Cyclodextrins are cyclic oligosaccharides, consisting of 6, 7 or 8 glucose units, designated by the Greek letter α, β, or γ, respectively. The glucose units are linked by α-1,4-glucosidic bonds. As a consequence of the chair conformation of the sugar units, all secondary hydroxyl groups (at C-2, C-3) are located on one side of the ring, while all the primary hydroxyl groups at C-6 are situated on the other side. As a result, the external faces are hydrophilic, making the cyclodextrins water-soluble. In contrast, the cavities of the cyclodextrins are hydrophobic, since they are lined by the hydrogen of atoms C-3 and C-5, and by ether-like oxygens. These matrices allow complexation with a variety of relatively hydrophobic compounds, including, for instance, steroid compounds such as 17α-estradiol (see, e.g., van Uden et al. Plant Cell Tiss. Org. Cult. 38:1-3-113 (1994)). The complexation takes place by Van der Waals interactions and by hydrogen bond formation. For a general review of the chemistry of cyclodextrins, see, Wenz, Agnew. Chem. Int. Ed. Engl., 33:803-822 (1994).

The physico-chemical properties of the cyclodextrin derivatives depend strongly on the kind and the degree of substitution. For example, their solubility in water ranges from insoluble (e.g., triacetyl-beta-cyclodextrin) to 147% soluble (w/v) (G-2-beta-cyclodextrin). In addition, they are soluble in many organic solvents. The properties of the cyclodextrins enable the control over solubility of various formulation components by increasing or decreasing their solubility.

Numerous cyclodextrins and methods for their preparation have been described. For example, Parmeter (I), et al. (U.S. Pat. No. 3,453,259) and Gramera, et al. (U.S. Pat. No. 3,459,731) described electroneutral cyclodextrins. Other derivatives include cyclodextrins with cationic properties [Parmeter (II), U.S. Pat. No. 3,453,257], insoluble crosslinked cyclodextrins (Solms, U.S. Pat. No. 3,420,788), and cyclodextrins with anionic properties [Parmeter (III), U.S. Pat. No. 3,426,011]. Among the cyclodextrin derivatives with anionic properties, carboxylic acids, phosphorous acids, phosphinous acids, phosphonic acids, phosphoric acids,

thiophosphonic acids, thiosulphinic acids, and sulfonic acids have been appended to the parent cyclodextrin [see, Parmeter (III), supra]. Furthermore, sulfoalkyl ether cyclodextrin derivatives have been described by Stella, et al. (U.S. Pat. No. 5,134,127).

Liposomes consist of at least one lipid bilayer membrane enclosing an aqueous internal compartment. Liposomes may be characterized by membrane type and by size. Small unilamellar vesicles (SUVs) have a single membrane and typically range between 0.02 and 0.05 μm in diameter; large unilamellar vesicles (LUVS) are typically larger than 0.05 μm. Oligolamellar large vesicles and multilamellar vesicles have multiple, usually concentric, membrane layers and are typically larger than 0.1 μm. Liposomes with several nonconcentric membranes, i.e., several smaller vesicles contained within a larger vesicle, are termed multivesicular vesicles.

One aspect of the present invention relates to formulations comprising liposomes containing an oligomer of the present invention, where the liposome membrane is formulated to provide a liposome with increased carrying capacity. Alternatively or in addition, the compound of the present invention may be contained within, or adsorbed onto, the liposome bilayer of the liposome. An oligomer of the present invention may be aggregated with a lipid surfactant and carried within the liposome's internal space; in these cases, the liposome membrane is formulated to resist the disruptive effects of the active agent-surfactant aggregate.

According to one embodiment of the present invention, the lipid bilayer of a liposome contains lipids derivatized with polyethylene glycol (PEG), such that the PEG chains extend from the inner surface of the lipid bilayer into the interior space encapsulated by the liposome, and extend from the exterior of the lipid bilayer into the surrounding environment.

Active agents contained within liposomes of the present invention are in solubilized form. Aggregates of surfactant and active agent (such as emulsions or micelles containing the active agent of interest) may be entrapped within the interior space of liposomes according to the present invention. A surfactant acts to disperse and solubilize the active agent, and may be selected from any suitable aliphatic, cycloaliphatic or aromatic surfactant, including but not limited to biocompatible lysophosphatidylcholines (LPGs) of varying chain lengths (for example, from about C14 to about C20). Polymer-derivatized lipids such as PEG-lipids may also be utilized for micelle formation as they will act to inhibit micelle/membrane fusion, and as the addition of a polymer to surfactant molecules decreases the CMC of the surfactant and aids in

micelle formation. Preferred are surfactants with CMOs in the micromolar range; higher CMC surfactants may be utilized to prepare micelles entrapped within liposomes of the present invention.

Liposomes according to the present invention may be prepared by any of a variety of techniques that are known in the art. See, e.g., U.S. Pat. No. 4,235,871; Published PCT applications WO 96/14057; New RRC, Liposomes: A practical approach, IRL Press, Oxford (1990), pages 33-104; Lasic DD, Liposomes from physics to applications, Elsevier Science Publishers BV, Amsterdam, 1993. For example, liposomes of the present invention may be prepared by diffusing a lipid derivatized with a hydrophilic polymer into preformed liposomes, such as by exposing preformed liposomes to micelles composed of lipid-grafted polymers, at lipid concentrations corresponding to the final mole percent of derivatized lipid which is desired in the liposome. Liposomes containing a hydrophilic polymer can also be formed by homogenization, lipid-field hydration, or extrusion techniques, as are known in the art.

In another exemplary formulation procedure, the active agent is first dispersed by sonication in a lysophosphatidylcholine or other low CMC surfactant (including polymer grafted lipids) that readily solubilizes hydrophobic molecules. The resulting micellar suspension of active agent is then used to rehydrate a dried lipid sample that contains a suitable mole percent of polymer-grafted lipid, or cholesterol. The lipid and active agent suspension is then formed into liposomes using extrusion techniques as are known in the art, and the resulting liposomes separated from the unencapsulated solution by standard column separation.

In one aspect of the present invention, the liposomes are prepared to have substantially homogeneous sizes in a selected size range. One effective sizing method involves extruding an aqueous suspension of the liposomes through a series of polycarbonate membranes having a selected uniform pore size; the pore size of the membrane will correspond roughly with the largest sizes of liposomes produced by extrusion through that membrane. See e.g., U.S. Pat. No. 4,737,323 (Apr. 12, 1988). In certain embodiments, reagents such as DharmaFECT® and Lipofectamine® may be utilized to introduce polynucleotides or proteins into cells.

The release characteristics of a formulation of the present invention depend on the encapsulating material, the concentration of encapsulated drug, and the presence of release modifiers. For example, release can be manipulated to be pH dependent, for example, using a pH sensitive coating that releases only at a low pH, as in the stomach, or a higher pH, as in the

intestine. An enteric coating can be used to prevent release from occurring until after passage through the stomach. Multiple coatings or mixtures of cyanamide encapsulated in different materials can be used to obtain an initial release in the stomach, followed by later release in the intestine. Release can also be manipulated by inclusion of salts or pore forming agents, which can increase water uptake or release of drug by diffusion from the capsule. Excipients which modify the solubility of the drug can also be used to control the release rate. Agents which enhance degradation of the matrix or release from the matrix can also be incorporated. They can be added to the drug, added as a separate phase (i.e., as particulates), or can be co-dissolved in the polymer phase depending on the compound. In most cases the amount should be between 0.1 and thirty percent (w/w polymer). Types of degradation enhancers include inorganic salts such as ammonium sulfate and ammonium chloride, organic acids such as citric acid, benzoic acid, and ascorbic acid, inorganic bases such as sodium carbonate, potassium carbonate, calcium carbonate, zinc carbonate, and zinc hydroxide, and organic bases such as protamine sulfate, spermine, choline, ethanolamine, diethanolamine, and triethanolamine and surfactants such as Tween® and Pluronic®. Pore forming agents which add microstructure to the matrices (i.e., water soluble compounds such as inorganic salts and sugars) are added as particulates. The range is typically between one and thirty percent (w/w polymer).

Uptake can also be manipulated by altering residence time of the particles in the gut. This can be achieved, for example, by coating the particle with, or selecting as the encapsulating material, a mucosal adhesive polymer. Examples include most polymers with free carboxyl groups, such as chitosan, celluloses, and especially polyacrylates (as used herein, polyacrylates refers to polymers including acrylate groups and modified acrylate groups such as cyanoacrylates and methacrylates).

An oligomer may be formulated to be contained within, or, adapted to release by a surgical or medical device or implant. In certain aspects, an implant may be coated or otherwise treated with an oligomer. For example, hydrogels, or other polymers, such as biocompatible and/or biodegradable polymers, may be used to coat an implant with the compositions of the present invention (i.e., the composition may be adapted for use with a medical device by using a hydrogel or other polymer). Polymers and copolymers for coating medical devices with an agent are well-known in the art. Examples of implants include, but are not limited to, stents, drug-eluting stents, sutures, prosthesis, vascular catheters, dialysis catheters, vascular grafts, prosthetic

heart valves, cardiac pacemakers, implantable cardioverter defibrillators, IV needles, devices for bone setting and formation, such as pins, screws, plates, and other devices, and artificial tissue matrices for wound healing.

In addition to the methods provided herein, the oligomers for use according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals. The antisense oligomers and their corresponding formulations may be administered alone or in combination with other therapeutic strategies in the treatment of muscular dystrophy, such as myoblast transplantation, stem cell therapies, administration of aminoglycoside antibiotics, proteasome inhibitors, and up-regulation therapies (e.g., upregulation of utrophin, an autosomal paralogue of dystrophin).

The routes of administration described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and any dosage for any particular animal and condition. Multiple approaches for introducing functional new genetic material into cells, both in vitro and in vivo have been attempted (Friedmann (1989) Science, 244:1275-1280). These approaches include integration of the gene to be expressed into modified retroviruses (Friedmann (1989) supra; Rosenberg (1991) Cancer Research 51(18), suppl.: 5074S-5079S); integration into non-retrovirus vectors (e.g., adeno-associated viral vectors) (Rosenfeld, et al. (1992) Cell, 68:143-155; Rosenfeld, et al. (1991) Science, 252:431-434); or delivery of a transgene linked to a heterologous promoter-enhancer element via liposomes (Friedmann (1989), supra; Brigham, et al. (1989) Am. J. Med. Sci., 298:278-281; Nabel, et al. (1990) Science, 249:1285-1288; Hazinski, et al. (1991) Am. J. Resp. Cell Molec. Biol., 4:206-209; and Wang and Huang (1987) Proc. Natl. Acad. Sci. (USA), 84:7851-7855); coupled to ligand-specific, cation-based transport systems (Wu and Wu (1988) J. Biol. Chem., 263:14621-14624) or the use of naked DNA, expression vectors (Nabel et al. (1990), supra); Wolff et al. (1990) Science, 247:1465-1468). Direct injection of transgenes into tissue produces only localized expression (Rosenfeld (1992) supra); Rosenfeld et al. (1991) supra; Brigham et al. (1989) supra; Nabel (1990) supra; and Hazinski et al. (1991) supra). The Brigham et al. group (Am. J. Med. Sci. (1989) 298:278-281 and Clinical Research (1991) 39 (abstract)) have reported in vivo transfection only of lungs of mice following either intravenous or intratracheal administration of a DNA liposome complex. An example of a review article of human gene therapy procedures is: Anderson, Science (1992) 256:808-813.

V. Kits

The invention also provides kits for treatment of a patient with a genetic disease which kit comprises at least an antisense molecule (e.g.,one or more antisense oligonucleotides capable of specifically hybridizing to any one or more of exons 1-79 of the dystrophin gene; for example, Exon 51 as set forth in Tables 3 and 4 herein), packaged in a suitable container, together with instructions for its use. The kits may also contain peripheral reagents such as buffers, stabilizers, etc. Those of ordinary skill in the field should appreciate that applications of the above method has wide application for identifying antisense molecules suitable for use in the treatment of many other diseases.

VII. EXAMPLES

Materials and Methods

Patients

Eligible patients were between 7 and 13 years of age (inclusive), with out-of-frame deletions of the DMD gene that could be corrected by skipping exon 51. Patients were confirmed to have stable cardiac and pulmonary function and a stable dose of glucocorticoids for at least 24 weeks prior to enrollment. Only patients who could walk between 200 and 400 meters (±10%) on the 6-Minute Walk Test (6MWT) at baseline were enrolled.

Study Design

This one-year trial was conducted in two phases: (1) treatment was double-blind through week 24 and (2) open-label thereafter. Primary endpoints were change in percent dystrophin fibers and ambulation as measured by the 6-Minute Walk Test (6MWT).

Study 201 was a single-site, randomized, double-blind, placebo-controlled, multiple-dose efficacy, safety and tolerability trial of eteplirsen. Twelve patients with DMD were randomized to one of three groups: eteplirsen 30 mg/kg/week (Cohort 1); eteplirsen 50 mg/kg/week (Cohort 2); or placebo/delayed eteplirsen (Cohort 3). All patients received weekly intravenous eteplirsen or placebo/delayed eteplirsen dosing. Placebo-treated patients crossed over to weekly eteplirsen 30 (n=2) or 50 mg/kg (n=2) at week 25. Efficacy and safety were assessed at scheduled visits,

and an independent Data Safety Monitoring Board ensured the welfare of all patients. All patients had bicep biopsies at baseline. Follow-up biopsies were performed in the opposite arm (biceps) at week 12 for the 50 mg/kg group and two placebo-treated patients and at week 24 for the 30 mg/kg group and two placebo-treated patients.

Patients continued weekly dosing with 30 or 50 mg/kg eteplirsen under Study 202, a long-term, open-label extension study. All efficacy assessments continued to be performed during Study 202, including a third biopsy (in the left deltoid muscle) in all patients at week 48. Monitoring of adverse events continued throughout the study. A schematic of the study design is shown in Figure 2.

Study Drug

Eteplirsen [sequence 5'-CTCCAACATCAAGGAAGATGGCATTTCTAG-3'] (SEQ ID NO:1) was supplied by Sarepta Therapeutics, Inc. in single-use vials of phosphate-buffered saline (100 mg/ml). Eteplirsen was reconstituted with 150 ml normal saline and infused over 60 minutes. Placebo, administered during the first 24 weeks of Study 201, was supplied as identical vials of phosphate-buffered saline and was administered in the same manner as eteplirsen.

Safety and Tolerability Monitoring

Safety was assessed by evaluation of adverse events, vital signs, physical examinations, electrocardiograms, echocardiograms, and clinical laboratory testing. In addition, kidney function was monitored via regular assessments of serum cystatin C and urine cystatin C and KIM-1.

Pharmacokinetic and Immune Assessments

Pharmacokinetic parameters of eteplirsen were established from plasma and urine taken after the twelfth dose using a validated and sensitive anion exchange high-performance liquid chromatography with fluorescence detection bioanalytical method. Single samples for analysis of plasma concentrations were taken at weeks 24, 25, and 36. Immune response to novel dystrophin protein was measured every six weeks through week 24 with ELISPOT following methods previously published.

Biochemical Efficacy Assessments

Pre- and post-treatment dystrophin expression studies were based on MANDYS106 [a gift from Glen Morris, MDA Monoclonal Antibody Library], a highly sensitive marker for dystrophin used in prior studies of eteplirsen and other exon skipping candidates. Three 10 μm frozen sections, separated by at least 200 μm, were stained with MANDYS106, followed by a secondary antibody (Alexa Fluor 594 goat antimouse antibody). Percent dystrophin-positive fibers were calculated by dividing the number of positive fibers by the total fibers counted. As normal muscle samples have 100% dystrophin-positive fibers, percent dystrophin-positive fibers is expressed as a percentage of normal. The same antibody-stained sections were used for dystrophin quantification using Bioquant image analysis software. The total dystrophin fluorescence signal intensity was reported as a percentage of normal.

Supportive measurements included expression of the components of the sarcoglycan complex (β,γ), neuronal NOS, and Western blot (with the anti-dystrophin antibody NCL-Dys1 from Novacastra). RT-PCR analysis, for confirmation of exon skipping, was performed on 400 ng of total RNA using dystrophin-specific reverse primers as previously described.

Clinical Efficacy Assessments

The 6MWT was administered using the protocol established for patients with DMD by McDonald, et al. (Muscle Nerve, 2010; 42:966-74, herein incorporated by reference). Exploratory functional outcomes included the North Star Ambulatory Assessment, quantitative muscle testing, the 9-Hole Peg Test, pulmonary function testing (PFT), timed function tests, and assessment of quality of life.

Statistical Analysis

SAS version 9.3 (Cary, NC) was used for all statistical analyses. Mixed model with treatment as fixed effect, subject nested within treatment as random effect, with the baseline value and time since DMD diagnosis as covariates for the analysis of muscle biopsy data was used. Mixed model repeated measures (MMRM) with treatment, time, and treatment-by-time interaction terms as fixed effect, subject nested within treatment as random effect, and with the baseline value and time since DMD diagnosis as covariates for analysis of the 6MWT data was used. Safety and muscle biopsy analyses were performed on the intent-to-treat population;

analysis of ambulation-related outcomes, including the 6MWT, used a modified intent-to-treat (mITT) population that excluded two patients in Cohort 1 who showed signs of disease progression and significant decline on the 6MWT within weeks of enrollment and could not perform measures of ambulation at week 24 or beyond.

Example 1: Subject Characteristics

Baseline characteristics of the 12 patients in this study are summarized in Table 2. Five different genotypes amenable to exon 51 skipping were represented in the study population. Mean distances on the 6-Minute Walk Test (6MWT) at baseline were similar to those in other studies of children with DMD, and as expected, were well below the 600 plus meters typically observed in age-matched healthy children. Due to the stochastic nature of the sampling, the 30 mg/kg cohort was slightly older, heavier, and taller, relative to the other cohorts, and had a lower mean 6MWT distance at baseline. All patients received all infusions of study medication as planned and completed all assessments.

Table 2. Baseline Demography and Disease Characteristics

Treatment Arm	Placebo/ Delayed Eteplirsen N=4	Eteplirsen 30 mg/kg N=4	Eteplirsen 50 mg/kg N=4
Mutation			
45-50 n(%)	0	2 (50)	1 (25)
48-50 n(%)	0	1 (25)	0
49-50 n(%)	3 (75)	0	2 (50)
50 n(%)	1 (25)	0	0
52 n(%)	0	1 (25)	1 (25)
Gender n(%)			
Male	4 (100)	4 (100)	4 (100)
Age, years			
Mean	8.5	9.3	8.5
SD	1.73	0.50	1.29
Min, Max	7, 10	9, 10	7, 10
Height, cm			
Mean	119.3	130.5	121.3
SD	3.40	9.47	7.85
Min, Max	116, 124	117, 138	117, 133
Weight, kg			
Mean	30.6	34.8	29.0
SD	6.04	7.05	6.38
Min, Max	22.1, 36.2	24.8, 39.8	23.7, 38.3
Race, n(%)			
Asian	0	1 (25)	0
White	4 (100)	3 (75)	4 (100)
6MWT*, meters			
Mean	394.5	355.3	396.0
SD	42.25	74.78	26.61
Min, Max	364, 456	261, 442	365, 429

*6MWT results are the maximum observed value of two tests administered on two consecutive days during screening.

Abbreviations: 6MWT=6-Minute Walk Test; min=minimum; max=maximum; SD=standard deviation.

Table 2

Example 2: Safety and Lack of Adverse Events

Eteplirsen was well tolerated with no treatment-related adverse events, serious adverse events, discontinuations or missed doses through 48 weeks of treatment. Moreover, no clinically significant changes were observed on physical examination or in vital signs. Electrocardiograms, echocardiograms, and PFTs remained stable, and chemistries showed no clinically significant changes in hematologic, renal, coagulation or liver functions. Mild and transient proteinuria was observed in a single placebo-treated subject.

Example 3: Pharmacokinetic Profile

Analysis of PK parameters at week 12 revealed rapid absorption. Plasma clearance averaged 339 ± 75.8 mL/hr/kg for 30 mg/kg and 319 ± 125 mL/hr/kg for 50 mg/kg. Half-life averaged 3.30 ± 0.341 hr for 30 mg/kg and 3.17 ± 0.249 hr for 50 mg/kg, with renal clearance accounting for approximately 65-70% of total systemic clearance.

Example 4: Efficacy

At week 48, eteplirsen produced robust increases in the number and intensity of dystrophin-positive fibers. As shown in Fig. 3, patients who received 30 or 50 mg/kg eteplirsen without interruption for 48 weeks showed a mean increase in the percentage of dystrophin-positive fibers to 47% of normal ($p \leq 0.001$), relative to baseline. Increases were similar when the 30 (52%; $p \leq 0.001$) and 50 (43%; $p \leq 0.008$) mg/kg cohorts were analyzed separately, suggesting that eteplirsen's effect on the production of novel dystrophin is independent of dose within this range of doses.

Biopsies were taken at staggered time points (see Fig. 2) to evaluate the impact of treatment duration on novel dystrophin production. At week 12, the 50 mg/kg cohort had undetectable levels of novel dystrophin. At week 24, the 30 mg/kg cohort demonstrated an increase in the percentage of dystrophin-positive fibers to 23% of normal ($p \leq 0.002$), and at week 48, after 24 weeks of treatment with 30 or 50 mg/kg eteplirsen, the 4 patients in the placebo/delayed eteplirsen cohort showed an increase to 38% of normal, relative to baseline ($p \leq 0.009$). Together these data suggest that treatment duration plays an important role in eteplirsen's ability to uniformly restore novel dystrophin production. Consistent with these

findings, eteplirsen also significantly increased mean fluorescence signal intensity at week 48 in all three treatment groups (all p-values ≤0.023).

Fig.4 illustrates eteplirsen's time-dependent effect on the percentage of dystrophin-positive fibers (Panel A), which was accompanied by restoration of β– and γ-sarcoglycan and nNOSμ at the sarcolemma (Panel B). Dystrophin expression and exon skipping were confirmed by Western blot and RT-PCR in all patients. RT-PCR results from a representative patient are shown in Panel C. These data confirmed the increase in functional dystrophin in the patients.

Example 5: Functional Outcomes

The progressive loss of walking ability is a universal hallmark of DMD, with most patients showing functional compromise by 7 or 8 years of age and becoming wheelchair dependent by 10 to 14 years of age. Consistent with this, boys assigned to the placebo/delayed eteplirsen cohort in this study showed a decline in walking ability after week 12 at a rate predicted by prior studies, culminating in a loss of approximately 60 meters by week 48 (Fig. 5). In marked contrast, eteplirsen-treated patients maintained a stable walking distance over the duration of the study, with a mean increase from baseline of about 7 meters by week 48. The difference between the eteplirsen-treated patients and those in the placebo/delayed eteplirsen cohort first became statistically significant at week 32 (39-meter difference; p ≤0.05). Interestingly, patients in the placebo/delayed eteplirsen cohort appeared to stabilize after week 36, *i.e.*, between 12 and 24 weeks after initiating treatment with eteplirsen at week 25. As previously noted, two boys who showed signs of rapid disease progression and significant decline on the 6MWT within weeks of enrollment and were unable to perform measures of ambulation at 24 weeks or beyond, were excluded from this analysis. However, both remained on eteplirsen through week 48 with no treatment-related adverse events and maintained stable pulmonary and upper limb function as measured by PFT and the 9-Hole Peg Test, respectively.

Notably, patients receiving eteplirsen for 48 weeks, evaluable on the 6MWT (n=6), significantly (p ≤0.001) improved on the 6MWT (67.3m) compared to the placebo/delayed cohort.

Both cohorts have maintained stable 6MWT results over 120 weeks as described below. At 120 weeks, patients in the 30 mg/kg and 50 mg/kg eteplirsen cohorts who were able to perform the 6MWT (modified Intent-to-Treat or mITT population; n=6) experienced a general

stability with a slight decline of 13.9 meters, or less than 5 percent, from baseline in walking ability. A statistically significant treatment benefit of 64.9 meters ($p \leq 0.006$) was observed for the mITT population compared with the placebo/delayed-treatment cohort (n=4), which initiated treatment at Week 25 following 24 weeks of placebo. After experiencing a substantial decline earlier in the study (prior to treatment with etiplersen), the placebo/delayed-treatment cohort also demonstrated stabilization in walking ability for more than 1.5 years, from Week 36 through 120, the period from which meaningful levels of dystrophin were likely produced, with a decline of 9.5 meters over this timeframe. These analyses were based on the maximum 6MWT score when the test was performed on two consecutive days.

Example 6: Immune Response

There were no differences between the eteplirsen- and placebo-treated patients in the number of interferon-γ-induced spot forming colonies to dystrophin peptide pools (extended over the entire protein) at any time point assessed, including week 24, indicating that the newly expressed dystrophin in the eteplirsen-treated patients did not elicit a T-cell response.

Example 7: Pulmonary Function

Respiratory muscle function from baseline through Week 120 in both dosing cohorts, as measured by maximum inspiratory and expiratory pressure (MIP and MEP), showed a 14.6 percent mean increase in MIP and a 15.0 percent mean increase in MEP. Analyses of MIP percent predicted (MIP adjusted for weight) and MEP percent predicted (MEP adjusted for age) demonstrated a mean increase from 90.2 percent at baseline to 95.2 percent at Week 120 in MIP percent predicted, and a slight mean increase from 79.3 percent at baseline to 79.6 percent at Week 120 in MEP percent predicted. In addition, there was a mean increase in forced vital capacity (FVC), a measure of lung volume, of 8.7 percent from baseline to Week 120, and FVC percent predicted (FVC adjusted for age and height) was maintained above a mean of 90 percent through Week 120, with 101 percent at baseline and 93 percent at Week 120.

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All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering to the patient a dose of eteplirsen of about 30 mg/kg once a week over 120 weeks, thereby delaying the progression of the disease over 120 weeks as determined by one or both of the following:

(i) the patient maintaining stable walking ability over 120 weeks, as measured by a decline of less than 5 percent, or less than 13.9 meters, in walking ability, relative to baseline in the 6 Minute Walk Test (6MWT); and

(ii) the patient showing an increase in respiratory muscle function over 120 weeks relative to baseline, as measured by a 14.6 percent increase in maximum inspiratory pressure (MIP) and a 15 percent increase in maximum expiratory pressure (MEP).

2. The method according to claim 1, wherein eteplirsen is administered in a single dose.

3. The method according to claim 1, wherein eteplirsen is administered intravenously.

4. The method according to claim 1, wherein the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene.

5. The method according to claim 1, wherein the patient is a pediatric patient.

6. The method according to claim 1, wherein the patient is administered an oral corticosteroid for at least 24 weeks prior to the first dose of eteplirsen.

7. The method according to claim 6, wherein the corticosteroid is betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone.

8. A method for treating Duchenne muscular dystrophy in a patient in need thereof comprising administering to the patient a dose of eteplirsen of about 50 mg/kg once a week over 120 weeks, thereby delaying the progression of the disease over 120 weeks as determined by one or both of the following:

(i) the patient maintaining stable walking ability over 120 weeks, as measured by a decline of less than 5 percent, or less than 13.9 meters, in walking ability, relative to baseline in the 6 Minute Walk Test (6MWT); and

(ii) the patient showing an increase in respiratory muscle function over 120 weeks relative to baseline, as measured by a 14.6 percent increase in maximum inspiratory pressure (MIP) and a 15 percent increase in maximum expiratory pressure (MEP).

9. The method according to claim 8, wherein eteplirsen is administered in a single dose.

10. The method according to claim 8, wherein eteplirsen is administered intravenously.

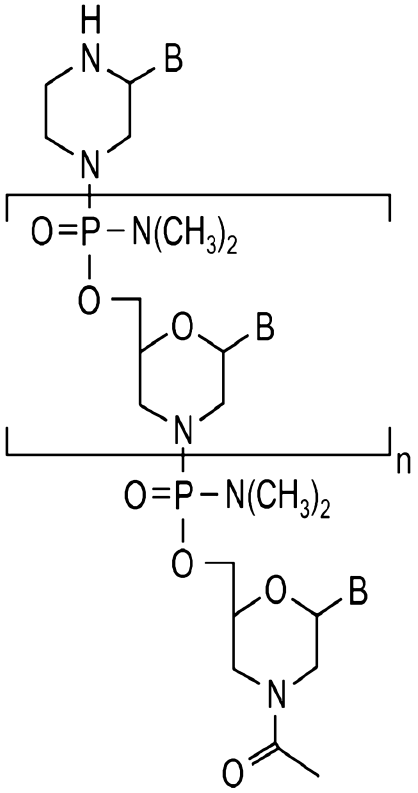
11. The method according to claim 8, wherein the patient has an out-of-frame deletion(s) that may be corrected by skipping exon 51 of the dystrophin gene.

12. The method according to claim 8, wherein the patient is a pediatric patient.

13. The method according to claim 8, wherein the patient is administered an oral corticosteroid for at least 24 weeks prior to the first dose of eteplirsen.

14. The method according to claim 13, wherein the corticosteroid is betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone.

FIG. 1A



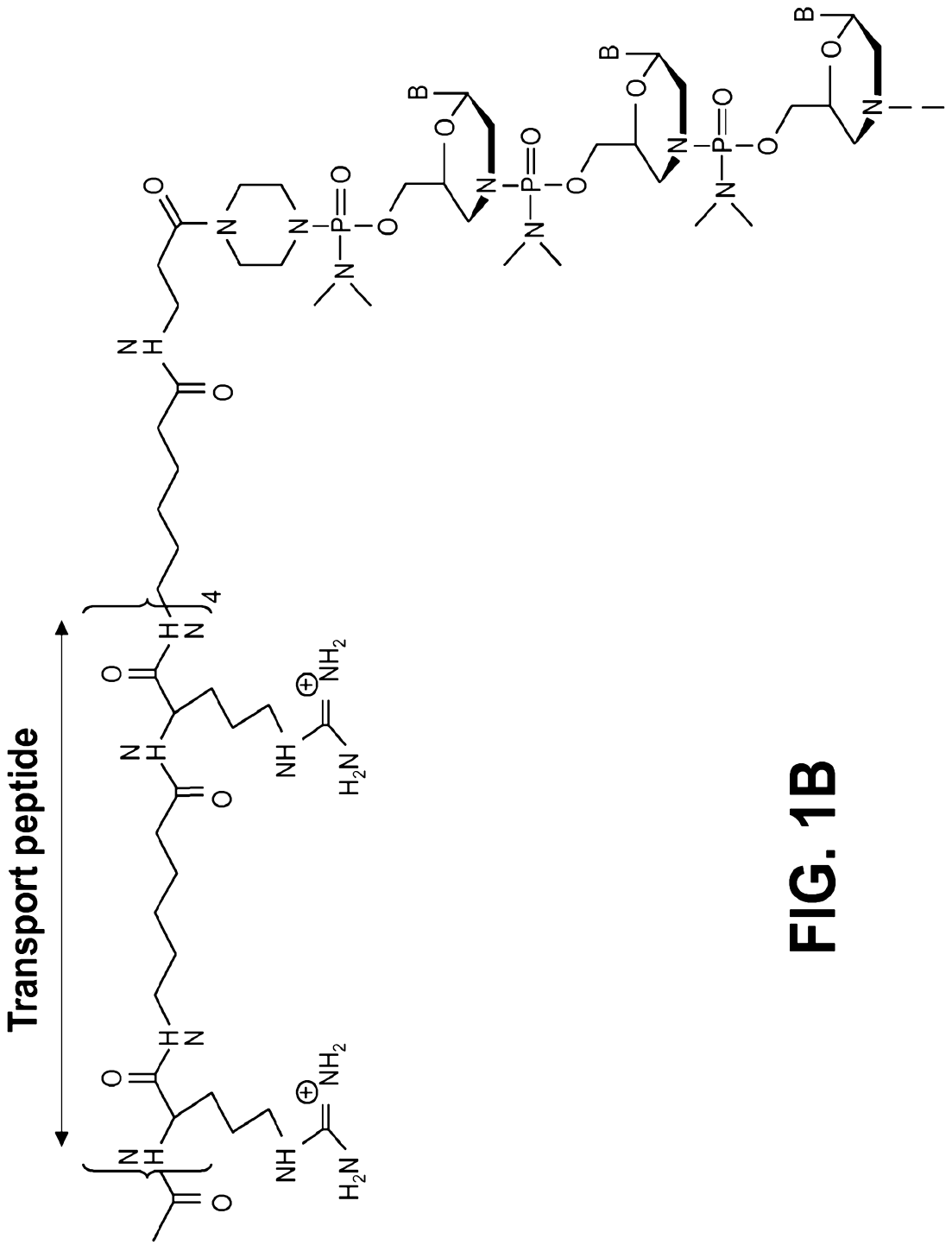


FIG. 1B

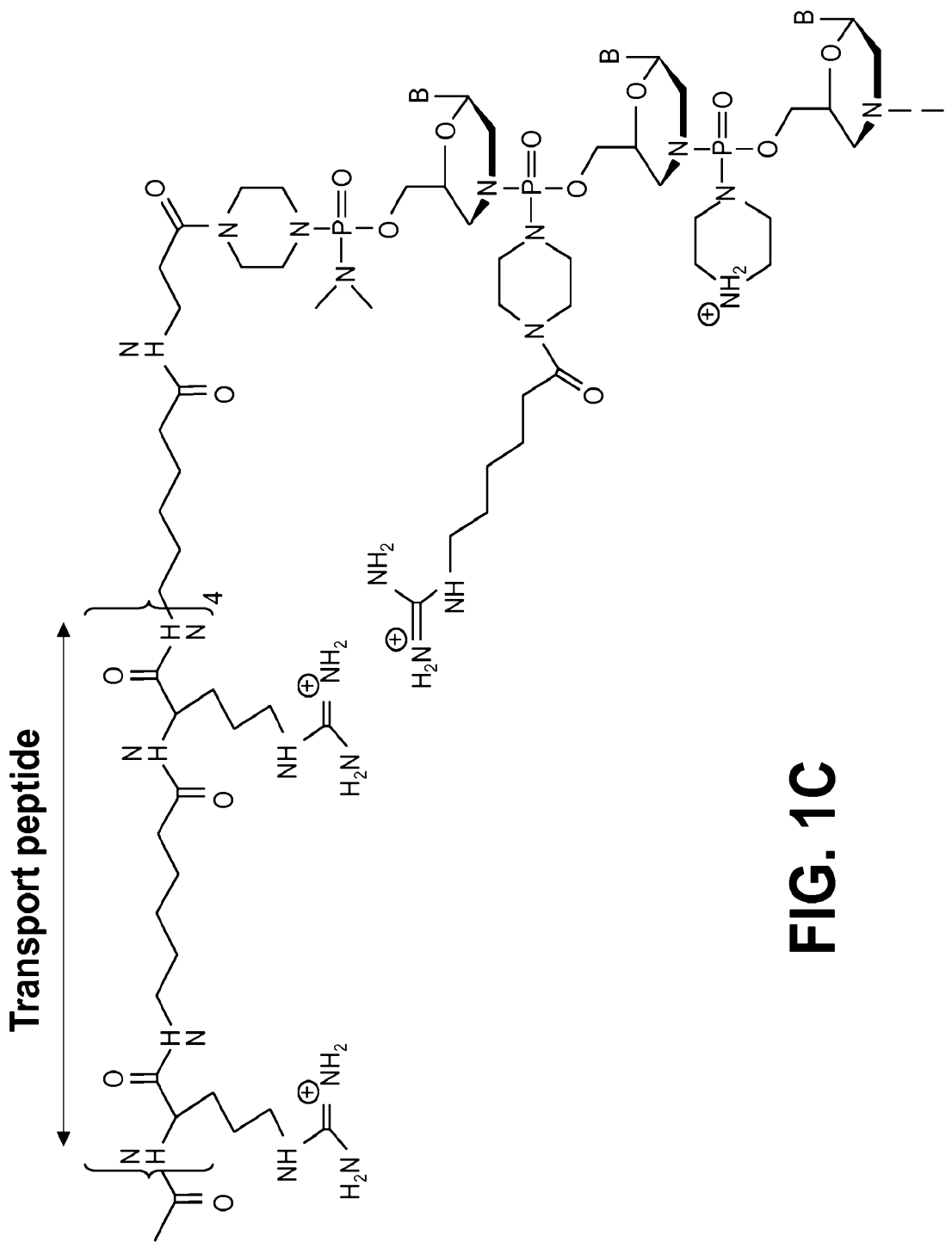


FIG. 1C

FIG. 1D

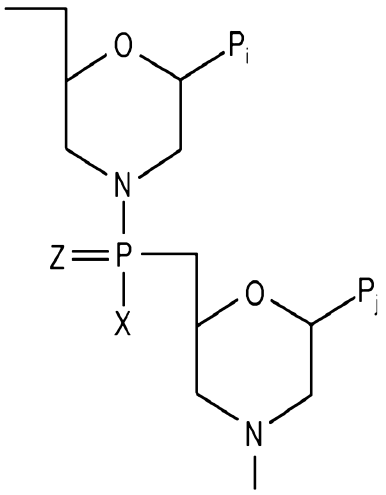


FIG. 1E

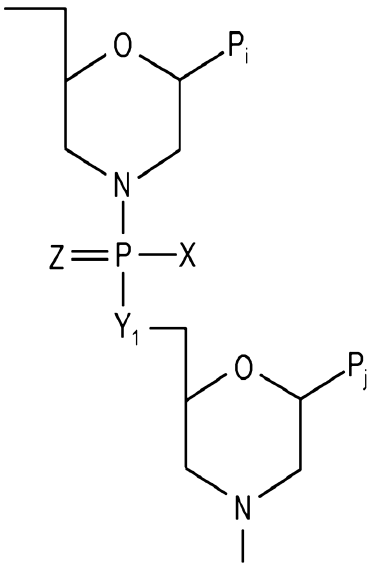


FIG. 1F

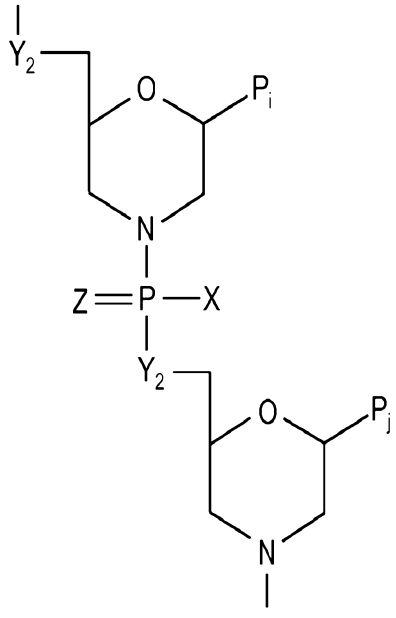
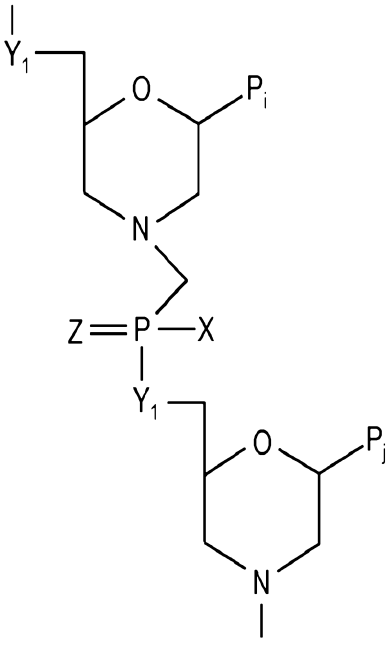


FIG. 1G



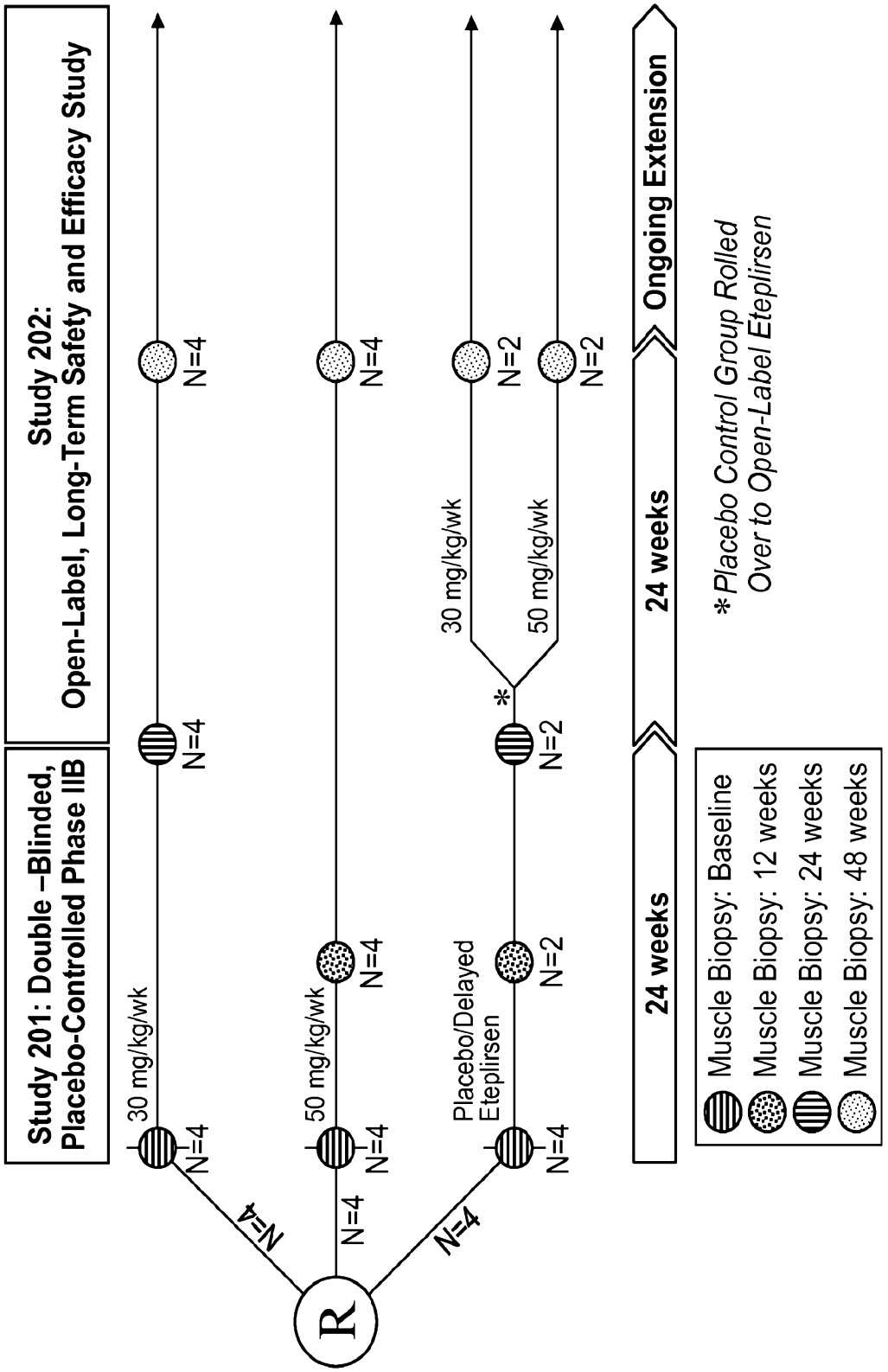

Study 201: Double –Blinded, Placebo-Controlled Phase IIB
Study 202: Open-Label, Long-Term Safety and Efficacy Study
30 mg/kg/wk
50 mg/kg/wk
Placebo/Delayed Eteplirsen
N=4
N=2
R
24 weeks
Ongoing Extension
* Placebo Control Group Rolled Over to Open-Label Eteplirsen
Muscle Biopsy: Baseline
Muscle Biopsy: 12 weeks
Muscle Biopsy: 24 weeks
Muscle Biopsy: 48 weeks

FIG. 2

A

Cohort (n)	Week 12 Mean Change from BL, SE (p-value) +	Week 24 Mean Change from BL, SE (p-value) +	Week 48 Mean Change from BL, S, SE (p-value) +
All Eteplirsen (8)	NA	NA	47.3, 3.89 (≤0.001)
Eteplirsen 30 mg/kg (4)	ND	22.9, 2.90 (≤0.002)	51.7, 3.54 (≤0.001)
Eteplirsen 50 mg/kg (4)	0.8, 3.55 (NS)	ND	42.9, 6.72 (≤0.008)
Placebo/Delayed Eteplirsen (4) +	-4.0, 2.92	-4.0, 2.92	37.7, 6.30 (≤0.009)
Eteplirsen 30 mg/kg (2)	ND	-7.48, 1.00	33.6, 5.23
Eteplirsen 50 mg/kg (2)	-0.6, 5.16	ND	41.8, 13.30

B

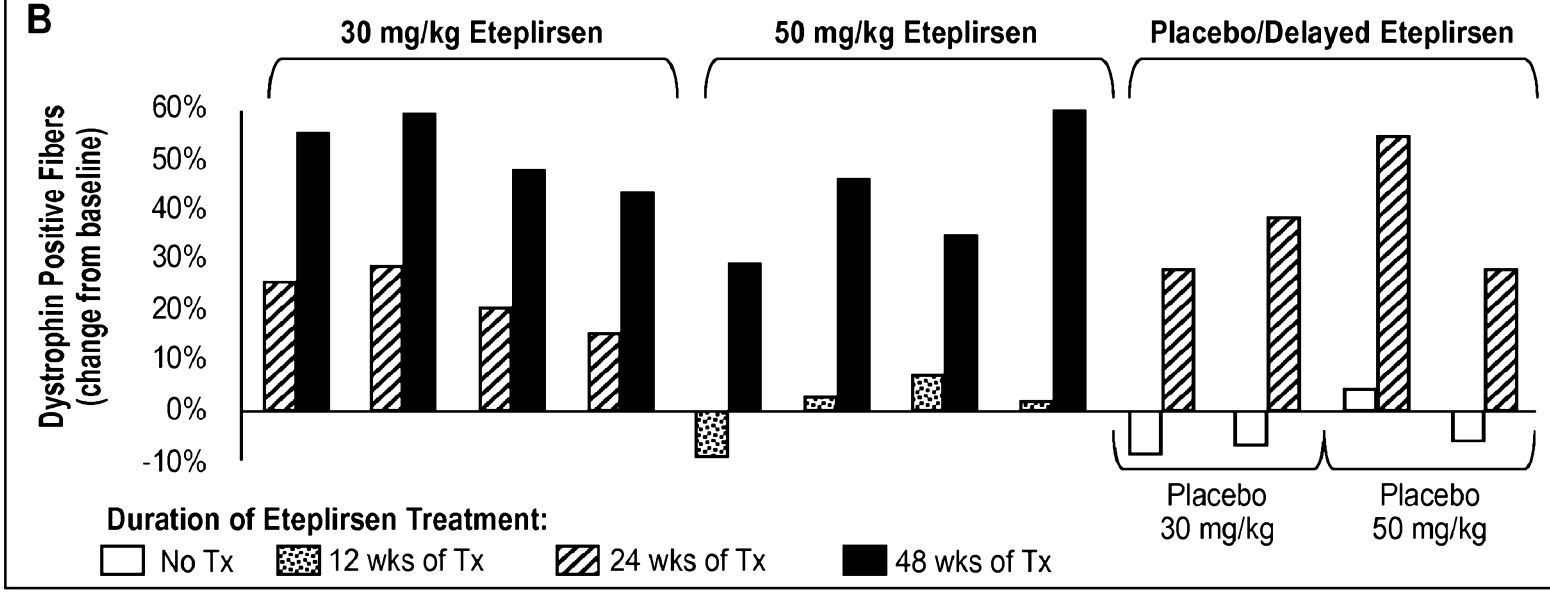


FIG. 3

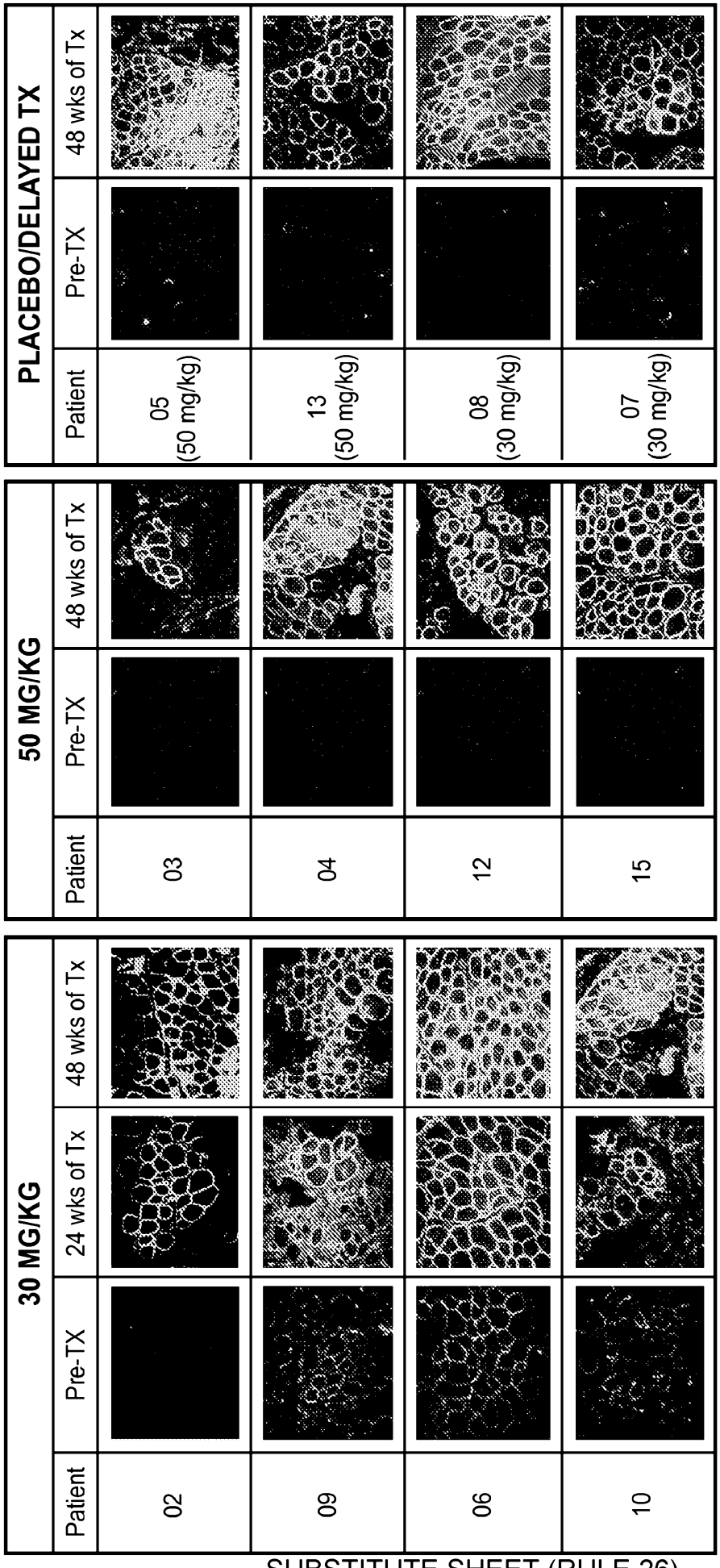

30 MG/KG
Patient
Pre-TX
24 wks of Tx
48 wks of Tx
02
09
06
10
50 MG/KG
Patient
Pre-TX
48 wks of Tx
03
04
12
15
PLACEBO/DELAYED TX
Patient
Pre-TX
48 wks of Tx
05 (50 mg/kg)
13 (50 mg/kg)
08 (30 mg/kg)
07 (30 mg/kg)

FIG. 4A

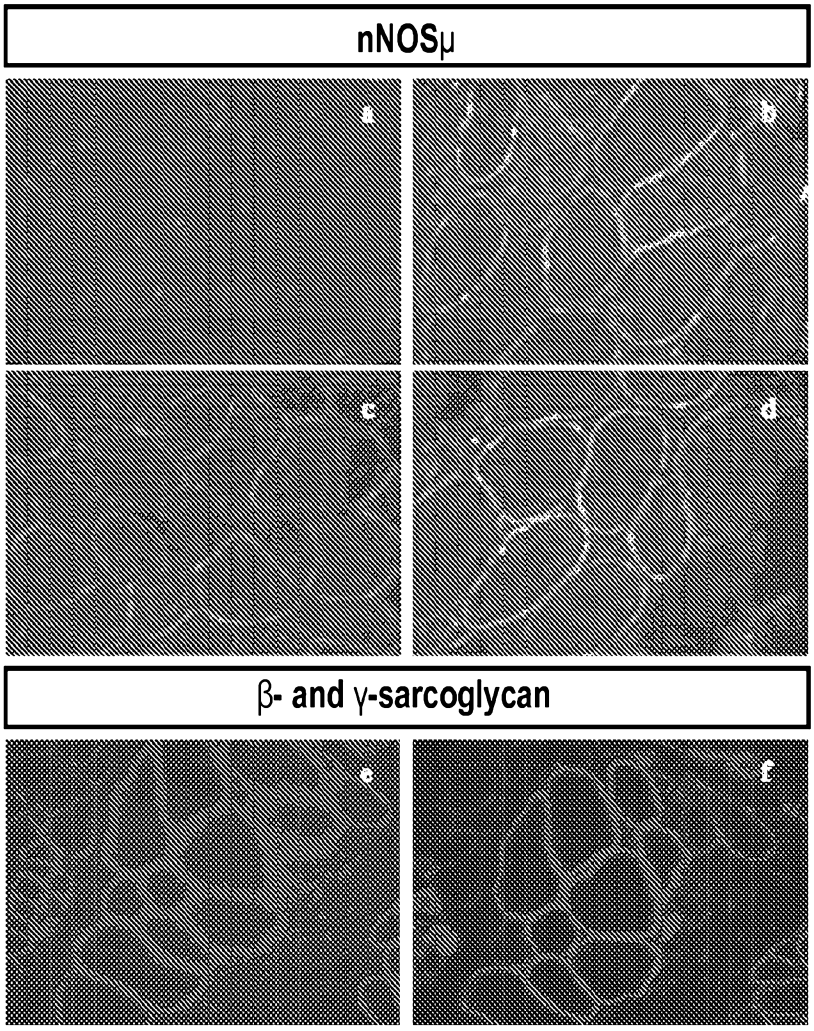

nNOSμ
a
b
c
d
β- and γ-sarcoglycan
e
f

FIG. 4B

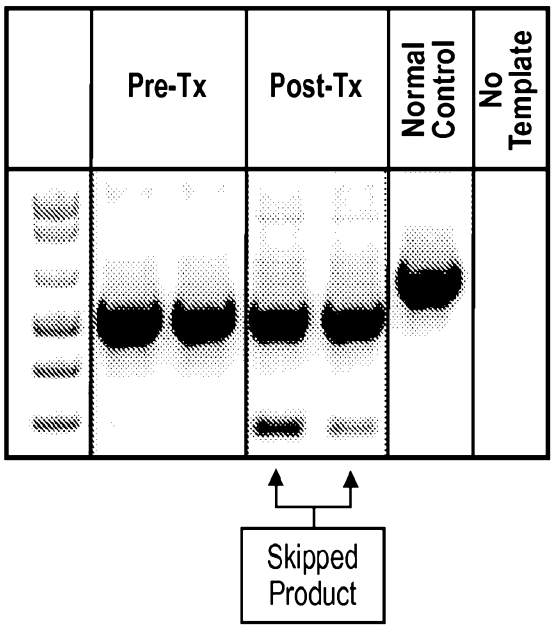

Pre-Tx
Post-Tx
Normal Control
No Template
Skipped Product

FIG. 4C

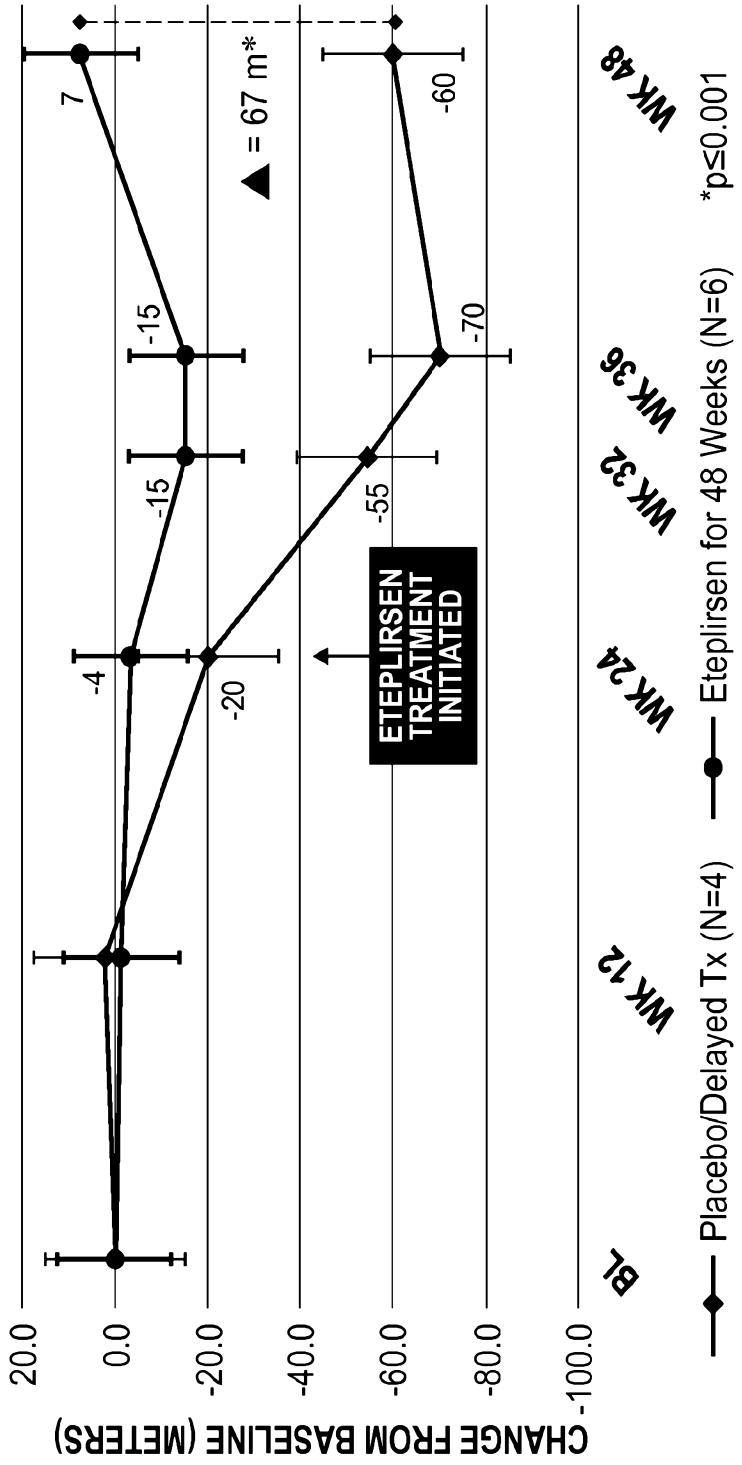

CHANGE FROM BASELINE (METERS)
20.0
0.0
-20.0
-40.0
-60.0
-80.0
-100.0
BL
WK 12
WK 24
WK 32
WK 36
WK 48
7
-4
-15
-15
-20
-55
-70
-60
▲ = 67 m*
ETEPLIRSEN TREATMENT INITIATED
Placebo/Delayed Tx (N=4)
Eteplirsen for 48 Weeks (N=6)
*p≤0.001

Fig. 5

SEQUENCE LISTING

With respect to the nucleic acid sequences provided in the application, persons skilled in the art will appreciate that depending on the use of the oligomers, Ts and Us are interchangeable.

TABLE 3

EXON	SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
51	1000	eteplirsen H51A(+66+95)	CTC CAA CAT CAA GGA AGA TGG CAT TTC TAG
51	1001	H51A(+66+90)	ACA UCA AGG AAG AUG GCA UUU CUA G
51	1002	H51A(+61+90)	ACA UCA AGG AAG AUG GCA UUU CUA GUU UGG
51	1003	Hu.DMD.exon51.25.001.2	GAG CAG GTA CCT CCA ACA TCA AGG AA
50	1004	H50D(+07-18)	GGG AUC CAG UAU ACU UAC AGG CUC C
50	1005	AVI-4038/5038	CTT ACA GGC TCC AAT AGT GGT CAG T
53	1006	H53A27(+30+56)	CCT CCG GTT CTG AAG GTG TTC TTG TAC
53	1007	H53A(+36+60)	GTT GCC TCC GGT TCT GAA GGT GTT C
45	1008	H45A (-03+19)	CAA TGC CAT CCT GGA GTT CCT G

TABLE 4

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
1	H8A(-06+18)	GAU AGG UGG UAU CAA CAU CUG UAA
2	H8A (-03+18)	GAU AGG UGG UAU CAA CAU CUG
3	H8A(-07+18)	GAU AGG UGG UAU CAA CAU CUG UAA G
4	H8A(-06+14)	GGU GGU AUC AAC AUC UGU AA
5	H8A(-10+10)	GUA UCA ACA UCU GUA AGC AC
6	H7A(+45+67)	UGC AUG UUC CAG UCG UUG UGU GG
7	H7A(+02+26)	CAC UAU UCC AGU CAA AUA GGU CUG G
8	H7D(+15-10)	AUU UAC CAA CCU UCA GGA UCG AGU A
9	H7A(-18+03)	GGC CUA AAA CAC AUA CAC AUA

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
10	C6A(-10+10)	CAU UUU UGA CCU ACA UGU GG
11	C6A(-14+06)	UUU GAC CUA CAU GUG GAA AG
12	C6A(-14+12)	UAC AUU UUU GAC CUA CAU GUG GAA AG
13	C6A(-13+09)	AUU UUU GAC CUA CAU GGG AAA G
14	CH6A(+69+91)	UAC GAG UUG AUU GUC GGA CCC AG
15	C6D(+12-13)	GUG GUC UCC UUA CCU AUG ACU GUG G
16	C6D(+06-11)	GGU CUC CUU ACC UAU GA
17	H6D(+04-21)	UGU CUC AGU AAU CUU CUU ACC UAU
18	H6D(+18-04)	UCU UAC CUA UGA CUA UGG AUG AGA
19	H4A(+13+32)	GCA UGA ACU CUU GUG GAU CC
20	H4D(+04-16)	CCA GGG UAC UAC UUA CAU UA
21	H4D(-24-44)	AUC GUG UGU CAC AGC AUC CAG
22	H4A(+11+40)	UGU UCA GGG CAU GAA CUC UUG UGG AUC CUU
23	H3A(+30+60)	UAG GAG GCG CCU CCC AUC CUG UAG GUC ACU G
24	H3A(+35+65)	AGG UCU AGG AGG CGC CUC CCA UCC UGU AGG U
25	H3A(+30+54)	GCG CCU CCC AUC CUG UAG GUC ACU G
26	H3D(+46-21)	CUU CGA GGA GGU CUA GGA GGC GCC UC
27	H3A(+30+50)	CUC CCA UCC UGU AGG UCA CUG
28	H3D(+19-03)	UAC CAG UUU UUG CCC UGU CAG G
29	H3A(-06+20)	UCA AUA UGC UGC UUC CCA AAC UGA AA
30	H3A(+37+61)	CUA GGA GGC GCC UCC CAU CCU GUA G
31	H5A(+20+50)	UUA UGA UUU CCA UCU ACG AUG UCA GUA CUU C
32	H5D(+25-05)	CUU ACC UGC CAG UGG AGG AUU AUA UUC CAA A
33	H5D(+10-15)	CAU CAG GAU UCU UAC CUG CCA GUG G
34	H5A(+10+34)	CGA UGU CAG UAC UUC CAA UAU UCA C
35	H5D(-04-21)	ACC AUU CAU CAG GAU UCU
36	H5D(+16-02)	ACC UGC CAG UGG AGG AUU
37	H5A(-07+20)	CCA AUA UUC ACU AAA UCA ACC UGU UAA
38	H5D(+18-12)	CAG GAU UGU UAC CUG CCA GUG GAG GAU UAU
39	H5A(+05+35)	ACG AUG UCA GUA CUU CCA AUA UUC ACU AAA U
40	H5A(+15+45)	AUU UCC AUC UAC GAU GUC AGU ACU UCC AAU A
41	H10A(-05+16)	CAG GAG CUU CCA AAU GCU GCA
42	H10A(-05+24)	CUU GUC UUC AGG AGC UUC CAA AUG CUG CA
43	H10A(+98+119)	UCC UCA GCA GAA AGA AGC CAC G

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
44	H10A(+130+149)	UUA GAA AUC UCU CCU UGU GC
45	H10A(-33-14)	UAA AUU GGG UGU UAC ACA AU
46	H11D(+26+49)	CCC UGA GGC AUU CCC AUC UUG AAU
47	H11D(+11-09)	AGG ACU UAC UUG CUU UGU UU
48	H11A(+118+140)	CUU GAA UUU AGG AGA UUC AUC UG
49	H11A(+75+97)	CAU CUU CUG AUA AUU UUC CUG UU
50	H12A(+52+75)	UCU UCU GUU UUU GUU AGC CAG UCA
51	H12A(-10+10)	UCU AUG UAA ACU GAA AAU UU
52	H12A(+11+30)	UUC UGG AGA UCC AUU AAA AC
53	H13A(+77+100)	CAG CAG UUG CGU GAU CUC CAC UAG
54	H13A(+55+75)	UUC AUC AAC UAC CAC CAC CAU
55	H13D(+06-19)	CUA AGC AAA AUA AUC UGA CCU UAA G
56	H14A(+37+64)	CUU GUA AAA GAA CCC AGC GGU CUU CUG U
57	H14A(+14+35)	CAU CUA CAG AUG UUU GCC CAU C
58	H14A(+51+73)	GAA GGA UGU CUU GUA AAA GAA CC
59	H14D(-02+18)	ACC UGU UCU UCA GUA AGA CG
60	H14D(+14-10)	CAU GAC ACA CCU GUU CUU CAG UAA
61	H14A(+61+80)	CAU UUG AGA AGG AUG UCU UG
62	H14A(-12+12)	AUC UCC CAA UAC CUG GAG AAG AGA
63	H15A(-12+19)	GCC AUG CAC UAA AAA GGC ACU GCA AGA CAU U
64	H15A(+48+71)	UCU UUA AAG CCA GUU GUG UGA AUC
65	H15A(+08+28)	UUU CUG AAA GCC AUG CAC UAA
66	H15D(+17-08)	GUA CAU ACG GCC AGU UUU UGA AGA C
67	H16A(-12+19)	CUA GAU CCG CUU UUA AAA CCU GUU AAA ACA A
68	H16A(-06+25)	UCU UUU CUA GAU CCG CUU UUA AAA CCU GUU A
69	H16A(-06+19)	CUA GAU CCG CUU UUA AAA CCU GUU A
70	H16A(+87+109)	CCG UCU UCU GGG UCA CUG ACU UA
71	H16A(-07+19)	CUA GAU CCG CUU UUA AAA CCU GUU AA
72	H16A(-07+13)	CCG CUU UUA AAA CCU GUU AA
73	H16A(+12+37)	UGG AUU GCU UUU UCU UUU CUA GAU CC
74	H16A(+92+116)	CAU GCU UCC GUC UUC UGG GUC ACU G
75	H16A(+45+67)	G AUC UUG UUU GAG UGA AUA CAG U
76	H16A(+105+126)	GUU AUC CAG CCA UGC UUC CGU C
77	H16D(+05-20)	UGA UAA UUG GUA UCA CUA ACC UGU G
78	H16D(+12-11)	GUA UCA CUA ACC UGU GCU GUA C
79	H19A(+35+53)	CUG CUG GCA UCU UGC AGU U
80	H19A(+35+65)	GCC UGA GCU GAU CUG CUG GCA UCU UGC AGU U
81	H20A(+44+71)	CUG GCA GAA UUC GAU CCA CCG GCU GUU C
82	H20A(+147+168)	CAG CAG UAG UUG UCA UCU GCU C

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
83	H20A(+185+203)	UGA UGG GGU GGU GGG UUG G
84	H20A(-08+17)	AUC UGC AUU AAC ACC CUC UAG AAA G
85	H20A(+30+53)	CCG GCU GUU CAG UUG UUC UGA GGC
86	H20A(-11+17)	AUC UGC AUU AAC ACC CUC UAG AAA GAA A
87	H20D(+08-20)	GAA GGA GAA GAG AUU CUU ACC UUA CAA A
88	H20A(+44+63)	AUU CGA UCC ACC GGC UGU UC
89	H20A(+149+168)	CAG CAG UAG UUG UCA UCU GC
90	H21A(-06+16)	GCC GGU UGA CUU CAU CCU GUG C
91	H21A(+85+106)	CUG CAU CCA GGA ACA UGG GUC C
92	H21A(+85+108)	GUC UGC AUC CAG GAA CAU GGG UC
93	H21A(+08+31)	GUU GAA GAU CUG AUA GCC GGU UGA
94	H21D(+18-07)	UAC UUA CUG UCU GUA GCU CUU UCU
95	H22A(+22+45)	CAC UCA UGG UCU CCU GAU AGC GCA
96	H22A(+125+146)	CUG CAA UUC CCC GAG UCU CUG C
97	H22A(+47+69)	ACU GCU GGA CCC AUG UCC UGA UG
98	H22A(+80+101)	CUA AGU UGA GGU AUG GAG AGU
99	H22D(+13-11)	UAU UCA CAG ACC UGC AAU UCC CC
100	H23A(+34+59)	ACA GUG GUG CUG AGA UAG UAU AGG CC
101	H23A(+18+39)	UAG GCC ACU UUG UUG CUC UUG C
102	H23A(+72+90)	UUC AGA GGG CGC UUU CUU C
103	H24A(+48+70)	GGG CAG GCC AUU CCU CCU UCA GA
104	H24A(-02+22)	UCU UCA GGG UUU GUA UGU GAU UCU
105	H25A(+9+36)	CUG GGC UGA AUU GUC UGA AUA UCA CUG
106	H25A(+131+156)	CUG UUG GCA CAU GUG AUC CCA CUG AG
107	H25D(+16-08)	GUC UAU ACC UGU UGG CAC AUG UGA
108	H26A(+132+156)	UGC UUU CUG UAA UUC AUC UGG AGU U
109	H26A(-07+19)	CCU CCU UUC UGG CAU AGA CCU UCC AC
110	H26A(+68+92)	UGU GUC AUC CAU UCG UGC AUC UCU G
111	H27A(+82+106)	UUA AGG CCU CUU GUG CUA CAG GUG G
112	H27A(-4+19)	GGG GCU CUU CUU UAG CUC UCU GA
113	H27D(+19-03)	GAC UUC CAA AGU CUU GCA UUU C
114	H28A(-05+19)	GCC AAC AUG CCC AAA CUU CCU AAG
115	H28A(+99+124)	CAG AGA UUU CCU CAG CUC CGC CAG GA
116	H28D(+16-05)	CUU ACA UCU AGC ACC UCA GAG
117	H29A(+57+81)	UCC GCC AUC UGU UAG GGU CUG UGC C
118	H29A(+18+42)	AUU UGG GUU AUC CUC UGA AUG UCG C
119	H29D(+17-05)	CAU ACC UCU UCA UGU AGU UCC C
120	H30A(+122+147)	CAU UUG AGC UGC GUC CAC CUU GUC UG
121	H30A(+25+50)	UCC UGG GCA GAC UGG AUG CUC UGU UC
122	H30D(+19-04)	UUG CCU GGG CUU CCU GAG GCA UU
123	H31D(+06-18)	UUC UGA AAU AAC AUA UAC CUG UGC
124	H31D(+03-22)	UAG UUU CUG AAA UAA CAU AUA CCU G
125	H31A(+05+25)	GAC UUG UCA AAU CAG AUU GGA

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
126	H31D(+04-20)	GUU UCU GAA AUA ACA UAU ACC UGU
127	H32D(+04-16)	CAC CAG AAA UAC AUA CCA CA
128	H32A(+151+170)	CAA UGA UUU AGC UGU GAC UG
129	H32A(+10+32)	CGA AAC UUC AUG GAG ACA UCU UG
130	H32A(+49+73)	CUU GUA GAC GCU GCU CAA AAU UGG C
131	H33D(+09-11)	CAU GCA CAC ACC UUU GCU CC
132	H33A(+53+76)	UCU GUA CAA UCU GAC GUC CAG UCU
133	H33A(+30+56)	GUC UUU AUC ACC AUU UCC ACU UCA GAC
134	H33A(+64+88)	CCG UCU GCU UUU UCU GUA CAA UCU G
135	H34A(+83+104)	UCC AUA UCU GUA GCU GCC AGC C
136	H34A(+143+165)	CCA GGC AAC UUC AGA AUC CAA AU
137	H34A(-20+10)	UUU CUG UUA CCU GAA AAG AAU UAU AAU GAA
138	H34A(+46+70)	CAU UCA UUU CCU UUC GCA UCU UAC G
139	H34A(+95+120)	UGA UCU CUU UGU CAA UUC CAU AUC UG
140	H34D(+10-20)	UUC AGU GAU AUA GGU UUU ACC UUU CCC CAG
141	H34A(+72+96)	CUG UAG CUG CCA GCC AUU CUG UCA AG
142	H35A(+141+161)	UCU UCU GCU CGG GAG GUG ACA
143	H35A(+116+135)	CCA GUU ACU AUU CAG AAG AC
144	H35A(+24+43)	UCU UCA GGU GCA CCU UCU GU
145	H36A(+26+50)	UGU GAU GUG GUC CAC AUU CUG GUC A
146	H36A(-02+18)	CCA UGU GUU UCU GGU AUU CC
147	H37A(+26+50)	CGU GUA GAG UCC ACC UUU GGG CGU A
148	H37A(+82+105)	UAC UAA UUU CCU GCA GUG GUC ACC
149	H37A(+134+157)	UUC UGU GUG AAA UGG CUG CAA AUC
150	H38A(-01+19)	CCU UCA AAG GAA UGG AGG CC
151	H38A(+59+83)	UGC UGA AUU UCA GCC UCC AGU GGU U
152	H38A(+88+112)	UGA AGU CUU CCU CUU UCA GAU UCA C
153	H39A(+62+85)	CUG GCU UUC UCU CAU CUG UGA UUC
154	H39A(+39+58)	GUU GUA AGU UGU CUC CUC UU
155	H39A(+102+121)	UUG UCU GUA ACA GCU GCU GU
156	H39D(+10-10)	GCU CUA AUA CCU UGA GAG CA
157	H40A(-05+17)	CUU UGA GAC CUC AAA UCC UGU U
158	H40A(+129+153)	CUU UAU UUU CCU UUC AUC UCU GGG C
159	H42A(-04+23)	AUC GUU UCU UCA CGG ACA GUG UGC UGG
160	H42A(+86+109)	GGG CUU GUG AGA CAU GAG UGA UUU
161	H42D(+19-02)	A CCU UCA GAG GAC UCC UCU UGC
162	H43D(+10-15)	UAU GUG UUA CCU ACC CUU GUC GGU C
163	H43A(+101+120)	GGA GAG AGC UUC CUG UAG CU
164	H43A(+78+100)	UCA CCC UUU CCA CAG GCG UUG CA
165	H44A(+85+104)	UUU GUG UCU UUC UGA GAA AC
166	H44D(+10-10)	AAA GAC UUA CCU UAA GAU AC

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
167	H44A(-06+14)	AUC UGU CAA AUC GCC UGC AG
168	H46D(+16-04)	UUA CCU UGA CUU GCU CAA GC
169	H46A(+90+109)	UCC AGG UUC AAG UGG GAU AC
170	H47A(+76+100)	GCU CUU CUG GGC UUA UGG GAG CAC U
171	H47D(+25-02)	ACC UUU AUC CAC UGG AGA UUU GUC UGC
172	H47A(-9+12)	UUC CAC CAG UAA CUG AAA CAG
173	H50A(+02+30)	CCA CUC AGA GCU CAG AUC UUC UAA CUU CC
174	H50A(+07+33)	CUU CCA CUC AGA GCU CAG AUC UUC UAA
175	H50D(+07-18)	GGG AUC CAG UAU ACU UAC AGG CUC C
176	H51A(-01+25)	ACC AGA GUA ACA GUC UGA GUA GGA GC
177	H51D(+16-07)	CUC AUA CCU UCU GCU UGA UGA UC
178	H51A(+111 +134)	UUC UGU CCA AGC CCG GUU GAA AUC
179	H51A(+61+90)	ACA UCA AGG AAG AUG GCA UUU CUA GUU UGG
180	H51A(+66+90)	ACA UCA AGG AAG AUG GCA UUU CUA G
181	H51A(+66+95)	CUC CAA CAU CAA GGA AGA UGG CAU UUC UAG
182	H51D(+08-17)	AUC AUU UUU UCU CAU ACC UUC UGC U
183	H51A/D(+08-17) & (-15+)	AUC AUU UUU UCU CAU ACC UUC UGC UAG GAG CUA AAA
184	H51A(+175+195)	CAC CCA CCA UCA CCC UCU GUG
185	H51A(+199+220)	AUC AUC UCG UUG AUA UCC UCA A
186	H52A(-07+14)	UCC UGC AUU GUU GCC UGU AAG
187	H52A(+12+41)	UCC AAC UGG GGA CGC CUC UGU UCC AAA UCC
188	H52A(+17+37)	ACU GGG GAC GCC UCU GUU CCA
189	H52A(+93+112)	CCG UAA UGA UUG UUC UAG CC
190	H52D(+05-15)	UGU UAA AAA ACU UAC UUC GA
191	H53A(+45+69)	CAU UCA ACU GUU GCC UCC GGU UCU G
192	H53A(+39+62)	CUG UUG CCU CCG GUU CUG AAG GUG
193	H53A(+39+69)	CAU UCA ACU GUU GCC UCC GGU UCU GAA GGU G
194	H53D(+14-07)	UAC UAA CCU UGG UUU CUG UGA
195	H53A(+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
196	H53A(+150+176)	UGU AUA GGG ACC CUC CUU CCA UGA CUC
197	H53D(+20-05)	CUA ACC UUG GUU UCU GUG AUU UUC U
198	H53D(+09-18)	GGU AUC UUU GAU ACU AAC CUU GGU UUC
199	H53A(-12+10)	AUU CUU UCA ACU AGA AUA AAA G
200	H53A(-07+18)	GAU UCU GAA UUC UUU CAA CUA GAA U
201	H53A(+07+26)	AUC CCA CUG AUU CUG AAU UC
202	H53A(+124+145)	UUG GCU CUG GCC UGU CCU AAG A
203	H46A(+86+115)	CUC UUU UCC AGG UUC AAG UGG GAU ACU

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5' - 3')
		AGC
204	H46A(+107+137)	CAA GCU UUU CUU UUA GUU GCU GCU CUU UUC C
205	H46A(-10+20)	UAU UCU UUU GUU CUU CUA GCC UGG AGA AAG
206	H46A(+50+77)	CUG CUU CCU CCA ACC AUA AAA CAA AUU C
207	H45A(-06+20)	CCA AUG CCA UCC UGG AGU UCC UGU AA
208	H45A(+91 +110)	UCC UGU AGA AUA CUG GCA UC
209	H45A(+125+151)	UGC AGA CCU CCU GCC ACC GCA GAU UCA
210	H45D(+16 -04)	CUA CCU CUU UUU UCU GUC UG
211	H45A(+71+90)	UGU UUU UGA GGA UUG CUG AA

TABLE 4 (Continued)

Description	Sequence	SEQ ID NO
H53A(+33+60)	GTTGCCTCCGGTTCTGAAGGTGTTCTTG	1
H53A(+23+47)	CTGAAGGTGTTCTTGTACTTCATCC	6
H53A(+33+62)	CTGTTGCCTCCGGTTCTGAAGGTGTTCTTG	7
H53A(+33+65)	CAACTGTTGCCTCCGGTTCTGAAGGTGTTCTTG	8
H53A(+31+55)	CTCCGGTTCTGAAGGTGTTCTTGTA	9
H53A(+46+73)	ATTTCATTCAACTGTTGCCTCCGGTTCT	10
H53A(+22+46)	TGAAGGTGTTCTTGTACTTCATCCC	11
H53A(+46+69)	CATTCAACTGTTGCCTCCGGTTCT	12
H53A(+40+61)	TGTTGCCTCCGGTTCTGAAGGT	13
Description	Sequence	SEQ ID NO
H53A(+30+60)	GTTGCCTCCGGTTCTGAAGGTGTTC	14
H53A(+30+57)	GCCTCCGGTTCTGAAGGTGTTCTTGTAC	15
H53A(+30+56)	CCTCCGGTTCTGAAGGTGTTCTTGTAC	16
H53A(+30+55)	CTCCGGTTCTGAAGGTGTTCTTGTAC	17
H53A(+33+57)	GCCTCCGGTTCTGAAGGTGTTCTTG	18

TABLE 4 (Continued)

Description	Sequence	SEQ ID NO
H44A(-07+17)	CAGATCTGTCAAATCGCCTGCAGG	1
H44A(-07+20)	CAACAGATCTGTCAAATCGCCTGCAGG	2
H44A(-07+22)	CTCAACAGATCTGTCAAATCGCCTGCAGG	3
H44A(+77+101)	GTGTCTTTCTGAGAAACTGTTCAGC	4
H44A(+64+91)	GAGAAACTGTTCAGCTTCTGTTAGCCAC	5
H44A(+62+89)	GAAACTGTTCAGCTTCTGTTAGCCACTG	6
H44A(+62+85)	CTGTTCAGCTTCTGTTAGCCACTG	7
H44A(-06+14)	ATCTGTCAAATCGCCTGCAG	8
H44A(+85+104)	TTTGTGTCTTTCTGAGAAAC	9
H44A(+61+84)	TGTTCAGCTTCTGTTAGCCACTGA	10
H44A(-10+15)	GATCTGTCAAATCGCCTGCAGGTAA	11
H44A(+64+88)	AAACTGTTCAGCTTCTGTTAGCCAC	12
H44A(+79+103)	TTGTGTCTTTCTGAGAAACTGTTCA	13
H44A(-06+20)	CAACAGATCTGTCAAATCGCCTGCAG	14
H44A(-09+17)	CAGATCTGTCAAATCGCCTGCAGGTA	15
H44A(+59+85)	CTGTTCAGCTTCTGTTAGCCACTGATT	16
H44A(+59+89)	GAAACTGTTCAGCTTCTGTTAGCCACTGATT	17
H44A(+65+90)	AGAAACTGTTCAGCTTCTGTTAGCCA	18

TABLE 4 (Continued)

Name	Sequences	SEQ ID NO.
Oligomer Targeting Sequences (5' to 3'):		
Hu.DMD.Exon44.25.001	CTGCAGGTAAAAGCATATGGATCAA	1
Hu.DMD.Exon44.25.002	ATCGCCTGCAGGTAAAAGCATATGG	2
Hu.DMD.Exon44.25.003	GTCAAATCGCCTGCAGGTAAAAGCA	3
Hu.DMD.Exon44.25.004	GATCTGTCAAATCGCCTGCAGGTAA	4
Hu.DMD.Exon44.25.005	CAACAGATCTGTCAAATCGCCTGCA	5
Hu.DMD.Exon44.25.006	TTTCTCAACAGATCTGTCAAATCGC	6
Hu.DMD.Exon44.25.007	CCATTTCTCAACAGATCTGTCAAAT	7
Hu.DMD.Exon44.25.008	ATAATGAAAACGCCGCCATTTCTCA	8
Hu.DMD.Exon44.25.009	AAATATCTTTATATCATAATGAAAA	9
Hu.DMD.Exon44.25.010	TGTTAGCCACTGATTAAATATCTTT	10
Hu.DMD.Exon44.25.011	AAACTGTTCAGCTTCTGTTAGCCAC	11
Hu.DMD.Exon44.25.012	TTGTGTCTTTCTGAGAAACTGTTCA	12
Hu.DMD.Exon44.25.013	CCAATTCTCAGGAATTTGTGTCTTT	13
Hu.DMD.Exon44.25.014	GTATTTAGCATGTTCCCAATTCTCA	14
Hu.DMD.Exon44.25.015	CTTAAGATACCATTTGTATTTAGCA	15
Hu.DMD.Exon44.25.016	CTTACCTTAAGATACCATTTGTATT	16
Hu.DMD.Exon44.25.017	AAAGACTTACCTTAAGATACCATTT	17
Hu.DMD.Exon44.25.018	AAATCAAAGACTTACCTTAAGATAC	18
Hu.DMD.Exon44.25.019	AAAACAAATCAAAGACTTACCTTAA	19
Hu.DMD.Exon44.25.020	TCGAAAAAACAAATCAAAGACTTAC	20

TABLE 4 (Continued)

Hu.DMD.Exon45.25.001	CTGTAAGATACCAAAAAGGCAAAAC	21
Hu.DMD.Exon45.25.002	CCTGTAAGATACCAAAAAGGCAAAA	22
Hu.DMD.Exon45.25.002.2	AGTTCCTGTAAGATACCAAAAAGGC	23
Hu.DMD.Exon45.25.003	GAGTTCCTGTAAGATACCAAAAAGG	24
Hu.DMD.Exon45.25.003.2	CCTGGAGTTCCTGTAAGATACCAAA	25
Hu.DMD.Exon45.25.004	TCCTGGAGTTCCTGTAAGATACCAA	26
Hu.DMD.Exon45.25.004.2	GCCATCCTGGAGTTCCTGTAAGATA	27
Hu.DMD.Exon45.25.005	TGCCATCCTGGAGTTCCTGTAAGAT	28
Hu.DMD.Exon45.25.005.2	CCAATGCCATCCTGGAGTTCCTGTA	29
Hu.DMD.Exon45.25.006	CCCAATGCCATCCTGGAGTTCCTGT	30
Hu.DMD.Exon45.25.006.2	GCTGCCCAATGCCATCCTGGAGTTC	31
Hu.DMD.Exon45.25.007	CGCTGCCCAATGCCATCCTGGAGTT	32
Hu.DMD.Exon45.25.008	AACAGTTTGCCGCTGCCCAATGCCA	33
Hu.DMD.Exon45.25.008.2	CTGACAACAGTTTGCCGCTGCCCAA	34
Hu.DMD.Exon45.25.009	GTTGCATTCAATGTTCTGACAACAG	35
Hu.DMD.Exon45.25.010	GCTGAATTATTTCTTCCCCAGTTGC	36
Hu.DMD.Exon45.25.010.2	ATTATTTCTTCCCCAGTTGCATTCA	37
Hu.DMD.Exon45.25.011	GGCATCTGTTTTTGAGGATTGCTGA	38
Hu.DMD.Exon45.25.011.2	TTTGAGGATTGCTGAATTATTTCTT	39
Hu.DMD.Exon45.25.012	AATTTTTCCTGTAGAATACTGGCAT	40
Hu.DMD.Exon45.25.012.	ATACTGGCATCTGTTTTTGAGGATT	41

TABLE 4 (Continued)

2		
Hu.DMD.Exon45.25.013	ACCGCAGATTCAGGCTTCCCAATTT	42
Hu.DMD.Exon45.25.013.2	AATTTTTCCTGTAGAATACTGGCAT	43
Hu.DMD.Exon45.25.014	CTGTTTGCAGACCTCCTGCCACCGC	44
Hu.DMD.Exon45.25.014.2	AGATTCAGGCTTCCCAATTTTTCCT	45
Hu.DMD.Exon45.25.015	CTCTTTTTTCTGTCTGACAGCTGTT	46
Hu.DMD.Exon45.25.015.2	ACCTCCTGCCACCGCAGATTCAGGC	47
Hu.DMD.Exon45.25.016	CCTACCTCTTTTTTCTGTCTGACAG	48
Hu.DMD.Exon45.25.016.2	GACAGCTGTTTGCAGACCTCCTGCC	49
Hu.DMD.Exon45.25.017	GTCGCCCTACCTCTTTTTTCTGTCT	50
Hu.DMD.Exon45.25.018	GATCTGTCGCCCTACCTCTTTTTTC	51
Hu.DMD.Exon45.25.019	TATTAGATCTGTCGCCCTACCTCTT	52
Hu.DMD.Exon45.25.020	ATTCCTATTAGATCTGTCGCCCTAC	53
Hu.DMD.Exon45.20.001	AGATACCAAAAAGGCAAAAC	54
Hu.DMD.Exon45.20.002	AAGATACCAAAAAGGCAAAA	55
Hu.DMD.Exon45.20.003	CCTGTAAGATACCAAAAAGG	56
Hu.DMD.Exon45.20.004	GAGTTCCTGTAAGATACCAA	57
Hu.DMD.Exon45.20.005	TCCTGGAGTTCCTGTAAGAT	58
Hu.DMD.Exon45.20.006	TGCCATCCTGGAGTTCCTGT	59
Hu.DMD.Exon45.20.007	CCCAATGCCATCCTGGAGTT	60
Hu.DMD.Exon45.20.008	CGCTGCCCAATGCCATCCTG	61
Hu.DMD.Exon45.20.009	CTGACAACAGTTTGCCGCTG	62
Hu.DMD.Exon45.20.010	GTTGCATTCAATGTTCTGAC	63
Hu.DMD.Exon45.20.011	ATTATTTCTTCCCCAGTTGC	64
Hu.DMD.Exon45.20.012	TTTGAGGATTGCTGAATTAT	65

TABLE 4 (Continued)

Hu.DMD.Exon45.20.013	ATACTGGCATCTGTTTTTGA	66
Hu.DMD.Exon45.20.014	AATTTTTCCTGTAGAATACT	67
Hu.DMD.Exon45.20.015	AGATTCAGGCTTCCCAATTT	68
Hu.DMD.Exon45.20.016	ACCTCCTGCCACCGCAGATT	69
Hu.DMD.Exon45.20.017	GACAGCTGTTTGCAGACCTC	70
Hu.DMD.Exon45.20.018	CTCTTTTTTCTGTCTGACAG	71
Hu.DMD.Exon45.20.019	CCTACCTCTTTTTTCTGTCT	72
Hu.DMD.Exon45.20.020	GTCGCCCTACCTCTTTTTTC	73
Hu.DMD.Exon45.20.021	GATCTGTCGCCCTACCTCTT	74
Hu.DMD.Exon45.20.022	TATTAGATCTGTCGCCCTAC	75
Hu.DMD.Exon45.20.023	ATTCCTATTAGATCTGTCGC	76
Hu.DMD.Exon46.25.001	GGGGGATTTGAGAAAATAAAATTAC	77
Hu.DMD.Exon46.25.002	ATTTGAGAAAATAAAATTACCTTGA	78
Hu.DMD.Exon46.25.002.2	CTAGCCTGGAGAAAGAAGAATAAAA	79
Hu.DMD.Exon46.25.003	AGAAAATAAAATTACCTTGACTTGC	80
Hu.DMD.Exon46.25.003.2	TTCTTCTAGCCTGGAGAAAGAAGAA	81
Hu.DMD.Exon46.25.004	ATAAAATTACCTTGACTTGCTCAAG	82
Hu.DMD.Exon46.25.004.2	TTTTGTTCTTCTAGCCTGGAGAAAG	83
Hu.DMD.Exon46.25.005	ATTACCTTGACTTGCTCAAGCTTTT	84
Hu.DMD.Exon46.25.005.2	TATTCTTTTGTTCTTCTAGCCTGGA	85
Hu.DMD.Exon46.25.006	CTTGACTTGCTCAAGCTTTTCTTTT	86
Hu.DMD.Exon46.25.006.2	CAAGATATTCTTTTGTTCTTCTAGC	87
Hu.DMD.Exon46.25.007	CTTTTAGTTGCTGCTCTTTTCCAGG	88
Hu.DMD.Exon46.25.008	CCAGGTTCAAGTGGGATACTAGCAA	89

TABLE 4 (Continued)

Hu.DMD.Exon46.25.008.2	ATCTCTTTGAAATTCTGACAAGATA	90
Hu.DMD.Exon46.25.009	AGCAATGTTATCTGCTTCCTCCAAC	91
Hu.DMD.Exon46.25.009.2	AACAAATTCATTTAAATCTCTTTGA	92
Hu.DMD.Exon46.25.010	CCAACCATAAAACAAATTCATTTAA	93
Hu.DMD.Exon46.25.010.2	TTCCTCCAACCATAAAACAAATTCA	94
Hu.DMD.Exon46.25.011	TTTAAATCTCTTTGAAATTCTGACA	95
Hu.DMD.Exon46.25.012	TGACAAGATATTCTTTTGTTCTTCT	96
Hu.DMD.Exon46.25.012.2	TTCAAGTGGGATACTAGCAATGTTA	97
Hu.DMD.Exon46.25.013	AGATATTCTTTTGTTCTTCTAGCCT	98
Hu.DMD.Exon46.25.013.2	CTGCTCTTTTCCAGGTTCAAGTGGG	99
Hu.DMD.Exon46.25.014	TTCTTTTGTTCTTCTAGCCTGGAGA	100
Hu.DMD.Exon46.25.014.2	CTTTTCTTTTAGTTGCTGCTCTTTT	101
Hu.DMD.Exon46.25.015	TTGTTCTTCTAGCCTGGAGAAAGAA	102
Hu.DMD.Exon46.25.016	CTTCTAGCCTGGAGAAAGAAGAATA	103
Hu.DMD.Exon46.25.017	AGCCTGGAGAAAGAAGAATAAAATT	104
Hu.DMD.Exon46.25.018	CTGGAGAAAGAAGAATAAAATTGTT	105
Hu.DMD.Exon46.20.001	GAAAGAAGAATAAAATTGTT	106
Hu.DMD.Exon46.20.002	GGAGAAAGAAGAATAAAATT	107
Hu.DMD.Exon46.20.003	AGCCTGGAGAAAGAAGAATA	108
Hu.DMD.Exon46.20.004	CTTCTAGCCTGGAGAAAGAA	109
Hu.DMD.Exon46.20.005	TTGTTCTTCTAGCCTGGAGA	110
Hu.DMD.Exon46.20.006	TTCTTTTGTTCTTCTAGCCT	111
Hu.DMD.Exon46.20.007	TGACAAGATATTCTTTTGTT	112

TABLE 4 (Continued)

Hu.DMD.Exon46.20.008	ATCTCTTTGAAATTCTGACA	113
Hu.DMD.Exon46.20.009	AACAAATTCATTTAAATCTC	114
Hu.DMD.Exon46.20.010	TTCCTCCAACCATAAAACAA	115
Hu.DMD.Exon46.20.011	AGCAATGTTATCTGCTTCCT	116
Hu.DMD.Exon46.20.012	TTCAAGTGGGATACTAGCAA	117
Hu.DMD.Exon46.20.013	CTGCTCTTTTCCAGGTTCAA	118
Hu.DMD.Exon46.20.014	CTTTTCTTTTAGTTGCTGCT	119
Hu.DMD.Exon46.20.015	CTTGACTTGCTCAAGCTTTT	120
Hu.DMD.Exon46.20.016	ATTACCTTGACTTGCTCAAG	121
Hu.DMD.Exon46.20.017	ATAAAATTACCTTGACTTGC	122
Hu.DMD.Exon46.20.018	AGAAAATAAAATTACCTTGA	123
Hu.DMD.Exon46.20.019	ATTTGAGAAAATAAAATTAC	124
Hu.DMD.Exon46.20.020	GGGGGATTTGAGAAAATAAA	125
Hu.DMD.Exon47.25.001	CTGAAACAGACAAATGCAACAACGT	126
Hu.DMD.Exon47.25.002	AGTAACTGAAACAGACAAATGCAAC	127
Hu.DMD.Exon47.25.003	CCACCAGTAACTGAAACAGACAAAT	128
Hu.DMD.Exon47.25.004	CTCTTCCACCAGTAACTGAAACAGA	129
Hu.DMD.Exon47.25.005	GGCAACTCTTCCACCAGTAACTGAA	130
Hu.DMD.Exon47.25.006	GCAGGGGCAACTCTTCCACCAGTAA	131
Hu.DMD.Exon47.25.007	CTGGCGCAGGGGCAACTCTTCCACC	132
Hu.DMD.Exon47.25.008	TTTAATTGTTTGAGAATTCCCTGGC	133
Hu.DMD.Exon47.25.008.2	TTGTTTGAGAATTCCCTGGCGCAGG	134
Hu.DMD.Exon47.25.009	GCACGGGTCCTCCAGTTTCATTTAA	135
Hu.DMD.Exon47.25.009.2	TCCAGTTTCATTTAATTGTTTGAGA	136
Hu.DMD.Exon47.25.010	GCTTATGGGAGCACTTACAAGCACG	137
Hu.DMD.Exon47.25.010.2	TACAAGCACGGGTCCTCCAGTTTCA	138

TABLE 4 (Continued)

Hu.DMD.Exon47.25.011	AGTTTATCTTGCTCTTCTGGGCTTA	139
Hu.DMD.Exon47.25.012	TCTGCTTGAGCTTATTTTCAAGTTT	140
Hu.DMD.Exon47.25.012.2	ATCTTGCTCTTCTGGGCTTATGGGA	141
Hu.DMD.Exon47.25.013	CTTTATCCACTGGAGATTTGTCTGC	142
Hu.DMD.Exon47.25.013.2	CTTATTTTCAAGTTTATCTTGCTCT	143
Hu.DMD.Exon47.25.014	CTAACCTTTATCCACTGGAGATTTG	144
Hu.DMD.Exon47.25.014.2	ATTTGTCTGCTTGAGCTTATTTTCA	145
Hu.DMD.Exon47.25.015	AATGTCTAACCTTTATCCACTGGAG	146
Hu.DMD.Exon47.25.016	TGGTTAATGTCTAACCTTTATCCAC	147
Hu.DMD.Exon47.25.017	AGAGATGGTTAATGTCTAACCTTTA	148
Hu.DMD.Exon47.25.018	ACGGAAGAGATGGTTAATGTCTAAC	149
Hu.DMD.Exon47.20.001	ACAGACAAATGCAACAACGT	150
Hu.DMD.Exon47.20.002	CTGAAACAGACAAATGCAAC	151
Hu.DMD.Exon47.20.003	AGTAACTGAAACAGACAAAT	152
Hu.DMD.Exon47.20.004	CCACCAGTAACTGAAACAGA	153
Hu.DMD.Exon47.20.005	CTCTTCCACCAGTAACTGAA	154
Hu.DMD.Exon47.20.006	GGCAACTCTTCCACCAGTAA	155
Hu.DMD.Exon47.20.007	CTGGCGCAGGGGCAACTCTT	156
Hu.DMD.Exon47.20.008	TTGTTTGAGAATTCCCTGGC	157
Hu.DMD.Exon47.20.009	TCCAGTTTCATTTAATTGTT	158
Hu.DMD.Exon47.20.010	TACAAGCACGGGTCCTCCAG	159
Hu.DMD.Exon47.20.011	GCTTATGGGAGCACTTACAA	160
Hu.DMD.Exon47.20.012	ATCTTGCTCTTCTGGGCTTA	161
Hu.DMD.Exon47.20.013	CTTATTTTCAAGTTTATCTT	162
Hu.DMD.Exon47.20.014	ATTTGTCTGCTTGAGCTTAT	163
Hu.DMD.Exon47.20.015	CTTTATCCACTGGAGATTTG	164

TABLE 4 (Continued)

Hu.DMD.Exon47.20.016	CTAACCTTTATCCACTGGAG	165
Hu.DMD.Exon47.20.017	AATGTCTAACCTTTATCCAC	166
Hu.DMD.Exon47.20.018	TGGTTAATGTCTAACCTTTA	167
Hu.DMD.Exon47.20.019	AGAGATGGTTAATGTCTAAC	168
Hu.DMD.Exon47.20.020	ACGGAAGAGATGGTTAATGT	169
Hu.DMD.Exon48.25.001	CTGAAAGGAAAATACATTTTAAAAA	170
Hu.DMD.Exon48.25.002	CCTGAAAGGAAAATACATTTTAAAA	171
Hu.DMD.Exon48.25.002.2	GAAACCTGAAAGGAAAATACATTTT	172
Hu.DMD.Exon48.25.003	GGAAACCTGAAAGGAAAATACATTT	173
Hu.DMD.Exon48.25.003.2	CTCTGGAAACCTGAAAGGAAAATAC	174
Hu.DMD.Exon48.25.004	GCTCTGGAAACCTGAAAGGAAAATA	175
Hu.DMD.Exon48.25.004.2	TAAAGCTCTGGAAACCTGAAAGGAA	634
Hu.DMD.Exon48.25.005	GTAAAGCTCTGGAAACCTGAAAGGA	176
Hu.DMD.Exon48.25.005.2	TCAGGTAAAGCTCTGGAAACCTGAA	177
Hu.DMD.Exon48.25.006	CTCAGGTAAAGCTCTGGAAACCTGA	178
Hu.DMD.Exon48.25.006.2	GTTTCTCAGGTAAAGCTCTGGAAAC	179
Hu.DMD.Exon48.25.007	TGTTTCTCAGGTAAAGCTCTGGAAA	180
Hu.DMD.Exon48.25.007.2	AATTTCTCCTTGTTTCTCAGGTAAA	181
Hu.DMD.Exon48.25.008	TTTGAGCTTCAATTTCTCCTTGTTT	182
Hu.DMD.Exon48.25.008	TTTTATTTGAGCTTCAATTTCTCCT	183
Hu.DMD.Exon48.25.009	AAGCTGCCCAAGGTCTTTTATTTGA	184
Hu.DMD.Exon48.25.010	AGGTCTTCAAGCTTTTTTTCAAGCT	185
Hu.DMD.Exon48.25.010.	TTCAAGCTTTTTTTCAAGCTGCCCA	186

TABLE 4 (Continued)

2		
Hu.DMD.Exon48.25.011	GATGATTTAACTGCTCTTCAAGGTC	187
Hu.DMD.Exon48.25.011.2	CTGCTCTTCAAGGTCTTCAAGCTTT	188
Hu.DMD.Exon48.25.012	AGGAGATAACCACAGCAGCAGATGA	189
Hu.DMD.Exon48.25.012.2	CAGCAGATGATTTAACTGCTCTTCA	190
Hu.DMD.Exon48.25.013	ATTTCCAACTGATTCCTAATAGGAG	191
Hu.DMD.Exon48.25.014	CTTGGTTTGGTTGGTTATAAATTTC	192
Hu.DMD.Exon48.25.014.2	CAACTGATTCCTAATAGGAGATAAC	193
Hu.DMD.Exon48.25.015	CTTAACGTCAAATGGTCCTTCTTGG	194
Hu.DMD.Exon48.25.015.2	TTGGTTATAAATTTCCAACTGATTC	195
Hu.DMD.Exon48.25.016	CCTACCTTAACGTCAAATGGTCCTT	196
Hu.DMD.Exon48.25.016.2	TCCTTCTTGGTTTGGTTGGTTATAA	197
Hu.DMD.Exon48.25.017	AGTTCCCTACCTTAACGTCAAATGG	198
Hu.DMD.Exon48.25.018	CAAAAAGTTCCCTACCTTAACGTCA	199
Hu.DMD.Exon48.25.019	TAAAGCAAAAAGTTCCCTACCTTAA	200
Hu.DMD.Exon48.25.020	ATATTTAAAGCAAAAAGTTCCCTAC	201
Hu.DMD.Exon48.20.001	AGGAAAATACATTTTAAAAA	202
Hu.DMD.Exon48.20.002	AAGGAAAATACATTTTAAAA	203
Hu.DMD.Exon48.20.003	CCTGAAAGGAAAATACATTT	204
Hu.DMD.Exon48.20.004	GGAAACCTGAAAGGAAAATA	205
Hu.DMD.Exon48.20.005	GCTCTGGAAACCTGAAAGGA	206
Hu.DMD.Exon48.20.006	GTAAAGCTCTGGAAACCTGA	207
Hu.DMD.Exon48.20.007	CTCAGGTAAAGCTCTGGAAA	208
Hu.DMD.Exon48.20.008	AATTTCTCCTTGTTTCTCAG	209

TABLE 4 (Continued)

Hu.DMD.Exon48.20.009	TTTTATTTGAGCTTCAATTT	210
Hu.DMD.Exon48.20.010	AAGCTGCCCAAGGTCTTTTA	211
Hu.DMD.Exon48.20.011	TTCAAGCTTTTTTTCAAGCT	212
Hu.DMD.Exon48.20.012	CTGCTCTTCAAGGTCTTCAA	213
Hu.DMD.Exon48.20.013	CAGCAGATGATTTAACTGCT	214
Hu.DMD.Exon48.20.014	AGGAGATAACCACAGCAGCA	215
Hu.DMD.Exon48.20.015	CAACTGATTCCTAATAGGAG	216
Hu.DMD.Exon48.20.016	TTGGTTATAAATTTCCAACT	217
Hu.DMD.Exon48.20.017	TCCTTCTTGGTTTGGTTGGT	218
Hu.DMD.Exon48.20.018	CTTAACGTCAAATGGTCCTT	219
Hu.DMD.Exon48.20.019	CCTACCTTAACGTCAAATGG	220
Hu.DMD.Exon48.20.020	AGTTCCCTACCTTAACGTCA	221
Hu.DMD.Exon48.20.021	CAAAAAGTTCCCTACCTTAA	222
Hu.DMD.Exon48.20.022	TAAAGCAAAAAGTTCCCTAC	223
Hu.DMD.Exon48.20.023	ATATTTAAAGCAAAAAGTTC	224
Hu.DMD.Exon49.25.001	CTGGGGAAAAGAACCCATATAGTGC	225
Hu.DMD.Exon49.25.002	TCCTGGGGAAAAGAACCCATATAGT	226
Hu.DMD.Exon49.25.002.2	GTTTCCTGGGGAAAAGAACCCATAT	227
Hu.DMD.Exon49.25.003	CAGTTTCCTGGGGAAAAGAACCCAT	228
Hu.DMD.Exon49.25.003.2	TTTCAGTTTCCTGGGGAAAAGAACC	229
Hu.DMD.Exon49.25.004	TATTTCAGTTTCCTGGGGAAAAGAA	230
Hu.DMD.Exon49.25.004.2	TGCTATTTCAGTTTCCTGGGGAAAA	231
Hu.DMD.Exon49.25.005	ACTGCTATTTCAGTTTCCTGGGGAA	232
Hu.DMD.Exon49.25.005.2	TGAACTGCTATTTCAGTTTCCTGGG	233
Hu.DMD.Exon49.25.006	CTTGAACTGCTATTTCAGTTTCCTG	234

TABLE 4 (Continued)

Hu.DMD.Exon49.25.006.2	TAGCTTGAACTGCTATTTCAGTTTC	235
Hu.DMD.Exon49.25.007	TTTAGCTTGAACTGCTATTTCAGTT	236
Hu.DMD.Exon49.25.008	TTCCACATCCGGTTGTTTAGCTTGA	237
Hu.DMD.Exon49.25.009	TGCCCTTTAGACAAAATCTCTTCCA	238
Hu.DMD.Exon49.25.009.2	TTTAGACAAAATCTCTTCCACATCC	239
Hu.DMD.Exon49.25.010	GTTTTTCCTTGTACAAATGCTGCCC	240
Hu.DMD.Exon49.25.010.2	GTACAAATGCTGCCCTTTAGACAAA	241
Hu.DMD.Exon49.25.011	CTTCACTGGCTGAGTGGCTGGTTTT	242
Hu.DMD.Exon49.25.011.2	GGCTGGTTTTTCCTTGTACAAATGC	243
Hu.DMD.Exon49.25.012	ATTACCTTCACTGGCTGAGTGGCTG	244
Hu.DMD.Exon49.25.013	GCTTCATTACCTTCACTGGCTGAGT	245
Hu.DMD.Exon49.25.014	AGGTTGCTTCATTACCTTCACTGGC	246
Hu.DMD.Exon49.25.015	GCTAGAGGTTGCTTCATTACCTTCA	247
Hu.DMD.Exon49.25.016	ATATTGCTAGAGGTTGCTTCATTAC	248
Hu.DMD.Exon49.20.001	GAAAAGAACCCATATAGTGC	249
Hu.DMD.Exon49.20.002	GGGAAAAGAACCCATATAGT	250
Hu.DMD.Exon49.20.003	TCCTGGGGAAAAGAACCCAT	251
Hu.DMD.Exon49.20.004	CAGTTTCCTGGGGAAAAGAA	252
Hu.DMD.Exon49.20.005	TATTTCAGTTTCCTGGGGAA	253
Hu.DMD.Exon49.20.006	ACTGCTATTTCAGTTTCCTG	254
Hu.DMD.Exon49.20.007	CTTGAACTGCTATTTCAGTT	255
Hu.DMD.Exon49.20.008	TTTAGCTTGAACTGCTATTT	256
Hu.DMD.Exon49.20.009	TTCCACATCCGGTTGTTTAG	257
Hu.DMD.Exon49.20.010	TTTAGACAAAATCTCTTCCA	258
Hu.DMD.Exon49.20.011	GTACAAATGCTGCCCTTTAG	259

TABLE 4 (Continued)

Hu.DMD.Exon49.20.012	GGCTGGTTTTTCCTTGTACA	260
Hu.DMD.Exon49.20.013	CTTCACTGGCTGAGTGGCTG	261
Hu.DMD.Exon49.20.014	ATTACCTTCACTGGCTGAGT	262
Hu.DMD.Exon49.20.015	GCTTCATTACCTTCACTGGC	263
Hu.DMD.Exon49.20.016	AGGTTGCTTCATTACCTTCA	264
Hu.DMD.Exon49.20.017	GCTAGAGGTTGCTTCATTAC	265
Hu.DMD.Exon49.20.018	ATATTGCTAGAGGTTGCTTC	266
Hu.DMD.Exon50.25.001	CTTTAACAGAAAAGCATACACATTA	267
Hu.DMD.Exon50.25.002	TCCTCTTTAACAGAAAAGCATACAC	268
Hu.DMD.Exon50.25.002.2	TTCCTCTTTAACAGAAAAGCATACA	269
Hu.DMD.Exon50.25.003	TAACTTCCTCTTTAACAGAAAAGCA	270
Hu.DMD.Exon50.25.003.2	CTAACTTCCTCTTTAACAGAAAAGC	271
Hu.DMD.Exon50.25.004	TCTTCTAACTTCCTCTTTAACAGAA	272
Hu.DMD.Exon50.25.004.2	ATCTTCTAACTTCCTCTTTAACAGA	273
Hu.DMD.Exon50.25.005	TCAGATCTTCTAACTTCCTCTTTAA	274
Hu.DMD.Exon50.25.005.2	CTCAGATCTTCTAACTTCCTCTTTA	275
Hu.DMD.Exon50.25.006	AGAGCTCAGATCTTCTAACTTCCTC	276
Hu.DMD.Exon50.25.006.2 NG-08-0731	CAGAGCTCAGATCTTCTAACTTCCT	277
Hu.DMD.Exon50.25.007	CACTCAGAGCTCAGATCTTCTACT	278
Hu.DMD.Exon50.25.007.2	CCTTCCACTCAGAGCTCAGATCTTC	279
Hu.DMD.Exon50.25.008	GTAAACGGTTTACCGCCTTCCACTC	280
Hu.DMD.Exon50.25.009	CTTTGCCCTCAGCTCTTGAAGTAAA	281

TABLE 4 (Continued)

Hu.DMD.Exon50.25.009.2	CCCTCAGCTCTTGAAGTAAACGGTT	282
Hu.DMD.Exon50.25.010	CCAGGAGCTAGGTCAGGCTGCTTTG	283
Hu.DMD.Exon50.25.010.2	GGTCAGGCTGCTTTGCCCTCAGCTC	284
Hu.DMD.Exon50.25.011	AGGCTCCAATAGTGGTCAGTCCAGG	285
Hu.DMD.Exon50.25.011.2	TCAGTCCAGGAGCTAGGTCAGGCTG	286
Hu.DMD.Exon50.25.012 AVI-5038	CTTACAGGCTCCAATAGTGGTCAGT	287
Hu.DMD.Exon50.25.013	GTATACTTACAGGCTCCAATAGTGG	288
Hu.DMD.Exon50.25.014	ATCCAGTATACTTACAGGCTCCAAT	289
Hu.DMD.Exon50.25.015 NG-08-0741	ATGGGATCCAGTATACTTACAGGCT	290
Hu.DMD.Exon50.25.016 NG-08-0742	AGAGAATGGGATCCAGTATACTTAC	291
Hu.DMD.Exon50.20.001	ACAGAAAAGCATACACATTA	292
Hu.DMD.Exon50.20.002	TTTAACAGAAAAGCATACAC	293
Hu.DMD.Exon50.20.003	TCCTCTTTAACAGAAAAGCA	294
Hu.DMD.Exon50.20.004	TAACTTCCTCTTTAACAGAA	295
Hu.DMD.Exon50.20.005	TCTTCTAACTTCCTCTTTAA	296
Hu.DMD.Exon50.20.006	TCAGATCTTCTAACTTCCTC	297
Hu.DMD.Exon50.20.007	CCTTCCACTCAGAGCTCAGA	298
Hu.DMD.Exon50.20.008	GTAAACGGTTTACCGCCTTC	299
Hu.DMD.Exon50.20.009	CCCTCAGCTCTTGAAGTAAA	300
Hu.DMD.Exon50.20.010	GGTCAGGCTGCTTTGCCCTC	301
Hu.DMD.Exon50.20.011	TCAGTCCAGGAGCTAGGTCA	302
Hu.DMD.Exon50.20.012	AGGCTCCAATAGTGGTCAGT	303
Hu.DMD.Exon50.20.013	CTTACAGGCTCCAATAGTGG	304

TABLE 4 (Continued)

Hu.DMD.Exon50.20.014	GTATACTTACAGGCTCCAAT	305
Hu.DMD.Exon50.20.015	ATCCAGTATACTTACAGGCT	306
Hu.DMD.Exon50.20.016	ATGGGATCCAGTATACTTAC	307
Hu.DMD.Exon50.20.017	AGAGAATGGGATCCAGTATA	308
Hu.DMD.Exon51.25.001-44	CTAAAATATTTTGGGTTTTTGCAAAA	309
Hu.DMD.Exon51.25.002-45	GCTAAAATATTTTGGGTTTTTGCAAA	310
Hu.DMD.Exon51.25.002.2-46	TAGGAGCTAAAATATTTTGGGTTTTT	311
Hu.DMD.Exon51.25.003	AGTAGGAGCTAAAATATTTTGGGTT	312
Hu.DMD.Exon51.25.003.2	TGAGTAGGAGCTAAAATATTTTGGG	313
Hu.DMD.Exon51.25.004	CTGAGTAGGAGCTAAAATATTTTGGG	314
Hu.DMD.Exon51.25.004.2	CAGTCTGAGTAGGAGCTAAAATATT	315
Hu.DMD.Exon51.25.005	ACAGTCTGAGTAGGAGCTAAAATATT	316
Hu.DMD.Exon51.25.005.2	GAGTAACAGTCTGAGTAGGAGCTAAA	317
Hu.DMD.Exon51.25.006	CAGAGTAACAGTCTGAGTAGGAGCT	318
Hu.DMD.Exon51.25.006.2	CACCAGAGTAACAGTCTGAGTAGGAG	319
Hu.DMD.Exon51.25.007	GTCACCAGAGTAACAGTCTGAGTAG	320
Hu.DMD.Exon51.25.007.2	AACCACAGGTTGTGTCACCAGAGTAA	321
Hu.DMD.Exon51.25.008	GTTGTGTCACCAGAGTAACAGTCTG	322
Hu.DMD.Exon51.25.009	TGGCAGTTTCCTTAGTAACCACAGGT	323
Hu.DMD.Exon51.25.010	ATTTCTAGTTTGGAGATGGCAGTTTC	324
Hu.DMD.Exon51.25.010.	GGAAGATGGCATTTCTAGTTTGGAG	325

TABLE 4 (Continued)

2		
Hu.DMD.Exon51.25.011	CATCAAGGAAGATGGCATTTCTAGTT	326
Hu.DMD.Exon51.25.011.2	GAGCAGGTACCTCCAACATCAAGGAA	327
Hu.DMD.Exon51.25.012	ATCTGCCAGAGCAGGTACCTCCAAC	328
Hu.DMD.Exon51.25.013	AAGTTCTGTCCAAGCCCGGTTGAAAT	329
Hu.DMD.Exon51.25.013.2	CGGTTGAAATCTGCCAGAGCAGGTAC	330
Hu.DMD.Exon51.25.014	GAGAAAGCCAGTCGGTAAGTTCTGTC	331
Hu.DMD.Exon51.25.014.2	GTCGGTAAGTTCTGTCCAAGCCCGG	332
Hu.DMD.Exon51.25.015	ATAACTTGATCAAGCAGAGAAAGCCA	333
Hu.DMD.Exon51.25.015.2	AAGCAGAGAAAGCCAGTCGGTAAGT	334
Hu.DMD.Exon51.25.016	CACCCTCTGTGATTTTATAACTTGAT	335
Hu.DMD.Exon51.25.017	CAAGGTCACCCACCATCACCCTCTGT	336
Hu.DMD.Exon51.25.017.2	CATCACCCTCTGTGATTTTATAACT	337
Hu.DMD.Exon51.25.018	CTTCTGCTTGATGATCATCTCGTTGA	338
Hu.DMD.Exon51.25.019	CCTTCTGCTTGATGATCATCTCGTTG	339
Hu.DMD.Exon51.25.019.2	ATCTCGTTGATATCCTCAAGGTCACC	340
Hu.DMD.Exon51.25.020	TCATACCTTCTGCTTGATGATCATCT	341
Hu.DMD.Exon51.25.020.2	TCATTTTTTCTCATACCTTCTGCTTG	342
Hu.DMD.Exon51.25.021	TTTTCTCATACCTTCTGCTTGATGAT	343
Hu.DMD.Exon51.25.022	TTTTATCATTTTTTCTCATACCTTCT	344
Hu.DMD.Exon51.25.023	CCAACTTTTATCATTTTTTCTCATAC	345
Hu.DMD.Exon51.20.001	ATATTTTGGGTTTTTGCAAA	346

TABLE 4 (Continued)

Hu.DMD.Exon51.20.002	AAAATATTTTGGGTTTTTGC	347
Hu.DMD.Exon51.20.003	GAGCTAAAATATTTTGGGTT	348
Hu.DMD.Exon51.20.004	AGTAGGAGCTAAAATATTTT	349
Hu.DMD.Exon51.20.005	GTCTGAGTAGGAGCTAAAAT	350
Hu.DMD.Exon51.20.006	TAACAGTCTGAGTAGGAGCT	351
Hu.DMD.Exon51.20.007	CAGAGTAACAGTCTGAGTAG	352
Hu.DMD.Exon51.20.008	CACAGGTTGTGTCACCAGAG	353
Hu.DMD.Exon51.20.009	AGTTTCCTTAGTAACCACAG	354
Hu.DMD.Exon51.20.010	TAGTTTGGAGATGGCAGTTT	355
Hu.DMD.Exon51.20.011	GGAAGATGGCATTTCTAGTT	356
Hu.DMD.Exon51.20.012	TACCTCCAACATCAAGGAAG	357
Hu.DMD.Exon51.20.013	ATCTGCCAGAGCAGGTACCT	358
Hu.DMD.Exon51.20.014	CCAAGCCCGGTTGAAATCTG	359
Hu.DMD.Exon51.20.015	GTCGGTAAGTTCTGTCCAAG	360
Hu.DMD.Exon51.20.016	AAGCAGAGAAAGCCAGTCGG	361
Hu.DMD.Exon51.20.017	TTTTATAACTTGATCAAGCA	362
Hu.DMD.Exon51.20.018	CATCACCCTCTGTGATTTTA	363
Hu.DMD.Exon51.20.019	CTCAAGGTCACCCACCATCA	364
Hu.DMD.Exon51.20.020	CATCTCGTTGATATCCTCAA	365
Hu.DMD.Exon51.20.021	CTTCTGCTTGATGATCATCT	366
Hu.DMD.Exon51.20.022	CATACCTTCTGCTTGATGAT	367
Hu.DMD.Exon51.20.023	TTTCTCATACCTTCTGCTTG	368
Hu.DMD.Exon51.20.024	CATTTTTTCTCATACCTTCT	369
Hu.DMD.Exon51.20.025	TTTATCATTTTTTCTCATAC	370
Hu.DMD.Exon51.20.026	CAACTTTTATCATTTTTTCT	371
Hu.DMD.Exon52.25.001	CTGTAAGAACAAATATCCCTTAGTA	372
Hu.DMD.Exon52.25.002	TGCCTGTAAGAACAAATATCCCTTA	373
Hu.DMD.Exon52.25.002.2	GTTGCCTGTAAGAACAAATATCCCT	374

TABLE 4 (Continued)

Hu.DMD.Exon52.25.003	ATTGTTGCCTGTAAGAACAAATATC	375
Hu.DMD.Exon52.25.003.2	GCATTGTTGCCTGTAAGAACAAATA	376
Hu.DMD.Exon52.25.004	CCTGCATTGTTGCCTGTAAGAACAA	377
Hu.DMD.Exon52.25.004.2	ATCCTGCATTGTTGCCTGTAAGAAC	378
Hu.DMD.Exon52.25.005	CAAATCCTGCATTGTTGCCTGTAAG	379
Hu.DMD.Exon52.25.005.2	TCCAAATCCTGCATTGTTGCCTGTA	380
Hu.DMD.Exon52.25.006	TGTTCCAAATCCTGCATTGTTGCCT	381
Hu.DMD.Exon52.25.006.2	TCTGTTCCAAATCCTGCATTGTTGC	382
Hu.DMD.Exon52.25.007	AACTGGGGACGCCTCTGTTCCAAAT	383
Hu.DMD.Exon52.25.007.2	GCCTCTGTTCCAAATCCTGCATTGT	384
Hu.DMD.Exon52.25.008	CAGCGGTAATGAGTTCTTCCAACTG	385
Hu.DMD.Exon52.25.008.2	CTTCCAACTGGGGACGCCTCTGTTC	386
Hu.DMD.Exon52.25.009	CTTGTTTTTCAAATTTTGGGCAGCG	387
Hu.DMD.Exon52.25.010	CTAGCCTCTTGATTGCTGGTCTTGT	388
Hu.DMD.Exon52.25.010.2	TTTTCAAATTTTGGGCAGCGGTAAT	389
Hu.DMD.Exon52.25.011	TTCGATCCGTAATGATTGTTCTAGC	390
Hu.DMD.Exon52.25.011.2	GATTGCTGGTCTTGTTTTTCAAATT	391
Hu.DMD.Exon52.25.012	CTTACTTCGATCCGTAATGATTGTT	392
Hu.DMD.Exon52.25.012.2	TTGTTCTAGCCTCTTGATTGCTGGT	393
Hu.DMD.Exon52.25.013	AAAAACTTACTTCGATCCGTAATGA	394

TABLE 4 (Continued)

Hu.DMD.Exon52.25.014	TGTTAAAAAACTTACTTCGATCCGT	395
Hu.DMD.Exon52.25.015	ATGCTTGTTAAAAAACTTACTTCGA	396
Hu.DMD.Exon52.25.016	GTCCCATGCTTGTTAAAAAACTTAC	397
Hu.DMD.Exon52.20.001	AGAACAAATATCCCTTAGTA	398
Hu.DMD.Exon52.20.002	GTAAGAACAAATATCCCTTA	399
Hu.DMD.Exon52.20.003	TGCCTGTAAGAACAAATATC	400
Hu.DMD.Exon52.20.004	ATTGTTGCCTGTAAGAACAA	401
Hu.DMD.Exon52.20.005	CCTGCATTGTTGCCTGTAAG	402
Hu.DMD.Exon52.20.006	CAAATCCTGCATTGTTGCCT	403
Hu.DMD.Exon52.20.007	GCCTCTGTTCCAAATCCTGC	404
Hu.DMD.Exon52.20.008	CTTCCAACTGGGGACGCCTC	405
Hu.DMD.Exon52.20.009	CAGCGGTAATGAGTTCTTCC	406
Hu.DMD.Exon52.20.010	TTTTCAAATTTTGGGCAGCG	407
Hu.DMD.Exon52.20.011	GATTGCTGGTCTTGTTTTTC	408
Hu.DMD.Exon52.20.012	TTGTTCTAGCCTCTTGATTG	409
Hu.DMD.Exon52.20.013	TTCGATCCGTAATGATTGTT	410
Hu.DMD.Exon52.20.014	CTTACTTCGATCCGTAATGA	411
Hu.DMD.Exon52.20.015	AAAAACTTACTTCGATCCGT	412
Hu.DMD.Exon52.20.016	TGTTAAAAAACTTACTTCGA	413
Hu.DMD.Exon52.20.017	ATGCTTGTTAAAAAACTTAC	414
Hu.DMD.Exon52.20.018	GTCCCATGCTTGTTAAAAAA	415
Hu.DMD.Exon53.25.001	CTAGAATAAAAGGAAAAATAAATAT	416
Hu.DMD.Exon53.25.002	AACTAGAATAAAAGGAAAAATAAAT	417
Hu.DMD.Exon53.25.002.2	TTCAACTAGAATAAAAGGAAAAATA	418
Hu.DMD.Exon53.25.003	CTTTCAACTAGAATAAAAGGAAAAA	419
Hu.DMD.Exon53.25.003.2	ATTCTTTCAACTAGAATAAAAGGAA	420
Hu.DMD.Exon53.25.004	GAATTCTTTCAACTAGAATAAAAGG	421

TABLE 4 (Continued)

Hu.DMD.Exon53.25.004.2	TCTGAATTCTTTCAACTAGAATAAA	422
Hu.DMD.Exon53.25.005	ATTCTGAATTCTTTCAACTAGAATA	423
Hu.DMD.Exon53.25.005.2	CTGATTCTGAATTCTTTCAACTAGA	424
Hu.DMD.Exon53.25.006	CACTGATTCTGAATTCTTTCAACTA	425
Hu.DMD.Exon53.25.006.2	TCCCACTGATTCTGAATTCTTTCAA	426
Hu.DMD.Exon53.25.007	CATCCCACTGATTCTGAATTCTTTC	427
Hu.DMD.Exon53.25.008	TACTTCATCCCACTGATTCTGAATT	428
Hu.DMD.Exon53.25.008.2	CTGAAGGTGTTCTTGTACTTCATCC	429
Hu.DMD.Exon53.25.009	CGGTTCTGAAGGTGTTCTTGTACT	430
Hu.DMD.Exon53.25.009.2	CTGTTGCCTCCGGTTCTGAAGGTGT	431
Hu.DMD.Exon53.25.010	TTTCATTCAACTGTTGCCTCCGGTT	432
Hu.DMD.Exon53.25.010.2	TAACATTTCATTCAACTGTTGCCTC	433
Hu.DMD.Exon53.25.011	TTGTGTTGAATCCTTTAACATTTCA	434
Hu.DMD.Exon53.25.012	TCTTCCTTAGCTTCCAGCCATTGTG	435
Hu.DMD.Exon53.25.012.2	CTTAGCTTCCAGCCATTGTGTTGAA	436
Hu.DMD.Exon53.25.013	GTCCTAAGACCTGCTCAGCTTCTTC	437
Hu.DMD.Exon53.25.013.2	CTGCTCAGCTTCTTCCTTAGCTTCC	438
Hu.DMD.Exon53.25.014	CTCAAGCTTGGCTCTGGCCTGTCCT	439
Hu.DMD.Exon53.25.014.2	GGCCTGTCCTAAGACCTGCTCAGCT	440
Hu.DMD.Exon53.25.015	TAGGGACCCTCCTTCCATGACTCAA	441

TABLE 4 (Continued)

Hu.DMD.Exon53.25.016	TTTGGATTGCATCTACTGTATAGGG	442
Hu.DMD.Exon53.25.016.2	ACCCTCCTTCCATGACTCAAGCTTG	443
Hu.DMD.Exon53.25.017	CTTGGTTTCTGTGATTTTCTTTTGG	444
Hu.DMD.Exon53.25.017.2	ATCTACTGTATAGGGACCCTCCTTC	445
Hu.DMD.Exon53.25.018	CTAACCTTGGTTTCTGTGATTTTCT	446
Hu.DMD.Exon53.25.018.2	TTTCTTTTGGATTGCATCTACTGTA	447
Hu.DMD.Exon53.25.019	TGATACTAACCTTGGTTTCTGTGAT	448
Hu.DMD.Exon53.25.020	ATCTTTGATACTAACCTTGGTTTCT	449
Hu.DMD.Exon53.25.021	AAGGTATCTTTGATACTAACCTTGG	450
Hu.DMD.Exon53.25.022	TTAAAAAGGTATCTTTGATACTAAC	451
Hu.DMD.Exon53.20.001	ATAAAAGGAAAAATAAATAT	452
Hu.DMD.Exon53.20.002	GAATAAAAGGAAAAATAAAT	453
Hu.DMD.Exon53.20.003	AACTAGAATAAAAGGAAAAA	454
Hu.DMD.Exon53.20.004	CTTTCAACTAGAATAAAAGG	455
Hu.DMD.Exon53.20.005	GAATTCTTTCAACTAGAATA	456
Hu.DMD.Exon53.20.006	ATTCTGAATTCTTTCAACTA	457
Hu.DMD.Exon53.20.007	TACTTCATCCCACTGATTCT	458
Hu.DMD.Exon53.20.008	CTGAAGGTGTTCTTGTACT	459
Hu.DMD.Exon53.20.009	CTGTTGCCTCCGGTTCTGAA	460
Hu.DMD.Exon53.20.010	TAACATTTCATTCAACTGTT	461
Hu.DMD.Exon53.20.011	TTGTGTTGAATCCTTTAACA	462
Hu.DMD.Exon53.20.012	CTTAGCTTCCAGCCATTGTG	463
Hu.DMD.Exon53.20.013	CTGCTCAGCTTCTTCCTTAG	464
Hu.DMD.Exon53.20.014	GGCCTGTCCTAAGACCTGCT	465
Hu.DMD.Exon53.20.015	CTCAAGCTTGGCTCTGGCCT	466
Hu.DMD.Exon53.20.016	ACCCTCCTTCCATGACTCAA	467

TABLE 4 (Continued)

Hu.DMD.Exon53.20.017	ATCTACTGTATAGGGACCCT	468
Hu.DMD.Exon53.20.018	TTTCTTTTGGATTGCATCTA	469
Hu.DMD.Exon53.20.019	CTTGGTTTCTGTGATTTTCT	470
Hu.DMD.Exon53.20.020	CTAACCTTGGTTTCTGTGAT	471
Hu.DMD.Exon53.20.021	TGATACTAACCTTGGTTTCT	472
Hu.DMD.Exon53.20.022	ATCTTTGATACTAACCTTGG	473
Hu.DMD.Exon53.20.023	AAGGTATCTTTGATACTAAC	474
Hu.DMD.Exon53.20.024	TTAAAAAGGTATCTTTGATA	475
Hu.DMD.Exon54.25.001	CTATAGATTTTTATGAGAAAGAGA	476
Hu.DMD.Exon54.25.002	AACTGCTATAGATTTTTATGAGAAA	477
Hu.DMD.Exon54.25.003	TGGCCAACTGCTATAGATTTTTATG	478
Hu.DMD.Exon54.25.004	GTCTTTGGCCAACTGCTATAGATTT	479
Hu.DMD.Exon54.25.005	CGGAGGTCTTTGGCCAACTGCTATA	480
Hu.DMD.Exon54.25.006	ACTGGCGGAGGTCTTTGGCCAACTG	481
Hu.DMD.Exon54.25.007	TTTGTCTGCCACTGGCGGAGGTCTT	482
Hu.DMD.Exon54.25.008	AGTCATTTGCCACATCTACATTTGT	483
Hu.DMD.Exon54.25.008.2	TTTGCCACATCTACATTTGTCTGCC	484
Hu.DMD.Exon54.25.009	CCGGAGAAGTTTCAGGGCCAAGTCA	485
Hu.DMD.Exon54.25.010	GTATCATCTGCAGAATAATCCCGGA	486
Hu.DMD.Exon54.25.010.2	TAATCCCGGAGAAGTTTCAGGGCCA	487
Hu.DMD.Exon54.25.011	TTATCATGTGGACTTTTCTGGTATC	488
Hu.DMD.Exon54.25.012	AGAGGCATTGATATTCTCTGTTATC	489
Hu.DMD.Exon54.25.012.2	ATGTGGACTTTTCTGGTATCATCTG	490
Hu.DMD.Exon54.25.013	CTTTTATGAATGCTTCTCCAAGAGG	491
Hu.DMD.Exon54.25.013.2	ATATTCTCTGTTATCATGTGGACTT	492

TABLE 4 (Continued)

Hu.DMD.Exon54.25.014	CATACCTTTTATGAATGCTTCTCCA	493
Hu.DMD.Exon54.25.014.2	CTCCAAGAGGCATTGATATTCTCTG	494
Hu.DMD.Exon54.25.015	TAATTCATACCTTTTATGAATGCTT	495
Hu.DMD.Exon54.25.015.2	CTTTTATGAATGCTTCTCCAAGAGG	496
Hu.DMD.Exon54.25.016	TAATGTAATTCATACCTTTTATGAA	497
Hu.DMD.Exon54.25.017	AGAAATAATGTAATTCATACCTTTT	498
Hu.DMD.Exon54.25.018	GTTTTAGAAATAATGTAATTCATAC	499
Hu.DMD.Exon54.20.001	GATTTTTATGAGAAAGAGA	500
Hu.DMD.Exon54.20.002	CTATAGATTTTTATGAGAAA	501
Hu.DMD.Exon54.20.003	AACTGCTATAGATTTTTATG	502
Hu.DMD.Exon54.20.004	TGGCCAACTGCTATAGATTT	503
Hu.DMD.Exon54.20.005	GTCTTTGGCCAACTGCTATA	504
Hu.DMD.Exon54.20.006	CGGAGGTCTTTGGCCAACTG	505
Hu.DMD.Exon54.20.007	TTTGTCTGCCACTGGCGGAG	506
Hu.DMD.Exon54.20.008	TTTGCCACATCTACATTTGT	507
Hu.DMD.Exon54.20.009	TTCAGGGCCAAGTCATTTGC	508
Hu.DMD.Exon54.20.010	TAATCCCGGAGAAGTTTCAG	509
Hu.DMD.Exon54.20.011	GTATCATCTGCAGAATAATC	510
Hu.DMD.Exon54.20.012	ATGTGGACTTTTCTGGTATC	511
Hu.DMD.Exon54.20.013	ATATTCTCTGTTATCATGTG	512
Hu.DMD.Exon54.20.014	CTCCAAGAGGCATTGATATT	513
Hu.DMD.Exon54.20.015	CTTTTATGAATGCTTCTCCA	514
Hu.DMD.Exon54.20.016	CATACCTTTTATGAATGCTT	515
Hu.DMD.Exon54.20.017	TAATTCATACCTTTTATGAA	516
Hu.DMD.Exon54.20.018	TAATGTAATTCATACCTTTT	517
Hu.DMD.Exon54.20.019	AGAAATAATGTAATTCATAC	518
Hu.DMD.Exon54.20.020	GTTTTAGAAATAATGTAATT	519

TABLE 4 (Continued)

Hu.DMD.Exon55.25.001	CTGCAAAGGACCAAATGTTCAGATG	520
Hu.DMD.Exon55.25.002	TCACCCTGCAAAGGACCAAATGTTC	521
Hu.DMD.Exon55.25.003	CTCACTCACCCTGCAAAGGACCAAA	522
Hu.DMD.Exon55.25.004	TCTCGCTCACTCACCCTGCAAAGGA	523
Hu.DMD.Exon55.25.005	CAGCCTCTCGCTCACTCACCCTGCA	524
Hu.DMD.Exon55.25.006	CAAAGCAGCCTCTCGCTCACTCACC	525
Hu.DMD.Exon55.25.007	TCTTCCAAAGCAGCCTCTCGCTCAC	526
Hu.DMD.Exon55.25.007.2	TCTATGAGTTTCTTCCAAAGCAGCC	527
Hu.DMD.Exon55.25.008	GTTGCAGTAATCTATGAGTTTCTTC	528
Hu.DMD.Exon55.25.008.2	GAACTGTTGCAGTAATCTATGAGTT	529
Hu.DMD.Exon55.25.009	TTCCAGGTCCAGGGGGAACTGTTGC	530
Hu.DMD.Exon55.25.010	GTAAGCCAGGCAAGAAACTTTTCCA	531
Hu.DMD.Exon55.25.010.2	CCAGGCAAGAAACTTTTCCAGGTCC	532
Hu.DMD.Exon55.25.011	TGGCAGTTGTTTCAGCTTCTGTAAG	533
Hu.DMD.Exon55.25.011.2	TTCAGCTTCTGTAAGCCAGGCAAGA	635
Hu.DMD.Exon55.25.012	GGTAGCATCCTGTAGGACATTGGCA	534
Hu.DMD.Exon55.25.012.2	GACATTGGCAGTTGTTTCAGCTTCT	535
Hu.DMD.Exon55.25.013	TCTAGGAGCCTTTCCTTACGGGTAG	536
Hu.DMD.Exon55.25.014	CTTTTACTCCCTTGGAGTCTTCTAG	537
Hu.DMD.Exon55.25.014.2	GAGCCTTTCCTTACGGGTAGCATCC	538
Hu.DMD.Exon55.25.015	TTGCCATTGTTTCATCAGCTCTTTT	539
Hu.DMD.Exon55.25.015.2	CTTGGAGTCTTCTAGGAGCCTTTCC	540

TABLE 4 (Continued)

Hu.DMD.Exon55.25.016	CTTACTTGCCATTGTTTCATCAGCT	541
Hu.DMD.Exon55.25.016.2	CAGCTCTTTTACTCCCTTGGAGTCT	542
Hu.DMD.Exon55.25.017	CCTGACTTACTTGCCATTGTTTCAT	543
Hu.DMD.Exon55.25.018	AAATGCCTGACTTACTTGCCATTGT	544
Hu.DMD.Exon55.25.019	AGCGGAAATGCCTGACTTACTTGCC	545
Hu.DMD.Exon55.25.020	GCTAAAGCGGAAATGCCTGACTTAC	546
Hu.DMD.Exon55.20.001	AAGGACCAAATGTTCAGATG	547
Hu.DMD.Exon55.20.002	CTGCAAAGGACCAAATGTTC	548
Hu.DMD.Exon55.20.003	TCACCCTGCAAAGGACCAAA	549
Hu.DMD.Exon55.20.004	CTCACTCACCCTGCAAAGGA	550
Hu.DMD.Exon55.20.005	TCTCGCTCACTCACCCTGCA	551
Hu.DMD.Exon55.20.006	CAGCCTCTCGCTCACTCACC	552
Hu.DMD.Exon55.20.007	CAAAGCAGCCTCTCGCTCAC	553
Hu.DMD.Exon55.20.008	TCTATGAGTTTCTTCCAAAG	554
Hu.DMD.Exon55.20.009	GAACTGTTGCAGTAATCTAT	555
Hu.DMD.Exon55.20.010	TTCCAGGTCCAGGGGGAACT	556
Hu.DMD.Exon55.20.011	CCAGGCAAGAAACTTTTCCA	557
Hu.DMD.Exon55.20.012	TTCAGCTTCTGTAAGCCAGG	558
Hu.DMD.Exon55.20.013	GACATTGGCAGTTGTTTCAG	559
Hu.DMD.Exon55.20.014	GGTAGCATCCTGTAGGACAT	560
Hu.DMD.Exon55.20.015	GAGCCTTTCCTTACGGGTAG	561
Hu.DMD.Exon55.20.016	CTTGGAGTCTTCTAGGAGCC	562
Hu.DMD.Exon55.20.017	CAGCTCTTTTACTCCCTTGG	563
Hu.DMD.Exon55.20.018	TTGCCATTGTTTCATCAGCT	564
Hu.DMD.Exon55.20.019	CTTACTTGCCATTGTTTCAT	565
Hu.DMD.Exon55.20.020	CCTGACTTACTTGCCATTGT	566
Hu.DMD.Exon55.20.021	AAATGCCTGACTTACTTGCC	567
Hu.DMD.Exon55.20.022	AGCGGAAATGCCTGACTTAC	568

TABLE 4 (Continued)

Hu.DMD.Exon55.20.023	GCTAAAGCGGAAATGCCTGA	569
H50A(+02+30)-AVI-5656	CCACTCAGAGCTCAGATCTTCTAACTTCC	584
H50D(+07-18)-AVI-5915	GGGATCCAGTATACTTACAGGCTCC	585
H50A(+07+33)	CTTCCACTCAGAGCTCAGATCTTCTAA	586
H51A(+61+90)-AVI-4657	ACATCAAGGAAGATGGCATTTCTAGTTTGG	587
H51A(+66+95)-AVI-4658	CTCCAACATCAAGGAAGATGGCATTTCTAG	588
H51A(+111+134)	TTCTGTCCAAGCCCGGTTGAAATC	589
H51A(+175+195)	CACCCACCATCACCCTCYGTG	590
H51A(+199+220)	ATCATCTCGTTGATATCCTCAA	591
H51A(+66+90)	ACATCAAGGAAGATGGCATTTCTAG	592
H51A(-01+25)	ACCAGAGTAACAGTCTGAGTAGGAGC	593
h51AON1	TCAAGGAAGATGGCATTTCT	594
h51AON2	CCTCTGTGATTTTATAACTTGAT	595
H51D(+08-17)	ATCATTTTTTCTCATACCTTCTGCT	596
H51D(+16-07)	CTCATACCTTCTGCTTGATGATC	597
hAON#23	TGGCATTTCTAGTTTGG	598
hAON#24	CCAGAGCAGGTACCTCCAACATC	599
H44A(+61+84)	TGTTCAGCTTCTGTTAGCCACTGA	600
H44A(+85+104)	TTTGTGTCTTTCTGAGAAAC	601
h44AON1	CGCCGCCATTTCTCAACAG	602
H44A(-06+14)	ATCTGTCAAATCGCCTGCAG	603
H45A(+71+90)	TGTTTTTGAGGATTGCTGAA	604
h45AON1	GCTGAATTATTTCTTCCCC	605
h45AON5	GCCCAATGCCATCCTGG	606
H45A(-06+20)	CCAATGCCATCCTGGAGTTCCTGTAA	607
H53A(+39+69)	CATTCAACTGTTGCCTCCGGTTCTGAAGGTG	608

TABLE 4 (Continued)

H53A(+23+47)	CTGAAGGTGTTCTTGTACTTCATCC	609
h53AON1	CTGTTGCCTCCGGTTCTG	610
H53A(-12+10)	ATTCTTTCAACTAGAATAAAAG	611
huEx45.30.66	GCCATCCTGGAGTTCCTGTAAGATACC AAA	612
huEx45.30.71	CCAATGCCATCCTGGAGTTCCTGTAAG ATA	613
huEx45.30.79	GCCGCTGCCCAATGCCATCCTGGAGTT CCT	614
huEx45.30.83	GTTTGCCGCTGCCCAATGCCATCCTGG AGT	615
huEx45.30.88	CAACAGTTTGCCGCTGCCCAATGCCAT CCT	616
huEx45.30.92	CTGACAACAGTTTGCCGCTGCCCAATG CCA	617
huEx45.30.96	TGTTCTGACAACAGTTTGCCGCTGCCC AAT	618
huEx45.30.99	CAATGTTCTGACAACAGTTTGCCGCTG CCC	619
huEx45.30.103	CATTCAATGTTCTGACAACAGTTTGCCG CT	620
huEx45.30.120	TATTTCTTCCCCAGTTGCATTCAATGTT CT	621
huEx45.30.127	GCTGAATTATTTCTTCCCCAGTTGCATT CA	622
huEx45.30.132	GGATTGCTGAATTATTTCTTCCCCAGTT GC	623
huEx45.30.137	TTTGAGGATTGCTGAATTATTTCTTCCC CA	624
huEx53.30.84	GTACTTCATCCCACTGATTCTGAATTCT TT	625
huEx53.30.88	TCTTGTACTTCATCCCACTGATTCTGAA TT	626
huEx53.30.91	TGTTCTTGTACTTCATCCCACTGATTCT GA	627
huEx53.30.103	CGGTTCTGAAGGTGTTCTTGTACTTCAT CC	628
huEx53.30.106	CTCCGGTTCTGAAGGTGTTCTTGTACTT CA	629
huEx53.30.109	TGCCTCCGGTTCTGAAGGTGTTCTTGTA CT	630

TABLE 4 (Continued)

huEx53.30.112	TGTTGCCTCCGGTTCTGAAGGTGTTCTTGT	631
huEx53.30.115	AACTGTTGCCTCCGGTTCTGAAGGTGTTCT	632
huEx53.30.118	TTCAACTGTTGCCTCCGGTTCTGAAGGTGT	633
h50AON1		
h50AON2		
Peptide Transporters (NH_2 to COOH)*:		
rTAT	RRRQRRKKRC	570
R_9F_2	RRRRRRRRRFFC	571
$(RRAhx)_4B$	RRAhxRRAhxRRAhxRRAhxB	572
$(RAhxR)_4AhxB$; (P007)	RAhxRRAhxRRAhxRRAhxRAhxB	573
$(AhxRR)_4AhxB$	AhxRRAhxRRAhxRRAhxRRAhxB	574
$(RAhx)_6B$	RAhxRAhxRAhxRAhxRAhxRAhxB	575
$(RAhx)_8B$	RAhxRAhxRAhxRAhxRAhxRAhxRAhxRAhxB	576
$(RAhxR)_5AhxB$	RAhxRRAhxRRAhxRRAhxRRAhxRAhxB	577
$(RAhxRRBR)_2AhxB$; (CPO6062)	RAhxRRBRRAhxRRBRAhxB	578
MSP	ASSLNIA	579
Cell Penetrating Peptide / Homing Peptide / PMO Conjugates (NH_2 to COOH and 5' to 3')		
MSP-PMO	ASSLNIA-XB-GGCCAAACCTCGGCTTACCTGAAAT	580 636
CP06062-MSP-PMO	RXRRBRRXRRBR-XB-ASSLNIA-X-GGCCAAACCTCGGCTTACCTGAAAT	581 636
MSP-CP06062-PMO	ASSLNIA-X-RXRRBRRXRRBR-B-GGCCAAACCTCGGCTTACCTGAAAT	582 636
CP06062-PMO	RXRRBRRXRRBR-XB-GGCCAAACCTCGGCTTACCTGAAAT	583 636

*Ahx is 6-aminohexanoic acid and B is beta-alanine.