(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 February 2021 (11.02.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/025899 A1

(51) International Patent Classification:
A61K 9/00 (2006.01) *A61K 47/02* (2006.01)
A61K 9/08 (2006.01) *A61P 21/00* (2006.01)
A61K 31/00 (2006.01)

(21) International Application Number:
PCT/US2020/043824

(22) International Filing Date:
28 July 2020 (28.07.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/881,996 02 August 2019 (02.08.2019) US
63/056,772 27 July 2020 (27.07.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2021/025899 A1

(54) Title: PHOSPHORODIAMIDATE MORPHOLINO OLIGOMER PHARMACEUTICAL COMPOSITIONS

(57) Abstract: Provided herein are pharmaceutical compositions comprising golodirsen or casimersen. Also provided herein are methods of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the disclosure.

PHOSPHORODIAMIDATE MORPHOLINO OLIGOMER PHARMACEUTICAL COMPOSITIONS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No.: 63/056,772, filed on July 27, 2020 and U.S. Provisional Application No.: 62/881,996, filed on August 2, 2019. The entire teachings of the above-referenced applications are incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 27, 2020, is named 8175_53_WO00_SL.txt and is 677 bytes in size.

BACKGROUND

Antisense technology provides a means for modulating the expression of one or more specific gene products, including alternative splice products, and is uniquely useful in a number of therapeutic, diagnostic, and research applications. The principle behind antisense technology is that an antisense compound, e.g., an oligonucleotide, which hybridizes to a target nucleic acid, modulates gene expression activities such as transcription, splicing or translation through any one of a number of antisense mechanisms. The sequence specificity of antisense compounds makes them attractive as tools for target validation and gene functionalization, as well as therapeutics to selectively modulate the expression of genes involved in disease.

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.

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.

Golodirsen is a phosphorodiamidate morpholino oligomer (PMO) designed to skip exon 53 of the human dystrophin gene in patients with DMD who are amendable to exon 53 skipping to restore the read frame and produce a functional shorter form of the dystrophin protein. Sarepta Therapeutics, Inc., submitted a New Drug Application (NDA) to the United States Food and Drug Administration (FDA) seeking approval for the treatment of DMD in patients amendable to exon 53 skipping. Sarepta's NDA was approved by the FDA in December 2019.

Casimersen is a phosphorodiamidate morpholino oligomer (PMO) designed to skip exon 45 of the human dystrophin gene in patients with DMD who are amendable to exon 45 skipping to restore the read frame and produce a functional shorter form of the dystrophin protein. Sarepta Therapeutics, Inc., submitted a New Drug Application (NDA) to the United States Food and Drug Administration (FDA) seeking approval for the treatment of DMD in patients amendable to exon 45 skipping. Sarepta's NDA is currently under review by the FDA.

Although significant progress has been made in the field of antisense technology, there remains a need in the art for pharmaceutical formulations comprising oligonucleotides.

SUMMARY

Provided herein are pharmaceutical compositions comprising golodirsen wherein the concentration of golodirsen is about 50 mg/mL of the pharmaceutical composition. Also provided herein are methods of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the disclosure.

Accordingly, in one aspect, provided herein is a pharmaceutical composition, comprising:

a) golodirsen;

b) sodium chloride;

c) potassium chloride;

d) potassium phosphate monobasic;

e) sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50 mg/mL of the pharmaceutical composition.

Other formulation substances that are known in general to one skilled in the art are also conceived.

In yet another aspect, provided herein is a pharmaceutical composition, comprising:

a) 40-60 mg of golodirsen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 80-120 mg of golodirsen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water.

In still another aspect, provided herein is a pharmaceutical composition, comprising:

a) 400-600 mg of golodirsen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water.

Pharmaceutical compositions of the disclosure comprise a concentration of golodirsen of about 50 mg/mL of the pharmaceutical composition.

Provided herein are also pharmaceutical compositions comprising casimersen wherein the concentration of casimersen is about 50 mg/mL of the pharmaceutical composition. Also provided herein are methods of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the disclosure.

Accordingly, in one aspect, provided herein is a pharmaceutical composition, comprising:

a) casimersen;

b) sodium chloride;

c) potassium chloride;

d) potassium phosphate monobasic;

e) sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50 mg/mL of the pharmaceutical composition.

Other formulation substances that are known in general to one skilled in the art are also conceived.

In yet another aspect, provided herein is a pharmaceutical composition, comprising:

a) 40-60 mg of casimersen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 80-120 mg of casimersen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water.

In still another aspect, provided herein is a pharmaceutical composition, comprising:

a) 400-600 mg of casimersen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water.

Pharmaceutical compositions of the disclosure comprise a concentration of casimersen of about 50 mg/mL of the pharmaceutical composition.

In another aspect, provided herein is a method of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition provided herein.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 and **Fig. 2** show a representative analytical high performance liquid chromatography (HPLC) chromatogram of a synthesized and deprotected golodirsen (SRP-4053) crude drug substance (see Example 1).

Fig.3 and **Fig. 4** show a representative analytical HPLC chromatogram of a purified golodirsen drug substance solution (see Example 2).

Fig. 5 and **Fig. 6** show a representative analytical HPLC chromatogram of a desalted and lyophilized golodirsen drug substance (see Example 2).

Fig. 7 and **Fig. 8** show a representative analytical high performance liquid chromatography (HPLC) chromatogram of a synthesized and deprotected casimersen (SRP-4045) crude drug substance (see Example 4).

Fig.9 and **Fig. 10** show a representative analytical HPLC chromatogram of a purified casimersen drug substance solution (see Example 5).

Fig. 11 and **Fig. 12** show a representative analytical HPLC chromatogram of a desalted and lyophilized casimersen drug substance (see Example 5).

DETAILED DESCRIPTION

Provided herein are pharmaceutical compositions comprising golodirsen or casimersen. Also provided herein are methods of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the disclosure. The morpholino oligonucleotides described herein (golodirsen and casimersen) display stronger affinity for DNA and RNA without compromising sequence selectivity, relative to native or unmodified oligonucleotides. In some embodiments, the oligonucleotides of the disclosure minimize or prevent cleavage by RNase H. In some embodiments, the antisense oligonucleotides of the disclosure do not activate RNase H.

Definitions

Listed below are definitions of various terms used to describe this disclosure. These definitions apply to the terms as they are used throughout this specification and claims, unless otherwise limited in specific instances, either individually or as part of a larger group.

The term “support-bound” refers to a chemical moiety that is covalently linked to a support-medium.

The term "support-medium" refers to any material including, for example, any particle, bead, or surface, upon which an oligomer can be attached or synthesized upon, or can be modified for attachment or synthesis of an oligomer. Representative substrates include, but are not limited to, inorganic supports and organic supports such as glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, TEFLON, etc.), polysaccharides, nylon or nitrocellulose, ceramics, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, optical fiber bundles, and a variety of other polymers. Particularly useful solid supports and solid surfaces for some embodiments are located within a flow cell apparatus. In some embodiments of the processes described herein, the support-medium comprises polystyrene with 1% crosslinked divinylbenzene.

In some embodiments, representative support-medium comprise at least one reactive site for attachment or synthesis of an oligomer. For example, in some embodiments, a support-medium of the disclosure comprises one or more terminal amino or hydroxyl groups capable of forming a chemical bond with an incoming nucleoside or other activated group for attaching or synthesizing an oligomer.

Some representative support-medium that are amenable to the processes described herein include, but are not limited to, the following: controlled pore glass (CPG); oxalyl-controlled pore glass (see, e.g., Alul et al., *Nucleic Acids Research* 1991, 19, 1527); silica-containing particles, such as porous glass beads and silica gel such as that formed by the reaction of trichloro-[3-(4-chloromethyl)phenyl]propylsilane and porous glass beads (see Parr and Grohmann, *Angew. Chem. Internal. Ed.* 1972, 11, 314; sold under the trademark "PORASIL E" by Waters Associates, Framingham, Mass., USA); a mono ester of 1,4-dihydroxymethylbenzene and silica (see Bayer and Jung, *Tetrahedron Lett.* 1970, 51, 4503; sold under the trademark "BIOPAK" by Waters Associates); TENTAGEL (see, e.g., Wright et al., *Tetrahedron Lett.* 1993, 34, 3373); cross-linked styrene/divinylbenzene copolymer beaded matrix, or POROS, a copolymer of polystyrene/divinylbenzene (available from PerSeptive Biosystems); soluble support-medium such as polyethylene glycol PEG's (see Bonora et al., *Organic Process Research & Development* 2000, 4, 225–231); PEPS support, which is a polyethylene (PE) film with pendant long-chain polystyrene (PS) grafts (see Berg et al., *J. Am. Chem. Soc.* 1989, 111, 8024 and International Patent Application WO 1990/02749); copolymers of dimethylacrylamide cross-linked with N,N′-bisacryloylethylenediamine, including a known amount of N-tertbutoxycarbonyl-beta-alanyl-

N′-acryloylhexamethylenediamine (see Atherton et al., *J. Am. Chem. Soc.* 1975, 97, 6584; Atherton et al., *Bioorg. Chem.* 1979, 8, 351; and Atherton et al., *J. Chem. Soc. Perkin I* 1981, 538); glass particles coated with a hydrophobic cross-linked styrene polymer (see Scott et al., *J. Chrom. Sci.* 1971, 9, 577); fluorinated ethylene polymer onto which has been grafted polystyrene (see Kent and Merrifield, *Israel J. Chem.* 1978, 17, 243 and van Rietschoten in *Peptides* 1974, Y. Wolman, Ed., Wiley and Sons, New York, 1975, pp. 113–116); hydroxypropylacrylate-coated polypropylene membranes (Daniels et al., *Tetrahedron Lett.* 1989, 30, 4345); acrylic acid-grafted polyethylene-rods (Geysen et al., *Proc. Natl. Acad. Sci. USA* 1984, 81, 3998); a "tea bag" containing traditionally-used polymer beads (Houghten, *Proc. Natl. Acad. Sci. USA* 1985, 82, 5131); and combinations thereof.

The term "flow cell apparatus" refers to a chamber comprising a surface (e.g., solid surface) across which one or more fluid reagents (e.g., liquid or gas) can be flowed.

The term "treating" or "treatment" as used herein comprises a treatment relieving, reducing or alleviating at least one symptom in a subject or effecting a delay of progression of a disease. For example, treatment can be the diminishment of one or several symptoms of a disorder or complete eradication of a disorder, such as muscular dystrophy, e.g., Duchenne muscular dystrophy. Within the meaning of the present disclosure, the term "treat" also denotes to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) or reduce the risk of developing or worsening a disease. The term "protect" is used herein to mean prevent, delay, or treat, or all, as appropriate, development, continuance or aggravation of a disease in a subject, e.g., a mammal or human. The term "prevent," "preventing" or "prevention" as used herein comprises the prevention of at least one symptom associated with or caused by the state, disease or disorder being prevented.

The term "subject" or "patient" as used herein is intended to include animals, which are capable of suffering from or afflicted with a muscle disease or any disorder involving, directly or indirectly, a muscle disease. Examples of subjects include mammals, e.g., humans, apes, monkeys, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In an embodiment, the subject is a human, e.g., a human suffering from, at risk of suffering from, or potentially capable of suffering from muscle diseases.

"Amendable to exon 53 skipping" as used herein with regard to a subject or patient is intended to include subjects and patients having various mutations in the dystrophin gene which are amenable to exon 53 skipping. Non-limiting examples of mutations in the following exons of the dystrophin gene are amenable to exon 53 skipping include, e.g.: exons 3 to 52, 4 to 52, 5 to 52, 6 to 52, 9 to 52, 10 to 52, 11 to 52, 13 to 52, 14 to 52, 15 to 52, 16 to

52, 17 to 52, 19 to 52, 21 to 52, 23 to 52, 24 to 52, 25 to 52, 26 to 52, 27 to 52, 28 to 52, 29 to 52, 30 to 52, 31 to 52, 32 to 52, 33 to 52, 34 to 52, 35 to 52, 36 to 52, 37 to 52, 38 to 52, 39 to 52, 40 to 52, 41 to 52, 43 to 52, 42 to 52, 45 to 52, 47 to 52, 48 to 52, 49 to 52, 50 to 52, 54 to 58, 54 to 61, 54 to 63, 54 to 64, 54 to 66, 54 to 76, 54 to 77, or exon 52. *See*, for example, the Leiden Duchenne muscular dystrophy mutation database, Leiden University Medical Center, The Netherlands. Determining whether a patient has a mutation in the dystrophin gene that is amenable to exon skipping is within the purview of one of skill in the art (see, e.g., Aartsma-Rus et al., *Hum Mut* 2009, 30, 293-299; Gurvich et al., Hum Mutat. 2009; 30(4) 633-640, and Fletcher et al. (2010) Molecular Therapy 18(6) 1218-1223).

"Amendable to exon 45 skipping" as used herein with regard to a subject or patient is intended to include subjects and patients having various mutations in the dystrophin gene which are amenable to exon 45 skipping. Non-limiting examples of mutations in the following exons of the dystrophin gene are amenable to exon 45 skipping include, e.g.: exons 7 to 42, 12 to 42, 18 to 42, 44 to 46, 44 to 47, 44 to 48, 44 to 49, 44 to 51, 44 to 53, 44 to 55, 44 to 57, or 44 to 59, or exon 44. exons 7 to 42, 12 to 42, 18 to 42, 44 to 46, 44 to 47, 44 to 48, 44 to 49, 44 to 51, 44 to 53, 44 to 55, 44 to 57, or 44 to 59, or exon 44.

The terms "comprising" and "including" are used herein in their open-ended and non-limiting sense unless otherwise noted.

The terms "a" and "an" and "the" and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like.

The terms "about" or "approximately" are generally understood by persons knowledgeable in the relevant subject area, but in certain circumstances can mean within ±10%, or within ±5%, of a given value or range.

"USP" refers to United States Pharmacopeia, incorporated herein by reference in its entirety, and indicates that the material so identified conforms to USP specification.

"NF" refers to National Formulary, incorporated herein by reference in its entirety, and indicates that the material so identified conforms to NF specifications.

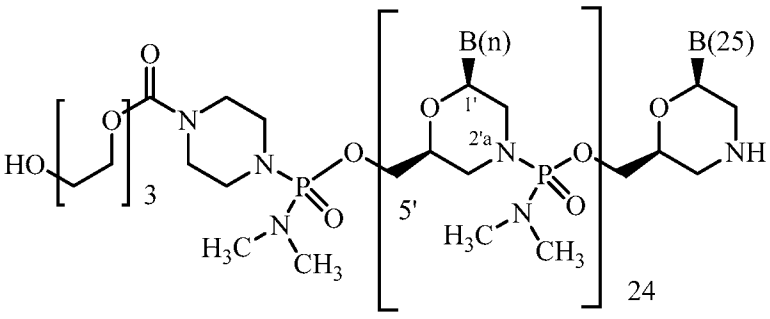
Oligomers

Morpholino-based oligomers (including antisense oligomers) are detailed, for example, in 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, 8,299,206, and 8,076,476, U.S. Patent Application Publication Nos. US 2016/0040162 and US 2019/0262375 A1, International Patent Application Publication Nos. WO/2009/064471 and WO/2012/043730, and Summerton et al., *Antisense Nucleic Acid Drug Dev.* 1997, 7, 187-195, each of which are hereby incorporated by reference in their entirety.

Golodirsen (see e.g., Example 2 of U.S. Patent Application Publication No. US 2016/0040162 , incorporated herein by reference in its entirety) has been the subject of clinical studies to test its safety and efficacy, and clinical development is ongoing. Golodirsen is a phosphorodiamidate morpholino (PMO) antisense oligonucleotide. The dystrophin therapeutic "golodirsen," is also known as "SRP-4053." Golodirsen is a PMO having the base sequence 5'-GTTGCCTCCGGTTCTGAAGGTGTTC-3' (SEQ ID NO: 1). Golodirsen is registered under CAS Registry Number 1422959-91-8. Chemical names include: RNA, [P-deoxy-P-(dimethylamino)](2',3'-dideoxy-2',3'-imino-2',3'-seco)(2'a→5')(G-m5U-m5U-G-C-C-m5U-C-C-G-G-m5U-m5U-C-m5U-G-A-A-G-G-m5U-G-m5U-m5U-C), 5'-[P-[4-[[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]carbonyl]-1-piperazinyl]-N,N-dimethylphosphonamidate], *all-P-ambo*-[*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-imino-2',3'-seco](2'a→5')(G-T-T-G-C-C-T-C-C-G-G-T-T-C-T-G-A-A-G-G-T-G-T-T-C) 5'-[4-({2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}carbonyl)-*N*,*N*-dimethylpiperazine-1-phosphonamidate].

Golodirsen has the following structure:

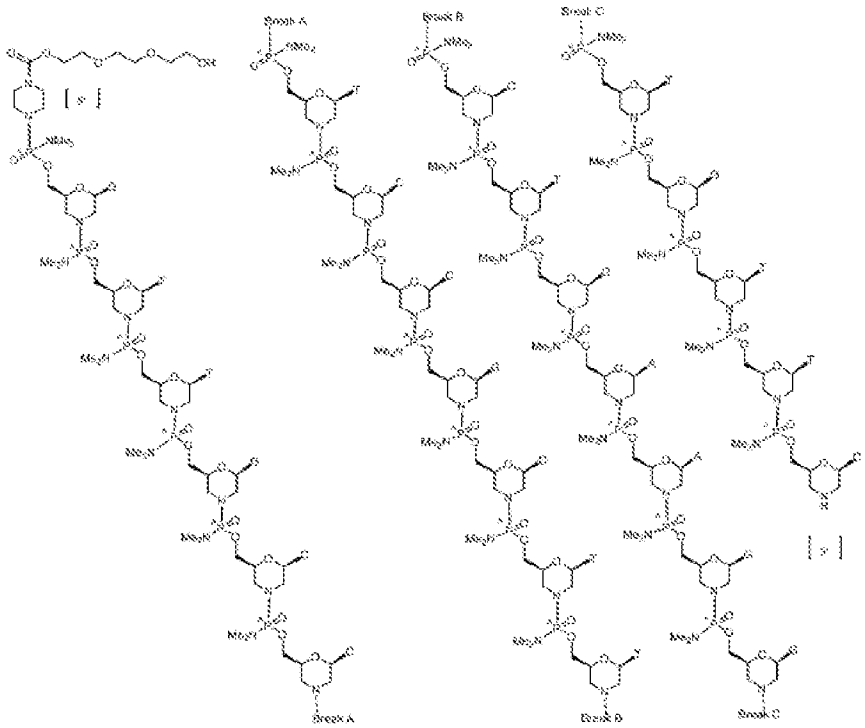


n = 1 - 24

B(1-25):

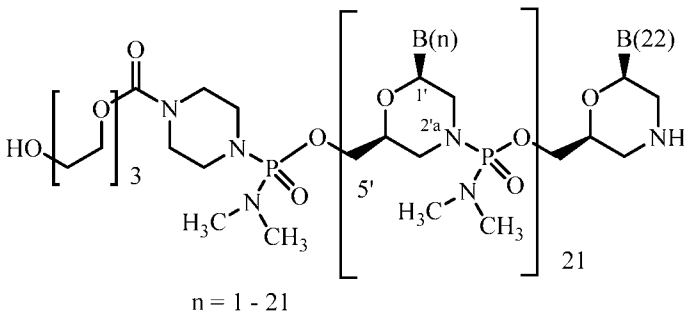
G-T-T-G-C-C-T-C-C-G-G-T-T-C-T-G-A-A-G-G-T-G-T-T-C (SEQ ID NO:1)

Golodirsen can also be depicted as shown below:



Casimersen (see e.g., Example 3 of U.S. Patent Application Publication No. US 2019/0262375, incorporated herein by reference in its entirety) has been the subject of clinical studies to test its safety and efficacy, and clinical development is ongoing. Casimersen is a phosphorodiamidate morpholino (PMO) antisense oligonucleotide. The dystrophin therapeutic "casimersen," is also known as "SRP-4045." Casimersen is a PMO having the base sequence 5'-CAATGCCATCCTGGAGTTCCTG-3' (SEQ ID NO: 2). Casimersen is registered under CAS Registry Number 1422958-19-7. Chemical names include: *all-P-ambo*-[P,2',3'-trideoxy-P-(dimethylamino)-2',3'-imino-2',3'-seco](2'a→5')(C-A-A-T-G-C-C-A-T-C-C-T-G-G-A-G-T-T-C-C-T-G) 5'-[4-({2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}carbonyl)-N,N-dimethylpiperazine-1-phosphonamidate].

Casimersen has the following structure:

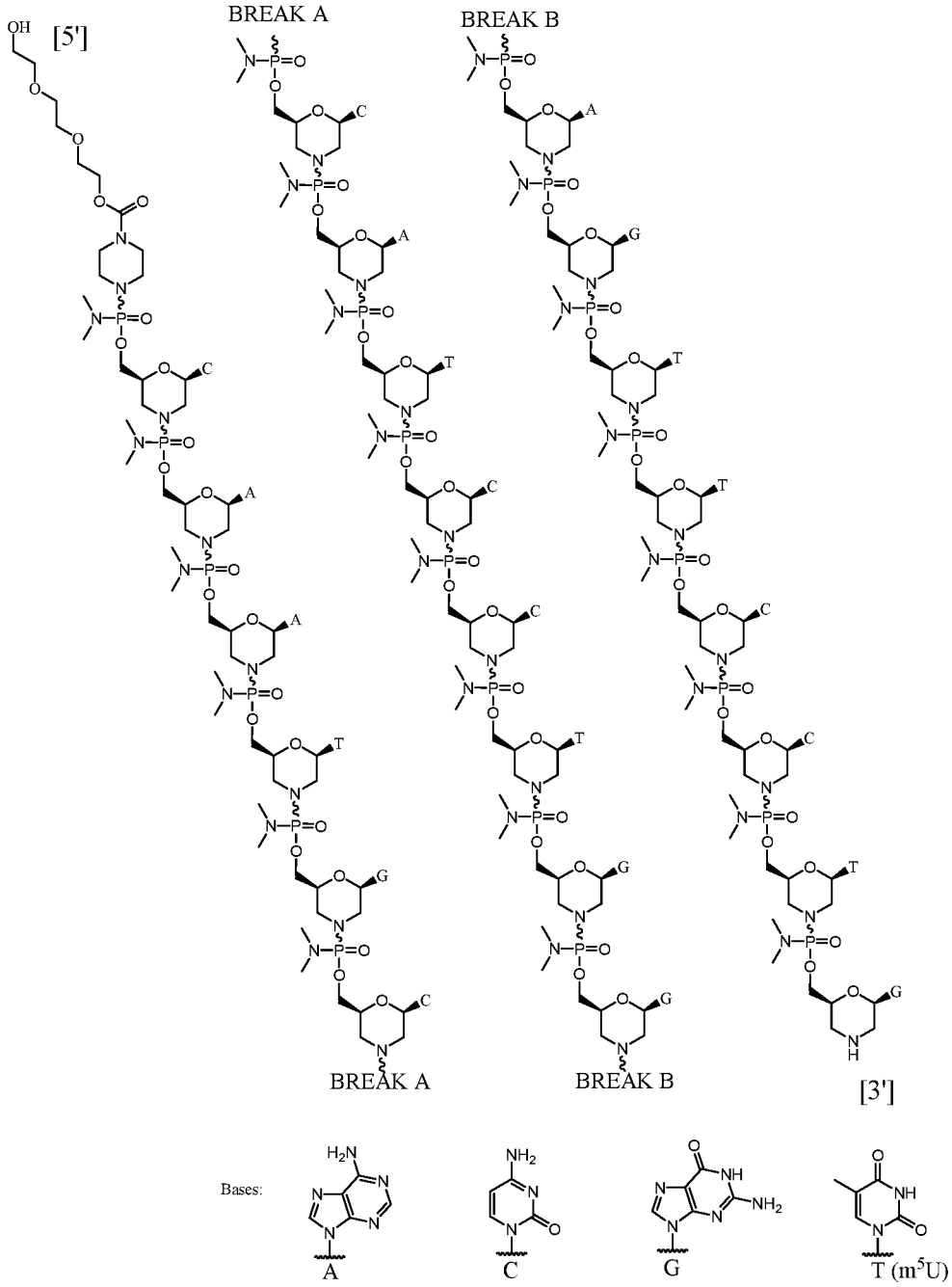


n = 1 - 21

B(1-22):

C-A-A-T-G-C-C-A-T-C-C-T-G-G-A-G-T-T-C-C-T-G (SEQ ID NO:2).

Casimersen can also be depicted as shown below:



The sequence of bases from the 5' end to the 3' end is:

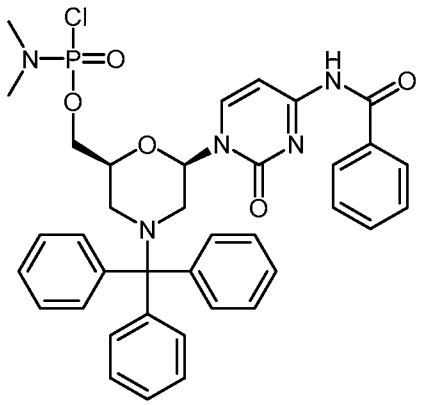
CAATGCCATCCTGGAGTTCCTG

For clarity, the structural formulae of golodirsen and casimersen is a continuous structural formula from 5' to 3', and, for the convenience of depicting the entire formula in a compact form in the above structural formulae, Applicants have included various illustration breaks labeled "BREAK A," "BREAK B," and "BREAK C." As would be understood by the

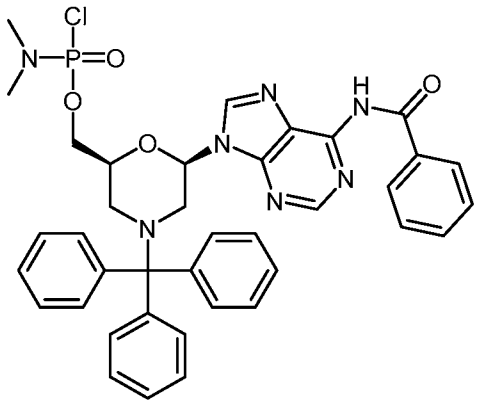
skilled artisan, for example, each indication of "BREAK A" shows a continuation of the illustration of the structural formula at these points. The skilled artisan understands that the same is true for each instance of "BREAK B," and "BREAK C" in the structural formulae of golodirsen and casimersen above. None of the illustration breaks, however, are intended to indicate, nor would the skilled artisan understand them to mean, an actual discontinuation of the structural formula of golodirsen and casimersen above.

Oligomeric compounds of the disclosure may have asymmetric centers, chiral axes, and chiral planes (as described, for example, in: E. L. Eliel and S. H. Wilen, *Stereochemistry of Carbon Compounds*, John Wiley & Sons, New York, 1994, pages 1119-1190, and March, J., *Advanced Organic Chemistry*, 3d. Ed., Chap. 4, John Wiley & Sons, New York (1985)), and may occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers and mixtures thereof, including optical isomers. Oligomeric compounds of the disclosure herein specifically mentioned, without any indication of their stereochemistry, are intended to represent all possible isomers and mixtures thereof.

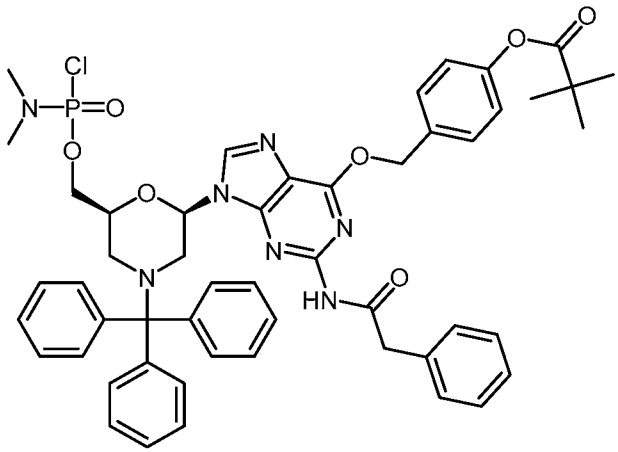
Specifically, without wishing to be bound by any particular theory, oligomeric compounds of the disclosure are prepared, as discussed herein, from activated morpholino subunits including Compound C, Compound D, Compound E, and Compound F:



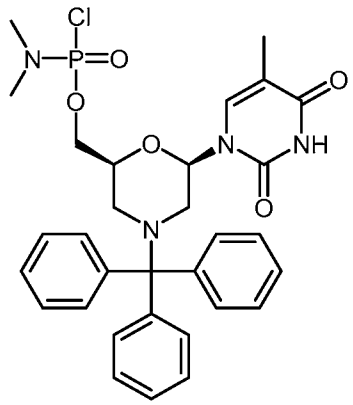
Compound (C)



Compound (D)

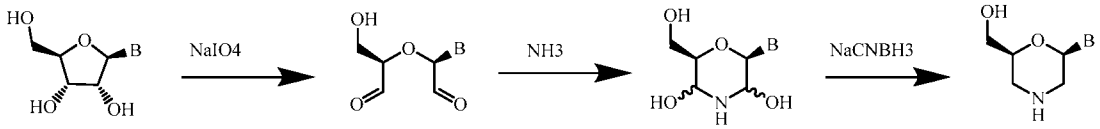


Compound (E)



Compound (F)

Each of Compound C, Compound D, Compound E, and Compound F may be prepared, for example, from the corresponding beta-D-ribofuranosyl as depicted below:



See Summerton et al., *Antisense Nucleic Acid Drug Dev.* 1997, 7, 187-195. Without being bound by any particular theory, the stereochemistry of the two chiral carbons is retained under the synthetic conditions. Without being bound by any particular theory, a number of possible additional stereoisomers of each morpholino subunit may otherwise be produced based on selection of, for example, an alpha-L- ribofuranosyl, alpha-D-ribofuranosyl, beta-L-ribofuranosyl, or beta-D-ribofuranosyl starting material. Without being bound by any particular theory, incorporation of 10 to 40 compounds independently selected from the group consisting of Compound C, Compound D, Compound E and Compound F, and the additional stereoisomers of each morpholino subunit for example, into an oligomeric compound may result in numerous possible stereoisomers. Without wishing to be bound by any particular theory, oligomeric compounds of the disclosure comprise one or more phosphorous-containing intersubunit linkages, which create a chiral center at each phosphorus, each of which is designated as either an "Sp" or "Rp" configuration as understood in the art. Without wishing to be bound by any particular theory, this chirality creates stereoisomers, which have identical chemical composition but different three-dimensional arrangement of their atoms. Without wishing to be bound by any particular theory, the configuration of each phosphorous intersubunit linkage occurs randomly during synthesis of, for example, oligomeric compounds of the disclosure. Without wishing to be bound by any particular theory, the synthesis process generates an exponentially large number of stereoisomers of an oligomeric compound of the disclosure because oligomeric compounds of the disclosure are comprised of numerous phosphorous-containing intersubunit linkages – with each phosphorous-containing intersubunit linkage having a random chiral configuration. Specifically, without wishing to be bound by any particular theory, each intersubunit linkage of an additional morpholino subunit doubles the number of stereoisomers of the product, so that a conventional preparation of an oligomeric compound of the disclosure is in fact a highly heterogeneous mixture of 2^N stereoisomers, where N represents the number of phosphorous-containing intersubunit linkages.

Pharmaceutical Compositions

Provided herein are pharmaceutical compositions comprising golodirsen, or a pharmaceutically acceptable salt thereof, wherein the concentration of golodirsen is about 50 mg/mL of the pharmaceutical composition. Provided herein are also pharmaceutical compositions comprising casimersen, or a pharmaceutically acceptable salt thereof, wherein the concentration of casimersen is about 50 mg/mL of the pharmaceutical composition. In certain embodiments, the compositions are suitable for use in treating a muscle disease.

Accordingly, in one aspect, provided herein is a pharmaceutical composition, comprising:

a) golodirsen;

b) sodium chloride;

c) potassium chloride;

d) potassium phosphate monobasic;

e) sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50 mg/mL of the pharmaceutical composition.

In yet another aspect, provided herein is a pharmaceutical composition, comprising:

a) 40-60 mg of golodirsen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water.

In one embodiment of this aspect, the pharmaceutical composition comprises about 50 mg of golodirsen. In another embodiment of this aspect, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 50 mg of golodirsen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of golodirsen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 80-120 mg of golodirsen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment of this aspect, the total volume of the pharmaceutical composition is about 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 100 mg of golodirsen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is about 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of golodirsen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 400-600 mg of golodirsen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the pharmaceutical composition comprises about 500 mg of golodirsen. In another embodiment, the total volume of the pharmaceutical composition is about 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 500 mg of golodirsen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is about 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of golodirsen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is 10 mL.

In embodiments including, for example, some embodiments of the pharmaceutical compositions discussed above, the concentration of golodirsen is about 50 mg/mL of the pharmaceutical composition. In some embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from about 45 mg/mL to about 55 mg/mL. In some embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from 45 mg/mL to 55 mg/mL. In certain embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from about 47.5 mg/mL to about 52.5 mg/mL. In certain embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from 47.5 mg/mL to 52.5 mg/mL. In some embodiments, the concentration of golodirsen in the pharmaceutical composition is about 50 mg/mL ± 10%. In some embodiments, the concentration of golodirsen in the pharmaceutical composition is 50 mg/mL ± 10%. In certain embodiments, the concentration of golodirsen in the pharmaceutical composition is within ± 10% of 50 mg/mL. In some embodiments, the concentration of golodirsen in the pharmaceutical composition is about 50 mg/mL ± 5%. In some embodiments, the concentration of golodirsen in the pharmaceutical composition is 50 mg/mL ± 5%.

In certain embodiments, the concentration of golodirsen in the pharmaceutical composition is within ± % of 50 mg/mL. In some embodiments, the concentration of golodirsen ranges from about 45.5 mg/mL to 55 mg/mL, about 46 mg/mL to about 54.5 mg/mL, about 46.5 mg/mL to about 54 mg/mL, about 47 mg/mL to about 53.5 mg/mL, about 47.5 mg/mL to about 53 mg/mL, about 45.5 mg/mL to about 52.5 mg/mL, about 45.5 mg/mL to about 52 mg/mL, about 48 mg/mL to about 51.5 mg/mL, about 48.5 mg/mL to about 51 mg/mL, about 49 mg/mL to about 50.5 mg/mL, or about 49.5 mg/mL to about 50 mg/mL of the pharmaceutical composition.

In some embodiments, the concentration of golodirsen in the pharmaceutical composition is about 45 mg/mL, 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition. In certain embodiments, the concentration of golodirsen is 45 mg/mL, 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 5 w/v % golodirsen;

b) about 0.8 w/v % sodium chloride;

c) about 0.02 w/v % potassium chloride;

d) about 0.02 w/v % potassium phosphate monobasic;

e) about 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the total volume of the composition is 10 mL.

In another embodiment of this aspect that specifies certain w/v percentages, the pharmaceutical composition comprises about 50 mg of golodirsen. In some embodiments, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 500 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 5 w/v % golodirsen;

b) 0.8 w/v % sodium chloride;

c) 0.02 w/v % potassium chloride;

d) 0.02 w/v % potassium phosphate monobasic;

e) 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment of this aspect that specifies certain w/v percentages, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 50 mg/mL golodirsen;

b) about 8 mg/mL sodium chloride;

c) about 0.2 mg/mL potassium chloride;

d) about 0.2 mg/mL potassium phosphate monobasic;

e) about 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the total volume of the composition is 10 mL.

In another embodiment, the pharmaceutical composition comprises about 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 500 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg/mL golodirsen;

b) 8 mg/mL sodium chloride;

c) 0.2 mg/mL potassium chloride;

d) 0.2 mg/mL potassium phosphate monobasic;

e) 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 50 mg of golodirsen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of golodirsen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 100 mg of golodirsen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is about 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of golodirsen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 500 mg of golodirsen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is about 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of golodirsen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of golodirsen;

b) 8 mg of sodium chloride, USP;

c) 0.2 mg of potassium chloride, USP;

d) 0.2 mg of potassium phosphate monobasic, NF;

e) 1.14 mg of sodium phosphate dibasic anhydrous, USP; and

f) water for injection, USP,

wherein the total volume of the pharmaceutical composition is 1 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of golodirsen;

b) 16 mg of sodium chloride, USP;

c) 0.4 mg of potassium chloride, USP;

d) 0.4 mg of potassium phosphate monobasic, NF;

e) 2.28 mg of sodium phosphate dibasic anhydrous, USP; and

f) water for injection, USP,

wherein the total volume of the pharmaceutical composition is 2 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of golodirsen;

b) 80 mg of sodium chloride, USP;

c) 2 mg of potassium chloride, USP;

d) 2 mg of potassium phosphate monobasic, NF;

e) 11.4 mg of sodium phosphate dibasic anhydrous, USP; and

f) water for injection, USP,

wherein the total volume of the pharmaceutical composition is 10 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of golodirsen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic anhydrous; and

f) water for injection,

wherein the total volume of the pharmaceutical composition is 1 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of golodirsen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic anhydrous; and

f) water for injection,

wherein the total volume of the pharmaceutical composition is 2 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of golodirsen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic anhydrous; and

f) water for injection,

wherein the total volume of the pharmaceutical composition is 10 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) casimersen;

b) sodium chloride;

c) potassium chloride;

d) potassium phosphate monobasic;

e) sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50 mg/mL of the pharmaceutical composition.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 40-60 mg of casimersen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water.

In one embodiment of this aspect, the pharmaceutical composition comprises about 50 mg of casimersen. In another embodiment of this aspect, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 50 mg of casimersen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of casimersen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 80-120 mg of casimersen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment of this aspect, the total volume of the pharmaceutical composition is about 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 100 mg of casimersen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is about 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of casimersen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 400-600 mg of casimersen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the pharmaceutical composition comprises about 500 mg of casimersen. In another embodiment, the total volume of the pharmaceutical composition is about 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 500 mg of casimersen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is about 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of casimersen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect, the total volume of the pharmaceutical composition is 10 mL.

In embodiments including, for example, some embodiments of the pharmaceutical compositions discussed above, the concentration of casimersen is about 50 mg/mL of the pharmaceutical composition. In some embodiments, the concentration of casimersen in the pharmaceutical composition ranges from about 45 mg/mL to about 55 mg/mL. In some

embodiments, the concentration of casimersen in the pharmaceutical composition ranges from 45 mg/mL to 55 mg/mL. In certain embodiments, the concentration of casimersen in the pharmaceutical composition ranges from about 47.5 mg/mL to about 52.5 mg/mL. In certain embodiments, the concentration of casimersen in the pharmaceutical composition ranges from 47.5 mg/mL to 52.5 mg/mL. In some embodiments, the concentration of casimersen in the pharmaceutical composition is about 50 mg/mL ± 10%. In some embodiments, the concentration of casimersen in the pharmaceutical composition is 50 mg/mL ± 10%. In certain embodiments, the concentration of casimersen in the pharmaceutical composition is within ± 10% of 50 mg/mL. In some embodiments, the concentration of casimersen in the pharmaceutical composition is about 50 mg/mL ± 5%. In some embodiments, the concentration of casimersen in the pharmaceutical composition is 50 mg/mL ± 5%.

In certain embodiments, the concentration of casimersen in the pharmaceutical composition is within ± % of 50 mg/mL. In some embodiments, the concentration of casimersen ranges from about 45.5 mg/mL to 55 mg/mL, about 46 mg/mL to about 54.5 mg/mL, about 46.5 mg/mL to about 54 mg/mL, about 47 mg/mL to about 53.5 mg/mL, about 47.5 mg/mL to about 53 mg/mL, about 45.5 mg/mL to about 52.5 mg/mL, about 45.5 mg/mL to about 52 mg/mL, about 48 mg/mL to about 51.5 mg/mL, about 48.5 mg/mL to about 51 mg/mL, about 49 mg/mL to about 50.5 mg/mL, or about 49.5 mg/mL to about 50 mg/mL of the pharmaceutical composition.

In some embodiments, the concentration of casimersen in the pharmaceutical composition is about 45 mg/mL, 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition. In certain embodiments, the concentration of casimersen is 45 mg/mL, 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 5 w/v % casimersen;

b) about 0.8 w/v % sodium chloride;

c) about 0.02 w/v % potassium chloride;

d) about 0.02 w/v % potassium phosphate monobasic;

e) about 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the total volume of the composition is 10 mL.

In another embodiment of this aspect that specifies certain w/v percentages, the pharmaceutical composition comprises about 50 mg of casimersen. In some embodiments, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 500 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 5 w/v % casimersen;

b) 0.8 w/v % sodium chloride;

c) 0.02 w/v % potassium chloride;

d) 0.02 w/v % potassium phosphate monobasic;

e) 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment of this aspect that specifies certain w/v percentages, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 50 mg/mL casimersen;

b) about 8 mg/mL sodium chloride;

c) about 0.2 mg/mL potassium chloride;

d) about 0.2 mg/mL potassium phosphate monobasic;

e) about 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the total volume of the composition is 10 mL.

In another embodiment, the pharmaceutical composition comprises about 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 500 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg/mL casimersen;

b) 8 mg/mL sodium chloride;

c) 0.2 mg/mL potassium chloride;

d) 0.2 mg/mL potassium phosphate monobasic;

e) 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 50 mg of casimersen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of casimersen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 1 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 100 mg of casimersen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is about 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of casimersen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 2 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) about 500 mg of casimersen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is about 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of casimersen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 10 mL.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of casimersen;

b) 8 mg of sodium chloride, USP;

c) 0.2 mg of potassium chloride, USP;

d) 0.2 mg of potassium phosphate monobasic, NF;

e) 1.14 mg of sodium phosphate dibasic anhydrous, USP; and

f) water for injection, USP,

wherein the total volume of the pharmaceutical composition is 1 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of casimersen;

b) 16 mg of sodium chloride, USP;

c) 0.4 mg of potassium chloride, USP;

d) 0.4 mg of potassium phosphate monobasic, NF;

e) 2.28 mg of sodium phosphate dibasic anhydrous, USP; and

f) water for injection, USP,

wherein the total volume of the pharmaceutical composition is 2 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of casimersen;

b) 80 mg of sodium chloride, USP;

c) 2 mg of potassium chloride, USP;

d) 2 mg of potassium phosphate monobasic, NF;

e) 11.4 mg of sodium phosphate dibasic anhydrous, USP; and

f) water for injection, USP,

wherein the total volume of the pharmaceutical composition is 10 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 50 mg of casimersen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic anhydrous; and

f) water for injection,

wherein the total volume of the pharmaceutical composition is 1 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 100 mg of casimersen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic anhydrous; and

f) water for injection,

wherein the total volume of the pharmaceutical composition is 2 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In another aspect, provided herein is a pharmaceutical composition, comprising:

a) 500 mg of casimersen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic anhydrous; and

f) water for injection,

wherein the total volume of the pharmaceutical composition is 10 mL, the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In some embodiments including, for example, some embodiments discussed above, the pH of the pharmaceutical composition is about 7.5 or is 7.5. In some embodiments, the pH of the pharmaceutical composition is adjusted to about pH 7.5 with NaOH, NF, HCl, NF, or a combination thereof.

In certain embodiments including, for example, some embodiments discussed above, the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm. In some embodiments, the pH of the pharmaceutical composition is about 7.5 and the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In a further embodiment, pharmaceutical compositions of the disclosure may additionally comprise a carbohydrate as provided in Han et al., *Nat. Comms.* 2016, 7, 10981, the entirety of which is incorporated herein by reference. In some embodiments, pharmaceutical compositions of the disclosure may comprise 5% of a hexose carbohydrate. For example, pharmaceutical composition of the disclosure may comprise 5% glucose, 5% fructose, or 5% mannose. In certain embodiments, pharmaceutical compositions of the disclosure may comprise 2.5% glucose and 2.5% fructose. In some embodiments, pharmaceutical compositions of the disclosure may comprises a carbohydrate selected from: arabinose present in an amount of 5% by volume, glucose present in an amount of 5% by volume, sorbitol present in an amount of 5% by volume, galactose present in an amount of 5% by volume, fructose present in an amount of 5% by volume, xylitol present in an amount of 5% by volume, mannose present in an amount of 5% by volume, a combination of glucose and fructose each present in an amount of 2.5% by volume, and a combination of glucose present in an amount of 5.7% by volume, fructose present in an amount of 2.86% by volume, and xylitol present in an amount of 1.4% by volume.

Methods

Provided herein are methods of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the disclosure.

Accordingly, in one aspect, provided herein is a method of treating a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition disclosed herein. In one embodiment, the muscle disease is Duchenne muscular dystrophy.

In another aspect, provided herein is a method of preventing a muscle disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition disclosed herein. In one embodiment, the muscle disease is Duchenne muscular dystrophy.

In an additional aspect, provided herein is a method for treating Duchenne muscular dystrophy in a subject in need thereof wherein the subject has a mutation of the dystrophin gene that is amenable to exon 53 skipping, comprising administering to the subject a pharmaceutical composition of the disclosure comprising golodirsen.

In an additional aspect, provided herein is a method for treating Duchenne muscular dystrophy in a subject in need thereof wherein the subject has a mutation of the dystrophin gene that is amenable to exon 45 skipping, comprising administering to the subject a pharmaceutical composition of the disclosure comprising casimersen.

The subject considered herein is typically a human. However, the subject can be any mammal for which treatment is desired. Thus, the methods described herein can be applied to both human and veterinary applications.

It will be appreciated that pharmaceutical compositions provided herein may be administered by any means known in the art. The pharmaceutical compositions provided herein are more preferably delivered by intravenous, intra-arterial, intraperitoneal, intramuscular, or subcutaneous routes of administration.

Accordingly, in one aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 40-60 mg of golodirsen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water.

In one embodiment of this method, the pharmaceutical composition comprises about 50 mg of golodirsen. In another embodiment of this method, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 50 mg of golodirsen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 80-120 mg of golodirsen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment of this method, the total volume of the pharmaceutical composition is about 2 mL. In another embodiment of this method, the total volume of the pharmaceutical composition is 2 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 100 mg of golodirsen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the total volume of the pharmaceutical composition is about 2 mL. In an embodiment of this method, the total volume of the pharmaceutical composition is 2 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 400-600 mg of golodirsen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the pharmaceutical composition comprises about 500 mg of golodirsen. In another embodiment, the total volume of the pharmaceutical composition is about 10 mL. In an embodiment of this method, the pharmaceutical composition comprises 500 mg of golodirsen. In another embodiment, the total volume of the pharmaceutical composition is 10 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 500 mg of golodirsen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the total volume of the pharmaceutical composition is about 10 mL. In an embodiment of this method, the total volume of the pharmaceutical composition is 10 mL.

In embodiments including, for example, some embodiments of the pharmaceutical compositions discussed above, the concentration of golodirsen is about 50 mg/mL of the pharmaceutical composition. In some embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from about 45 mg/mL to about 55 mg/mL. In some embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from 45 mg/mL to 55 mg/mL. In certain embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from about 47.5 mg/mL to about 52.5 mg/mL. In certain embodiments, the concentration of golodirsen in the pharmaceutical composition ranges from 47.5 mg/mL to 52.5 mg/mL. For example, the concentration of golodirsen in the pharmaceutical composition ranges from

In some embodiments, the concentration of golodirsen in the pharmaceutical composition is about 50 mg/mL ± 10%. In some embodiments, the concentration of golodirsen in the pharmaceutical composition is 50 mg/mL ± 10%. In certain embodiments, the concentration of golodirsen in the pharmaceutical composition is within ± 10% of 50 mg/mL. In some embodiments, the concentration of golodirsen in the pharmaceutical

composition is about 50 mg/mL ± 5%. In some embodiments, the concentration of golodirsen in the pharmaceutical composition is 50 mg/mL ± 5%.

In certain embodiments, the concentration of golodirsen in the pharmaceutical composition is within ± 5% of 50 mg/mL. In some embodiments, the concentration of golodirsen ranges from about 45.5 mg/mL to 55 mg/mL, about 46 mg/mL to about 54.5 mg/mL, about 46.5 mg/mL to about 54 mg/mL, about 47 mg/mL to about 53.5 mg/mL, about 47.5 mg/mL to about 53 mg/mL, about 45.5 mg/mL to about 52.5 mg/mL, about 45.5 mg/mL to about 52 mg/mL, about 48 mg/mL to about 51.5 mg/mL, about 48.5 mg/mL to about 51 mg/mL, about 49 mg/mL to about 50.5 mg/mL, or about 49.5 mg/mL to about 50 mg/mL of the pharmaceutical composition.

In some embodiments, the concentration of golodirsen in the pharmaceutical composition is about 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition. In certain embodiments, the concentration of golodirsen is 45 mg/mL, 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 5 w/v % golodirsen;

b) about 0.8 w/v % sodium chloride;

c) about 0.02 w/v % potassium chloride;

d) about 0.02 w/v % potassium phosphate monobasic;

e) about 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this method that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the total volume of the composition is 10 mL.

In another embodiment of this method that specifies certain w/v percentages, the pharmaceutical composition comprises about 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 500 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 5 w/v % golodirsen;

b) 0.8 w/v % sodium chloride;

c) 0.02 w/v % potassium chloride;

d) 0.02 w/v % potassium phosphate monobasic;

e) 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment of this aspect that specifies certain w/v percentages, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another embodiment of this method that specifies certain w/v percentages, the pharmaceutical composition comprises about 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 500 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 50 mg/mL golodirsen;

b) about 8 mg/mL sodium chloride;

c) about 0.2 mg/mL potassium chloride;

d) about 0.2 mg/mL potassium phosphate monobasic;

e) about 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the pharmaceutical composition comprises about 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises about 500 mg of golodirsen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 50 mg/mL golodirsen;

b) 8 mg/mL sodium chloride;

c) 0.2 mg/mL potassium chloride;

d) 0.2 mg/mL potassium phosphate monobasic;

e) 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment, the pharmaceutical composition comprises 50 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 100 mg of golodirsen. In another embodiment, the pharmaceutical composition comprises 500 mg of golodirsen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 50 mg of golodirsen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 1 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 100 mg of golodirsen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 2 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 500 mg of golodirsen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 10 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 40-60 mg of casimersen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water.

In one embodiment of this method, the pharmaceutical composition comprises about 50 mg of casimersen. In another embodiment of this method, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 50 mg of casimersen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the total volume of the pharmaceutical composition is about 1 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 80-120 mg of casimersen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment of this method, the total volume of the pharmaceutical composition is about 2 mL. In another embodiment of this method, the total volume of the pharmaceutical composition is 2 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 100 mg of casimersen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the total volume of the pharmaceutical composition is about 2 mL. In an embodiment of this method, the total volume of the pharmaceutical composition is 2 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 400-600 mg of casimersen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the pharmaceutical composition comprises about 500 mg of casimersen. In another embodiment, the total volume of the pharmaceutical composition is about 10 mL. In an embodiment of this method, the pharmaceutical composition comprises 500 mg of casimersen. In another embodiment, the total volume of the pharmaceutical composition is 10 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 500 mg of casimersen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water.

In an embodiment of this method, the total volume of the pharmaceutical composition is about 10 mL. In an embodiment of this method, the total volume of the pharmaceutical composition is 10 mL.

In embodiments including, for example, some embodiments of the pharmaceutical compositions discussed above, the concentration of casimersen is about 50 mg/mL of the pharmaceutical composition. In some embodiments, the concentration of casimersen in the pharmaceutical composition ranges from about 45 mg/mL to about 55 mg/mL. In some embodiments, the concentration of casimersen in the pharmaceutical composition ranges from 45 mg/mL to 55 mg/mL. In certain embodiments, the concentration of casimersen in the pharmaceutical composition ranges from about 47.5 mg/mL to about 52.5 mg/mL. In certain embodiments, the concentration of casimersen in the pharmaceutical composition ranges from 47.5 mg/mL to 52.5 mg/mL. For example, the concentration of casimersen in the pharmaceutical composition ranges from

In some embodiments, the concentration of casimersen in the pharmaceutical composition is about 50 mg/mL ± 10%. In some embodiments, the concentration of casimersen in the pharmaceutical composition is 50 mg/mL ± 10%. In certain embodiments, the concentration of casimersen in the pharmaceutical composition is within ± 10% of 50

mg/mL. In some embodiments, the concentration of casimersen in the pharmaceutical composition is about 50 mg/mL ± 5%. In some embodiments, the concentration of casimersen in the pharmaceutical composition is 50 mg/mL ± 5%.

In certain embodiments, the concentration of casimersen in the pharmaceutical composition is within ± 5% of 50 mg/mL. In some embodiments, the concentration of casimersen ranges from about 45.5 mg/mL to 55 mg/mL, about 46 mg/mL to about 54.5 mg/mL, about 46.5 mg/mL to about 54 mg/mL, about 47 mg/mL to about 53.5 mg/mL, about 47.5 mg/mL to about 53 mg/mL, about 45.5 mg/mL to about 52.5 mg/mL, about 45.5 mg/mL to about 52 mg/mL, about 48 mg/mL to about 51.5 mg/mL, about 48.5 mg/mL to about 51 mg/mL, about 49 mg/mL to about 50.5 mg/mL, or about 49.5 mg/mL to about 50 mg/mL of the pharmaceutical composition.

In some embodiments, the concentration of casimersen in the pharmaceutical composition is about 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition. In certain embodiments, the concentration of casimersen is 45 mg/mL, 45.5 mg/mL, 46 mg/mL, 46.5 mg/mL, 47 mg/mL, 47.5 mg/mL, 48 mg/mL, 48.5 mg/mL, 49 mg/mL, 49.5 mg/mL, 50 mg/mL, 50.5 mg/mL, 51 mg/mL, 51.5 mg/mL, 52 mg/mL, 52.5 mg/mL, 53 mg/mL, 53.5 mg/mL, 54 mg/mL, 54.5 mg/mL, or 55 mg/mL of the pharmaceutical composition.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 5 w/v % casimersen;

b) about 0.8 w/v % sodium chloride;

c) about 0.02 w/v % potassium chloride;

d) about 0.02 w/v % potassium phosphate monobasic;

e) about 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this method that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the total volume of the composition is 10 mL.

In another embodiment of this method that specifies certain w/v percentages, the pharmaceutical composition comprises about 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 500 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 5 w/v % casimersen;

b) 0.8 w/v % sodium chloride;

c) 0.02 w/v % potassium chloride;

d) 0.02 w/v % potassium phosphate monobasic;

e) 0.114 w/v % sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain w/v percentages, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment of this aspect that specifies certain w/v percentages, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another embodiment of this method that specifies certain w/v percentages, the pharmaceutical composition comprises about 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 500 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) about 50 mg/mL casimersen;

b) about 8 mg/mL sodium chloride;

c) about 0.2 mg/mL potassium chloride;

d) about 0.2 mg/mL potassium phosphate monobasic;

e) about 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is about 1 mL. In another embodiment, the total volume of the composition is about 2 mL. In another embodiment, the total volume of the composition is about 10 mL. In another embodiment, the pharmaceutical composition comprises about 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises about 500 mg of casimersen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 50 mg/mL casimersen;

b) 8 mg/mL sodium chloride;

c) 0.2 mg/mL potassium chloride;

d) 0.2 mg/mL potassium phosphate monobasic;

e) 1.14 mg/mL sodium phosphate dibasic; and

f) water.

In an embodiment of this aspect that specifies certain mg/mL ratios, the total volume of the composition is 1-10 mL. In another embodiment, the total volume of the composition is 1 mL. In another embodiment, the total volume of the composition is 2 mL. In another embodiment, the total volume of the composition is 10 mL. In another embodiment, the pharmaceutical composition comprises 50 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 100 mg of casimersen. In another embodiment, the pharmaceutical composition comprises 500 mg of casimersen.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 50 mg of casimersen;

b) 8 mg of sodium chloride;

c) 0.2 mg of potassium chloride;

d) 0.2 mg of potassium phosphate monobasic;

e) 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 1 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 100 mg of casimersen;

b) 16 mg of sodium chloride;

c) 0.4 mg of potassium chloride;

d) 0.4 mg of potassium phosphate monobasic;

e) 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 2 mL.

In another aspect, methods of the disclosure comprise administering to the subject a pharmaceutical composition, the pharmaceutical composition comprising:

a) 500 mg of casimersen;

b) 80 mg of sodium chloride;

c) 2 mg of potassium chloride;

d) 2 mg of potassium phosphate monobasic;

e) 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the total volume of the pharmaceutical composition is 10 mL.

In some embodiments including, for example, some embodiments discussed above, the pH of the pharmaceutical composition is about 7.5 or is 7.5. In some embodiments, the pH of the pharmaceutical composition is adjusted to about pH 7.5 with NaOH, NF, HCl, NF, or a combination thereof.

In certain embodiments including, for example, some embodiments discussed above, the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm. In some embodiments, the pH of the pharmaceutical composition is about 7.5 and the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

In a further embodiment, the pharmaceutical compositions of the disclosure may be co-administered with a carbohydrate in the methods of the disclosure, either in the same formulation or is a separate formulation, as provided in Han et al., *Nat. Comms.* 2016, 7, 10981, the entirety of which is incorporated herein by reference. In some embodiments, pharmaceutical compositions of the disclosure may be co-administered with 5% of a hexose carbohydrate. For example, pharmaceutical compositions of the disclosure may be co-

administered with 5% glucose, 5% fructose, or 5% mannose. In certain embodiments, pharmaceutical compositions of the disclosure may be co-administered with 2.5% glucose and 2.5% fructose. In some embodiments, pharmaceutical composition of the disclosure may be co-administered with a carbohydrate selected from: arabinose present in an amount of 5% by volume, glucose present in an amount of 5% by volume, sorbitol present in an amount of 5% by volume, galactose present in an amount of 5% by volume, fructose present in an amount of 5% by volume, xylitol present in an amount of 5% by volume, mannose present in an amount of 5% by volume, a combination of glucose and fructose each present in an amount of 2.5% by volume, and a combination of glucose present in an amount of 5.7% by volume, fructose present in an amount of 2.86% by volume, and xylitol present in an amount of 1.4% by volume.

Kits

In other embodiments, kits are provided. Kits according to the disclosure include package(s) comprising golodirsen or casimersen, or pharmaceutical compositions of the disclosure. In some embodiments, kits comprise golodirsen, or a pharmaceutically acceptable salt thereof. In some embodiments, kits comprise casimersen, or a pharmaceutically acceptable salt thereof.

The phrase "package" means any vessel containing oligonucleotides or compositions presented herein. In some embodiments, the package can be a box or wrapping. Packaging materials for use in packaging pharmaceutical products are well-known to those of skill in the art. Examples of pharmaceutical packaging materials include, but are not limited to, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

The kit can also contain items that are not contained within the package, but are attached to the outside of the package, for example, pipettes.

Kits can further contain instructions for administering golodirsen, casimersen, or pharmaceutical compositions of the disclosure to a patient. Kits also can comprise instructions for approved uses of golodirsen or casimersen by regulatory agencies, such as the United States Food and Drug Administration. Kits can also contain labeling or product inserts for golodirsen or casimersen. The package(s) or any product insert(s), or both, may themselves be approved by regulatory agencies. The kits can include golodirsen or casimersen in the solid phase or in a liquid phase (such as buffers provided) in a package. The

kits can also include buffers for preparing solutions for conducting the methods, and pipettes for transferring liquids from one container to another.

EXAMPLES

Examples have been set forth below for the purpose of illustration and to describe certain specific embodiments of the disclosure. However, the scope of the claims is not to be in any way limited by the examples set forth herein. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art and such changes and modifications including, without limitation, those relating to the chemical structures, substituents, derivatives, formulations or methods of the disclosure may be made without departing from the spirit of the disclosure and the scope of the appended claims. Definitions of the variables in the structures in the schemes herein are commensurate with those of corresponding positions in the formulae presented herein.

Example 1: 50L Solid-phase Synthesis of Golodirsen Crude Drug Substance

1. Materials

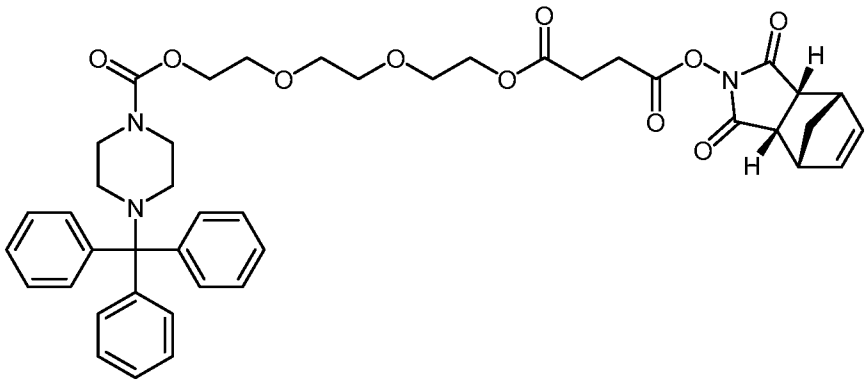
Table 1: Starting Materials

Material Name	Chemical Name	CAS Number	Chemical Formula	Molecular Weight
Activated A Subunit	Phosphoramidochloridic acid, *N*,*N*-dimethyl-,[6-[6-(benzoylamino)-9H-purin-9-yl]-4-(triphenylmethyl)-2-morpholinyl]methyl ester	1155373-30-0	$C_{38}H_{37}ClN_7O_4P$	722.2
Activated C Subunit	Phosphoramidochloridic acid, *N*,*N*-dimethyl-,[6-[4-(benzoylamino)-2-oxo-1(2H)-pyrimidinyl]-4-(triphenylmethyl)-2-morpholinyl]methyl ester	1155373-31-1	$C_{37}H_{37}ClN_5O_5P$	698.2

Activated DPG Subunit	Propanoic Acid, 2,2-dimethyl-,4-[[[9-[6-[[[chloro(dimethylamino)phosphinyl]oxy]methyl]-4-(triphenylmethyl)-2-morpholinyl]-2-[(2-phenylacetyl)amino]-9H-purin-6-yl]oxy]methyl]phenyl ester	1155309-89-9	$C_{51}H_{53}ClN_7O_7P$	942.2
Activated T Subunit	Phosphoramidochloridic acid, *N*,*N*-dimethyl-,[6-(3,4-dihydro-5-methyl-2,4-dioxo-1(2H)-pyrimidinyl)]-4-(triphenylmethyl)-2-morpholinyl]methyl ester	1155373-34-4	$C_{31}H_{34}ClN_4O_5P$	609.1
Activated EG3 Tail	Butanedioic acid, 1-[3aR,4S,7R,7aS)-1,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-methano-2H-isoindol-2-yl] 4-[2-[2-[2-[[[4-(triphenylmethyl)-1-piperazinyl]carbonyl]oxy]ethoxy]ethoxy]ethyl] ester	1380600-06-5	$C_{43}H_{47}N_3O_{10}$	765.9

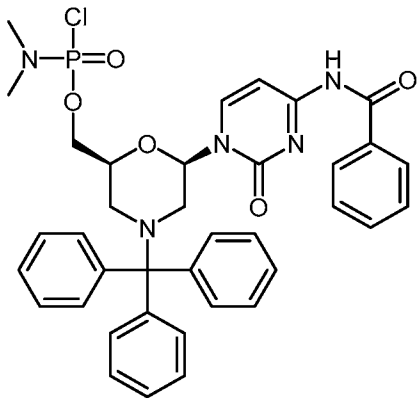
Chemical Structures of Starting Materials:

A. Activated EG3 Tail (For preparation, see WO 2017/205880)



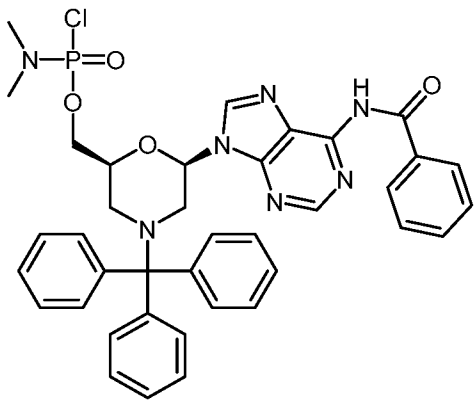
Compound (B)

B. Activated C Subunit (For preparation, see U.S. Patent No. 8,067,571)



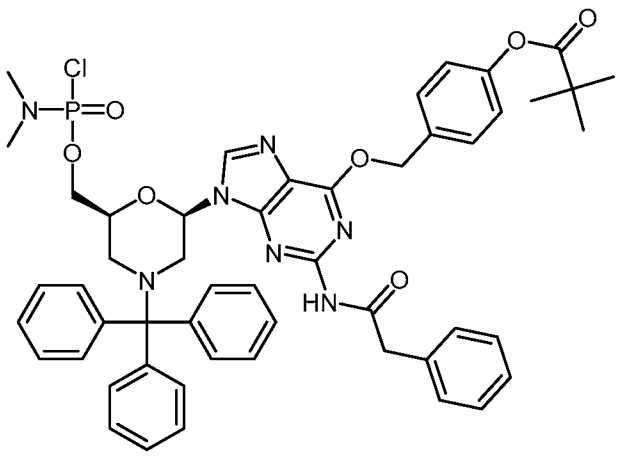
Compound (C1)

C. Activated A Subunit (For preparation, see U.S. Patent No. 8,067,571)



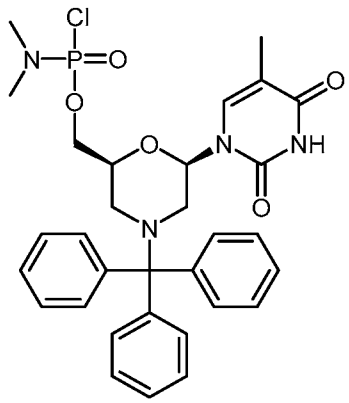
Compound (D1)

D. Activated DPG Subunit (For preparation, see WO 2009/064471)



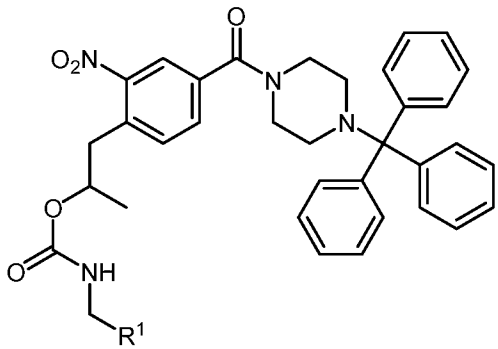
Compound (DPG1)

E. Activated T Subunit (For preparation, see WO 2013/082551)



Compound (T1)

F. Anchor Loaded Resin (For preparation, see WO 2017/205880)



Formula (I)

wherein R^1 is a support-medium.

Table 2: Description of Solutions for Solid Phase Oligomer Synthesis of Golodirsen Crude Drug Substance

Solution Name	Solution Composition
NCP2 Anchor Solution	37.5L NMP and 1292g NCP2 Anchor
DEDC Capping Solution	4.16L Diethyl Dicarbonate (DEDC), 3.64L NEM, and 33.8L DCM
CYTFA Solution	2.02 kg 4-cyanopyridine, 158 L DCM, 1.42 L TFA, 39 L TFE, and 2 L purified water
Neutralization Solution	35.3 L IPA, 7.5 L DIPEA, and 106.5 L DCM
Cleavage Solution	1,530.04 g DTT, 6.96 L NMP, and 2.98 L DBU

2. Synthesis of Golodirsen Crude Drug Substance

A. Resin swelling

750 g of Anchor Loaded Resin and 10.5 L of NMP were charged to a 50 L silanized reactor and stirred for 3 hours. The NMP was drained and the Anchor Loaded Resin was washed twice with 5.5L each of DCM and twice with 5.5 L each of 30% TFE/DCM.

B. Cycle 0: EG3 Tail Coupling

The Anchor Loaded Resin was washed three times with 5.5 L each of 30% TFE/DCM and drained, washed with 5.5 L of CYFTA solution for 15 minutes and drained, and again washed with 5.5 L of CYTFA Solution for 15 minutes without draining to which 122 mL of 1:1 NEM/DCM was charged and the suspension stirred for 2 minutes and drained. The resin was washed once with 5.5L Neutralization Solution for 10 minutes and drained, twice with 5.5 L of Neutralization Solution for 5 minutes and drained, then twice with 5.5 L each of DCM and drained. A solution of 706.2 g of activated EG3 Tail (MW 765.85) and 234 mL of NEM in 3 L of DMI was charged to the resin and stirred for 3 hours at RT and drained. The resin was washed once with 5.5 L of Neutralization Solution for 10 minutes and drained, once with 5.5 L of Neutralization Solution for 5 minutes and drained, and once with 5.5 L of DCM and drained. A solution of 374.8 g of benzoic anhydride and 195 mL NEM in 2680 mL NMP was charged and stirred for 15 minutes and drained. The resin was once with 5.5 L of Neutralization Solution for 10 minutes and drained, once with 5.5 L of Neutralization Solution for 5 minutes and drained, and once with 5.5 L of DCM and drained and twice with 5.5 L each of 30% TFE/DCM. The resin was suspended in 5.5 L of 30% TFE/DCM and held for 14 hours.

C. Subunit Coupling Cycles 1-30

i. Pre-coupling treatments

Prior to each coupling cycle as described in **Table 3**, the resin was: 1) washed with 30% TFE/DCM; 2) a) treated with CYTFA Solution 15 minutes and drained, and b) treated with CYTFA solution for 15 minutes to which was added 1:1 NEM/DCM, stirred, and drained; 3) stirred three times with Neutralization Solution; and 4) washed twice with DCM. See **Table 3**.

ii. Post Coupling Treatments

After each subunit solution was drained as described in **Table 3**, the resin was: 1) washed with DCM; and 2) washed three times with 30% TFE/DCM. If the resin was held for a time period prior to the next coupling cycle, the third TFE/DCM wash was not drained and the resin was retained in said TFE/DCM wash solution. See **Table 3**.

iii. Activated Subunit Coupling Cycles

The coupling cycles were performed as described in **Table 3**.

iv. Final IPA Washing

After the final coupling step was performed as described in **Table 3**, the resin was washed 8 times with 19.5 L each of IPA, and dried under vacuum at room temperature for about 63.5 hours to a dried weight of 4857.9 g.

D. Cleavage

The above resin bound golodirsen Crude Drug Substance was divided into two lots, each lot was treated as follows. A 1619.3g lot of resin was: 1) stirred with 10L of NMP for 2hrs, then the NMP was drained; 2) washed tree times with 10L each of 30% TFE/DCM; 3) treated with 10L CYTFA Solution for 15 minutes; and 4) 10L of CYTFA Solution for 15 minutes to which 130ml 1:1 NEM/DCM was then added and stirred for 2 minutes and drained. The resin was treated three times with 10L each of Neutralization Solution, washed six times with 10L of DCM, and eight times with 10L each of NMP. The resin was treated with a Cleaving Solution of 1530.4g DTT and 2980 DBU in 6.96L NMP for 2 hours to detach the golodirsen Crude Drug Substance from the resin. The Cleaving Solution was drained and retained in a separate vessel. The reactor and resin were washed with 4.97L of NMP which was combined with the Cleaving Solution.

Table 3:

Cycle No.: Subunit (SU)	Pre-coupling Treatment				Coupling Cycle		Post-Coupling Treatment	
	1	2	3	4			1	2
	30% TFE/DCM Wash	CYTFA Solution[1]	Neutralization Solution	DCM Wash	Quantity SU (g) NEM (L) DMI (L)	RT Coupling Time (Hrs.)	DCM Wash	30% TFE/DCM Wash
1:DPG	5.5L	a) 5,5L b) 5.5L, 122ml	3x5.5L	5.5L	536.7g; 195 ml NEM; 3.2L DMI	5	5.5L	3x5.5L
2:T	7.0L	a) 7L b) 7L, 158ml	3x7L	2x7L	468.2g and 195ml NEM 3.2L DMI	4.25	7L	3x7L
3:T	8L	a) 8L b) 8L, 182ml	3x8L	2x8L	536.7g; 195ml NEM; 3.4L DMI	4.25	8L	3x8L
4:DPG	9L	a) 9L b) 9L, 206ml	3x9L	2x9L	536.7g; 195ml NEM; 3.6L DMI	4.25	9L	3x9L
5:C	9.5L	a) 9.5L b) 9.5L, 220ml	3x9.5L	2x9.5L	555.2g; 195ml NEM; 3.4L DMI	4.25	9.5L	3x9.5L
6:C	10L	a) 10L b) 10L, 232ml	3x10L	2x10L	555.2g; 195ml NEM; 3.45L DMI	4.25	10L	3x10L
7:T	11L	a) 11L b) 11L, 256ml	3x11L	2x11L	536.7g; 195ml NEM; 3.57L DMI	4.25	11L	3x11L
8:C	11L	a) 11L b) 11L, 256ml	3x11L	2x11L	555.2g; 195ml NEM; 3.64L DMI	4.25	11L	3x11L

[1] ml indicates the amount of 1:1 NEM/DCM

Cycle No.: Subunit (SU)	Pre-coupling Treatment				Coupling Cycle		Post-Coupling Treatment	
	1	2	3	4			1	2
	30% TFE/DCM Wash	CYTFA Solution[1]	Neutralization Solution	DCM Wash	Quantity SU (g) NEM (L) DMI (L)	RT Coupling Time (Hrs.)	DCM Wash	30% TFE/DCM Wash
9:C	11.5L	a) 11.5L b) 11.5L 268ml	3x11.5L	2x 11.5L	468.2g; 195ml NEM; 3.72L DMI	4.25	11.5L	3x11.5L
10:DPG	12L	a) 12L b) 12L, 280ml	3x12L	2x12L	536.7g; 195ml NEM; 3.96L DMI	4.25	12L	3x12L
11:DPG	13.5L	a) 13.5L b) 13.5L, 204ml	3x13.5L	2x 13.5L	721.7g; 253ml NEM; 4.02L DMI	4.25	13.5L	3x13.5L
12:T	13.5L	a) 13.5L b) 13.5L, 204ml	3x13.5L	2x 13.5L	721.7g; 253ml NEM; 4.02L DMI	4.25	13.5L	3x13.5L
13:T	14L	a) 14L b) 14L, 216ml	3x14L	2x14L	941.9g; 253ml NEM; 4.02L DMI	4.25	14L	3x14L
14:C	14.5L	a) 14.5L b) 14.5L, 228ml	3x14.5L	2x 14.5L	941.9g; 253ml NEM; 4.1L DMI	4.25	14.5L	3x14.5L
15:T	15.5L	a) 15.5L b) 15.5L, 254ml	3x15.5L	2x 15.5L	721.7g; 253ml NEM; 4.26L DMI	4.25	15.5L	3x15.5L
16:DPG	15.5L	a) 15.5L b) 15.5L, 254ml	3x15.5L	2x 15.5L	721.7g; 253ml NEM; 4.26L DMI	4.25	15.5L	3x15.5L
17:A	16L	a) 16L b) 16L, 366ml	3x16L	2x16L	941.9g; 253ml NEM; 4.4L DMI	4.75	16L	3x16L

Cycle No.: Subunit (SU)	Pre-coupling Treatment				Coupling Cycle		Post-Coupling Treatment	
	1	2	3	4			1	2
	30% TFE/DCM Wash	CYTFA Solution[1]	Neutralization Solution	DCM Wash	Quantity SU (g) NEM (L) DMI (L)	RT Coupling Time (Hrs.)	DCM Wash	30% TFE/DCM Wash
18:A	16.5L	a) 16.5L b) 16.5L, 378ml	3x16.5L	2x 16.5L	721.7g; 253ml NEM; 4.4L DMI	4.25	16.5L	3x16.5L
19: DPG	16.5L	a) 16.5L b) 16.5L, 378ml	3x16.5L	2x 16.5L	608.7g; 253ml NEM; 4.57L DMI	4.25	16.5L	3x16.5L
20:DPG	17L	a) 17L b) 17L, 390ml	3x17L	2x17L	941.9g; 253ml NEM; 4.57L DMI	4.75	17L	3x17L
21:T	17L	a) 17L b) 17L, 390ml	3x17L	2x17L	1159.2g; 311ml NEM; 4.72L DMI	4.25	17L	3x17L
22: DPG	17.5L	a) 17.5L b) 17.5L, 402ml	3x17.5L	2x 17.5L	858.7g; 311ml NEM; 4.72L DMI	4.75	17.5L	3x17.5L
23:T	17.5L	a) 17.5L b) 17.5L, 402ml	3x17.5L	2x 17.5L	888.3g; 311ml NEM; 4.88L DMI	4.25	17.5L	3x17.5L
24:T	18L	a) 18L b) 18L, 414ml	3x18L	2x18L	749.1g; 311ml NEM; 4.95L DMI	4.25	18L	3x18L
25:C	18L	a) 18L b) 18L, 414ml	3x18L	2x18L	749.1g; 311ml NEM; 5.1L DMI	4.25	18L	3x18L

E. Deprotection

The combined Cleaving Solution and NMP wash were transferred to a pressure vessel to which was added 39.8L of NH_4OH (NH_3•H_2O) that had been chilled to a temperature of -

10° to -25° C in a freezer. The pressure vessel was sealed and heated to 45° C for 16hrs then allowed to cool to 25° C. This deprotection solution containing the golodirsen crude drug substance was diluted 3:1 with purified water prior to solvent removal. During solvent removal, the deprotection solution was pH adjusted to 3.0 with 2M phosphoric acid, then to pH 8.03 with NH_4OH. HPLC: C18 77.552% (**Fig. 1**) and SCX-10 73.768% (**Fig. 2**).

Example 2: Purification of Golodirsen Crude Drug Substance

The deprotection solution from Example 1, part E, containing the golodirsen crude drug substance was loaded onto a column of ToyoPearl Super-Q 650S anion exchange resin (Tosoh Bioscience) and eluted with a gradient of 0-35% B over 17 column volume (Buffer A: 10 mM sodium hydroxide; Buffer B: 1 M sodium chloride in 10 mM sodium hydroxide) and fractions of acceptable purity (C18 and SCX HPLC) were pooled to a purified drug product solution. HPLC: 93.571% (**C18; Fig. 3**) 88.270% (SCX; **Fig. 4**).

The purified drug substance solution was desalted and lyophilized to 1450.72 g purified golodirsen drug substance. Yield 54.56 %; HPLC: 93.531% (**Fig. 5**; C18) 88.354% (**Fig. 6**; SCX).

Table 4. Acronyms

Acronym	**Name**
DBU	1,8-Diazabicycloundec-7-ene
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMI	1,3-Dimethyl-2-imidazolidinone
DTT	Dithiothreitol
IPA	Isopropyl alcohol
MW	Molecular weight
NEM	N-Ethylmorpholine
NMP	N-Methyl-2-pyrrolidone
RT	Room temperature
TFA	2,2,2-Trifluoroacetic acid
TFE	2,2,2-Trifluoroethanol

Example 3: Exemplary Golodirsen Pharmaceutical Compositions

Table 5.

Exemplary Composition 1: Total volume of 1 mL	
Golodirsen	50 mg
sodium chloride, USP	8 mg
potassium chloride, USP	0.2 mg
potassium phosphate monobasic, NF	0.2 mg
sodium phosphate dibasic anhydrous, USP	1.14 mg
Water for injection, USP	q.s.

Exemplary Composition 2: Total volume of 2 mL	
Golodirsen	100 mg
sodium chloride, USP	16 mg
potassium chloride, USP	0.4 mg
potassium phosphate monobasic, NF	0.4 mg
sodium phosphate dibasic anhydrous, USP	2.28 mg
water for injection, USP	q.s.

Exemplary Composition 3: Total volume of 10 mL	
Golodirsen	500 mg
sodium chloride, USP	80 mg
potassium chloride, USP	2 mg
potassium phosphate monobasic, NF	2 mg
sodium phosphate dibasic anhydrous, USP	11.4 mg
water for injection, USP	q.s.

5

Exemplary Composition 4: Total volume of 1 mL	
Golodirsen	50 mg
sodium chloride	8 mg
potassium chloride	0.2 mg
potassium phosphate monobasic	0.2 mg
sodium phosphate dibasic anhydrous	1.14 mg
Water for injection	q.s.

Exemplary Composition 5: Total volume of 2 mL	
Golodirsen	100 mg
sodium chloride	16 mg
potassium chloride	0.4 mg
potassium phosphate monobasic	0.4 mg
sodium phosphate dibasic anhydrous	2.28 mg
water for injection	q.s.

Exemplary Composition 6: Total volume of 10 mL	
Golodirsen	500 mg
sodium chloride	80 mg
potassium chloride	2 mg

potassium phosphate monobasic	2 mg
sodium phosphate dibasic anhydrous	11.4 mg
water for injection	q.s.

For each of Exemplary Compositions 1-6 (Table 5), the amount of water present is sufficient to achieve a concentration of golodirsen of about 50 mg/mL of the pharmaceutical composition. Further, the pH of the Exemplary Compositions is about 7.5, and the osmolality of the Exemplary Compositions ranges from about 260 mOsm to about 320 mOsm.

Example 4: 50L Solid-phase Synthesis of Casimersen Crude Drug Substance

1. Materials

The materials, including the starting materials and the solutions used for solid phase oligomer synthesis of casimersen crude drug substance are the same as those used above in Example 1 for solid phase oligomer synthesis of golodirsen crude drug substance and described above in **Table 1** and **Table 2**.

2. Synthesis of Casimersen Crude Drug Substance

A. Resin swelling

750 g of Anchor Loaded Resin and 10.5 L of NMP were charged to a 50 L silanized reactor and stirred for 3 hours. The NMP was drained and the Anchor Loaded Resin was washed twice with 5.5L each of DCM and twice with 5.5 L each of 30% TFE/DCM.

B. Cycle 0: EG3 Tail Coupling

The Anchor Loaded Resin was washed three times with 5.5 L each of 30% TFE/DCM and drained, washed with 5.5 L of CYFTA solution for 15 minutes and drained, and again washed with 5.5 L of CYTFA Solution for 15 minutes without draining to which 122 mL of 1:1 NEM/DCM was charged and the suspension stirred for 2 minutes and drained. The resin was washed once with 5.5L Neutralization Solution for 10 minutes and drained, twice with 5.5 L of Neutralization Solution for 5 minutes and drained, then twice with 5.5 L each of DCM and drained. A solution of 706.2 g of activated EG3 Tail (MW 765.85) and 234 mL of NEM in 3 L of DMI was charged to the resin and stirred for 3 hours at RT and drained. The resin was washed once with 5.5 L of Neutralization Solution for 10 minutes and drained, once with 5.5 L of Neutralization Solution for 5 minutes and drained, and once with 5.5 L of DCM and drained. A solution of 374.8 g of benzoic anhydride and 195 mL NEM in 2680 mL NMP was charged and stirred for 15 minutes and drained. The resin was washed once

with 5.5 L of Neutralization Solution for 10 minutes and drained, once with 5.5 L of Neutralization Solution for 5 minutes and drained, and once with 5.5 L of DCM and drained and twice with 5.5 L each of 30% TFE/DCM. The resin was suspended in 5.5 L of 30% TFE/DCM and held for 14 hours.

C. Subunit Coupling Cycles 1-22

i. Pre-coupling treatments

Prior to each coupling cycle as described in **Table 6**, the resin was: 1) washed with 30% TFE/DCM; 2) a) treated with CYTFA Solution 15 minutes and drained, and b) treated with CYTFA solution for 15 minutes to which was added 1:1 NEM/DCM, stirred, and drained; 3) stirred three times with Neutralization Solution; and 4) washed twice with DCM. See **Table 6**.

ii. Post Coupling Treatments

After each subunit solution was drained as described in **Table 6**, the resin was: 1) washed with DCM; and 2) washed three times with 30% TFE/DCM. If the resin was held for a time period prior to the next coupling cycle, the third TFE/DCM wash was not drained and the resin was retained in said TFE/DCM wash solution. See **Table 6**.

iii. Activated Subunit Coupling Cycles

The coupling cycles were performed as described in **Table 6**.

iv. Final IPA Washing

After the final coupling step was performed as described in **Table 6**, the resin was washed 8 times with 19.5 L each of IPA, and dried under vacuum at room temperature for about 63.5 hours to a dried weight of 4523 g.

D. Cleavage

The above resin bound Casimersen Crude Drug Substance was divided into two lots, each lot was treated as follows. Two 2261.5 g lots of resin were each: 1) stirred with 10L of NMP for 2hrs, then the NMP was drained; 2) washed tree times with 10L each of 30% TFE/DCM; 3) treated with 10L CYTFA Solution for 15 minutes; and 4) 10L of CYTFA Solution for 15 minutes to which 130ml 1:1 NEM/DCM was then added and stirred for 2 minutes and drained. The resin was treated three times with 10L each of Neutralization Solution, washed six times with 10L of DCM, and eight times with 10L each of NMP. The resin was treated with a Cleaving Solution of 1530.4g DTT and 2980 DBU in 6.96L NMP for 2 hours to detach the Casimersen Crude Drug Substance from the resin. The Cleaving Solution was drained and retained in a separate vessel. The reactor and resin were washed with 4.97L of NMP which was combined with the Cleaving Solution.

Table 6:

Cycle No.: Subunit (SU)	Pre-coupling Treatment				Coupling Cycle		Post-Coupling Treatment	
	1	2	3	4			1	2
	30% TFE/DCM Wash	CYTFA Solution[2]	Neutralization Solution	DCM Wash	Quantity SU (g) NEM (L) DMI (L)	RT Coupling Time (Hrs.)	DCM Wash	30% TFE/DCM Wash
1:C	5.5L	a) 5,5L b) 5.5L, 122ml	3x5.5L	5.5L	584g; 195 ml NEM; 3.2L DMI	5	5.5L	3x5.5L
2:A	7.0L	a) 7L b) 7L, 158ml	3x7L	2x7L	592.2g and 195ml NEM 3.2L DMI	4.25	7L	3x7L
3:A	8L	a) 8L b) 8L, 182ml	3x8L	2x8L	592.2g; 195ml NEM; 3.4L DMI	4.25	8L	3x8L
4:T	9L	a) 9L b) 9L, 206ml	3x9L	2x9L	514.2g; 195ml NEM; 3.6L DMI	4.25	9L	3x9L
5:G	9.5L	a) 9.5L b) 9.5L, 220ml	3x9.5L	2x9.5L	755.5g; 195ml NEM; 3.4L DMI	4.25	9.5L	3x9.5L
6:C	10L	a) 10L b) 10L, 232ml	3x10L	2x10L	584.4g; 195ml NEM; 3.45L DMI	4.25	10L	3x10L
7:C	11L	a) 11L b) 11L, 256ml	3x11L	2x11L	584.5g; 195ml NEM; 3.57L DMI	4.25	11L	3x11L

[2] ml indicates the amount of 1:1 NEM/DCM

Cycle No.: Subunit (SU)	Pre-coupling Treatment				Coupling Cycle		Post-Coupling Treatment	
	1	2	3	4			1	2
	30% TFE/DCM Wash	CYTFA Solution[2]	Neutralization Solution	DCM Wash	Quantity SU (g) NEM (L) DMI (L)	RT Coupling Time (Hrs.)	DCM Wash	30% TFE/DCM Wash
8:A	11L	a) 11L b) 11L, 256ml	3x11L	2x11L	592.5g; 195ml NEM; 3.64L DMI	4.25	11L	3x11L
9:T	11.5L	a) 11.5L b) 11.5L 268ml	3x11.5L	2x 11.5L	514.5g; 195ml NEM; 3.72L DMI	4.25	11.5 L	3x11.5L
10:C	12L	a) 12L b) 12L, 280ml	3x12L	2x12 L	338.5g; 195ml NEM; 3.96L DMI	4.25	12L	3x12L
11:C	13.5L	a) 13.5L b) 13.5L, 204ml	3x13.5L	2x 13.5L	770.4g; 253ml NEM; 4.02L DMI	4.25	13.5 L	3x13.5L
12:T	13.5L	a) 13.5L b) 13.5L, 204ml	3x13.5L	2x 13.5L	668.7g; 253ml NEM; 4.02L DMI	4.25	13.5 L	3x13.5L
13:DPG	14L	a) 14L b) 14L, 216ml	3x14L	2x14 L	982.3g; 253ml NEM; 4.02L DMI	4.25	14L	3x14L
14:DPG	14.5L	a) 14.5L b) 14.5L, 228ml	3x14.5L	2x 14.5L	982.3g; 253ml NEM; 4.1L DMI	4.25	14.5 L	3x14.5L
15:A	15.5L	a) 15.5L b) 15.5L, 254ml	3x15.5L	2x 15.5L	770.1g; 253ml NEM; 4.26L DMI	4.25	15.5 L	3x15.5L
16:DPG	15.5L	a) 15.5L	3x15.5L	2x 15.5L	982.4g; 253ml NEM;	4.25	15.5 L	3x15.5L

Cycle No.: Subunit (SU)	Pre-coupling Treatment				Coupling Cycle		Post-Coupling Treatment	
	1	2	3	4			1	2
	30% TFE/DCM Wash	CYTFA Solution[2]	Neutralization Solution	DCM Wash	Quantity SU (g) NEM (L) DMI (L)	RT Coupling Time (Hrs.)	DCM Wash	30% TFE/DCM Wash
		b) 15.5L, 254ml			4.26L DMI			
17:T	16L	a) 16L b) 16L, 366ml	3x16L	2x16L	549.6g; 253ml NEM; 4.4L DMI	4.75	16L	3x16L
18:T	16.5L	a) 16.5L b) 16.5L, 378ml	3x16.5L	2x 16.5L	630.7g; 253ml NEM; 4.4L DMI	4.25	16.5L	3x16.5L
19:C	16.5L	a) 16.5L b) 16.5L, 378ml	3x16.5L	2x 16.5L	770.4g; 253ml NEM; 4.57L DMI	4.25	16.5L	3x16.5L
20:C	17L	a) 17L b) 17L, 390ml	3x17L	2x17L	770.4g; 253ml NEM; 4.57L DMI	4.75	17L	3x17L
21:T	17L	a) 17L b) 17L, 390ml	3x17L	2x17L	776.4g; 311ml NEM; 4.72L DMI	4.25	17L	3x17L
22:DPG	17.5L	a) 17.5L b) 17.5L, 402ml	3x17.5L	2x 17.5L	1208.7g; 311ml NEM; 4.72L DMI	4.75	17.5L	3x17.5L

E. Deprotection

The combined Cleaving Solution and NMP wash were transferred to a pressure vessel to which was added 39.8L of NH_4OH ($NH_3 \cdot H_2O$) that had been chilled to a temperature of -10° to -25° C in a freezer. The pressure vessel was sealed and heated to 45° C for 16hrs then allowed to cool to 25° C. This deprotection solution containing the Casimersen crude drug substance was diluted 3:1 with purified water prior to solvent removal. During solvent

removal, the deprotection solution was pH adjusted to 3.0 with 2M phosphoric acid, then to pH 8.03 with NH_4OH. HPLC: C18 80.93% **(Fig. 7)** and SCX-10 84.4% **(Fig. 8)**.

Example 5: Purification of Casimersen Crude Drug Substance

The deprotection solution from Example 4, part E, containing the Casimersen crude drug substance was loaded onto a column of ToyoPearl Super-Q 650S anion exchange resin (Tosoh Bioscience) and eluted with a gradient of 0-35% B over 17 column volume (Buffer A: 10 mM sodium hydroxide; Buffer B: 1 M sodium chloride in 10 mM sodium hydroxide) and fractions of acceptable purity (C18 and SCX HPLC) were pooled to a purified drug product solution. HPLC: 97.74% **(C18; Fig. 9)** 94.58% (SCX; **Fig. 10**).

The purified drug substance solution was desalted and lyophilized to 1477.82 g purified Casimersen drug substance. Yield 63.37%; HPLC: 96.045% **(Fig. 11**; C18) 96.346% **(Fig. 12**; SCX).

Example 6: Exemplary Casimersen Pharmaceutical Compositions

Table 7.

Exemplary Composition 7: Total volume of 1 mL	
Casimersen	50 mg
sodium chloride, USP	8 mg
potassium chloride, USP	0.2 mg
potassium phosphate monobasic, NF	0.2 mg
sodium phosphate dibasic anhydrous, USP	1.14 mg
Water for injection, USP	q.s.

Exemplary Composition 8: Total volume of 2 mL	
Casimersen	100 mg
sodium chloride, USP	16 mg
potassium chloride, USP	0.4 mg
potassium phosphate monobasic, NF	0.4 mg
sodium phosphate dibasic anhydrous, USP	2.28 mg
water for injection, USP	q.s.

Exemplary Composition 9: Total volume of 10 mL	
Casimersen	500 mg
sodium chloride, USP	80 mg
potassium chloride, USP	2 mg
potassium phosphate monobasic, NF	2 mg
sodium phosphate dibasic anhydrous, USP	11.4 mg
water for injection, USP	q.s.

Exemplary Composition 10: Total volume of 1 mL	
Casimersen	50 mg
sodium chloride	8 mg
potassium chloride	0.2 mg
potassium phosphate monobasic	0.2 mg
sodium phosphate dibasic anhydrous	1.14 mg
Water for injection	q.s.

Exemplary Composition 11: Total volume of 2 mL	
Casimersen	100 mg
sodium chloride	16 mg
potassium chloride	0.4 mg
potassium phosphate monobasic	0.4 mg
sodium phosphate dibasic anhydrous	2.28 mg
water for injection	q.s.

Exemplary Composition 12: Total volume of 10 mL	
Casimersen	500 mg
sodium chloride	80 mg
potassium chloride	2 mg
potassium phosphate monobasic	2 mg
sodium phosphate dibasic anhydrous	11.4 mg
water for injection	q.s.

For each of Exemplary Compositions 7-12 (Table 7), the amount of water present is sufficient to achieve a concentration of casimersen of about 50 mg/mL of the pharmaceutical composition. Further, the pH of the Exemplary Compositions is about 7.5, and the osmolality of the Exemplary Compositions ranges from about 260 mOsm to about 320 mOsm.

Incorporation by Reference

The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

What is claimed is:

1. A pharmaceutical composition, comprising:

a) golodirsen;

b) sodium chloride;

c) potassium chloride;

d) potassium phosphate monobasic;

e) sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

2. The pharmaceutical composition of claim 1, comprising:

a) 40-60 mg of golodirsen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

3. The pharmaceutical composition of claim 2, comprising about 50 mg of golodirsen.

4. The pharmaceutical composition of claim 1, comprising:

a) about 50 mg of golodirsen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

5. The pharmaceutical composition of claim 1, comprising:

a) 80-120 mg of golodirsen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

6. The pharmaceutical composition of claim 5, comprising about 100 mg of golodirsen.

7. The pharmaceutical composition of claim 5, comprising:

a) about 100 mg of golodirsen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

8. The pharmaceutical composition of claim 1, comprising:

a) 400-600 mg of golodirsen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

9. The pharmaceutical composition of claim 8, comprising about 500 mg of golodirsen.

10. The pharmaceutical composition of claim 8, comprising:

a) about 500 mg of golodirsen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of golodirsen is about 50mg/mL of the pharmaceutical composition.

11. The pharmaceutical composition of claim 1, comprising:

a) about 5 w/v % golodirsen;

b) about 0.8 w/v % sodium chloride;

c) about 0.02 w/v % potassium chloride;

d) about 0.02 w/v % potassium phosphate monobasic;

e) about 0.114 w/v % sodium phosphate dibasic; and

f) water.

12. The pharmaceutical composition of claim 1, comprising:

a) about 50 mg/mL golodirsen;

b) about 8 mg/mL sodium chloride;

c) about 0.2 mg/mL potassium chloride;

d) about 0.2 mg/mL potassium phosphate monobasic;

e) about 1.14 mg/mL sodium phosphate dibasic; and

f) water.

13. The pharmaceutical composition according to any one of claims 1-12, wherein the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

14. A method for treating Duchenne muscular dystrophy (DMD) in a subject in need thereof wherein the subject has a mutation of the dystrophin gene that is amenable to exon 53

skipping, comprising administering to the subject a pharmaceutical composition of any one of claims 1-13.

15. Use of the pharmaceutical composition of any one of claims 1-13 for the manufacture of a medicament for the treatment Duchenne muscular dystrophy (DMD) in a subject in need thereof, wherein the subject has a mutation of the dystrophin gene that is amenable to exon 53 skipping.

16. A pharmaceutical composition, comprising:

a) casimersen;

b) sodium chloride;

c) potassium chloride;

d) potassium phosphate monobasic;

e) sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

17. The pharmaceutical composition of claim 16, comprising:

a) 40-60 mg of casimersen;

b) 6.4-9.6 mg of sodium chloride;

c) 0.16-0.24 mg of potassium chloride;

d) 0.16-0.24 mg of potassium phosphate monobasic;

e) 0.91-1.37 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

18. The pharmaceutical composition of claim 17, comprising about 50 mg of casimersen.

19. The pharmaceutical composition of claim 16, comprising:

a) about 50 mg of casimersen;

b) about 8 mg of sodium chloride;

c) about 0.2 mg of potassium chloride;

d) about 0.2 mg of potassium phosphate monobasic;

e) about 1.14 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

20. The pharmaceutical composition of claim 16, comprising:

a) 80-120 mg of casimersen;

b) 12.8-19.2 mg of sodium chloride;

c) 0.32-0.48 mg of potassium chloride;

d) 0.32-0.48 mg of potassium phosphate monobasic;

e) 1.82-2.74 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

21. The pharmaceutical composition of claim 20, comprising about 100 mg of casimersen.

22. The pharmaceutical composition of claim 20, comprising:

a) about 100 mg of casimersen;

b) about 16 mg of sodium chloride;

c) about 0.4 mg of potassium chloride;

d) about 0.4 mg of potassium phosphate monobasic;

e) about 2.28 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

23. The pharmaceutical composition of claim 16, comprising:

a) 400-600 mg of casimersen;

b) 64-96 mg of sodium chloride;

c) 1.6-2.4 mg of potassium chloride;

d) 1.6-2.4 mg of potassium phosphate monobasic;

e) 9.1-13.7 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

24. The pharmaceutical composition of claim 23, comprising about 500 mg of casimersen.

25. The pharmaceutical composition of claim 23, comprising:

a) about 500 mg of casimersen;

b) about 80 mg of sodium chloride;

c) about 2 mg of potassium chloride;

d) about 2 mg of potassium phosphate monobasic;

e) about 11.4 mg of sodium phosphate dibasic; and

f) water,

wherein the concentration of casimersen is about 50mg/mL of the pharmaceutical composition.

26. The pharmaceutical composition of claim 16, comprising:

a) about 5 w/v % casimersen;

b) about 0.8 w/v % sodium chloride;

c) about 0.02 w/v % potassium chloride;

d) about 0.02 w/v % potassium phosphate monobasic;

e) about 0.114 w/v % sodium phosphate dibasic; and

f) water.

27. The pharmaceutical composition of claim 16, comprising:

a) about 50 mg/mL casimersen;

b) about 8 mg/mL sodium chloride;

c) about 0.2 mg/mL potassium chloride;

d) about 0.2 mg/mL potassium phosphate monobasic;

e) about 1.14 mg/mL sodium phosphate dibasic; and

f) water.

28. The pharmaceutical composition according to any one of claims 16-27, wherein the pH of the pharmaceutical composition is about 7.5, and the osmolality of the pharmaceutical composition ranges from about 260 mOsm to about 320 mOsm.

29. A method for treating Duchenne muscular dystrophy (DMD) in a subject in need thereof wherein the subject has a mutation of the dystrophin gene that is amenable to exon 45 skipping, comprising administering to the subject a pharmaceutical composition of any one of claims 16-28.

30. Use of the pharmaceutical composition of any one of claims 16-28 for the manufacture of a medicament for the treatment Duchenne muscular dystrophy (DMD) in a subject in need thereof, wherein the subject has a mutation of the dystrophin gene that is amenable to exon 45 skipping.

Fig. 1

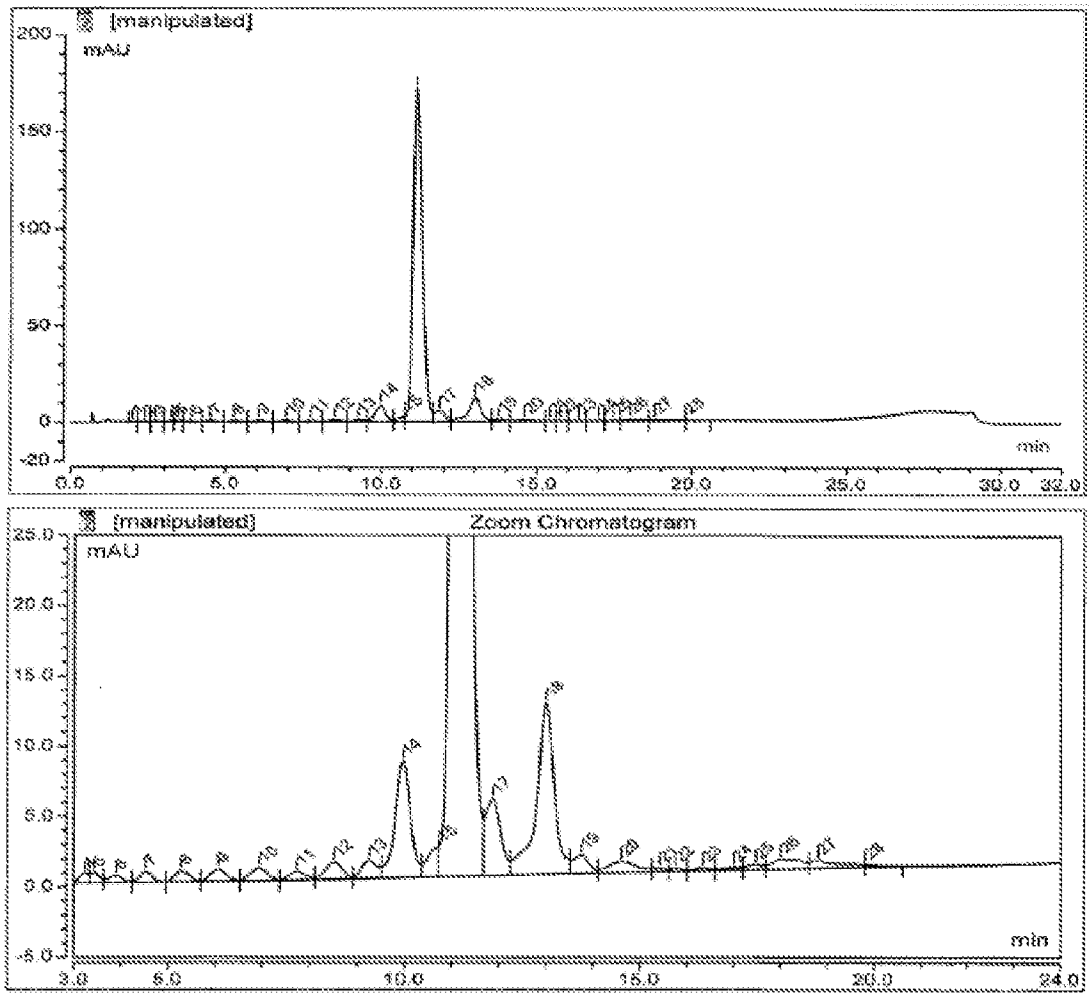

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Fig. 2

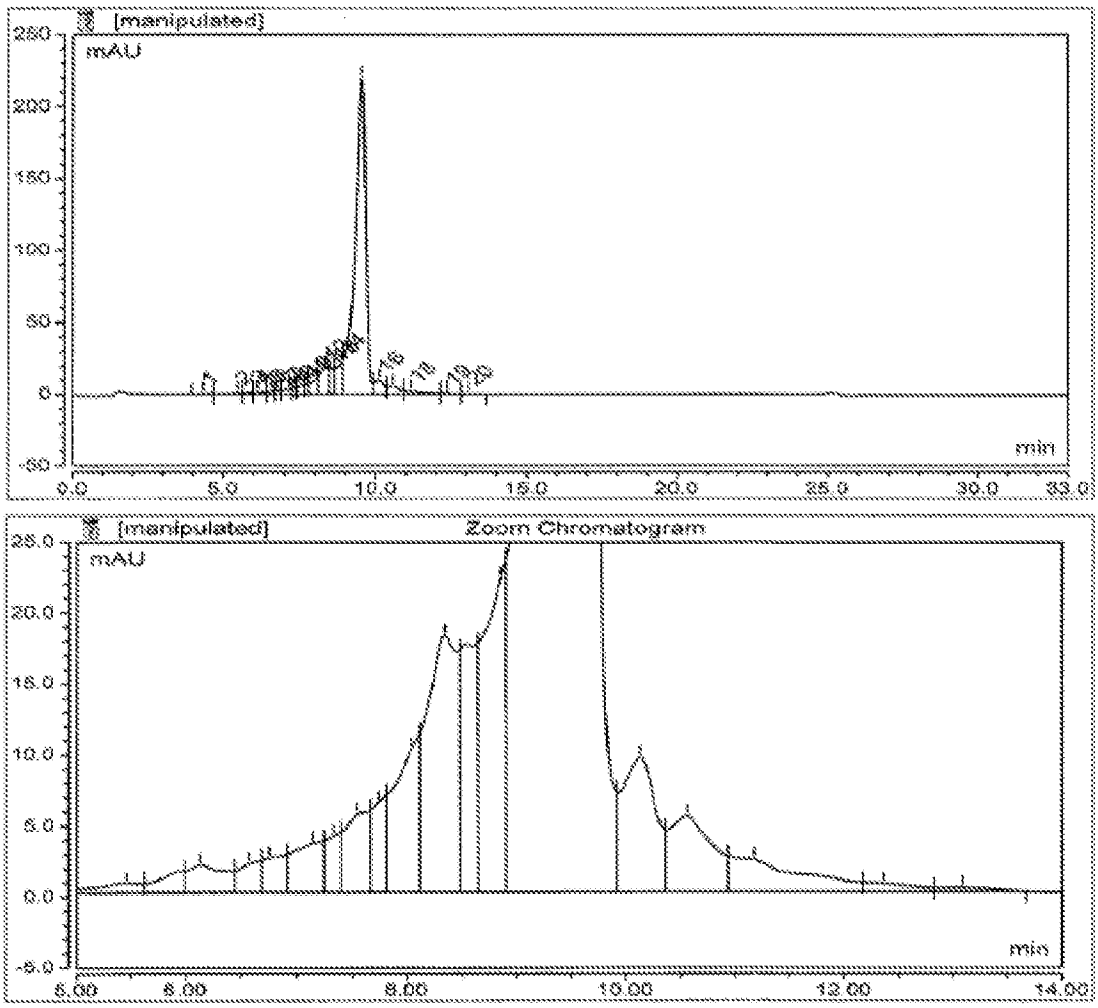
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Fig. 3

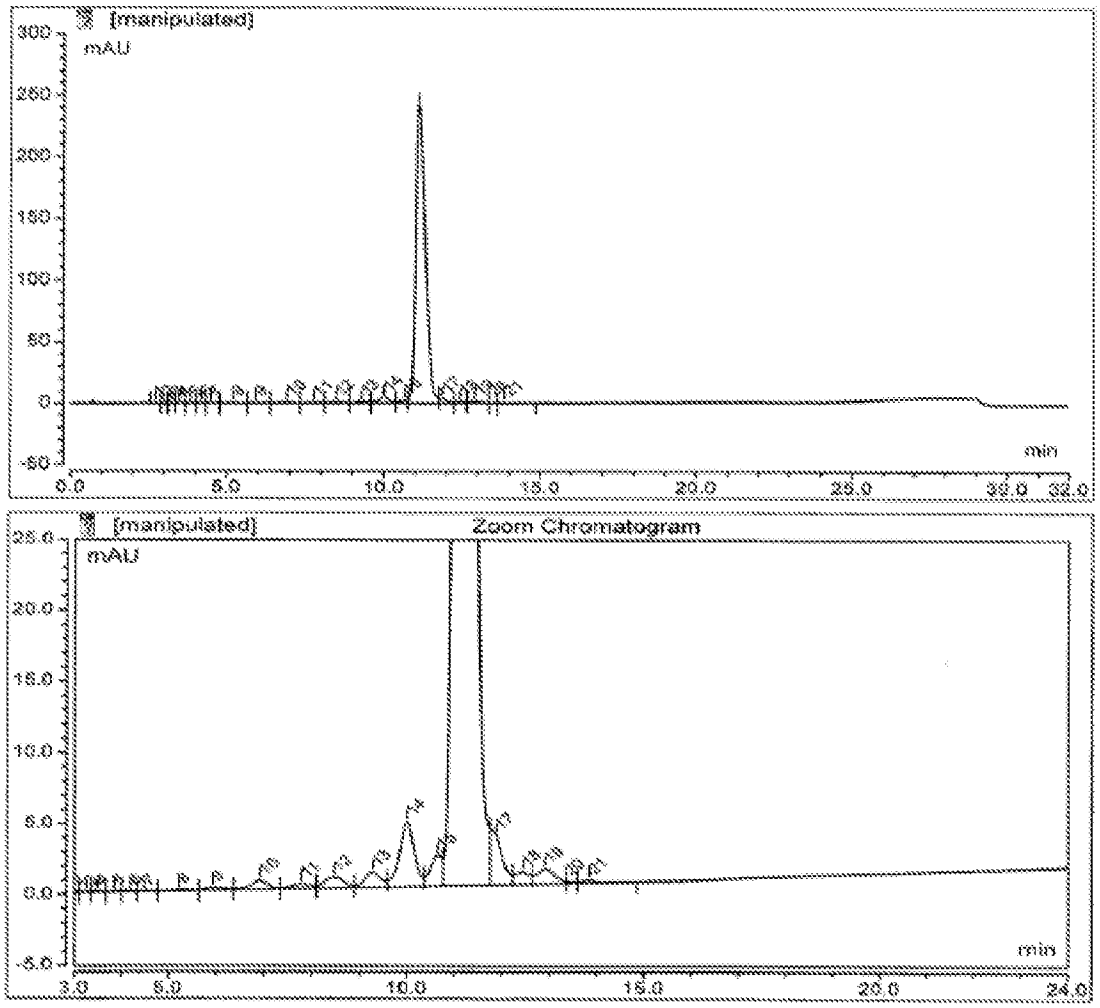
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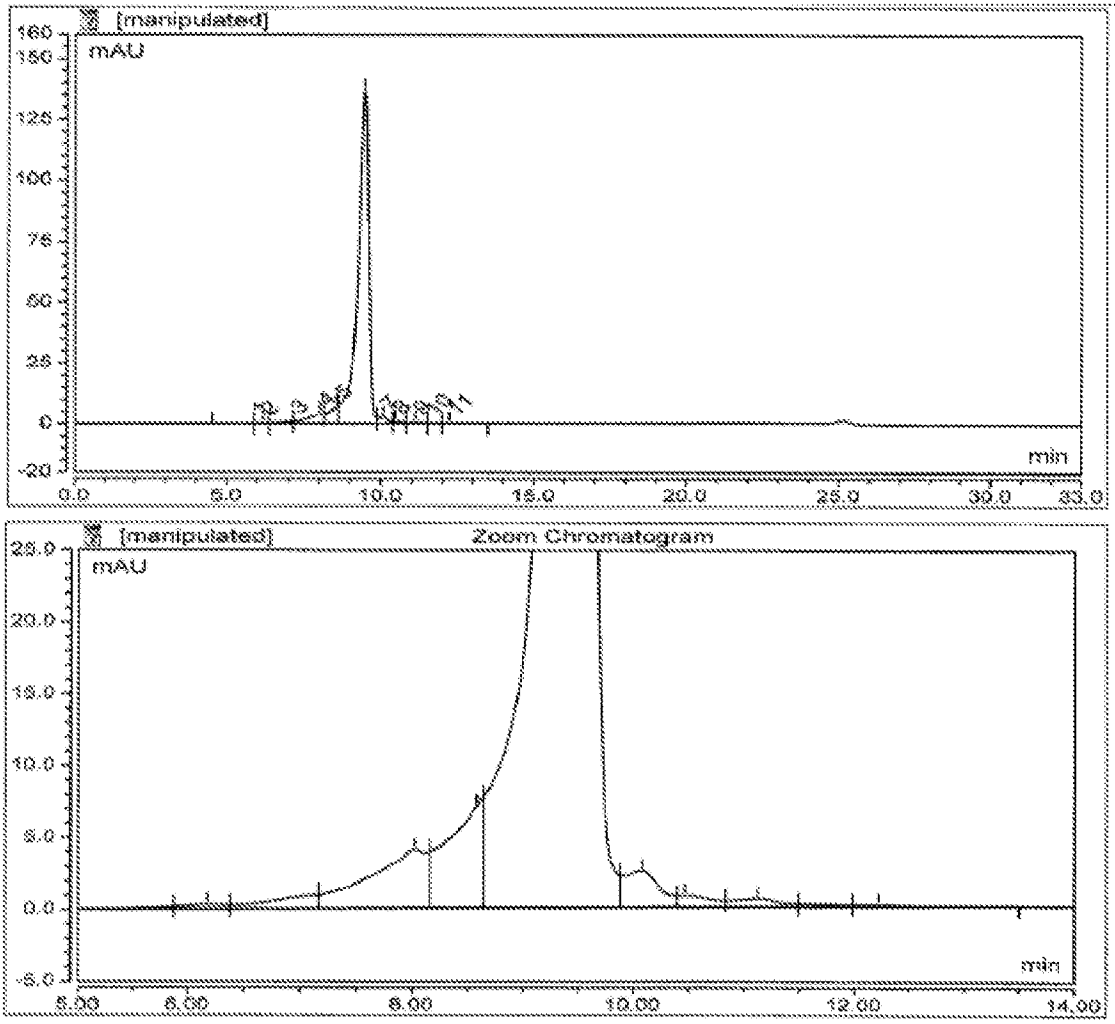
Fig. 4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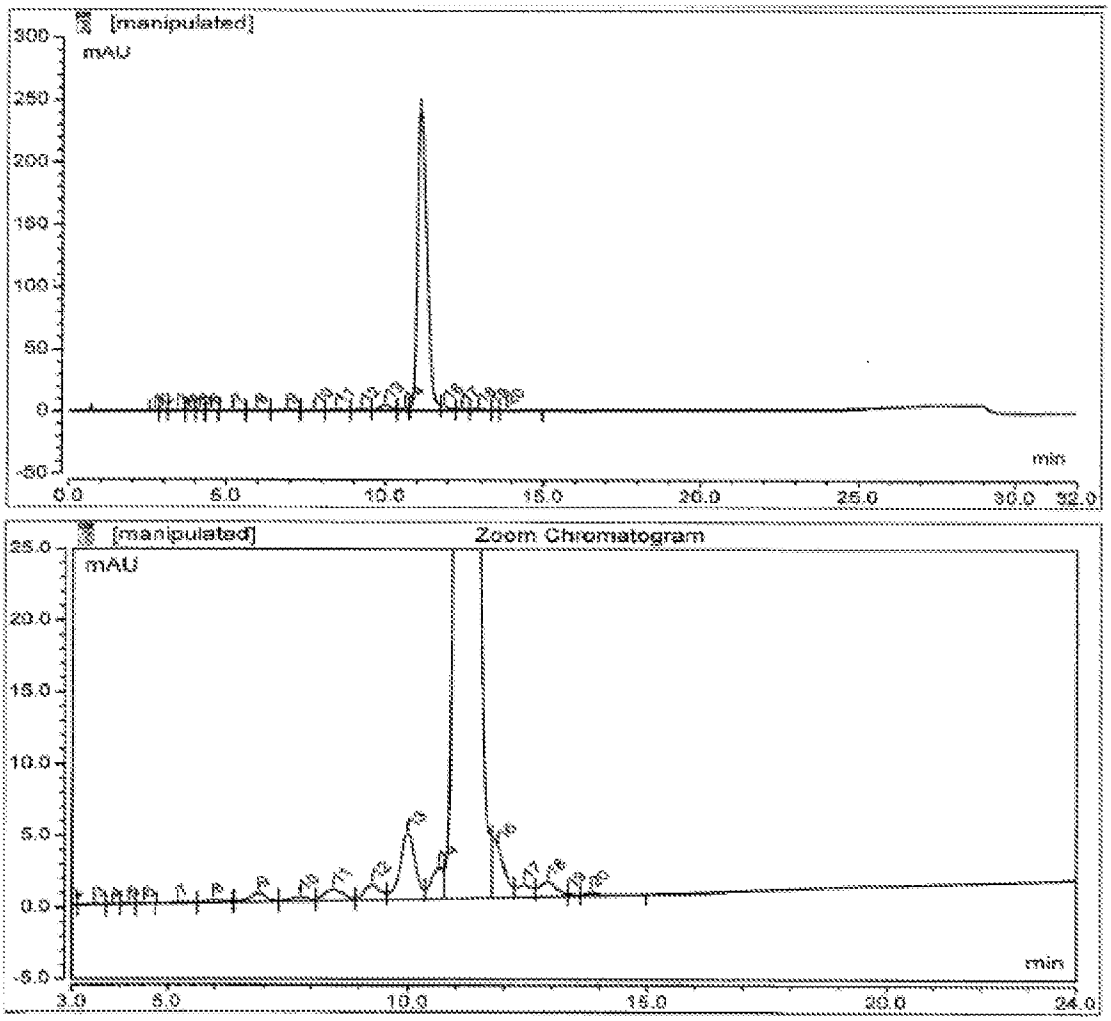
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Fig. 6

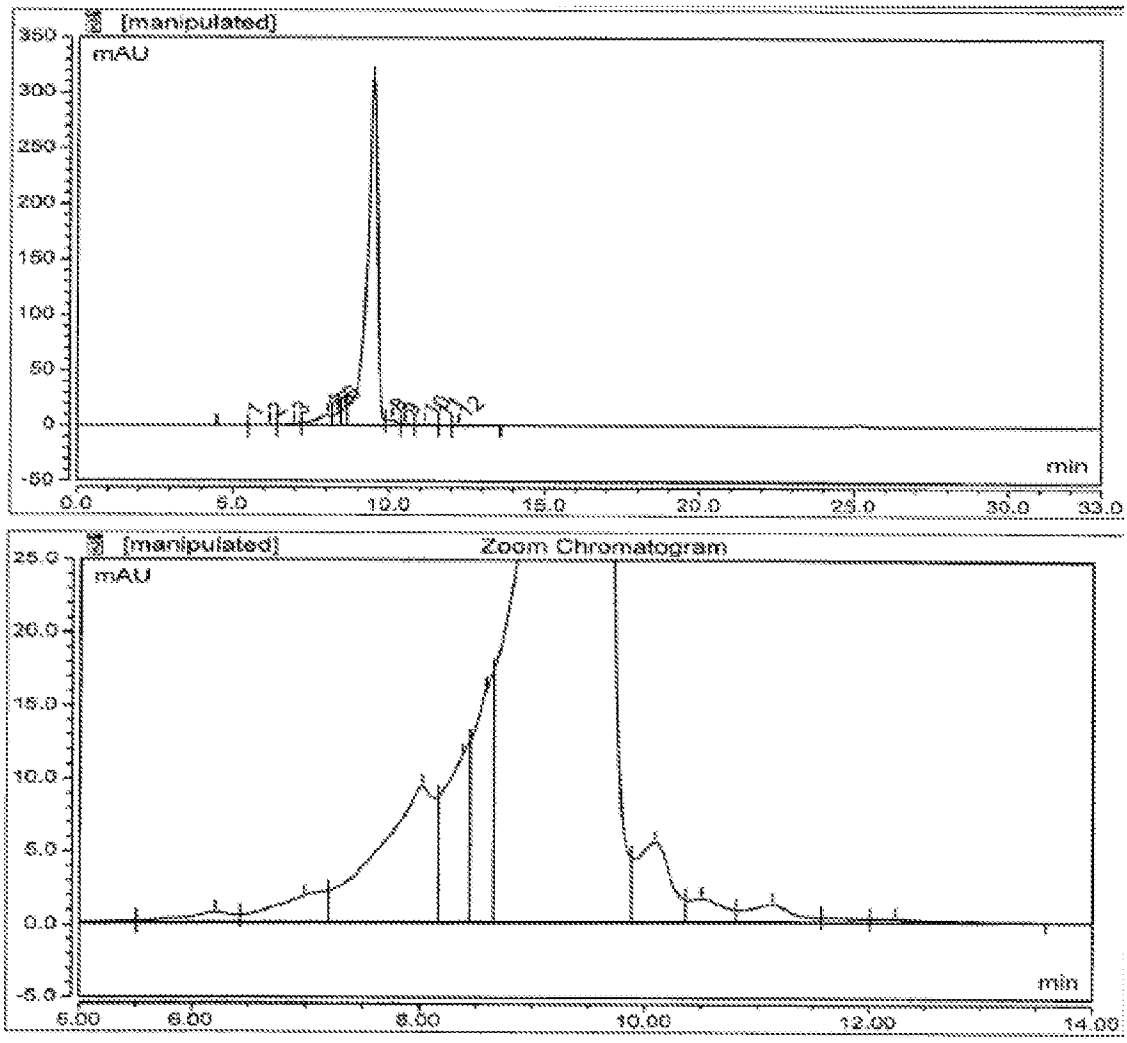
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Fig. 7

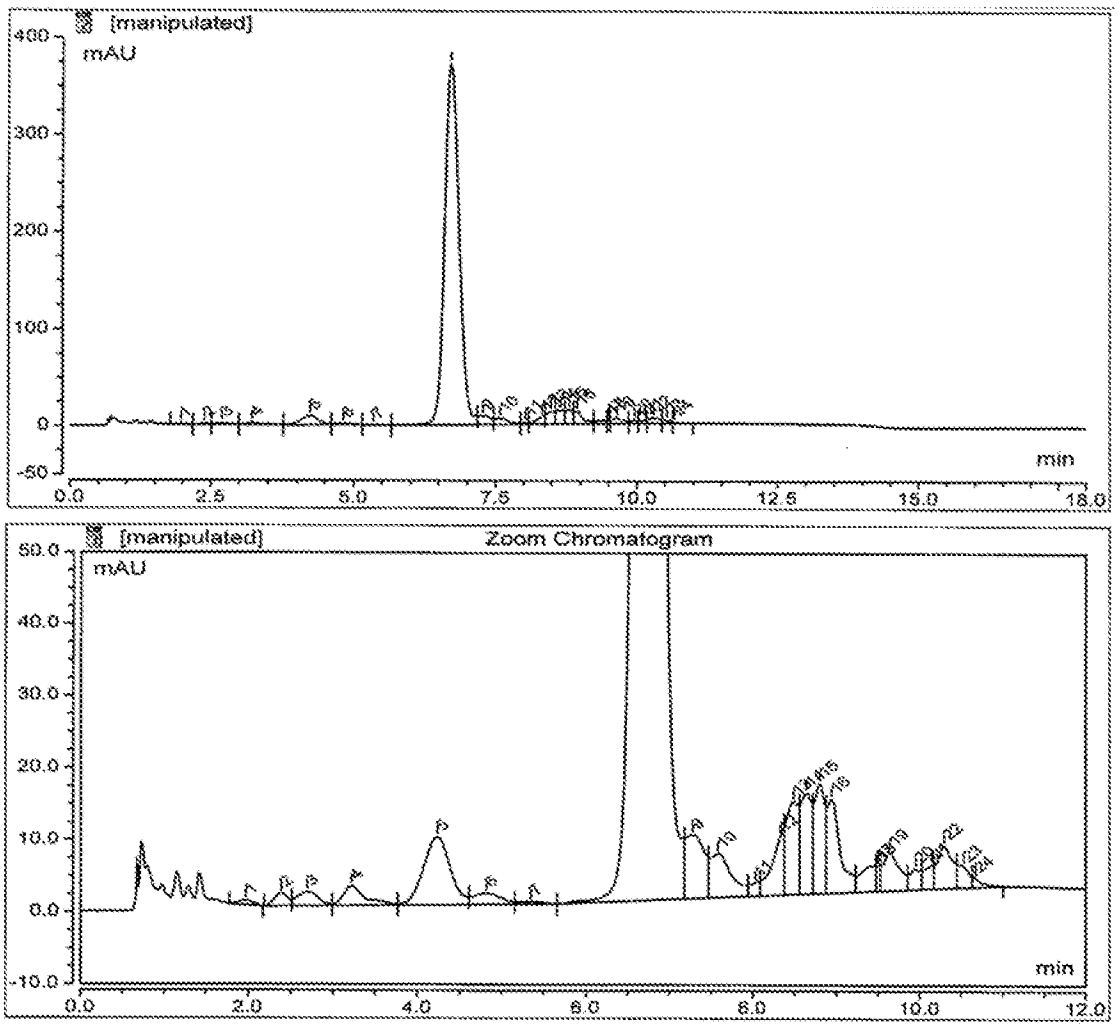
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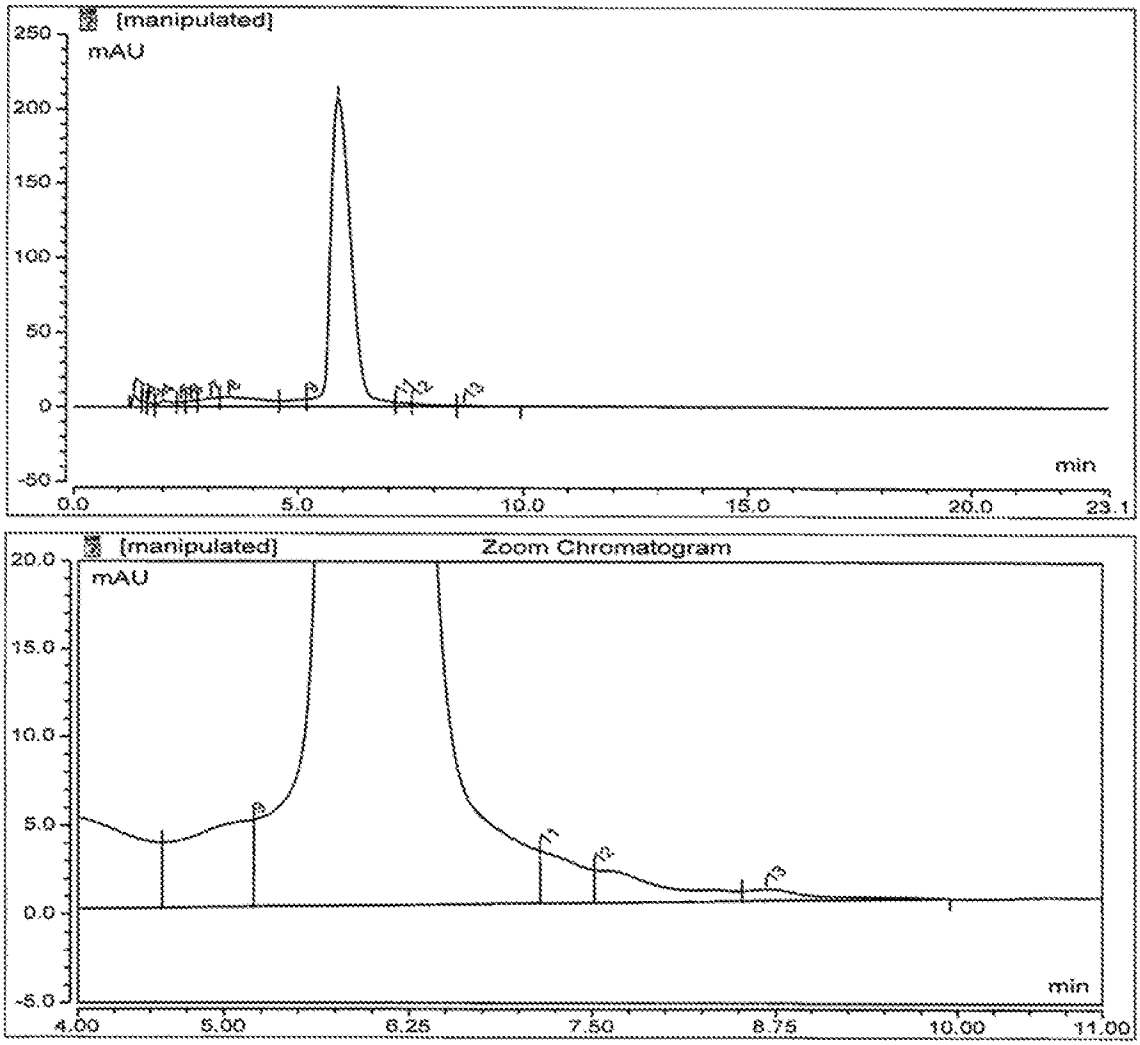
Fig. 8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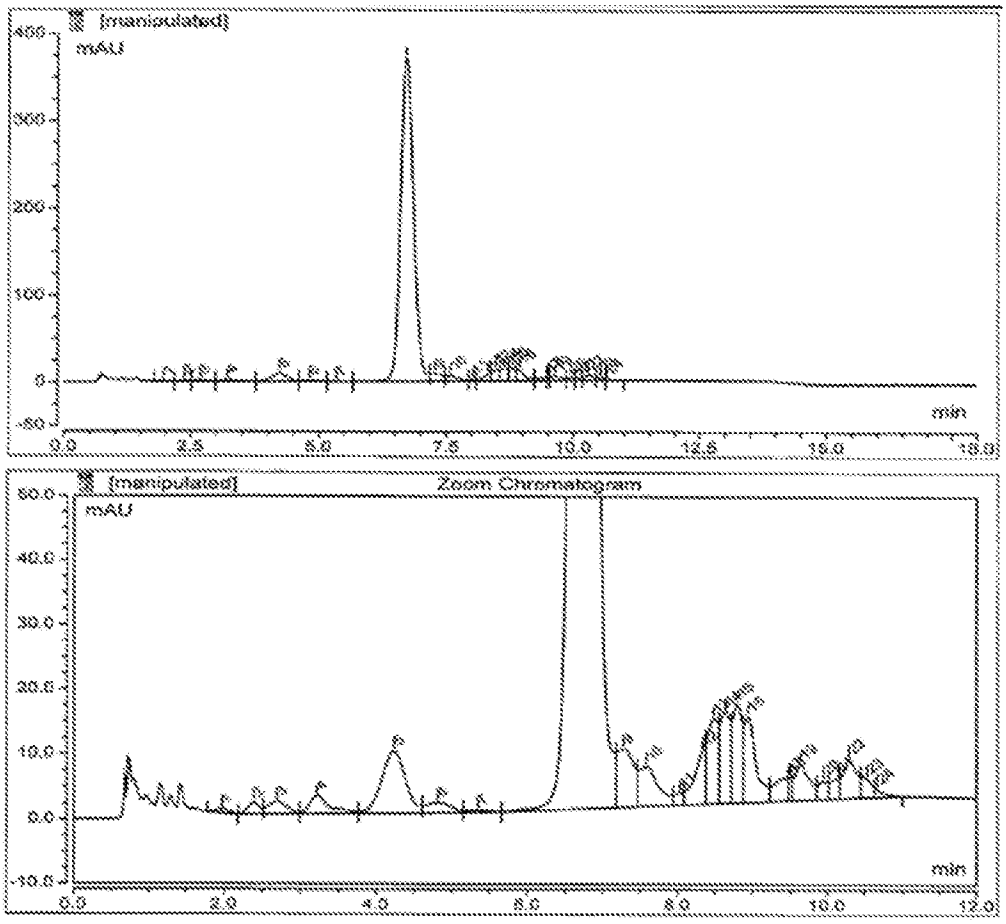
11.00

Fig. 9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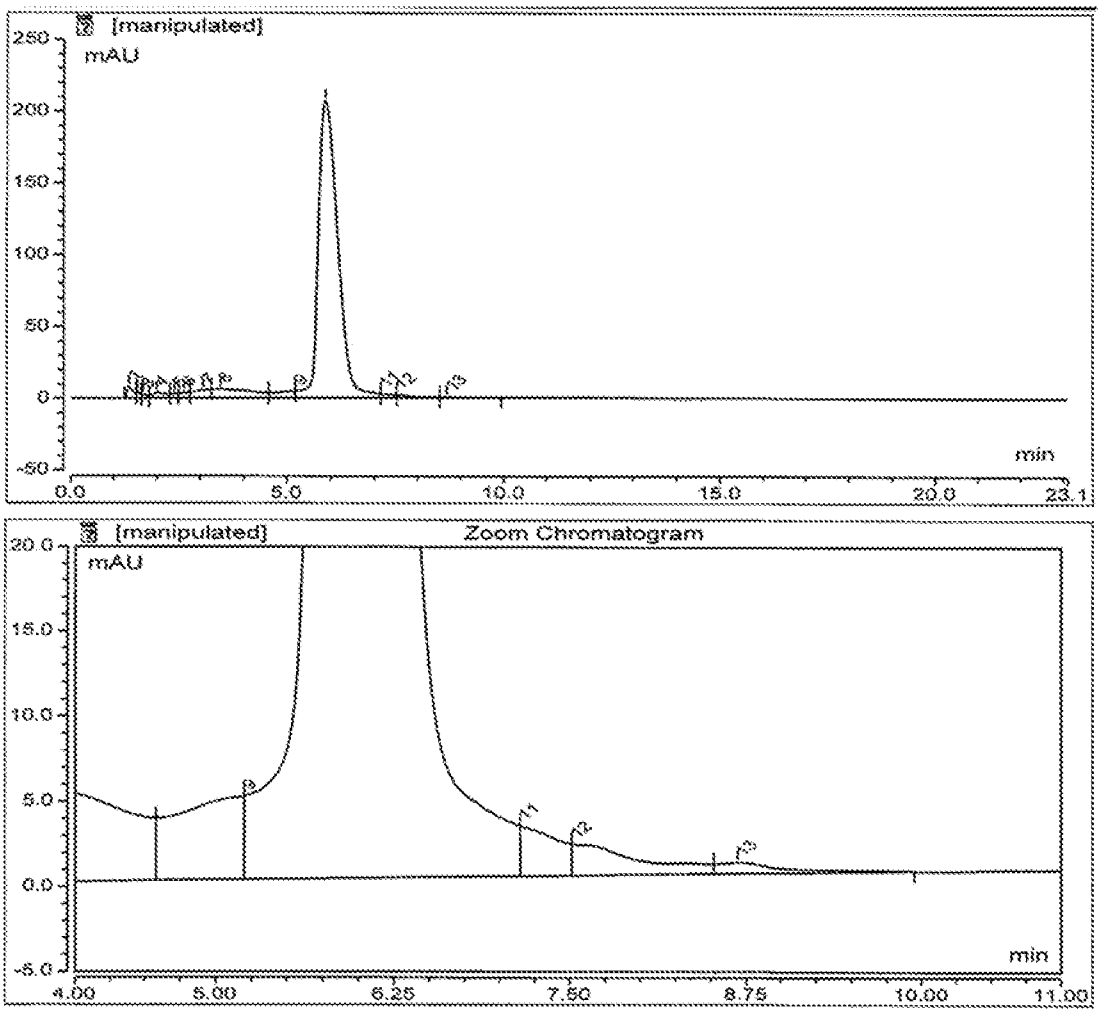
mAU
min
[manipulated]
Zoom Chromatogram
mAU
min

Fig. 10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Zoom Chromatogram
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-5.0
min
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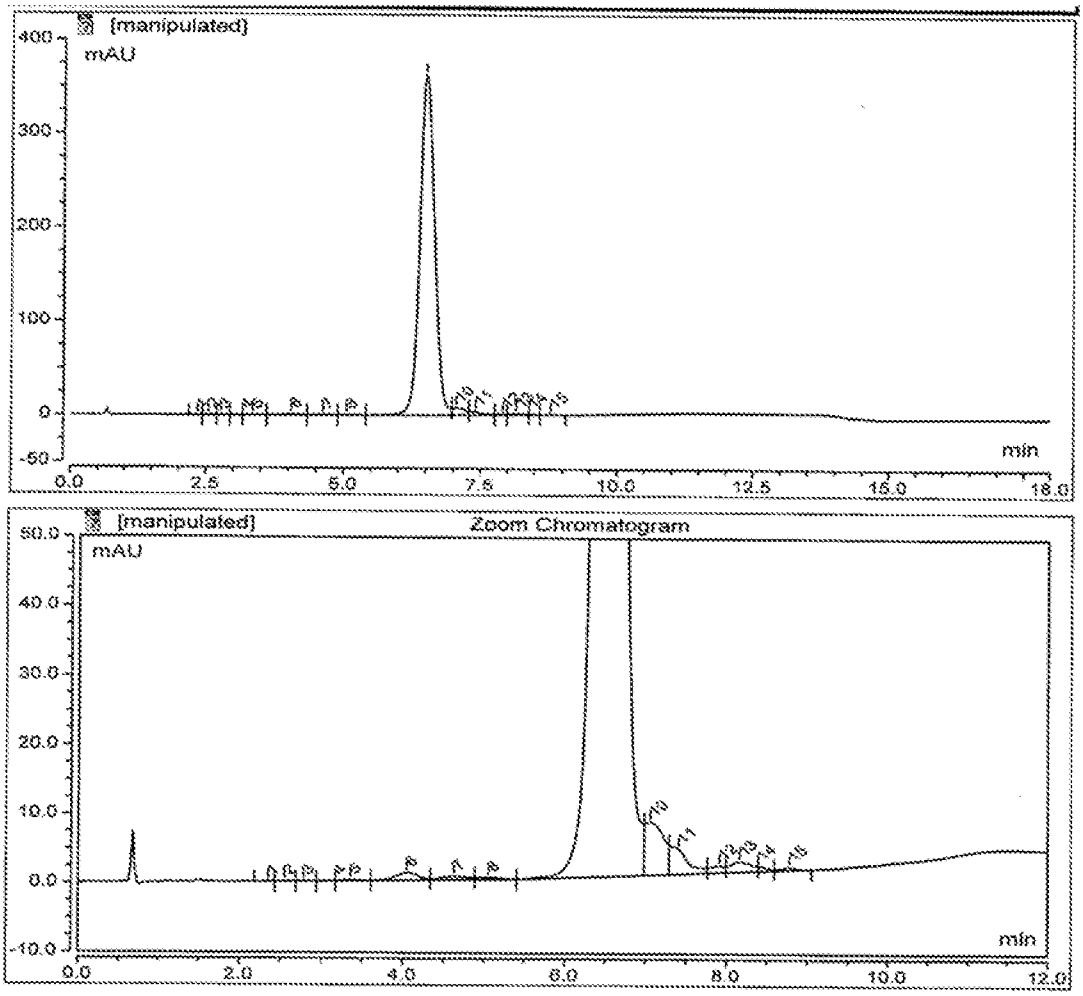
6.25
7.50
8.75
10.00
11.00

Fig. 11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Zoom Chromatogram
mAU
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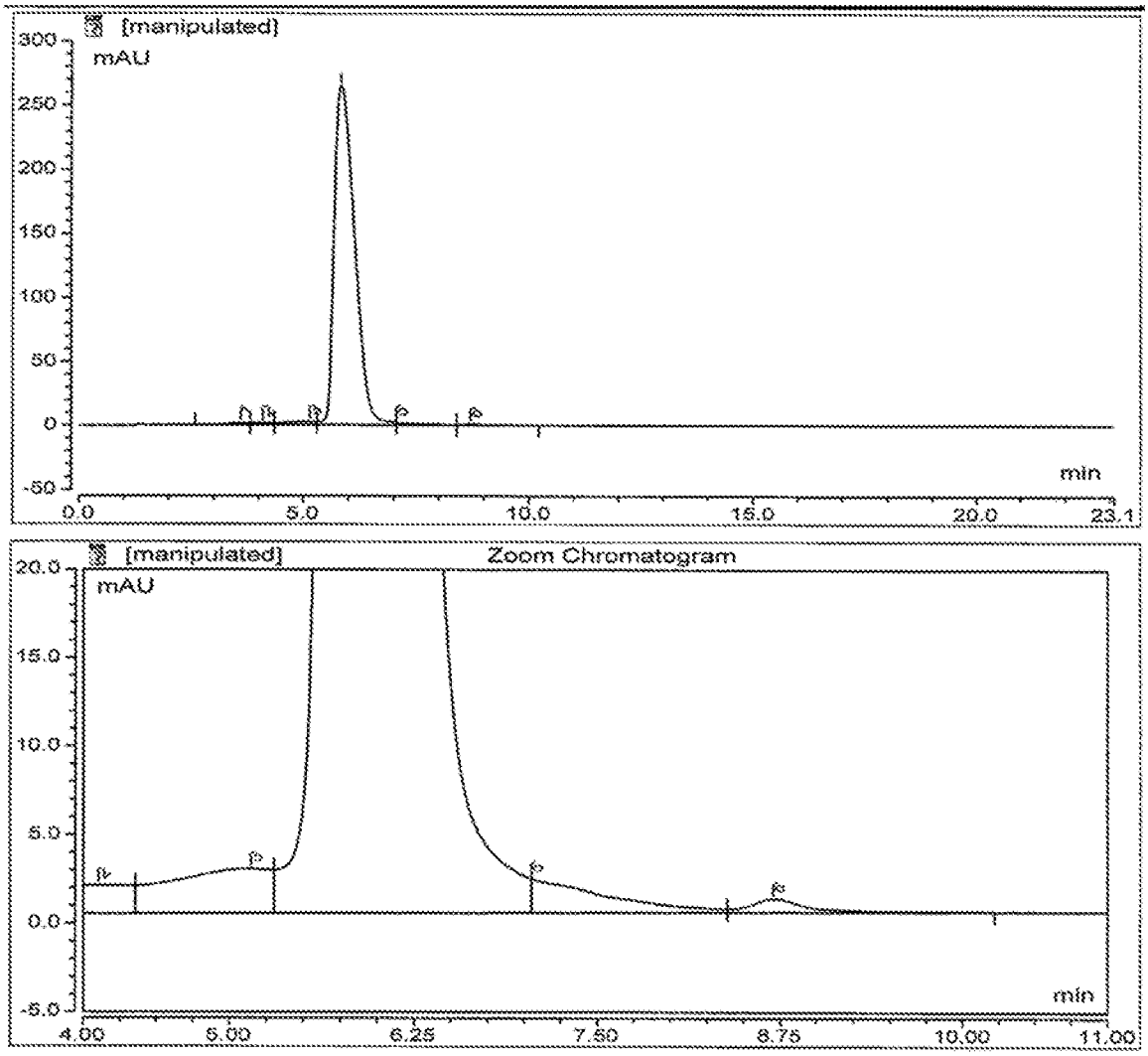
10.0
12.0

Fig. 12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min
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[manipulated]
Zoom Chromatogram
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0.0
-5.0
min
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5.00
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8.75
10.00
11.00

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/043824

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/00 A61K9/08 A61K31/00 A61K47/02 A61P21/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/005805 A1 (SAREPTA THERAPEUTICS INC [US]) 4 January 2018 (2018-01-04) cited in the application page 13, line 32 - page 14, line 3 page 52, lines 7-8 page 54, lines 5-7 page 55, lines 32-33 page 68, lines 31-33	1-30
E	WO 2020/198268 A1 (SAREPTA THERAPEUTICS INC [US]) 1 October 2020 (2020-10-01) paragraph [0166]	1-30

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 2020	05/11/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weiss, Marie-France

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/043824

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ANNEMIEKE AARTSMA-RUS ET AL: "Development of Exon Skipping Therapies for Duchenne Muscular Dystrophy: A Critical Review and a Perspective on the Outstanding Issues", NUCLEIC ACID THERAPEUTICS, vol. 27, no. 5, 1 October 2017 (2017-10-01), pages 251-259, XP055699369, US ISSN: 2159-3337, DOI: 10.1089/nat.2017.0682 the whole document -----	1-30

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/043824

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