

Description

Title of Invention:

ANTISENSE NUCLEIC ACID ENABLING EXON SKIPPING

Technical Field

[0001]

The present invention relates to an antisense oligomer capable of causing simultaneous skipping of a plurality of exons in a target gene, and a pharmaceutical composition comprising the oligomer.

Background Art

[0002]

In recent years, exon skipping therapy has received attention which involves causing exon skipping of a gene having a mutation that causes a disease so that a protein having partial functions arises, thereby treating the disease. Examples of the disease that may be treated by such exon skipping therapy include Duchenne muscular dystrophy (DMD).

[0003]

DMD is the most frequent form of hereditary progressive muscular disease that affects one in about 3,500 newborn boys. Although DMD patients exhibit motor functions rarely different from healthy humans in their infancy and childhood, muscle weakness is observed in

children from around 4 to 5 years old. Then, muscle weakness in DMD patients progresses with age to the loss of ambulation by about 12 years old and death due to cardiac or respiratory insufficiency in their twenties. Therefore, it has been strongly desired to develop an effective therapeutic agent.

[0004]

DMD is known to be caused by a mutation in the dystrophin gene. The dystrophin gene is located on X chromosome and is a huge gene consisting of 2.2 million DNA base pairs. DNA is transcribed into pre-mRNA, and introns are removed by splicing to form mRNA of 13,993 bases in which 79 exons are joined together. This mRNA is translated into 3,685 amino acids to produce the dystrophin protein. The dystrophin protein is associated with the maintenance of membrane stability in muscle cells and necessary to make muscle cells less fragile. Patients with DMD have a mutation in the dystrophin gene and hence, the functional dystrophin protein is rarely expressed in muscle cells of the patients. Therefore, the structure of muscle cells cannot be maintained at the time of muscle contraction in the body of the patients with DMD, leading to a large influx of calcium ions into muscle cells. Consequently, muscle cell necrosis and fibrosis progress so that muscle cells become progressively more difficult to regenerate.

[0005]

Becker muscular dystrophy (BMD) is also caused by a mutation in the dystrophin gene. The symptoms involve muscle weakness but are typically mild and slow in the progress of muscle weakness, when compared to DMD. In many cases, its onset is in adulthood. Differences in clinical symptoms between DMD and BMD are considered to reside in whether the reading frame for amino acids on the translation of dystrophin mRNA into the dystrophin protein is disrupted by the mutation or not (Non Patent Literature 1). More specifically, in DMD, the presence of mutation shifts the amino acid reading frame so that the expression of functional dystrophin protein is abolished, whereas in BMD the dystrophin protein that is capable of functioning, though imperfectly, is produced because the amino acid reading frame is preserved, while part of the exons are deleted by the mutation.

[0006]

Exon skipping is expected to serve as a method for treating DMD. This method involves modifying splicing to restore the amino acid reading frame of dystrophin mRNA and induce expression of the dystrophin protein having the function partially restored (Non Patent Literature 2). The amino acid sequence part to be translated from an exon, which is a target for exon skipping, will be lost. For this reason, the dystrophin protein expressed by this treatment becomes shorter than normal one but since the amino acid reading frame is maintained, the

function to stabilize muscle cells is partially retained. Consequently, it is expected that exon skipping will lead DMD to the similar symptoms to that of BMD which is milder. The exon skipping approach has passed the animal tests using mice or dogs and now is currently assessed in clinical trials on human DMD patients.

[0007]

The skipping of an exon can be induced by binding of antisense nucleic acids targeting site(s) surrounding either 5' or 3' splice site or both sites, or exon-internal sites. An exon will only be included in the mRNA when both splice sites thereof are recognized by the spliceosome complex. Thus, exon skipping can be induced by targeting the sites surrounding the splice sites with antisense nucleic acids. Furthermore, the binding of an SR protein rich in serine and arginine to an exonic splicing enhancer (ESE) is considered necessary for an exon to be recognized by the splicing mechanism. Accordingly, exon skipping can also be induced by targeting ESE.

[0008]

Since a mutation of the dystrophin gene may vary depending on DMD patients, antisense nucleic acids need to be designed based on the site or type of respective genetic mutation. There is a plurality of reports on an antisense nucleic acid that induces exon skipping targeting one sequence of consecutive bases for a single

exon in the dystrophin gene (Patent Literatures 1 to 6 and Non Patent Literatures 1 and 2). It has also been reported that when two types of antisense nucleic acids that target the same exon in the dystrophin gene are mixed and allowed to act (dual targeting), skipping activity may be enhanced as compared to use of each antisense nucleic acid alone (Patent Literature 7).

[0009]

A method called multi-exon skipping has received attention which involves causing skipping of a plurality of exons (exon group), not one exon as described above. This method enables a wide range of mutations in the dystrophin gene to be treated by exon skipping. For example, exons 45 to 55 in the dystrophin gene are known as hot spots of genetic mutation, and it has been reported that skipping of these 11 exons enables about 60% of DMD patients having a deletion mutation to be treated (Non Patent Literature 3). Most of patients congenitally lacking exons 45 to 55 are known to manifest no or mild symptoms, though developing BMD (Non Patent Literature 4). Thus, it is expected that drugs capable of inducing exon 45 to 55 skipping are promising as therapeutic agents for DMD.

[0010]

For example, a method using antisense nucleic acids respectively targeting all exons in a region which is the target of exon skipping (Non Patent Literatures 5, 7, 8,

and 10), and a method using antisense nucleic acids respectively targeting two different exons on the 3' side and 5' side of a region which is the target of exon skipping (Non Patent Literatures 6 and 9 and Patent Literature 8) have been reported as methods for inducing multi-exon skipping.

[0011]

However, there is still no report stating that an antisense nucleic acid targeting a region including the vicinity of a donor of an intron or the vicinity of an acceptor induces multi-exon skipping.

Citation List

Patent Literature

[0012]

Patent Literature 1: International Publication

WO2004/048570

Patent Literature 2: International Publication

WO2009/139630

Patent Literature 3: International Publication

WO2010/048586

Patent Literature 4: U.S. Patent Publication Nos.

2010/0168212

Patent Literature 5: International Publication

WO2011/057350

Patent Literature 6: International Publication

WO2006/000057

Patent Literature 7: International Publication

WO2007/135105

Patent Literature 8: International Publication

WO2004/083446

Non Patent Literature

[0013]

Non Patent Literature 1: Annemieke Aartsma-Rus et al.,

(2002) Neuromuscular Disorders 12: S71-S77

Non Patent Literature 2: Wilton S. D., et al., Molecular

Therapy 2007: 15: p. 1288-96

Non Patent Literature 3: Christophe Beroud et al., Human

Mutation, 28(2), 2007, 196-202

Non Patent Literature 4: Yusuke Echigoya et al.,

Molecular Therapy-Nucleic Acids, 4(2), 2015, e225

Non Patent Literature 5: Yoshitsugu Aoki et al., PNAS,

109(34), 2012, 13763-13768

Non Patent Literature 6: Laura van Vliet et al., BMC

Medical Genetics, 9, 105, 2008

Non Patent Literature 7: Joshua Lee et al., PLoS ONE,

13(5), e0197084, 2018

Non Patent Literature 8: Joshua Lee et al., Methods in

Molecular Biology, 1828, 141-150, 2018

Non Patent Literature 9: Annemieke Aartsma-Rus et al, Am.

J. Hum. Genet. 74(1), 83-92, 2004

Non Patent Literature 10: Yusuke Echigoya et al.,

Molecular Therapy, 27(11), 1-13, 2019

Summary of Invention

Technical Problem

[0014]

Under the foregoing circumstances, medicaments for treating patients having various mutations by causing simultaneous skipping of a plurality of exons (exon group) in pre-mRNA of interest have been desired.

Solution to Problem

[0015]

As a result of detailed studies of the technical contents of the above documents and the structure of the dystrophin gene, the present inventors have found that an antisense nucleic acid targeting a specific region in human dystrophin pre-mRNA is capable of causing simultaneous skipping of a plurality of exons among exons 45 to 55. Based on this finding, the present inventors have accomplished the present invention.

[0016]

Specifically, the present invention is as follows.

[1]

An antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which causes simultaneous skipping of any two or more numerically consecutive exons selected from the group consisting of

the 45th exon to the 55th exon in human dystrophin pre-mRNA,

the antisense oligomer comprising a base sequence complementary to a base sequence of at least one region selected from the group consisting of regions R1 to R24 represented by

region R_n (wherein n is an odd number of 1 to 23) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the N^Ath exon and a base sequence of NY bases in the downstream direction from the 5' end of the N^Bth intron in the human dystrophin pre-mRNA, and

region R_n (wherein n is an even number of 2 to 24) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the N^Ath intron and a base sequence of NY bases in the downstream direction from the 5' end of the N^Bth exon in the human dystrophin pre-mRNA,

or a partial base sequence thereof, wherein

when n is 1, NA = 44, NB = 44, NX = 20, and NY = 400,

when n is 2, NA = 44, NB = 45, NX = 600, and NY = 50,

when n is 3, NA = 45, NB = 45, NX = 20, and NY = 400,

when n is 4, NA = 45, NB = 46, NX = 400, and NY = 50,

when n is 5, NA = 46, NB = 46, NX = 20, and NY =
400,
when n is 6, NA = 46, NB = 47, NX = 400, and NY =
50,
when n is 7, NA = 47, NB = 47, NX = 20, and NY =
400,
when n is 8, NA = 47, NB = 48, NX = 400, and NY =
50,
when n is 9, NA = 48, NB = 48, NX = 20, and NY =
400,
when n is 10, NA = 48, NB = 49, NX = 400, and NY =
50,
when n is 11, NA = 49, NB = 49, NX = 20, and NY =
400,
when n is 12, NA = 49, NB = 50, NX = 400, and NY =
50,
when n is 13, NA = 50, NB = 50, NX = 20, and NY =
400,
when n is 14, NA = 50, NB = 51, NX = 400, and NY =
50,
when n is 15, NA = 51, NB = 51, NX = 20, and NY =
400,
when n is 16, NA = 51, NB = 52, NX = 400, and NY =
50,
when n is 17, NA = 52, NB = 52, NX = 20, and NY =
400,
when n is 18, NA = 52, NB = 53, NX = 400, and NY =

50,

when n is 19, NA = 53, NB = 53, NX = 20, and NY =

400,

when n is 20, NA = 53, NB = 54, NX = 400, and NY =

50,

when n is 21, NA = 54, NB = 54, NX = 20, and NY =

400,

when n is 22, NA = 54, NB = 55, NX = 400, and NY =

50,

when n is 23, NA = 55, NB = 55, NX = 20, and NY =

400, or

when n is 24, NA = 55, NB = 56, NX = 400, and NY =

50.

[2]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [1], wherein

the region R1 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 44th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 44th intron in the human dystrophin pre-mRNA,

the region R2 is a region that consists of a base sequence of 600 bases in the upstream direction from the 3' end of the 44th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 45th exon in the human dystrophin pre-mRNA,

the region R3 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 45th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 45th intron in the human dystrophin pre-mRNA,

the region R4 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 45th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 46th exon in the human dystrophin pre-mRNA,

the region R5 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 46th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 46th intron in the human dystrophin pre-mRNA,

the region R6 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 46th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 47th exon in the human dystrophin pre-mRNA,

the region R7 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 47th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 47th intron in the human dystrophin pre-mRNA,

the region R8 is a region that consists of a base sequence of 400 bases in the upstream direction from the

3' end of the 47th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 48th exon in the human dystrophin pre-mRNA,

the region R9 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 48th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 48th intron in the human dystrophin pre-mRNA,

the region R10 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 48th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 49th exon in the human dystrophin pre-mRNA,

the region R11 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 49th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 49th intron in the human dystrophin pre-mRNA,

the region R12 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 49th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 50th exon in the human dystrophin pre-mRNA,

the region R13 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 50th exon and a base sequence of 400 bases

in the downstream direction from the 5' end of the 50th intron in the human dystrophin pre-mRNA,

the region R14 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 50th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 51st exon in the human dystrophin pre-mRNA,

the region R15 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 51st exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 51st intron in the human dystrophin pre-mRNA,

the region R16 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 51st intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 52nd exon in the human dystrophin pre-mRNA,

the region R17 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 52nd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 52nd intron in the human dystrophin pre-mRNA,

the region R18 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 52nd intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 53rd exon in the human dystrophin pre-mRNA,

the region R19 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 53rd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 53rd intron in the human dystrophin pre-mRNA,

the region R20 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 53rd intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 54th exon in the human dystrophin pre-mRNA,

the region R21 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 54th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 54th intron in the human dystrophin pre-mRNA,

the region R22 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 54th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 55th exon in the human dystrophin pre-mRNA,

the region R23 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 55th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 55th intron in the human dystrophin pre-mRNA, or

the region R24 is a region that consists of a base sequence of 400 bases in the upstream direction from the

3' end of the 55th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 56th exon in the human dystrophin pre-mRNA.

[3]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [1] or [2], wherein

the antisense oligomer comprises a base sequence complementary to

(a) any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(b) a base sequence that hybridizes under stringent conditions to a base sequence complementary to any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(c) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or

(d) a partial base sequence of any one base sequence selected from the group consisting of the base sequences (a), (b), and (c).

[4]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [3], wherein

the antisense oligomer is an antisense oligomer comprising two or more unit oligomers linked to each other, wherein

each of the unit oligomers comprises a base sequence complementary to a base sequence of any one region selected from the group consisting of the regions R1 to R24, or a partial base sequence thereof, and the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[5]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [3], wherein

the antisense oligomer is an antisense oligomer comprising two or more unit oligomers linked to each other, wherein

each of the unit oligomers comprises a base sequence complementary to

(a) any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(b) a base sequence that hybridizes under stringent conditions to a base sequence complementary to any one

base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(c) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or

(d) a partial base sequence of any one base sequence selected from the group consisting of the base sequences (a), (b), and (c),

and the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[6]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [4] or [5], wherein each of the unit oligomers comprises a base sequence complementary to a consecutive base sequence of 5- to 20-base length in the region.

[7]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [3], wherein the antisense oligomer consists of (1) any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, or

(2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected.

[8]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [3] and [7], wherein the antisense oligomer consists of any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232.

[9]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [3], [7] and [8], wherein the antisense oligomer consists of any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64,

65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228.

[10]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [9], wherein the antisense oligomer is an oligonucleotide.

[11]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [10], wherein the sugar moiety and/or the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is modified.

[12]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [10] or [11], wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected from the group consisting of -OR, -R, -R'OR, -SH, -SR, -NH₂, -NHR, -NR₂, -N₃, -CN, -F, -Cl, -Br, and -I (wherein R is an alkyl or an aryl and R' is an alkylene).

[13]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [10] to [12], wherein the phosphate bond moiety of at least one nucleotide constituting the

oligonucleotide is any one selected from the group consisting of a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond and a boranophosphate bond.

[14]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [9], wherein the antisense oligomer is a morpholino oligomer.

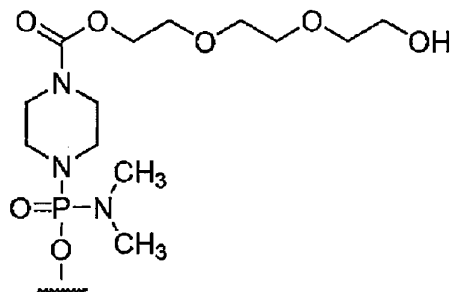
[15]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [14], wherein the antisense oligomer is a phosphorodiamidate morpholino oligomer.

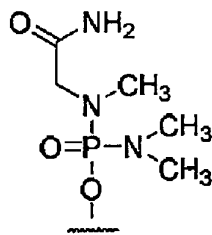
[16]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [14] or [15], wherein the 5' end is any one of chemical formulae (1) to (3) below:

[Formula 1]



(1)



(2)



(3)

[17]

A suppressor antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which suppresses single skipping of any one exon selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA,

the suppressor antisense oligomer comprising a base sequence complementary to

- (a) any one base sequence of a base sequence selected from the group consisting of SEQ ID NOs: 370 to 384,
- (b) a base sequence that has at least 85% identity with any one base sequence of a base sequence selected from the group consisting of SEQ ID NOs: 370 to 384, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or
- (c) a partial base sequence of the base sequence (a) or (b).

[18]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [17], wherein the suppressor antisense oligomer consists of

- (1) any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, or (2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID

NOs: 257 to 275, and has a length within $\pm 15\%$ of the length of the any one base sequence selected.

[19]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [17] or [18], wherein the suppressor antisense oligomer consists of any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263.

[20]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [17] to [19], wherein the suppressor antisense oligomer is an oligonucleotide.

[21]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [20], wherein the sugar moiety and/or the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is modified.

[22]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [20] or [21], wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected from the group consisting of

-OR, -R, -R'OR, -SH, -SR, -NH₂, -NHR, -NR₂, -N₃, -CN, -F, -Cl, -Br, and -I

(wherein R is an alkyl or an aryl and R' is an alkylene).

[23]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [20] to [22], wherein the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is any one selected from the group consisting of a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond and a boranophosphate bond.

[24]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [17] to [19], wherein the suppressor antisense oligomer is a morpholino oligomer.

[25]

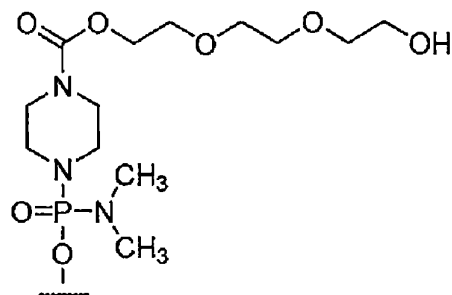
The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [24], wherein the suppressor antisense oligomer is a phosphorodiamidate morpholino oligomer.

[26]

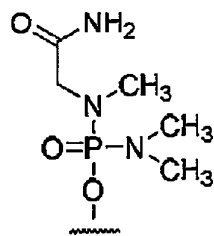
The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate

thereof according to [24] or [25], wherein the 5' end is any one of chemical formulae (1) to (3) below:

[Formula 2]



(1)



(2)



(3)

[27]

A pharmaceutical composition comprising the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [16].

[28]

The pharmaceutical composition according to [27], further comprising the suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [17] to [26].

[29]

A pharmaceutical composition comprising the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [16], and the suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [17] to [26].

[30]

The pharmaceutical composition according to [28] or [29], wherein

(1) the antisense oligomer is an oligomer consisting of SEQ ID NO: 75, and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 260,

(2) the antisense oligomer is an oligomer consisting of SEQ ID NO: 75, and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 261, or

(3) the antisense oligomer is an oligomer consisting of SEQ ID NO: 75, and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 263.

[31]

The pharmaceutical composition according to any one of [27] to [30], further comprising a pharmaceutically acceptable carrier.

[32]

The pharmaceutical composition according to any one of [27] to [31] for treatment of muscular dystrophy.

[33]

The pharmaceutical composition according to any one of [27] to [32] for being administered to a human patient.

[34]

A method for treatment of muscular dystrophy, which comprises administering to a patient with muscular dystrophy the antisense oligomer or the pharmaceutically

acceptable salt thereof, or hydrate thereof according to any one of [1] to [16], or the pharmaceutical composition according to any one of [27] to [33].

[35]

The method for treatment according to [34], wherein the patient with muscular dystrophy is a patient with a mutation that is amenable to exon 45 to 55 skipping in the dystrophin gene.

[36]

The method for treatment according to [34] or [35], wherein the patient is a human.

[37]

Use of the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [16], or the pharmaceutical composition according to any one of [27] to [33] in manufacturing of a medicament for the treatment of muscular dystrophy.

[38]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [16], or the pharmaceutical composition according to any one of [27] to [33] for use in the treatment of muscular dystrophy.

[39]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof, or the

pharmaceutical composition according to [38], wherein the treatment involves performing skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA.

[40]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof, or the pharmaceutical composition according to [38] or [39], wherein in the treatment, a patient with muscular dystrophy is a human.

[41]

A method for enhancing the efficiency of skipping of two or more numerically consecutive exons, which comprises

inhibiting a splicing silencer sequence, a splice site sequence, or a branch site sequence of pre-mRNA of interest when the two or more numerically consecutive exons are skipped from the pre-mRNA of interest.

[42]

The method according to [41], wherein the splicing silencer sequence is a recognition sequence of heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1).

[43]

The method according to [41] or [42], wherein the pre-mRNA of interest is human dystrophin pre-mRNA.

[44]

The method according to any one of [41] to [43], wherein the two or more numerically consecutive exons are selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA.

[45]

The method according to [44], wherein the skipping of the two or more numerically consecutive exons of the pre-mRNA of interest is performed using the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [1] to [16].

[46]

The method according to [44] or [45], wherein specific inhibition of the splicing silencer sequence, the splice site sequence, or the branch site sequence is performed using the suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of [17] to [26].

[0017]

The present invention may also include the following embodiments.

[47]

An antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which causes simultaneous skipping of two or more numerically consecutive exons from pre-mRNA of interest,

the antisense oligomer comprising a base sequence complementary to a base sequence of any one region selected from the group consisting of

(1) a region that is constituted by a base sequence of 20 bases in the upstream direction from the 3' end of any exon and a base sequence of 400 bases in the downstream direction from the 5' end of an intron which is adjacent to the 3' end of the exon in the pre-mRNA of interest, or

(2) a region that is constituted by a base sequence of 400 bases or 600 bases in the upstream direction from the 3' end of any intron and a base sequence of 50 bases in the downstream direction from the 5' end of an exon which is adjacent to the 3' end of the intron in the pre-mRNA of interest,
or a partial base sequence thereof.

[48]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to [47], wherein the pre-mRNA of interest is human dystrophin pre-mRNA.

Advantageous Effects of Invention

[0018]

The present invention provides an antisense oligomer that causes simultaneous skipping of a plurality of exons in a target. Another embodiment of the present invention

provides a pharmaceutical composition for treating patients having various mutations by causing simultaneous skipping of a plurality of exons in pre-mRNA of interest. An alternative embodiment of the present invention provides a suppressor antisense oligomer that suppresses single skipping of an exon in pre-mRNA of interest or a pharmaceutical composition comprising the oligomer. An alternative embodiment of the present invention enables simultaneous skipping of exons 45 to 55 in human dystrophin pre-mRNA to be caused with high efficiency.

Brief Description of Drawings

[0019]

[Figure 1] Figure 1 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells (human rhabdomyosarcoma cells) by RT-PCR.

[Figure 2] Figure 2 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 3] Figure 3 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 4] Figure 4 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 5] Figure 5 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 6] Figure 6 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 7] Figure 7 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 8] Figure 8 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 9] Figure 9 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 10] Figure 10 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 11] Figure 11 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 12] Figure 12 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 13] Figure 13 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 14] Figure 14 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 15] Figure 15 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 16] Figure 16 is a diagram showing results of studying exon 45 to 55 skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 17] Figure 17 is a diagram showing results of studying exon 45 to 55 multi-skipping and exon 45 single skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 18] Figure 18 is a diagram showing results of studying exon 45 to 55 multi-skipping and exon 45 single skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 19] Figure 19 is a diagram showing results of studying multi-skipping of exons selected from exons 45 to 49 and exon 45 single skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 20] Figure 20 is a diagram showing results of studying multi-skipping of exons selected from exons 45 to 49 in human dystrophin pre-mRNA in RD cells by RT-PCR, and calculating the total amounts of the respective skips of exons 45 and 46, exons 45 to 47, exons 45 to 48, and

exons 45 to 49 (total multi-skipping products). Each of these skips is considered to have therapeutic effects.

[Figure 21] Figure 21 is a diagram showing results of studying multi-skipping of exons selected from exons 45 to 52 and exon 45 single skipping in human dystrophin pre-mRNA in RD cells by RT-PCR.

[Figure 22] Figure 22 is a diagram showing results of studying multi-skipping of exons selected from exons 45 to 52 in human dystrophin pre-mRNA in RD cells by RT-PCR, and calculating the total amounts of the respective skips of exons 45 to 47, exons 45 to 48, exons 45 to 49, exons 45 to 50, exons 45 to 51, and exons 45 to 52 (left) or the total amounts of the respective skips of exons 45 to 47, exons 45 to 48, exons 45 to 49, and exons 45 to 51 (right). Each of the skips of exons 45 to 47, exons 45 to 48, exons 45 to 49, and exons 45 to 51 has therapeutic effects.

Description of Embodiments

[0020]

Hereinafter, the present invention is described in detail. The embodiments described below are intended to be presented by way of example merely to describe the invention but not limited only to the following embodiments. The present invention may be implemented in various ways without departing from the gist of the invention.

[0021]

1. Antisense oligomer

The present invention provides an antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which causes simultaneous skipping of two or more numerically consecutive exons from pre-mRNA of interest,

the antisense oligomer comprising a base sequence complementary to a base sequence of any one region selected from the group consisting of

(1) a region that is constituted by a base sequence of 20 bases in the upstream direction from the 3' end of any exon and a base sequence of 400 bases in the downstream direction from the 5' end of an intron which is adjacent to the 3' end of the exon in the pre-mRNA of interest, or

(2) a region that is constituted by a base sequence of 400 bases or 600 bases in the upstream direction from the 3' end of any intron and a base sequence of 50 bases in the downstream direction from the 5' end of an exon which is adjacent to the 3' end of the intron in the pre-mRNA of interest,
or a partial base sequence thereof.

[0022]

As used herein, the term "cause simultaneous skipping" of two or more numerically consecutive exons includes not only removal of the respective exons from pre-mRNA at completely the same timings but also

sequential removal of the respective exons within a period from pre-mRNA to mature mRNA. Specifically, the term "cause simultaneous skipping" of two or more numerically consecutive exons refers to removal of a plurality of (two or more) numerically consecutive exons from pre-mRNA.

[0023]

As used herein, the term "two or more numerically consecutive exons" means a plurality of exons that increase one by one in exon number among exons (the total number of exons is referred to as Texon) included in pre-mRNA of interest. The exon number means a number assigned to exons in order from the 5' end to the 3' end with an exon at the most upstream position of pre-mRNA defined as the first exon, followed by the second, the third, In the case of skipping of two or more numerically consecutive exons in a certain gene, its exon numbers $a_1, \dots, a_j$ can be represented by the sequence $\{a_j\}$. The general term a_j in the sequence $\{a_j\}$ is represented by the expression below:

[Expression 1]

$$a_j = m + (j - 1)$$

wherein m is a given natural number that satisfies $1 \leq m \leq (\text{Texon} - 1)$, and j is a natural number that satisfies $2 \leq (m + j) \leq \text{Texon} + 1$.

When the pre-mRNA of interest is, for example, human dystrophin pre-mRNA, Texon is 79.

In a certain embodiment, j is a given natural number selected from 1 to 11. In another embodiment, j is 11, j is 10, j is 9, j is 8, j is 7, j is 6, j is 5, j is 4, j is 3, j is 2, or j is 1.

[0024]

As used herein, the term "gene" is intended to mean a genomic gene and also include cDNA, pre-mRNA and mRNA. Preferably, the gene is pre-mRNA. As used herein, the term "pre-mRNA" is an RNA molecule comprising an exon and an intron transcribed from a target gene on the genome and is a mRNA precursor.

[0025]

In a certain embodiment, the present invention provides an antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which causes simultaneous skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA (hereinafter, the antisense oligomer and the pharmaceutically acceptable salt thereof and the hydrate of the antisense oligomer or the salt are also collectively referred to as the "antisense oligomer of the present invention"; the antisense oligomer of the present invention may refer to any of the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof).

[0026]

The human dystrophin pre-mRNA is an RNA molecule comprising an exon and an intron transcribed from the human dystrophin gene on the genome and is a mRNA precursor. Those skilled in the art can obtain information on the base sequence of the human dystrophin pre-mRNA by analogy from the genomic sequence of the human dystrophin gene (GenBank Accession Nos. NG_012232.1).

[0027]

In the human genome, the human dystrophin gene locates at locus Xp21.2. The human dystrophin gene has a size of about 3.0 Mbp and is the largest gene among known human genes. However, the coding regions of the human dystrophin gene are only about 14 kb, distributed as 79 exons throughout the human dystrophin gene (Roberts, RG, et al., Genomics, 16: 536-538 (1993)). The pre-mRNA, which is the transcript of the human dystrophin gene, undergoes splicing to form mature mRNA of about 14 kb. The base sequence of mature mRNA of human wild-type dystrophin gene is known (GenBank Accession Nos. NM_004006).

[0028]

The antisense oligomer of the present invention according to the foregoing embodiment targets at least any one region selected from the group consisting of regions R1 to R24 represented by

region R_n (wherein n is an odd number of 1 to 23) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the N^Ath exon and a base sequence of NY bases in the downstream direction from the 5' end of the N^Bth intron in the human dystrophin pre-mRNA, and

region R_n (wherein n is an even number of 2 to 24) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the N^Ath intron and a base sequence of NY bases in the downstream direction from the 5' end of the N^Bth exon in the human dystrophin pre-mRNA.

Hereinafter, the regions R₁ to R₂₄ are each or collectively referred to as a "target region of the antisense oligomer of the present invention" or interchangeably the "target region of the present invention".

As used herein, the term "targeting" means that an intended base sequence is a base sequence complementary to the base sequence of a target region or a partial base sequence of the target sequence.

[0029]

The antisense oligomer of the present invention according to the foregoing embodiment comprises a base sequence complementary to a base sequence of at least any one region selected from the group consisting of the

target regions R1 to R24 of the present invention, or a partial base sequence thereof.

[0030]

Examples of the target region R_n of the present invention according to the foregoing embodiment include regions wherein

when n is 1, NA = 44, NB = 44, NX = 20, and NY = 400,

when n is 2, NA = 44, NB = 45, NX = 600, and NY = 50,

when n is 3, NA = 45, NB = 45, NX = 20, and NY = 400,

when n is 4, NA = 45, NB = 46, NX = 400, and NY = 50,

when n is 5, NA = 46, NB = 46, NX = 20, and NY = 400,

when n is 6, NA = 46, NB = 47, NX = 400, and NY = 50,

when n is 7, NA = 47, NB = 47, NX = 20, and NY = 400,

when n is 8, NA = 47, NB = 48, NX = 400, and NY = 50,

when n is 9, NA = 48, NB = 48, NX = 20, and NY = 400,

when n is 10, NA = 48, NB = 49, NX = 400, and NY = 50,

when n is 11, NA = 49, NB = 49, NX = 20, and NY =
400,
when n is 12, NA = 49, NB = 50, NX = 400, and NY =
50,
when n is 13, NA = 50, NB = 50, NX = 20, and NY =
400,
when n is 14, NA = 50, NB = 51, NX = 400, and NY =
50,
when n is 15, NA = 51, NB = 51, NX = 20, and NY =
400,
when n is 16, NA = 51, NB = 52, NX = 400, and NY =
50,
when n is 17, NA = 52, NB = 52, NX = 20, and NY =
400,
when n is 18, NA = 52, NB = 53, NX = 400, and NY =
50,
when n is 19, NA = 53, NB = 53, NX = 20, and NY =
400,
when n is 20, NA = 53, NB = 54, NX = 400, and NY =
50,
when n is 21, NA = 54, NB = 54, NX = 20, and NY =
400,
when n is 22, NA = 54, NB = 55, NX = 400, and NY =
50,
when n is 23, NA = 55, NB = 55, NX = 20, and NY =
400, or

when n is 24, $NA = 55$, $NB = 56$, $NX = 400$, and $NY = 50$.

[0031]

In another embodiment, examples of the target region R_n of the present invention include, but not limited to, regions wherein

when n is 1, $NA = 44$, $NB = 44$, $NX = 20$, and $NY = 400$,

when n is 2, $NA = 44$, $NB = 45$, $NX = 600$, and $NY = 19$,

when n is 3, $NA = 45$, $NB = 45$, $NX = 20$, and $NY = 400$,

when n is 4, $NA = 45$, $NB = 46$, $NX = 400$, and $NY = 50$,

when n is 5, $NA = 46$, $NB = 46$, $NX = 20$, and $NY = 400$,

when n is 6, $NA = 46$, $NB = 47$, $NX = 400$, and $NY = 37$,

when n is 7, $NA = 47$, $NB = 47$, $NX = 20$, and $NY = 400$,

when n is 8, $NA = 47$, $NB = 48$, $NX = 400$, and $NY = 19$,

when n is 9, $NA = 48$, $NB = 48$, $NX = 20$, and $NY = 400$,

when n is 10, $NA = 48$, $NB = 49$, $NX = 400$, and $NY = 42$,

when n is 11, $NA = 49$, $NB = 49$, $NX = 20$, and $NY =$

400,

when n is 12, NA = 49, NB = 50, NX = 400, and NY =

44,

when n is 13, NA = 50, NB = 50, NX = 20, and NY =

400,

when n is 14, NA = 50, NB = 51, NX = 400, and NY =

25,

when n is 15, NA = 51, NB = 51, NX = 20, and NY =

400,

when n is 16, NA = 51, NB = 52, NX = 400, and NY =

24,

when n is 17, NA = 52, NB = 52, NX = 20, and NY =

400,

when n is 18, NA = 52, NB = 53, NX = 400, and NY =

34,

when n is 19, NA = 53, NB = 53, NX = 20, and NY =

400,

when n is 20, NA = 53, NB = 54, NX = 400, and NY =

43,

when n is 21, NA = 54, NB = 54, NX = 20, and NY =

400,

when n is 22, NA = 54, NB = 55, NX = 400, and NY =

25,

when n is 23, NA = 55, NB = 55, NX = 20, and NY =

400, or

when n is 24, NA = 55, NB = 56, NX = 400, and NY =

50.

[0032]

In another embodiment, R1 to R24 which are examples of the target region Rn of the present invention are, but not limited to, as follows:

for example, in a certain embodiment,

the region R1 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 44th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 44th intron in the human dystrophin pre-mRNA,

the region R2 is a region that consists of a base sequence of 600 bases in the upstream direction from the 3' end of the 44th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 45th exon in the human dystrophin pre-mRNA,

the region R3 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 45th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 45th intron in the human dystrophin pre-mRNA,

the region R4 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 45th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 46th exon in the human dystrophin pre-mRNA,

the region R5 is a region that consists of a base sequence of 20 bases in the upstream direction from the

3' end of the 46th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 46th intron in the human dystrophin pre-mRNA,

the region R6 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 46th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 47th exon in the human dystrophin pre-mRNA,

the region R7 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 47th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 47th intron in the human dystrophin pre-mRNA,

the region R8 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 47th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 48th exon in the human dystrophin pre-mRNA,

the region R9 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 48th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 48th intron in the human dystrophin pre-mRNA,

the region R10 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 48th intron and a base sequence of 50 bases

in the downstream direction from the 5' end of the 49th exon in the human dystrophin pre-mRNA,

the region R11 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 49th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 49th intron in the human dystrophin pre-mRNA,

the region R12 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 49th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 50th exon in the human dystrophin pre-mRNA,

the region R13 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 50th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 50th intron in the human dystrophin pre-mRNA,

the region R14 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 50th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 51st exon in the human dystrophin pre-mRNA,

the region R15 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 51st exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 51st intron in the human dystrophin pre-mRNA,

the region R16 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 51st intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 52nd exon in the human dystrophin pre-mRNA,

the region R17 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 52nd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 52nd intron in the human dystrophin pre-mRNA,

the region R18 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 52nd intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 53rd exon in the human dystrophin pre-mRNA,

the region R19 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 53rd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 53rd intron in the human dystrophin pre-mRNA,

the region R20 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 53rd intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 54th exon in the human dystrophin pre-mRNA,

the region R21 is a region that consists of a base sequence of 20 bases in the upstream direction from the

3' end of the 54th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 54th intron in the human dystrophin pre-mRNA,

the region R22 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 54th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 55th exon in the human dystrophin pre-mRNA,

the region R23 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 55th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 55th intron in the human dystrophin pre-mRNA, or

the region R24 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 55th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 56th exon in the human dystrophin pre-mRNA.

[0033]

In an alternative embodiment, R1 to R24 which are examples of the target region Rn of the present invention are, but not limited to, as follows:

for example, in a certain embodiment,

the region R1 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 44th exon and a base sequence of 400 bases

in the downstream direction from the 5' end of the 44th intron in the human dystrophin pre-mRNA,

the region R2 is a region that consists of a base sequence of 600 bases in the upstream direction from the 3' end of the 44th intron and a base sequence of 19 bases in the downstream direction from the 5' end of the 45th exon in the human dystrophin pre-mRNA,

the region R3 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 45th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 45th intron in the human dystrophin pre-mRNA,

the region R4 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 45th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 46th exon in the human dystrophin pre-mRNA,

the region R5 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 46th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 46th intron in the human dystrophin pre-mRNA,

the region R6 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 46th intron and a base sequence of 37 bases in the downstream direction from the 5' end of the 47th exon in the human dystrophin pre-mRNA,

the region R7 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 47th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 47th intron in the human dystrophin pre-mRNA,

the region R8 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 47th intron and a base sequence of 19 bases in the downstream direction from the 5' end of the 48th exon in the human dystrophin pre-mRNA,

the region R9 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 48th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 48th intron in the human dystrophin pre-mRNA,

the region R10 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 48th intron and a base sequence of 42 bases in the downstream direction from the 5' end of the 49th exon in the human dystrophin pre-mRNA,

the region R11 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 49th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 49th intron in the human dystrophin pre-mRNA,

the region R12 is a region that consists of a base sequence of 400 bases in the upstream direction from the

3' end of the 49th intron and a base sequence of 44 bases in the downstream direction from the 5' end of the 50th exon in the human dystrophin pre-mRNA,

the region R13 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 50th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 50th intron in the human dystrophin pre-mRNA,

the region R14 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 50th intron and a base sequence of 25 bases in the downstream direction from the 5' end of the 51st exon in the human dystrophin pre-mRNA,

the region R15 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 51st exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 51st intron in the human dystrophin pre-mRNA,

the region R16 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 51st intron and a base sequence of 24 bases in the downstream direction from the 5' end of the 52nd exon in the human dystrophin pre-mRNA,

the region R17 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 52nd exon and a base sequence of 400 bases

in the downstream direction from the 5' end of the 52nd intron in the human dystrophin pre-mRNA,

the region R18 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 52nd intron and a base sequence of 34 bases in the downstream direction from the 5' end of the 53rd exon in the human dystrophin pre-mRNA,

the region R19 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 53rd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 53rd intron in the human dystrophin pre-mRNA,

the region R20 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 53rd intron and a base sequence of 43 bases in the downstream direction from the 5' end of the 54th exon in the human dystrophin pre-mRNA,

the region R21 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 54th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 54th intron in the human dystrophin pre-mRNA,

the region R22 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 54th intron and a base sequence of 25 bases in the downstream direction from the 5' end of the 55th exon in the human dystrophin pre-mRNA,

the region R23 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 55th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 55th intron in the human dystrophin pre-mRNA, or

the region R24 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 55th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 56th exon in the human dystrophin pre-mRNA.

[0034]

In an alternative embodiment, R1 to R24 which are examples of the target region Rn of the present invention are, but not limited to, as follows:

(A1) Region R1

The region R1 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 44 and the 5' end of intron 44 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "+", and a base sequence region on the 3' side (downstream) therefrom is indicated by "-" (minus). In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 44, and the region indicated by the range of +1 base to +400 bases belongs to intron 44.

(A2) Region R2

The region R2 is a region indicated by the range of -600 bases to +50 bases when the boundary between the 3' end of intron 44 and the 5' end of exon 45 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -600 bases to -1 base belongs to intron 44, and the region indicated by the range of +1 base to +50 bases belongs to exon 45.

(A3) Region R3

The region R3 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 45 and the 5' end of intron 45 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 45, and the region indicated by the range of +1 base to +400 bases belongs to intron 45.

(A4) Region R4

The region R4 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 45 and the 5' end of exon 46 is defined as basing point 0, a base sequence region on the 5' side

(upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 45, and the region indicated by the range of +1 base to +50 bases belongs to exon 46.

(A5) Region R5

The region R5 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 46 and the 5' end of intron 46 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 46, and the region indicated by the range of +1 base to +400 bases belongs to intron 46.

(A6) Region R6

The region R6 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 46 and the 5' end of exon 47 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases

to -1 base belongs to intron 46, and the region indicated by the range of +1 base to +50 bases belongs to exon 47.

(A7) Region R7

The region R7 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 47 and the 5' end of intron 47 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 47, and the region indicated by the range of +1 base to +400 bases belongs to intron 47.

(A8) Region R8

The region R8 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 47 and the 5' end of exon 48 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 47, and the region indicated by the range of +1 base to +50 bases belongs to exon 48.

(A9) Region R9

The region R9 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 48 and the 5' end of intron 48 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 48, and the region indicated by the range of +1 base to +400 bases belongs to intron 48.

(A10) Region R10

The region R10 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 48 and the 5' end of exon 49 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 48, and the region indicated by the range of +1 base to +50 bases belongs to exon 49.

(A11) Region R11

The region R11 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 49 and the 5' end of intron 49 is defined as basing point 0, a base sequence region on the 5' side

(upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 49, and the region indicated by the range of +1 base to +400 bases belongs to intron 49.

(A12) Region R12

The region R12 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 49 and the 5' end of exon 50 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 49, and the region indicated by the range of +1 base to +50 bases belongs to exon 50.

(A13) Region R13

The region R13 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 50 and the 5' end of intron 50 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases

to -1 base belongs to exon 50, and the region indicated by the range of +1 base to +400 bases belongs to intron 50.

(A14) Region R14

The region R14 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 50 and the 5' end of exon 51 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 50, and the region indicated by the range of +1 base to +50 bases belongs to exon 51.

(A15) Region R15

The region R15 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 51 and the 5' end of intron 51 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 51, and the region indicated by the range of +1 base to +400 bases belongs to intron 51.

(A16) Region R16

The region R16 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 51 and the 5' end of exon 52 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 51, and the region indicated by the range of +1 base to +50 bases belongs to exon 52.

(A17) Region R17

The region R17 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 52 and the 5' end of intron 52 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 52, and the region indicated by the range of +1 base to +400 bases belongs to intron 52.

(A18) Region R18

The region R18 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 52 and the 5' end of exon 53 is defined as basing point 0, a base sequence region on the 5' side

(upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 52, and the region indicated by the range of +1 base to +50 bases belongs to exon 53.

(A19) Region R19

The region R19 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 53 and the 5' end of intron 53 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 53, and the region indicated by the range of +1 base to +400 bases belongs to intron 53.

(A20) Region R20

The region R20 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 53 and the 5' end of exon 54 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases

to -1 base belongs to intron 53, and the region indicated by the range of +1 base to +50 bases belongs to exon 54.

(A21) Region R21

The region R21 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 54 and the 5' end of intron 54 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 54, and the region indicated by the range of +1 base to +400 bases belongs to intron 54.

(A22) Region R22

The region R22 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 54 and the 5' end of exon 55 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 54, and the region indicated by the range of +1 base to +50 bases belongs to exon 55.

(A23) Region R23

The region R23 is a region indicated by the range of -20 bases to +400 bases when the boundary between the 3' end of exon 55 and the 5' end of intron 55 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -20 bases to -1 base belongs to exon 55, and the region indicated by the range of +1 base to +400 bases belongs to intron 55.

(A24) Region R24

The region R24 is a region indicated by the range of -400 bases to +50 bases when the boundary between the 3' end of intron 55 and the 5' end of exon 56 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 55, and the region indicated by the range of +1 base to +50 bases belongs to exon 56.

[0035]

In an alternative aspect, R2, R6, R8, R10, R12, R14, R16, R18, R20, and R22 which are examples of the target region R_n of the present invention are, but not limited to, as follows:

(A2) Region R2

The region R2 is a region indicated by the range of -600 bases to +19 bases when the boundary between the 3' end of intron 44 and the 5' end of exon 45 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -600 bases to -1 base belongs to intron 44, and the region indicated by the range of +1 base to +19 bases belongs to exon 45.

(A6) Region R6

The region R6 is a region indicated by the range of -400 bases to +37 bases when the boundary between the 3' end of intron 46 and the 5' end of exon 47 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 46, and the region indicated by the range of +1 base to +37 bases belongs to exon 47.

(A8) Region R8

The region R8 is a region indicated by the range of -400 bases to +19 bases when the boundary between the 3' end of intron 47 and the 5' end of exon 48 is defined as basing point 0, a base sequence region on the 5' side

(upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 47, and the region indicated by the range of +1 base to +19 bases belongs to exon 48.

(A10) Region R10

The region R10 is a region indicated by the range of -400 bases to +42 bases when the boundary between the 3' end of intron 48 and the 5' end of exon 49 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 48, and the region indicated by the range of +1 base to +42 bases belongs to exon 49.

(A12) Region R12

The region R12 is a region indicated by the range of -400 bases to +44 bases when the boundary between the 3' end of intron 49 and the 5' end of exon 50 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases

to -1 base belongs to intron 49, and the region indicated by the range of +1 base to +44 bases belongs to exon 50.

(A14) Region R14

The region R14 is a region indicated by the range of -400 bases to +25 bases when the boundary between the 3' end of intron 50 and the 5' end of exon 51 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 50, and the region indicated by the range of +1 base to +25 bases belongs to exon 51.

(A16) Region R16

The region R16 is a region indicated by the range of -400 bases to +24 bases when the boundary between the 3' end of intron 51 and the 5' end of exon 52 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 51, and the region indicated by the range of +1 base to +24 bases belongs to exon 52.

(A18) Region R18

The region R18 is a region indicated by the range of -400 bases to +34 bases when the boundary between the 3'

end of intron 52 and the 5' end of exon 53 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 52, and the region indicated by the range of +1 base to +34 bases belongs to exon 53.

(A20) Region R20

The region R20 is a region indicated by the range of -400 bases to +43 bases when the boundary between the 3' end of intron 53 and the 5' end of exon 54 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 53, and the region indicated by the range of +1 base to +43 bases belongs to exon 54.

(A22) Region R22

The region R22 is a region indicated by the range of -400 bases to +25 bases when the boundary between the 3' end of intron 54 and the 5' end of exon 55 is defined as basing point 0, a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus), and a base sequence region on the 3' side (downstream) therefrom is indicated by "+". In this

respect, the region indicated by the range of -400 bases to -1 base belongs to intron 54, and the region indicated by the range of +1 base to +25 bases belongs to exon 55.

[0036]

Specific examples of the base sequences of the regions R1 to R24 will be given below. As used herein, thymine "T" and uracil "U" are interchangeable with each other. It does not essentially affect the exon skipping activity of the antisense oligomer of the present invention whether it is "T" or "U". Therefore, as used herein, identical base sequences, even when "T" are replaced with "U", are also included and represented by the same SEQ ID NO. In the tables below, "U" may be described as "T" even in the base sequence of pre-mRNA. Those skilled in the art can understand an RNA sequence by appropriately replacing "T" with "U".

[0037]

[Table 1]

Table 1

Region	Base sequence of target region	SEQ ID NO
R1	<p>Region in vicinity of donor of intron 44 (in range of -20 to +400 bases from 5' end of intron 44 as basing point)</p> <p>AATACAAATGGTATCTTAAGGTAAGTCTTTGATTGTTTTTCGAAATTGTATTTATCTTC AGCACATCTGGACTCTTTAACTTCTTAAAGATCAGGTTCTGAAGGGTGATGGAAATTACTT TTGACTGTTGTTGTCATCATTATATTACTAGAAAAGAAAATTATCATAATGATAATATTAGA GCACGGTGCTATGGACTTTTTGTGTGAGGATGAGAGAGTTTGCCTGGACGGAGCTGGTTTA TCTGATAAACTGCAAAATATAATTGAATCTGTGACAGAGGGAAGCATCGTAACAGCAAGGT GTTTTGTGGCTTTGGGGCAGTGTGATTTCCGGCTTTATGTTGGAACCTTTCCAGAAGGAGA ACTTGTGGCATACTTAGCTAAATGAAGTTGCTAGAAATATCCATCATGATAAA</p>	SEQ No. 233
R2	<p>Region in vicinity of acceptor of intron 44 (in range of -600 to +50 bases from 3' end of intron 44 as basing point)</p> <p>TCTTGATGGGATGCTCCTGAAAGCAATTAAATTCAGTTTTTTGTGGCTTCTAATGCAAAA TACATTGACGCAGACAGAATTTGAAATGAATTTTCTTCTAATATAGCAATTAATTTTATTT AAATATCTCTAGAGTTTTTTTTTAATACTGTGACTAACCTATGTTTGTCTTTTTTCACTC TCGTATCCAGGATCACTAAGAAACCCAAATACTTTGTTTCTGTTTAAATTTTACAACATTT CATAGACTATTAACATGGAACATCCTTGTGGGGACAAGAAATCGAATTTGCTCTTGAAAA GGTTTCCAACATAATTGATTTGTAGGACATTATAACATCCTCTAGCTGACAAGCTTACAAAA ATAAAAAGCTGGAGCTAACCGAGAGGGTGCTTTTTTCCCTGACACATAAAAGGTGCTTTCT GTCTGTATCCTTTGGATATGGGCATGTGAGTTTCATAGGGAAATTTTACATGGAGCTTT TGTATTTCTTTCTTTGCCAGTACAACATGTCATGTGGTAGCACACTGTTTAATCTTTTCTCAA ATAAAAAGACATGGGGCTCATTTTTGTTTTGCCTTTTTGGTATCTTACAGGAACTCCAGG ATGGCATTGGGCAGCGGCAACCTGTTGTCAGAACATTGAA</p>	SEQ No. 234
R3	<p>Region in vicinity of donor of intron 45 (in range of -20 to +400 bases from 5' end of intron 45 as basing point)</p> <p>CTGTGACAGACAGAAAAAGAGGTAGGGCGACAGATCTAATAGGAATGAAAACATTTTAGCAG ACTTTTTAAGCTTTCTTTAGAAGAATATTTTCATGAGAGATTATAAGCAGGGTGAAAGGCAC TAACATTAAGAACCTATCAACCATTAAATCAACAGCAGTAAAGAAATTTTTTATTTCTTTT TTTCATATACTAAAAATATATACTTGTGGCTAGTTAGTGGTTTTCTGCTATTTTAACTTGA AGTTTGCTTTTAAAAATCACCCATGATTGCTTAAAGGTGAATATCTTCAATATATTTTAACT TCAACAAGCTGAATCTCAGTTGTTTTTCAAGAAGATTTTAGAAAGCAATTATAAATGATTG TTTTGTAGGAAAGACAGATCTTTGCTTAGTTTTTAAAAATAGCTATGAATATGAC</p>	SEQ No. 235
R4	<p>Region in vicinity of acceptor of intron 45 (in range of -400 to +50 bases from 3' end of intron 45 as basing point)</p> <p>ATTACAGGCGCCTGCCACCAACCTGGCAAAATTTTGTATTTTAGTGTAGACGGGGTTTC ACCATATTTGCCAGGCTGGTCCGAACTCCTGACCTCAAGTGATCCGCCACATCGGCCTC CCTAAGCGCTAGGGTTACAGGCATGAGCCACTGCGCCTGGCCAGGAATTTTGAATCAGAA TTTTTCTTGTTCGATTTAATCTCTTATCATTTAGAGATTCTTGAAATATTGAAATTACTT TGTTCAAAGTGAATGAATTTTCTTAAATTATGTATGGTTAACATCTTTTAAATTGCTTATT TTTAAATTGCCATGTTTGTGTCCAGTTTGCATTAACAAATAGTTTGAGAACTATGTTGGA AAAAAAAATAACAATTTTATTCTTCTCCAGGCTAGAAGAACAAAAGAATATCTTGTG AGAATTTCAAAGAGATTAAATG</p>	SEQ No. 236
R5	<p>Region in vicinity of donor of intron 46 (in range of -20 to +400 bases from 5' end of intron 46 as basing point)</p> <p>AAAAGCTTGAGCAAGTCAAGGTAATTTTATTTTCTCAAATCCCCAGGGCTGCTTGCATA AAGAAGTATATGAATCTATTTTTTAATTCATTCATTGGTTTTCTGCCCATTAGGTTATTCA TAGTTTCTTGTAAAGTGTTTTTCTCACAACCTTATTTCTTCTTAACCCTGCAGTTCTGAA CCAGTGCACATAAGAACATATGTATATATGTGTGTGTGATTTATATATACACACACAC</p>	SEQ No. 237

	ATATTGCATCTATACATCTACACATATAGATGTATAGATTCAATATGTCTAAATGTATATA ATTCACAGITTTTATCTTTGATTGAAATTAATTTAGATTTTACTTGAGAACTTCACAAC TTCATATAATTTTAACTGAAGACCAGATTGTGGAATCATAAAATCTAAATC	
R6	Region in vicinity of acceptor of intron 46 (in range of -400 to +50 bases from 3' end of intron 46 as basing point) ATAATGCAATTTCTCAGCAGCAAGCTACGGTATGCTATGGCATGCTATGATACCCAAGAGG CTCATGAATTTGTTTACATTGTTCTATTTCTGATAGAGAGATAGGTTTTCAGACACTAACT TTATTTGGAGTGTGCTTTACCATCTCACATTTTCTCTTAAAAAATTTATGAGGGATAAT ATAATCGTTTATTTTCTACAGAGATTTATCTACTGAGGGGGTGAAGTGTTCAGTCAATCAG CTCTGTGCTCAGATAGAAAACTGTTGGTATTTGAGGTACCACTGGGCCCTCGGTCAAGTCG CTTCATTTTGATAGACTAATCAATAGAAGCAAAGCAAGGTAGTTGGAATTGTGCTGTAAT TCATTTTAAACGTTGTTGCATTTGTCTGTTTCAGTTACTGGTGAAGAGTTGCCCTGCGC CAGGGAATTCCAAACAATTA	SEQ No. 238
R7	Region in vicinity of donor of intron 47 (in range of -20 to +400 bases from 5' end of intron 47 as basing point) CAAATCTCCAGTGGATAAAGGTTAGACATTAACCATCTCTCCGTCACATGTGTTAAATGT TGCAAGTATTTGTATGATTTTGTGTTTCCCTGGGTGCTTCATTGGTTCGGGGAGGAGGCTGTA TGTGGATTGTTGTTTGTGTTTGTGTTTAACTGACCGTTGCTTTGGCTATATGTTTG TTGTGGCTAGAAAAATGATGATGGTGAATGGCTTACATTAATGACCAAATGCCAAATTT TATACCACAATTTTTCATAAATATTCTGAAGAATCAGACTGAAGAAATGGCGAAGTAT TTAATTCAGTGGCCAGGCATGTACTGACAGTATTTAAGCTGAAAGGACGTGGTCTGGTCT AGTTAAACAAGTGTCTATAAATCAAATTAATTAATTCACACCTGTGGTATGGACT	SEQ No. 239
R8	Region in vicinity of acceptor of intron 47 (in range of -400 to +50 bases from 3' end of intron 47 as basing point) TATTCATTTTATAACTGCAAAGGAAGCGGTATGGCATATAATACACAACACACAGTAT ATTTAGTAAGTGAATAAATGAAAGATGTAITTTCTTTACTTTATCAGTTGCAGTTGGC TATGCCCTTTGTGAAGGTGTGTGTTTGAATTTCAAAAAGGTATTAGTTTCTTTAAAGCA AAGAATTTTGTAGCAGGTTAATGAATAATTTGAATACATTGGTTAAATCCCAACATGTA ATATATGTAAATAATCAATATTATGCTGCTAAATAACACAAATCAGTAAGATTCTGTAAT ATTTTCATGATAAACTTTTGAATAATATTTTAAACATTTTGGCTTATGCCCTTGAGAA TTATTTACCTTTTAAATGTATTTTCCCTTTCAGGTTTCCAGAGCTTTACCTGAGAAACAA GGAGAAATGAAGCTCAAATAA	SEQ No. 240
R9	Region in vicinity of donor of intron 48 (in range of -20 to +400 bases from 5' end of intron 48 as basing point) AAGGACCATTTGACGTTGAGGTAGGGAACCTTTTGTCTTTAAATATTTTGTCTTTTAAAG AAAAATGGCAATATCACTGAATTTTCTCATTGTTGATCATTATTAAGACAAAAATTAATCT TGTTAAAGTGTGGTAAGGAAGACTTTATTCAGGATAACCAATAGGCACAGGGACCACTG CAATGGAGTATTACAGGAGGTTGGATAGAGAGAGATTGGGCTCAACTCTAAATACAGCACA GTGGAAGTAGGAATTTATAGCCAAGGAGCAGTGTAGGAGTCAGTAGATGGAAAATTATTA GAGGAAACATCAGGGTAAGTGGGATTCTGGCTAAACCAACCTCACAGGATTCTTGCTGAA GATAGCCAGGCTTATCTTATCAGACAACCTTGGGGAATGTTGGAGAATACTG	SEQ No. 241
R10	Region in vicinity of acceptor of intron 48 (in range of -400 to +50 bases from 3' end of intron 48 as basing point) TACTAAACACAGAATTTGTAAAAAATAAGTGTATAAAGTAAAAATGAACATTAGGATTAT TGAGATTATTGTAGCTAAACTAGTGTATTATCATATAAATTATGTTAATAAATTGTATTG TCATTATTGCATTTTACTTTTTGAAAAGTAGTTAATGCCTGTGTTCTATATGAGTATTA TATAATTCAAGAAGATATTGGATGAATTTTTTTAAAGTTAATGTGTTTACATCTCTGTT TCTTTTCTCTGCACAAAAGCTACATTTTGTGCCCTTATGTACCAGGCAGAAAATTGATCT GCAATACATGTGGAGTCTCCAAGGGTATATTTAAATTTAGTAATTTTATTGCTAACTGTGA AGTTAATCTGCACTATATGGGTTCTTTTCCCAGGAACTGAAATAGCAGTTCAAGCTAAA CAACCGGATGTGAAGAGATTTT	SEQ No. 242

R11	<p>Region in vicinity of donor of intron 49 (in range of -20 to +400 bases from 5' end of intron 49 as basing point)</p> <p>CAGCCACTCAGCCAGTGAAGGTAATGAAGCAACCTCTAGCAATATCCATTACCTCATAATG GGTTATGCTTCCCCTGTTGTACATTTGCCATTGACGTGGACTATTTATAATCAGTGAAATA ACTTGTAAGGAAATACTGGCCATACTGTAATAGCAGAGGCAAAGCTGCTTTTGTATCAGC ATATCCTATTATATATTGTGATCTTAAGGCTATTAACGAGTCATTGCTTTAAAGGACTCA TTTCTGTCTGGTGTGCTGCCATCAATACAAAAGTAGTCCACCTTCAAGGTAGATTAAT TCTTTGGGGCTTTATTGCTTTGCTTGGCAGCCTTGATGCTTTTCATATTGTTGGTTTAAAT TCAAATCAAGCTACTGCATCATAGTGTCTGTCTCCAACAGCTGTAAAGAATCAC</p>	SEQ No. 243
R12	<p>Region in vicinity of acceptor of intron 49 (in range of -400 to +50 bases from 3' end of intron 49 as basing point)</p> <p>ATATAATTGACTGGGGGTGAGCCAGTACATTAGGATTTTCTAAAGTTATCTGGATAATTT TAGTATGCAACCACAATAGATACTCTTCAAGAATTAAGCTAGTTGCTGAGAGGGAACGTGT TTTTGTTGGTTTGTCTTCACTAATGTTTGCACCTACTTCTTTAAATAAAATTATGCCTG GAGAAAGGGTTTTGTATGGAGCAATTGATAAATTTGTAGGGTGGTTGGCTAAATAAT TATAATTCCTTTAAAGAAATTTCTACCCACTAAAGTTAATTTAGAAGTAAATAATAAGA AATCCAATAATATATTCACCAATGGATTAAGATGTTTCATGAATTATCTTCAAAGTGTTAA TCGAATAAGTAATGTGTATGCTTTTCTGTAAAGAGGAAGTTAGAAGATCTGAGCTCTGAG TGGAAAGCGGTAAACCGTTTACT</p>	SEQ No. 244
R13	<p>Region in vicinity of donor of intron 50 (in range of -20 to +400 bases from 5' end of intron 50 as basing point)</p> <p>ACTGACCACTATTGGAGCCTGTAAGTATACTGGATCCCATTCTCTTTGGCTCTAGCTATTT GTTCAAAGTGCAACTATGAAGTGATGACTGGGTGAGAGAGAAAATTTGTTTCAATTCTAA AGATAGAGATAAACCTTTGTGTATTGACTGTGCAAAAAGCTTTAGAGTACATTCCTTGA AATTGACTCTGATTCAAAGTGTTGCATGACAACGGGATATGGGGAGTGTTCTCTGGAGATA CACCACAAGGAAGAGAGAGCACAAGGGAGATTGTGGGAGAGTCTGAAATGTGATTGTCT TGCAGCAGAGGCCTAAGCCAGTCTCGCAGGAGCCCTACATCTGGGCTGGCTGTGCAGAGCT GTCTGAATTGCAGGCAGTGGGCCTGGCCCTTGTATTCTCTGATCCAGCCAGCCA</p>	SEQ No. 245
R14	<p>Region in vicinity of acceptor of intron 50 (in range of -400 to +50 bases from 3' end of intron 50 as basing point)</p> <p>TCTTGAATAAAAAAATAAGTAAATTTATTTCCCTGGCAAGGTCTGAAAACCTTTTGT TTCTTTACCCTTCCACAATGTATATGATTGTTACTGAGAAGGCTTATTTAACTTAAGTTA CTTGTCAGGCATGAGAATGAGCAAAATCGTTTTTTAAAAAATTTGTTAAATGTATATTAAT GAAAAGGTTGAATCTTTTCAATTTCTACCATGTATTGCTAAACAAAGTATCCACATTGTTA GAAAAAGATATATAATGTCATGAATAAGAGTTTGGCTCAAATTTGTTACTCTTCAATTAAT TTGACTTATTGTTATTGAAATTTGGCTCTTTAGCTGTGTTTCTAATTTTCTTTTCTTCT TTTTTCTTTTTCGAAAAACCCAAAATATTTTAGCTCCTACTCAGACTGTTACTCTGGTGA CACAACTGTGGTTACTAAGGAA</p>	SEQ No. 246
R15	<p>Region in vicinity of donor of intron 51 (in range of -20 to +400 bases from 5' end of intron 51 as basing point)</p> <p>AGATGATCATCAAGCAGAAGGTATGAGAAAAATGATAAAAGTTGGCAGAAGTTTTCTTT AAAATGAAGATTTTCCACCAATCACTTTACTCTCCTAGACCATTTCCCACCAAGTTCTTAGG CAACTGTTTCTCTCAGCAAAACACATTACTCTCACTATTGAGCCTAAGTATAATCAAGGA TATAAATTAATGCAAAATAACAAAAGTAGCCATACATTAATAAGGAAATATACAAAAA AAAAAAGGAGCAGAAACCTTACAAGAATAGTTGTCTCAGTTAAATTTACTAAACAACCT GGTATTTTAAAAATCTATTTTATACCAATAAGTCACTCACTGAGCTATTTACATTTAAA CTGTTTGTGTTTGGCACTACGAGCCCAACATATTGCAGAATCAAATATAATAGT</p>	SEQ No. 247
R16	<p>Region in vicinity of acceptor of intron 51 (in range of -400 to +50 bases from 3' end of intron 51 as basing point)</p> <p>TTTAAATAAATATACCTTAATTTTGACGTACACAGAATGATATTATAAGTATAAATAGT TATCTATCTTTTAAATACATTGTCGTAATTCAGAAATACATTTCTTACTCAAGGCATTGAG ACAGTGGTTTAAAGTAATCCGAGGTACTCCGGAATGTCTCCATTTGAGCCTTTAAATGAAGA</p>	SEQ No. 248

	AAATCTATAGTCAAGATTTTCATTTGAAATATTTTGTATCTAAGAATGAAACATATTTTC CTGTTAAATTTGTTTCTATAAACCCCTTATACAGTAACATCTTTTTTATTTCTAAAGTGT TTGGCTGGTCTCACAATTGTACTTTACTTTGTATTATGTAAGGAATACACAACGCTGAA GAACCTGATACTAAGGGATATTTGTTCTTACAGGCAACAATGCAGGATTTGGAACAGAGG CGTCCCCAGTTGGAAGAACTCAT	
R17	Region in vicinity of donor of intron 52 (in range of -20 to +400 bases from 5' end of intron 52 as basing point) AACAAATCATTACGGATCGAAGTAAGTTTTTAACAAGCATGGGACACACAAAGCAAGATGC ATGACAAGTTTCAATAAAAACTTAAGTTCATATATCCCCCTCACATTATAAAAAATAATGT GAAATAATTGTAATGATAACAATTGTGCTGAGATTTTCAGTCCATAATGTTACCTTTTAA TAAATGAATGTAATTCATTGAATAGAAGAAATACATTTTAAATCAATTCAGGGCTTATA TAGTTGCAAAGCATGCATTGATGGGTGTGGTGACCACAGTGTGGCAGAACATTTGTGGCAG AACATTTGTTCTTTAGTTGTCTCTGGGCTGGCATCCATGGAGATGCCAGTCTCTCCCTCA TATCCTTGGCTGTTGGTCCAAGCAGGCACTGGCTTCTTCTGGGCCATCTTTCA	SEQ No. 249
R18	Region in vicinity of acceptor of intron 52 (in range of -400 to +50 bases from 3' end of intron 52 as basing point) CAAACCTCCTGGGCAACAGAAAGCCTTCAGGCAATGAAATGCTGGCACTGGGAAATCAGGC TGATGGGTGCTGAAGTGGCAAGGATGAGGGGATATGGATATTCTGCTGTAGTGTCTTTCTA ACAGATGATTCATATTTGTTCTAGGGATCAAGAATTGAGTTAAAAATTTATATATATGTT GATGTTCTATGTCACCTTCAGGAAAATAATTTAACAGAACTAATATTTGCCATCAAAAA GCAAGAATCCTGTTGTTTCATCATCCTAGCCATAACACAATGAATAATTTTAAATAAGC AACATAAATGTGAGATAACGTTTGGAAAGTTACATTTAAAAATGTCTCTCCAGACTAGCATT TACTACTATATATTTATTTTCTTTTATTCTAGTTGAAAGAATTGAGAATCAGTGGGATG AAGTACAAGAACACCTTCAGAAC	SEQ No. 250
R19	Region in vicinity of donor of intron 53 (in range of -20 to +400 bases from 5' end of intron 53 as basing point) AGAAATCAGAGAAACCAAGGTTAGTATCAAAGATACCTTTTTAAAAATAAAATACTGGTTA CATTTGATAAAATTATACCATAGATTGTAATTTAATGATGTTAATGTAAAGTTATTAACA GAAAATCACGTTAAAGCTGAAATGAACAGTAGACTTTGTATATTTTCTTAGAGACAG AGTCTCACTGTCAACCCAGGCTAAAGTGCAGTGGCACAATCATAGCTCACTGAGCCTGAAC TCTGGGGCTCAAGCAGTCCTCTGCCTCAGCCTCCCTAGTAGCTGGGACTACTAGCCAGGC GTGTACCACCACGCCTGGCTAATTTTTTAAAAATTTTGTCTTCTAGAGATGGGTTCTT GAACCTTGGGCTCAAGCAATTCTCCTTCTTGGCCTCCCAAAGCACTAGGATT	SEQ No. 251
R20	Region in vicinity of acceptor of intron 53 (in range of -400 to +50 bases from 3' end of intron 53 as basing point) CAACAAGTTAATGAAGAGGGAAGAAATGTATGAGGTTTTTTTCGTTCAAATGTTGTTATA TGTCACATATTCAACAATTATATATGAGCTTATTTTGTAGTTTTTTCTCTGTGATAAA AACAAATTAAGCCCACTTTATTGCCAATTAATTGCTACTAAGTTGAAATACTTGATACTGGT TATTGCTCAAGATGCTGCATTGAAAAGTTTGTCTGAAAGGTGGGTACCTTATACTGTC ATGATTGACTAAATCATATGGTAGGTTAAAAGCAATCTAATATATGTATTCTGACCTGAGG ATTGAGAAGCTGTTTACGAAGTATTTTAAAGCACTCCAACTAGAGATTTCAAAAAAAAC TGACATTCATTCTCTTTCTATAAAAAATCTATAGCAGTTGGCCAAAGACCTCCGCCAGTGG CAGACAAATGTAGATGTGGCAAA	SEQ No. 252
R21	Region in vicinity of donor of intron 54 (in range of -20 to +400 bases from 5' end of intron 54 as basing point) TGGAGAAGCATTCAAAAAGGTATGAATTACATTATTTCTAAAACTACTGTTGGCTGTAAT AATGGGGTGGTGAACCTGGATGGACCATGAGGATTGTTTTTCCAATCCAGCTAAACTGGA GCTTGGGAGGTTCAAGACGATAAATACCAACTAACTCACGGACTTGGCTCAGACTTCTA TTTTAAAAACGAGGAACATAAGATCTCATTTGCCCGCTGTCAAAAAGTAGTGACATAACC AAGAGATTAACAAAAAGCAAAATACTGATTTATAGCTAGAAGAGCCATTTATCAGTCTAC TTTGATAACTCTATCCAAAGGAATATCTTTCTATCTCATCATGGCCACACTGCCTTACCT GTTATCTGATAAATAAGTCACITTTGGGATTCATGATAGAGTTATAGCTGTACAT	SEQ No. 253

R22	<p>Region in vicinity of acceptor of intron 54 (in range of -400 to +50 bases from 3' end of intron 54 as basing point)</p> <p>TCTCAAATTTGGCAGTATATTAATAAAGCTTTCAAATTTGACCAACAAAACTACAAAA TTGAAAAAAGGTACTTTGAACTTTTACATGTTCAAATATATGTATATATATTTTACATAT ATATATGAAACCTCCTCTGTGGAGAGGGGTTTATAGAAATCTGTAATTGTCATTCTTGCA GCCTTCCCCATACAAACGCCTTTAAGTTAAATAAAAAATGAAAGTAAATAGACTGCACAAT ATTATAGTTGTTGCTTAAAGGAAGAGCTGTAGCAACAACCTACCCCATTTGTTGGTATATTA CAATTTAGTTCCCTCCATCTTTCTCTTTTATGGAGTTTCACTAGGTGCACCATTCTGATATT TAATAATTGCATCTGAACATTTGGTCCTTTGCAGGGTGAGTGAGCGAGAGGCTGCTTTGGA AGAACTCATAGATTACTGCAAC</p>	SEQ No. 254
R23	<p>Region in vicinity of donor of intron 55 (in range of -20 to +400 bases from 5' end of intron 55 as basing point)</p> <p>AGCTGATGAAACAATGGCAAGTAAGTCAGGCATTTCCGCTTTAGCACTCTTGTGGATCCAA TTGAAACAATTCTCAGCATTTTGTACTTGTAACTGACAAGCCAGGGACAAAACAAAATAGTTG CTTTTATACAGCCTGATGTATTTTCGGTATTTGGACAAGGAGGAGAGAGGCAGAGGGAGAAG GAAACATCATTATAATTCCACTTAACACCCTCGCTTTAGAAAAAGTACATGCTCTGACCA GGAAAAATTTGCATATAAAACCAGAGCTTCGGTCAAGGAGAACTTTGCTCAGAGAAATA ACTTAGGGATTGGTTTATTAATTTTAAAGTTGACATTTTGTAGTGTATTTAATATTTT TACAGGGAAAGCATCTGTATGAATTGTCTGTTTTATTTAGCGTTGCTAACTGAA</p>	SEQ No. 255
R24	<p>Region in vicinity of acceptor of intron 55 (in range of -400 to +50 bases from 3' end of intron 55 as basing point)</p> <p>ACTTGATCCATATAGTAATGAAATTATTGGCACTGGGGTACACTTTATCATAGAATTTTAT TGCCATACACTTCCATAAAATAATACATTTTGTCCATAGACTAGAAGATATAACTTGTGAA CTTTATAAAGTTATAAATACATTACTTTCCAACCTCATAATGGCAAGGAATAAATCTATTAC AACTAATAAGATGCCCATTTTAAATCTACATAATAACAGGAGAAGGCAATACGCCAAGAAA AGGGATTTGAGATGTATCTTCTGTAGTTTAGCCTGATTGAAATGTCTTTTGAACATAA ATTATTTATATTTTGCAATTCTCCAAATTCACATTCATCGCTTGTCTTTTGTGTTGGTAA TTCTGCACATATTCTTCTTCTGCTGTCTGTAGGACCTCCAAGGTGAAATTGAAGCTCAC ACAGATGTTTATCACAACCTGGA</p>	SEQ No. 256

[0038]

In an alternative aspect, specific examples of the base sequences of the regions R2, R6, R8, R10, R12, R14, R16, R18, R20, and R22 are as follows.

[0039]

[Table 2]

Table 2

Region	Base sequence of target region	SEQ ID NO
R2	<p>Region in vicinity of acceptor of intron 44 (in range of -600 to +19 bases from 3' end of intron 44 as basing point)</p> <p>TCTTGATGGGATGCTCCTGAAAGCAATTAATTCAGTTTTTTGTGGCTTCTAATGCAAAA TACATTGACGCAGACAGAATTTGAAATGAATTTTCTTCTAATATAGCAATTAATTTTATTT AAATATCTCTAGAGTTTTTTTTTAATACTGTGACTAACCTATGTTTGTCTTTTTCACCTC TCGTATCCACGATCACTAAGAAACCCAAATACTTTGTTTCATGTTTAAATTTTACAACATTT CATAGACTATTAAACATGGAACATCCTTGTGGGGACAAGAAATCGAATTTGCTCTTGAAAA GGTTTCCAACATAATTGATTTGTAGGACATTATAACATCCTCTAGCTGACAAGCTTACAAAA ATAAAAACTGGAGCTAACCAGAGAGGGTGCCTTTTTCCCTGACACATAAAAGGTGTCTTCT GTCTTGATCCTTTGGATATGGGCATGTCAGTTTCATAGGGAAATTTTCACATGGAGCTTT TGTATTTCTTTCTTTGCCAGTACAACTGCATGTGGTAGCACACTGTTAATCTTTTCTCAA ATAAAAAGACATGGGGCTTCATTTTGTTCCTTTTGGTATCTTACAGGAACTCCAGG ATGGCATTG</p>	SEQ No. 341
R6	<p>Region in vicinity of acceptor of intron 46 (in range of -400 to +37 bases from 3' end of intron 46 as basing point)</p> <p>ATAATGCAATTTCTCAGCAGCAAGCTACGGTATGCTATGGCATGCTATGATACCCAAGAGG CTGATGAATTTGTTTACATTGTTCTATTTCTGATAGAGAGATAGGTTTTCAGACACTAACT TTATTTGGAGTGTGCTTTACCATCTCACATTTTCTCTTAAAAAATTTATGAGGGATAAT ATAATCGTTTATTTTCTACAGAGATTTATCTACTGAGGGGGTGAGTGTTCAGTCAATCAG CTCTGTGCTCAGATAGAAAACTGTTGGTATTTGAGGTACCACTGGGCCCTCGGTCAAGTCG CTTCATTTTGATAGACTAATCAATAGAAGCAAGCAAGGTAGTTGGAATTGTGCTGTAAT TCATTTTAAACGTTGTTGCATTTGTCTGTTTCAGTTACTGGTGAAGAGTTGCCCTGCGC CAGGGAATTC</p>	SEQ No. 385
R8	<p>Region in vicinity of acceptor of intron 47 (in range of -400 to +19 bases from 3' end of intron 47 as basing point)</p> <p>TATTCATTTTTATACTGCAAAGGAAGCGGTATGGCATATAATACACAACACACCAGTAT ATTTAGTAACAGAGTGAATAAATGAAAGATGTATTCTTTTACTTTATCAGTTGCAGTTGGC TATGCCCTTTGTGTAAGGTGTGTGTTTTGAAATTCAAAAAGGTATTAGTTTCTTTAAAGCA AAGAATTTTTGTAGCAGGTAAATGAATAATTTGAATACATTGGTTAAATCCCAACATGTA ATATATGTAATAATCAATATTATGCTGCTAAAATAACACAAATCAGTAAGATTCTGTAAT ATTTTCATGATAAATACTTTTGAATAATATTTTTAAACATTTTGGCTTATGCCCTTGAGAA TTATTTACCTTTTAAATGTATTTCTTTTTCAGGTTTCCAGAGCTTTACCTG</p>	SEQ No. 386
R10	<p>Region in vicinity of acceptor of intron 48 (in range of -400 to +42 bases from 3' end of intron 48 as basing point)</p> <p>TACTAAACACAGAATTTTGTAACAATAAGTGTATAAAGTAAAAATGAACATTAGGATTAT TGAGATTATTGTAGCTAAACTAGTGTATTATCATATAAATTATGTTAATAAATTGTATTG TCATTATTGCATTTTACTTTTTTGAAGTAGTTAATGCCGTGTGTTTCTATATGAGTATTA TATAATTCAAGAAGATATTGGATGAATTTTTTAAAGTTAATGTGTTTACATCTCTGTT TCTTTTCTCTGCACCAAAAGCTACATTTTGTGCCCTTATGTACCAGGCAGAAATTTGATCT GCAATACATGTGGAGTCTCCAAGGGTATATTTAAATTTAGTAATTTTATTGCTAACTGTGA AGTTAATCTGCACTATATGGGTTCTTTTCCCAGGAAACTGAAATAGCAGTTCAAGCTAAA CAACCGGATGTGGAA</p>	SEQ No. 387
R12	<p>Region in vicinity of acceptor of intron 49 (in range of -400 to +44 bases from 3' end of intron 49 as basing point)</p> <p>ATATAATTGACTGGGGGTGAGCCAGTACATTAGGATTTTCTAAAGTTATCTGGATAATTT TAGTATGCAACCACAATAGATACTCTTCAAGAATTAAGCTAGTTGCTGAGAGGGAACTGTT TTTTGTTGGTTTGTCTTCACTAATGTTTGCACCTACTTCTTTAAATAAAATTTATGCCCTG GAGAAAGGGTTTTGTATGGAGCAATTGATAAATATTTGTAGGGTGGTTGGCTAAAAATAAT TATAATTCCTTTAAAGAAATTTTACCCACTAAAGTTAATTTAGAAGTAAAAATAATAGA</p>	SEQ No. 388

	AATCCAATAATATATTACCAAATGGATTAAGATGTTTCATGAATTATCTTCAAAGTGTTAA TCGAATAAGTAATGTGTATGCTTTTCTGTAAAGAGGAAGTTAGAAGATCTGAGCTCTGAG TGGAAAGCGGGTAAACCG	
R14	Region in vicinity of acceptor of intron 50 (in range of -400 to +25 bases from 3' end of intron 50 as basing point) TCTTGAATAAAAAAAAAAATAAGTAAATTTATTTCCCTGGCAAGGTCTGAAAACCTTTTGTT TTCTTTACCACCTCCACAATGTATATGATTGTTACTGAGAAGGCTTATTTAACTTAAGTTA CTTGTCAGGCATGAGAATGAGCAAAATCGTTTTTTAAAAAATTGTTAAATGTATATTAAT GAAAAGGTTGAATCTTTTCATTTTCTACCATGTATTGCTAAACAAAGTATCCACATTGTTA GAAAAAGATATATAATGTCATGAATAAGAGCTTTGGCTCAAATTTGTTACTCTTCAATTAAT TTGACTTATTGTTATTGAAATTGGCTCTTAGCTGTGTTTCTAATTTTCTTTTCTTCT TTTTTCTTTTTTGCAAAACCCAAAATATTTTAGCTCCTACTCAGACTGTTACTCTGGTGA CAC	SEQ No. 342
R16	Region in vicinity of acceptor of intron 51 (in range of -400 to +24 bases from 3' end of intron 51 as basing point) TTTAAATAAATAATACCTTAATTTTGACGTCACACAGAATGATATTATAAGTATAAATAGT TATCTATCTTTTAAATACATTGTCGTAATTCAGAATAACATTTCTTACTCAAGGCATTGAG ACAGTGGTTTAAAGTAATCCGAGGTAAGTCCGAATGTCTCCATTTGAGCCTTTAAATGAAGA AAATCTATAGTCAAGATTTTCATTTGAAATATTTTGTATCTAAGAATGAAACATATTTTC CTGTTAAATGTTTTCTATAAACCCCTTATACAGTAACATCTTTTTTATTTCTAAAAGTGT TTGGCTGGTCTCACAATTGACTTTACTTTGTATTATGTAAGGAATACACAACGCTGAA GAACCTGATACTAAGGGATATTTGTTCTTACAGGCAACAATGCAGGATTTGGAACAGAGG CGTCCCAAGTTGGAAGAACTCA	SEQ No. 343
R18	Region in vicinity of acceptor of intron 52 (in range of -400 to +34 bases from 3' end of intron 52 as basing point) CAAACCTCTGTGGCAACAGAAAGCCTTCAGGCAATGAAATGCTGGCACTGGGAAATCAGGC TGATGGGTGCTGAAGTGGCAAGGATGAGGGGATATGGATATTTCTGCTGTAGTGCTTTTCTA ACAGATGATTCATATTTGGTTCTAGGGATCAAGAATTGAGTTAAATTTTATATATATGTT GATGTTCTATGTCACCTTCAGGAAAATAATTTAACAGAACTAATATTGGCCATCAAAAA GCAAAAGAAATCCTGTTGTTTCATCCTAGCCATAACACAATGAATAATTTTTTAAATAAGC AACATAAATGTGAGATAACGTTTGAAGTTACATTTAAATGTCTCCTCCAGACTAGCATT TACTACTATATATTTATTTTCTTTTATTCTAGTTGAAAGAATTCAGAATCAGTGGGATG AAGTACA	SEQ No. 344
R20	Region in vicinity of acceptor of intron 53 (in range of -400 to +43 bases from 3' end of intron 53 as basing point) CAACAAGTTAATGAAGAGGGAAAGAAATGTATGAGGTTTTTTTCGTTCAAATGTTGTTATA TGTCACATATTCACAATTATATATGAGCTTATTTTTGTAGTTTTTTCTCTGTGATAAAA AACAAATTAAGCCCACTTTATTGCCAATTAATTGCTACTAAGTTGAAATACTTGATACTGGT TATTGCTCAAGATGCTGCATTTGAAAAGTTTGCTCTGAAAGGTGGTTACCTTATACGTG ATGATTGACTAAATCATATGGTAGGTTAAAGCAATCTAATATATGTATTCTGACCTGAGG ATTCAGAAGCTGTTTACGAAGTATTTAAGACACTCCAAC TAGAGATTTCATAAAAAAAC TGACATTCAATCTCTTTCTCATAAAAAATCTATAGCAGTTGGCCAAAGACCTCCGCCAGTGG CAGACAAATGTAGATG	SEQ No. 389
R22	Region in vicinity of acceptor of intron 54 (in range of -400 to +25 bases from 3' end of intron 54 as basing point) TCTCAAATTTGGCAGTATATTAATAAAGCTTTCAAATTGACCAACAAAACTACAAAA TTGAAAAAAGGTACTTTGAACCTTCACATGTTCAAATATATGTATATATATTTACATAT ATATATGAAACCTCCTCTGTGGAGAGGGGTTATAGAAATCTGTAATTGTCTTCTTGAT GCCITCCCCATACAAACGCCCTTAAAGTTAAATAAAAAATGAAAGTAAATAGACTGCACAA ATTATAGTTCTTGCTTAAAGGAAGAGCTGTAGCAACAACTCACCCCATTTGTTGGTATATTA CAATTTAGTTCTCCTCTTTCTCTTTTATGGAGTTCACTAGGTGCACCATTTCTGATATT TAATAATTGCATCTGAACATTTGGTCTTTGCAGGGTGAGTGAGCGAGAGGCTGCTTTG	SEQ No. 345

[0040]

In a further alternative aspect, the antisense oligomer of the present invention targets at least any one region selected from the group consisting of regions R1 to R24 represented by

region R_n (wherein n is an odd number of 1 to 23) which consists of a base sequence of NY bases in the downstream direction from the 5' end of the NB_{th} intron in the human dystrophin pre-mRNA, and

region R_n (wherein n is an even number of 2 to 24) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the NA_{th} intron in the human dystrophin pre-mRNA.

[0041]

In the foregoing embodiment, examples of the target region R_n of the present invention include, but not limited to, regions wherein

when n is 1, NB = 44 and NY = 400,
when n is 2, NA = 44 and NX = 600,
when n is 3, NB = 45 and NY = 400,
when n is 4, NA = 45 and NX = 400,
when n is 5, NB = 46 and NY = 400,
when n is 6, NA = 46 and NX = 400,
when n is 7, NB = 47 and NY = 400,
when n is 8, NA = 47 and NX = 400,
when n is 9, NB = 48 and NY = 400,
when n is 10, NA = 48 and NX = 400,

when n is 11, NB = 49 and NY = 400,
when n is 12, NA = 49 and NX = 400,
when n is 13, NB = 50 and NY = 400,
when n is 14, NA = 50 and NX = 400,
when n is 15, NB = 51 and NY = 400,
when n is 16, NA = 51 and NX = 400,
when n is 17, NB = 52 and NY = 400,
when n is 18, NA = 52 and NX = 400,
when n is 19, NB = 53 and NY = 400,
when n is 20, NA = 53 and NX = 400,
when n is 21, NB = 54 and NY = 400,
when n is 22, NA = 54 and NX = 400,
when n is 23, NB = 55 and NY = 400, or
when n is 24, NA = 55 and NX = 400.

[0042]

R1 to R24 which are examples of the target region R_n of the present invention according to the foregoing embodiment are, but not limited to, as follows:

for example, in a certain embodiment,

the region R1 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 44th intron in the human dystrophin pre-mRNA,

the region R2 is a region that consists of a base sequence of 600 bases in the upstream direction from the 3' end of the 44th intron in the human dystrophin pre-mRNA,

the region R3 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 45th intron in the human dystrophin pre-mRNA,

the region R4 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 45th intron in the human dystrophin pre-mRNA,

the region R5 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 46th intron in the human dystrophin pre-mRNA,

the region R6 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 46th intron in the human dystrophin pre-mRNA,

the region R7 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 47th intron in the human dystrophin pre-mRNA,

the region R8 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 47th intron in the human dystrophin pre-mRNA,

the region R9 is a region that consists of a base sequence of 400 bases in the downstream direction from

the 5' end of the 48th intron in the human dystrophin pre-mRNA,

the region R10 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 48th intron in the human dystrophin pre-mRNA,

the region R11 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 49th intron in the human dystrophin pre-mRNA,

the region R12 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 49th intron in the human dystrophin pre-mRNA,

the region R13 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 50th intron in the human dystrophin pre-mRNA,

the region R14 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 50th intron in the human dystrophin pre-mRNA,

the region R15 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 51st intron in the human dystrophin pre-mRNA,

the region R16 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 51st intron in the human dystrophin pre-mRNA,

the region R17 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 52nd intron in the human dystrophin pre-mRNA,

the region R18 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 52nd intron in the human dystrophin pre-mRNA,

the region R19 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 53rd intron in the human dystrophin pre-mRNA,

the region R20 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 53rd intron in the human dystrophin pre-mRNA,

the region R21 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 54th intron in the human dystrophin pre-mRNA,

the region R22 is a region that consists of a base sequence of 400 bases in the upstream direction from the

3' end of the 54th intron in the human dystrophin pre-mRNA,

the region R23 is a region that consists of a base sequence of 400 bases in the downstream direction from the 5' end of the 55th intron in the human dystrophin pre-mRNA, or

the region R24 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 55th intron in the human dystrophin pre-mRNA.

[0043]

In another embodiment, R1 to R24 which are examples of the target region R_n of the present invention according to the foregoing embodiment are, but not limited to, as follows:

(A1) Region R1

The region R1 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 44 and the 5' end of intron 44 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 44.

(A2) Region R2

The region R2 is a region indicated by the range of -600 bases to -1 base when the boundary between the 3' end of intron 44 and the 5' end of exon 45 is defined as

basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -600 bases to -1 base belongs to intron 44.

(A3) Region R3

The region R3 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 45 and the 5' end of intron 45 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 45.

(A4) Region R4

The region R4 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 45 and the 5' end of exon 46 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 45.

(A5) Region R5

The region R5 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 46 and the 5' end of intron 46 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+".

In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 46.

(A6) Region R6

The region R6 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 46 and the 5' end of exon 47 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 46.

(A7) Region R7

The region R7 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 47 and the 5' end of intron 47 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 47.

(A8) Region R8

The region R8 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 47 and the 5' end of exon 48 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 47.

(A9) Region R9

The region R9 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 48 and the 5' end of intron 48 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 48.

(A10) Region R10

The region R10 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 48 and the 5' end of exon 49 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 48.

(A11) Region R11

The region R11 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 49 and the 5' end of intron 49 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 49.

(A12) Region R12

The region R12 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 49 and the 5' end of exon 50 is defined as

basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 49.

(A13) Region R13

The region R13 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 50 and the 5' end of intron 50 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 50.

(A14) Region R14

The region R14 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 50 and the 5' end of exon 51 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 50.

(A15) Region R15

The region R15 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 51 and the 5' end of intron 51 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+".

In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 51.

(A16) Region R16

The region R16 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 51 and the 5' end of exon 52 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 51.

(A17) Region R17

The region R17 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 52 and the 5' end of intron 52 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 52.

(A18) Region R18

The region R18 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 52 and the 5' end of exon 53 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 52.

(A19) Region R19

The region R19 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 53 and the 5' end of intron 53 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 53.

(A20) Region R20

The region R20 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 53 and the 5' end of exon 54 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 53.

(A21) Region R21

The region R21 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 54 and the 5' end of intron 54 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 54.

(A22) Region R22

The region R22 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 54 and the 5' end of exon 55 is defined as

basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 54.

(A23) Region R23

The region R23 is a region indicated by the range of +1 base to +400 bases when the boundary between the 3' end of exon 55 and the 5' end of intron 55 is defined as basing point 0 and a base sequence region on the 3' side (downstream) from the basing point is indicated by "+". In this respect, the region indicated by the range of +1 base to +400 bases belongs to intron 55.

(A24) Region R24

The region R24 is a region indicated by the range of -400 bases to -1 base when the boundary between the 3' end of intron 55 and the 5' end of exon 56 is defined as basing point 0 and a base sequence region on the 5' side (upstream) from the basing point is indicated by "-" (minus). In this respect, the region indicated by the range of -400 bases to -1 base belongs to intron 55.

[0044]

In the foregoing embodiment, specific examples of the base sequences of the regions R1 to R24 are as follows.

[0045]

[Table 3]

Table 3

Region	Base sequence of target region	SEQ ID NO
R1	<p>Region in vicinity of donor of intron 44 (in range of +1 to +400 bases from 5' end of intron 44 as basing point)</p> <p>GTAAGTCTTTGATTTGTTTTTCGAAATTGTATTTATCTTCAGCACATCTGGACTCTTTAACTTTCTTAAAGATCAGGTTCTGAAGGGTGATGGAATTACTTTTGACTGTTGTTGTCATCATATATTACTAGAAAGAAAATTATCATAATGATAAATTAGAGCACGGTGCTATGGACTTTTGTGTCAGGATGAGAGAGTTTGCCTGGACGGAGCTGGTTTATCTGATAAACTGCAAAATATAATTGAATCTGTGACAGAGGGAAGCATCGTAACAGCAAGGTGTTTTGTGGCTTTGGGGCAGTGTGATTTTGGGCTTTATGTTGGAACCTTTCCAGAAGGAGAACTTGTGGCATACTTAGCTAAAATGAAGTTGCTAGAAATATCCATCATGATAAA</p>	SEQ No. 346
R2	<p>Region in vicinity of acceptor of intron 44 (in range of -600 to -1 bases from 3' end of intron 44 as basing point)</p> <p>TCTTGATGGGATGCTCCTGAAAGCAATTAATTCAGTTTTTGTGGCTTCTAATGCAAAATACATTGACGCAGACAGAATTTGAAATGAATTTTCTTCTAATATAGCAATTAATTTATTTAAATATCTCTAGAGTTTTTTTTTAATACTGTGACTAACCTATGTTTGTCTTTTTCCACCTCTCGTATCCACGATCACTAAGAAACCCAAATACTTTGTTTCATGTTTAAATTTTACAACATTTTCATAGACTATTAAACATGGAACATCCTTGTGGGACAAGAAATCGAATTTGCTCTTGAAGAAAGTTTCCAATAATTGATTTGTAGGACATTATAACATCCTTAGCTGACAAGCTTACAAAAATAAAAACTGGAGCTAACCGAGAGGGTGCTTTTTTCCCTGACACATAAAAGGTGTCTTTCTGTCTTGATCCTTTGGATATGGGCATGTCAGTTTCATAGGGAAATTTTACATGGAGCTTTTGTATTTCTTTCTTTGCCAGTACAACCTGCATGTGGTAGCACACTGTTAATCTTTCTCAAATAAAAAAGACATGGGGCTTCATTTTGTGTTTGGTATCTTACAG</p>	SEQ No. 347
R3	<p>Region in vicinity of donor of intron 45 (in range of +1 to +400 bases from 5' end of intron 45 as basing point)</p> <p>GTAGGGCGACAGATCTAATAGGAATGAAAAATTTTAGCAGACTTTTAAAGCTTTCTTTAGAAGAATATTTTCATGAGAGATTATAAGCAGGGTGAAAGGCCTAACATTAAAGAACCTATCAACCATTAATCAACAGCAGTAAAGAAATTTTATTTCTTTTTTTCATATACTAAAAATATATCTTTGTGGCTAGTTAGTGGTTTTCTGCTATTTTAACTTGAAGTTTGCTTTAAAAATCACCATGATTGCTTAAAGGTGAATATCTTCAATATATTTAACTTCAACAAGCTGAATCTCAGTTGTTTTCAAGAAGATTTAGAAAGCAATTATAAATGATTGTTTTGTAGGAAAGACAGATCTTTGCTTAGTTTTAAAAATAGCTATGAATATGAC</p>	SEQ No. 348
R4	<p>Region in vicinity of acceptor of intron 45 (in range of -400 to -1 bases from 3' end of intron 45 as basing point)</p> <p>ATTACAGGCGCCTGCCACCAAACCTGGCAAATTTTGTATTTTGTAGTGTAGACGGGGTTTCAACCATATTTGCCAGGCTGGTTCGCAAACTCCTGACCTCAAGTGATCCGCCCACATCGGCCTCCCTAAGCGCTAGGGTTACAGGCATGAGCCACTGCGCCTGGCCAGGAATTTTGAATCAGAAATTTTCTTGTTCGATTTAATCTCTTATCATTTAGAGATTCTTGAAATATTGAAATTACTTTGTTCAAAGTGAATGAATTTTCTTAAATATGTATGGTTAACATCTTTTAAATTGCTTATTATAAATTGCCATGTTTGTGTCGCCAGTTTGCAATTAACAAATAGTTTGAGAACTATGTTGGAATAAAAAATAACAATTTATTCTTTCTCCAG</p>	SEQ No. 349
R5	<p>Region in vicinity of donor of intron 46 (in range of +1 to +400 bases from 5' end of intron 46 as basing point)</p> <p>GTAATTTTATTTTCTCAAATCCCCAGGGCCTGCTTGCATAAAGAAGTATATGAATCTATTTTTAATTCAATCATTGGTTTTCTGCCCATTAGGTTATTCATAGTTCCTTGCTAAAGTGTTTCTCACAACCTTTATTTCTTCTTAAACCTGCAGTTCTGAACAGTGACATAAGAACATATGTATATATGTGTGTGTGTATTTATATACACACACATATTGCATCTATACATCTACATATAGATGTATAGATTCAATATGTCTAAATGTATATAATTACAGTTTTTATCTTTGATTTGAAATTAATTTTAGATTTTACTTGAGAACCTCACAACTTCATATAATTTTAAAAACTGAAGACCAGATTGTGGAATCATAAAATCTAAATC</p>	SEQ No. 350

R6	Region in vicinity of acceptor of intron 46 (in range of -400 to -1 n bases from 3' end of intron 46 as basing point) ATAATGCAATTTCTCAGCAGCAAGCTACGGTATGCTATGGCATGCTATGATACCCAAGAGG CTGATGAATTTGTTTCACATTGTTCTATTTCTGATAGAGAGATAGGTTTTAGACACTAACT TTATTTGGAGTGTGCTTTACCATCTCACATTTTTCTCTTAAAAAATTTATGAGGGATAAT ATAATCGTTTATTTTCTACAGAGATTTCTACTGAGGGGTGAGTGTTCAGTCAATCAG CTCTGCTCAGATAGAAAAGTGTGGTATTTGAGGTACCACTGGGCCCTCGGTCAAGTCG CTTCATTTTGATAGACTAATCAATAGAAGCAAGACAAGGTAGTTGGAATTGTGCTGTAAT TCATTTAAACGTTGTTGCATTTGTCTGTTTCAG	SEQ No. 351
R7	Region in vicinity of donor of intron 47 (in range of +1 to +400 bases from 5' end of intron 47 as basing point) GTTAGACATTAACCATCTCTTCCGTCACATGTGTTAAATGTTGCAAGTATTTGTATGTATT TTGTTTCCTGGGTGCTTCATTGGTCGGGGAGGAGGCTGGTATGTGGATTGTTGTTTTGTTT TGTTTTTTTAACTGACCGTTTGGCTTTGGCTATATGTTTTGTTGTGGCTAGAAAAATGAT GATGGTGAATGGCTTTACATTAATGACCAATGCCAAAATTTATACCACAATTTTTTGCAT AAATTATTCTGAAGAATCAGACTGAAGAAATGGCGAAGTATTTAATTCAGTGGCCAGGCAT GTACTGACAGTATTTAAGCTGAAAGGACGTGGTCTGGTTCTAGTTAAACAAGTGCATAAA TCAAAATTAATTATTCACACCTGTGGTATGGACT	SEQ No. 352
R8	Region in vicinity of acceptor of intron 47 (in range of -400 to -1 bases from 3' end of intron 47 as basing point) TATTCATTTTTATACTGCAAAGGAAGCGGTATGGCATATAATACACAACACACCAGTAT ATTTAGTAAGTGAATAAATGAAAGATGTATTTCTTTACTTTATCAGTTGCAGTTGGC TATGCCTTTGTGTAAGGTGTGTGTTTTGAAATTCAAAAAGGTATTAGTTTCTTTAAAGCA AAGAATTTTTGTAGCAGGTAAATGAATAATTTGAATACATTGGTTAAATCCCAACATGTA ATATATGTAATAATCAATATTATGCTGCTAAAATAACACAAATCAGTAAGATTCTGTAAT ATTTTCATGATAAATAACTTTTGAATAATATTTTTAAACATTTTGGCTTATGCCTTGAGAA TTATTTACCTTTTAAATGTATTTCTTTTCAG	SEQ No. 353
R9	Region in vicinity of donor of intron 48 (in range of +1 to +400 bases from 5' end of intron 48 as basing point) GTAGGGAACCTTTTGCTTTAAATATTTTGTCTTTTTTAAAGAAAAATGGCAATATCACTGA ATTTTCTCAITTTGGTATCATTATTAAGACAAAATATTACTTGTAAAGTGTGGTAAGGAA GACTTTATTCAGGATAACCAATAGGCACAGGGACCACTGCAATGGAGTATTACAGGAGG TTGGATAGAGAGAGATTGGGCTCAACTTAAATACAGCACAGTGGAAAGTAGGAATTTATAG CCAAGGAGCAGTGTAGGAGTCAGTAGATGGAATAATTAAGAGGAAACATCAGGGGTAG TGGGATTCTGGCTAAACCAACCTCACAGGATTCTTGTGAAGATAGGCCAGGGTTATCTTA TCAGACAACCCCTTGGGAATGGTGGAGAATACTG	SEQ No. 354
R10	Region in vicinity of acceptor of intron 48 (in range of -400 to -1 bases from 3' end of intron 48 as basing point) TACTAAACACAGAATTTGTAAACAATAAGTGTATAAAGTAAATGAACATTAGGATTAT TGAGATTATTTAGCTAAACCTAGTGTATTATCATATAAATTATGTTAATAAATTGTATTG TCATTATTGCATTTTACTTTTTTGAAGTAGTTAATGCCTGTGTTTCTATATGAGTATTA TATAATTCAAGAAGATATTGGATGAATTTTTTTTAAAGTTTAAATGTGTTTACATCTCTGTT TCTTTTCTCTGCACCAAAAGCTACATTTTTGTGCCCTTATGTACCAGGCAGAAATTGATCT GCAATACATGTGGAGTCTCAAGGGTATATTTAAATTTAGTAATTTTATTGCTAACTGIGA AGTTAATCTGCACTATATGGGTTCTTTTCCCCAG	SEQ No. 355
R11	Region in vicinity of donor of intron 49 (in range of +1 to +400 bases from 5' end of intron 49 as basing point) GTAATGAAGCAACCTCTAGCAATATCCATTACCTCATAATGGGTTATGCTTCCCCTGTTGT ACATTTGCCATTGACGTGGACTATTTATAATCAGTGAAATAACTTGAAGGAAATACTGGC CATACTGTAATAGCAGAGGCAAGCTGTCTTTTATGATCAGCATATCTATTTATATATTGT GATCTTAAGGCTATTAACGAGTCATTGCTTTAAAGGACTCATTTCTGTCCTGGTGTGCTGC CATCAATACAAAAGTAGTCCACCTTCAAGGTAGATTAAATTCCTTTGGGGCTTATTGCTT	SEQ No. 356

	TGCTTGCCAGCCTTGATGCTTTTCATATTGTTGGTTTAATTCAAATCAAGCTACTGCATC ATAGTGTCTGTCTCCAACAGCTGTAAAGAATCAC	
R12	Region in vicinity of acceptor of intron 49 (in range of -400 to -1 bases from 3' end of intron 49 as basing point) ATATAATTGACTGGGGGTGAGCCAGTACATTAGGATTTTCCTAAAGTTATCTGGATAATTT TAGTATGCAACCACAATAGATACTCTTCAAGAATTAAGCTAGTTGCTGAGAGGGAACIGTT TTTTGTTGGTTTGTTCCTAATGTTTGCCTCTACTTCCTTTAAATAAAATTATGCCTG GAGAAAGGGTTTTGTATGGAGCAATTGATAAATATTTGTAGGGTGGTTGGCTAAAAATAAT TATAATTCCTTTAAAGAAATCTACCCACTAAAGTTAATTTAGAAGTAAAAATAATAGA AATCCAATAATATATTCACCAAATGGATTAAGATGTTTCATGAATTATCTTCAAAGTGTAA TCGAATAAGTAATGTGTATGCTTTTCTGTAAAG	SEQ No. 357
R13	Region in vicinity of donor of intron 50 (in range of +1 to +400 bases from 5' end of intron 50 as basing point) GTAAGTATACTGGATCCCATTCCTTTGGCTCTAGCTATTTGTTCAAAAGTGAACCTATGA AGTGATCACTGGGTGAGAGAGAAAAATTTGTTTCAATTCTAAAGATAGAGATAAACCTTGT GTTATTGACTGTGCAAAAGTCTTAGAGTACATTCTTGGAAATTGACTCTGATTCAAAGT GTTGCATGACAACGGGATATGGGGAGTGTCTCTGGAGATACCCCAAGGAAGAGAAGA GCACAAGGGAGATTGTGGGAGAGTCTGAAATGTGATTTGTCTGCAGCAGAGGCCAAGCCA GTCTCGCAGGAGCCCTACATCTGGGCTGGCTGTGCAGAGCTGTCTGAATTGCAGGCACTG GGCCTGGCCCTTGTATTCTGATCCAGCCAGCCA	SEQ No. 358
R14	Region in vicinity of acceptor of intron 50 (in range of -400 to -1 bases from 3' end of intron 50 as basing point) TCTTGAATAAAAAAATAAGTAAATTTATTTCCCTGGCAAGGTCTGAAAACTTTTGT TTCTTTACCACTTCCACAATGTATATGATTGTTACTGAGAAGGCTTATTTAACTTAAGTTA CTTGTCAGGCATGAGAATGAGCAAAATCGTTTTTAAAAAATTGTTAAATGTATATTAAT GAAAAGGTTGAATCTTTTCATTTCTACCATGTATTGCTAAACAAAGTATCCACATTGTTA GAAAAGATATATAATGTATGAATAAGAGTTTGGCTCAAATTGTTACTCTTCAATTAAT TTGACTTATTGTTATTGAAATTGGCTCTTTAGCTGTGTTTCTAATTTTCTTTTCTTCT TTTTTCCTTTTGCAAAAACCCAAATATTTTAG	SEQ No. 359
R15	Region in vicinity of donor of intron 51 (in range of +1 to +400 bases from 5' end of intron 51 as basing point) GTATGAGAAAAATGATAAAAGTTGGCAGAAGTTTTCTTTAAATGAAGATTTCCACCA ATCACTTTACTCTCCTAGACCATTTCACCCAGTTCTTAGGCACTGTTTCTCTCAGCA AACACATTACTCTCACTATTTCAGCCTAAGTATAATCAAGGATATAAATTAATGCAAAATAC AAAAGTAGCCATACATTAAAAAGGAAATATACAAAAAAGCAAGCAAGCAAGCAAGCA TTACAAGAATAGTTGTCTCAGTTAAATTTACTAAACAACCTGGTATTTTAAAAATCTATTT TATACCAAATAAGTCACTCACTGAGCTATTTACATTTAACTGTTTGTGTTTGGCACTACG CAGCCCAACATATTGCAGAATCAAAATATAATAGT	SEQ No. 360
R16	Region in vicinity of acceptor of intron 51 (in range of -400 to -1 bases from 3' end of intron 51 as basing point) TTTAAATAAATATACCTTAATTTTGACGTACACAGAATGATATTATAAGTATAAATAGT TATCTATCTTTAAATACATTGTCGTAATTCAGAATAACATTCTTACTCAAGGCATTGAG ACAGTGGTTTAAAGTAATCCGAGGTACTCCGGAATGTCTCCATTTGAGCCTTTAAATGAAGA AAATCTATAGTCAAGATTTTCATTTGAAATATTTTGATACTAAGAATGAAACATATTTT CTGTTAAATGTTTCTATAAACCTTATACAGTAACATCTTTTTTATTTCTAAAAGTGTT TTGGCTGGTCTCACAATTGTACTTTACITTTGATTATGTAAGGAATACACAACGCTGAA GAACCTGATACTAAGGGATATTGTTCTTACAG	SEQ No. 361
R17	Region in vicinity of donor of intron 52 (in range of +1 to +400 bases from 5' end of intron 52 as basing point) GTAAGTTTTTTAAAGCATGGGACACACAAGCAAGATGCATGACAAGTTTCAATAAAAA CTTAAGTTCATATATCCCCCTCACATTTATAAAAAAATGTGAAATAATTGTAAATGATAA CAATTGTGCTGAGATTTTCAGTCCATAATGTTACCTTTTAAATAAATGAATGTAATTCATT	SEQ No. 362

	GAATAGAAGAAATACATTTTTTAAATCAATTCAGGGCTTATATAGTTGCAAAGCATGCATTG ATGGGTGTGGTGACCACAGTGTGGCAGAACATTTGTGGCAGAACATTTGTTCTTTAGTTGT CATCTGGGCTGGCATCCATGGAGATGCCAGTCTCTCCCTCATATCCTTGGCTGTTGGTCCA AGCAGGCAGTGGCTTCTTCTGGGCCATCTTTCA	
R18	Region in vicinity of acceptor of intron 52 (in range of -400 to -1 bases from 3' end of intron 52 as basing point) CAAACCTCTGTGGCAACAGAAAGCCTTCAGGCAATGAAATGCTGGCACTGGGAAATCAGGC TGATGGGTGCTGAAGTGGCAAGGATGAGGGGATATGGATATTCTGCTGTAGTGCTTTTCTA ACAGATGATTCATATTTGGTTCTAGGGATCAAGAATTGAGTTAAAAATTTATATATATGTT GATGTTCTATGTCACCTTCAGGAAAAATAATTTAACAGAACTAATATTTGCCATCAAAAAA GCAAAGAATCCTGTTTTCATCATCCTAGCCATAACACAATGAATAATTTTTTAAATAAGC AACATAAATGTGAGATAACGTTTGAAGTTACATTTAAAAATGTCTCCTCCAGACTAGCATT TACTACTATATATTTATTTTCTTTTATTCTAG	SEQ No. 363
R19	Region in vicinity of donor of intron 53 (in range of +1 to +400 bases from 5' end of intron 53 as basing point) GTTAGTATCAAAGATACCTTTTTTAAATAAATACTGGTTACATTTGATAAAATTATACCA TAGATTGTAATTTAATGATGTTAATGTAAGTTATTAACAGAAATCAGTTAAAGCTGA AATGAACAGTAGACTTTGTATATTTATTTCTTAGAGACAGAGTCTCACTGTCAACCCAGGC TAAAGTGCAGTGGCACAATCATAGCTCACTGAGCCTTGAACCTCTGGGGCTCAAGCAGTCCT CCTGCCCTCAGCCTCCCTAGTAGCTGGGACTACTAGCCAGGCGTGTACCACCACGCCTGGCT AATTTTTTAAAAATTTTGTCTTCTGTAGAGATGGGTTCTTGAACCTTGGCCTCAAGCAA TTCTCCTTCTTGGCCTCCCAAAGCACTAGGATT	SEQ No. 364
R20	Region in vicinity of acceptor of intron 53 (in range of -400 to -1 bases from 3' end of intron 53 as basing point) CAACAAGTTAATGAAGAGGGAAGAAATGTATGAGGTTTTTTTCGTTCAAATGTTGTTATA TGTCACATATTCAACAATTATATATGAGCTTATTTTGTAGTTTTTTCTCTTGTGATAAA AACAAATTAAGCCCACTTATTGCCAATTAATTGCTACTAAGTTGAAATACTTGATACTGGT TATTGCTCAAGATGCTGCATTTGAAAAAGTTTGTCTGAAAGGTGGGTACCTTATACTGTC ATGATTGACTAAATCATATGGTAGGTTAAAGCAATCTAATATATGATTCTGACCTGAGG ATTGAGAAGCTGTTTACGAAGTATTTTAAAGCACTCCAACCTAGAGATTTCAAAAAAAAC TGACATTCATTCTCTTCTCATAAAAATCTATAG	SEQ No. 365
R21	Region in vicinity of donor of intron 54 (in range of +1 to +400 bases from 5' end of intron 54 as basing point) GTATGAATTACATTATTTCTAAACTACTGTTGGCTGTAATAATGGGGTGGTGAACCTGGA TGGACCATGAGGATTTGTTTTTCCAATCCAGCTAAACTGGAGCTTGGGAGGGTTCAAGACG ATAAATACCAACTAAACTCACGGACTTGGCTCAGACTTCTATTTTAAAAACGAGGAACATA AGATCTCATTGCCCCGTGTCACAAAAGTAGTGACATAACCAAGAGATTAACAAAAAGCA AAATACTGATTTATAGCTAGAAGAGCCATTTATCAGTCTACTTTGATAACTCTATCCAAAG GAATATCTTTCTATCTCATCATGGCGCACACTGCCTTACCTGTTATCTGATAAATAAGTCA CTTTGGGATTCATGATAGAGTTATAGCTGTACAT	SEQ No. 366
R22	Region in vicinity of acceptor of intron 54 (in range of -400 to -1 bases from 3' end of intron 54 as basing point) TCTCAAATTTGGCAGTATATTAATAAAGCTTTCAAATTTGACCAACAAAACTACAAAA TTGAAAAAAGGTACTTTGAACCTTCACATGTTCAAATATATGTATATATATTTACATAT ATATATGAAACCTCCTCTGTGGAGAGGGGTTTATAGAAATCTGTAATTGTCATTCTTGCA GCCTTCCCCATACAAACGCCCTTTAAGTTAAATAAAAAATGAAAGTAAATAGACTGCACAAT ATTATAGTTGTTGCTTAAAGGAAGAGCTGTAGCAACAACTCACCCTATTGTTGCTATATTA CAATTTAGTTCCCTCCATCTTTCTTTTATGGAGTTCACTAGGTGCACCATTCTGATATT TAATAATTGCATCTGAACATTTGGTCTTTGCGAG	SEQ No. 367
R23	Region in vicinity of donor of intron 55 (in range of +1 to +400 nucleotides from 5' end of intron 55 as basing point)	

	GTAAGTCAGGCATTTCGCTTTAGCACTCTTGTGGATCCAATTGAACAATTCTCAGCATT GTACTTGTAAGTGACAAGCCAGGGACAAAAAAGTTGCTTTTATACAGCCTGATGTA TTTCGGTATTGGAACAAGGAGGAGAGAGGCAGAGGGAGAAGGAAACATCATTTATAATTCC ACTTAACACCCTCGTCTTAGAAAAAGTACATGCTCTGACCAGGAAAACATTTGCATATAAA ACCAGAGCTTCGGTCAAGGAGAAAACCTTGCTCAGAGAAATAACTTAGGGATTGGTTTATTA AATTTTAAAAGTTGACATTTTTGAGTGTTTATTTAATATTTACAGGGAAAGCATCTGTAT GAATTGCTGTGTTTTATTAGCGTTGCTAACTGAA	SEQ No. 368
R24	Region in vicinity of acceptor of intron 55 (in range of -400 to -1 bases from 3' end of intron 55 as basing point) ACTTGATCCATATAGTAATGAAATTATTGGCACTGGGGTACACTTTATCATAGAATTTTAT TGCCATCACTTCCATAAAAATAATACATTTTGTCCATAGACTAGAAGATATAAAGTTGTA CTTTATAAAGTTATAAATACATTACTTTCCAACTCATAATGGCAAGGAATAAATCTATTAC AACTAATAAGATGCCCATTTTTAAATCTACATAATAACAGGAGAAGGCAATACGCCAAGAAA AGGGATTGTGAGATGATCTCTTGTAGTTAGCCTGATTGAAATGCTTTTGAACATAA ATTATTTATATTTTGAATTTCTCCAAATTCACATTCATCGCTTGTTCTTTTGTGGTAA TTCTGCACATATTCTTCTCTGCTGCTGCTGAG	SEQ No. 369

[0046]

The target regions R1 to R24 of the antisense oligomer of the present invention include both wild (e.g., the regions represented by SEQ ID NOS: 233 to 256, 341 to 369, and 385 to 389) and mutant types in relation to the human dystrophin pre-mRNA. Such a mutant type specifically has any one base sequence selected from the group consisting of base sequences (B0) and (B1) to (B16) below:

(B0) a base sequence that hybridizes under stringent conditions to a base sequence complementary to any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389;

(B1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected;

(B2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 14\%$ of the length of the any one base sequence selected;

(B3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 13\%$ of the length of the any one base sequence selected;

(B4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 12\%$ of the length of the any one base sequence selected;

(B5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 11\%$ of the length of the any one base sequence selected;

(B6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 10\%$ of the length of the any one base sequence selected;

(B7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting

of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 9\%$ of the length of the any one base sequence selected;

(B8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 8\%$ of the length of the any one base sequence selected;

(B9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 7\%$ of the length of the any one base sequence selected;

(B10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 6\%$ of the length of the any one base sequence selected;

(B11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 5\%$ of the length of the any one base sequence selected;

(B12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

and has a length within $\pm 4\%$ of the length of the any one base sequence selected;

(B13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 3\%$ of the length of the any one base sequence selected;

(B14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 2\%$ of the length of the any one base sequence selected;

(B15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 1\%$ of the length of the any one base sequence selected;

and

(B16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0047]

As used herein, the term "base sequence that hybridizes under stringent conditions" refers to, for example, a base sequence obtained by colony

hybridization, plaque hybridization, Southern hybridization or the like, using as a probe all or part of a base sequence complementary to, e.g., any one base sequence selected from the group consisting of SEQ ID NOS: 233 to 256, 341 to 369, and 385 to 389. The hybridization method which may be used includes methods described in, for example, "Sambrook & Russell, Molecular Cloning: A Laboratory Manual Vol. 3, Cold Spring Harbor, Laboratory Press, 2001," "Ausubel, Current Protocols in Molecular Biology, John Wiley & Sons, 1987-1997," etc.

[0048]

As used herein, the term "complementary base sequence" is not limited to a base sequence that forms Watson-Crick pairs with an intended base sequence, and also includes a base sequence that forms wobble base pairs therewith. Herein, the Watson-Crick pair means a base pair that forms a hydrogen bond between adenine and thymine, between adenine and uracil, or between guanine and cytosine, and the wobble base pair means a base pair that forms a hydrogen bond between guanine and uracil, between inosine and uracil, between inosine and adenine, or between inosine and cytosine. The term "complementary base sequence" does not have to have 100% complementarity with the intended base sequence and may contain, for example, 1, 2, 3, 4, or 5 noncomplementary bases to the intended base sequence or may be a base sequence shorter

by 1 base, 2 bases, 3 bases, 4 bases, or 5 bases than the intended base sequence.

[0049]

As used herein, the term "stringent conditions" may be any of low stringent conditions, moderate stringent conditions or high stringent conditions. The term "low stringent condition" is, for example, 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, 50% formamide at 32°C. The term "moderate stringent condition" is, for example, 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, 50% formamide at 42°C, or 5 × SSC, 1% SDS, 50 mM Tris-HCl (pH 7.5), 50% formamide at 42°C. The term "high stringent condition" is, for example, 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, 50% formamide at 50°C, or 0.2 × SSC, 0.1% SDS at 65°C. Under these conditions, base sequences with higher identity are expected to be obtained efficiently at higher temperatures, although multiple factors are involved in hybridization stringency including temperature, probe concentration, probe length, ionic strength, time, salt concentration and others, and those skilled in the art may appropriately select these factors to achieve similar stringency.

When commercially available kits are used for hybridization, for example, an Alkphos Direct Labelling and Detection System (GE Healthcare) may be used. In this case, according to the attached protocol, after incubation with a labeled probe overnight, the membrane

can be washed with a primary wash buffer containing 0.1% (w/v) SDS at 55°C, thereby detecting hybridization. Alternatively, when the probe is labeled with digoxigenin (DIG) using a commercially available reagent (e.g., a PCR Labelling Mix (Roche Diagnostics), etc.) in producing a probe based on all or part of the complementary sequence to any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, hybridization can be detected with a DIG Nucleic Acid Detection Kit (Roche Diagnostics) or the like.

[0050]

The identity between base sequences may be determined using algorithm BLAST (Basic Local Alignment Search Tool) by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87:2264-2268, 1990; Proc. Natl. Acad. Sci. USA 90: 5873, 1993). Programs called BLASTN and BLASTX based on the BLAST algorithm have been developed (Altschul SF, et al: J. Mol. Biol. 215: 403, 1990). When a base sequence is analyzed using BLASTN, the parameters are, for example, score = 100 and wordlength = 12. When BLAST and Gapped BLAST programs are used, the default parameters for each program are employed.

[0051]

The antisense oligomer of the present invention comprises a base sequence complementary to a base sequence of at least any one region selected from the group consisting of the target regions R1 to R24 of the

present invention, or a partial base sequence thereof. The term "partial" means a region, except for the full length, of at least any one region selected from the group consisting of the target regions R1 to R24, i.e., a partial region of at least any one region selected from the group consisting of the target regions R1 to R24. The partial region may be 10 to 60 bases long, 10 to 55 bases long, 10 to 50 bases long, 10 to 45 bases long, 10 to 40 bases long, 10 to 35 bases long, 10 to 30 bases long, 10 to 25 bases long, 15 to 60 bases long, 15 to 55 bases long, 15 to 50 bases long, 15 to 45 bases long, 15 to 40 bases long, 15 to 35 bases long, 15 to 30 bases long, 15 to 25 bases long, 16 to 60 bases long, 16 to 55 bases long, 16 to 50 bases long, 16 to 45 bases long, 16 to 40 bases long, 16 to 35 bases long, 16 to 30 bases long, 16 to 25 bases long, 17 to 60 bases long, 17 to 55 bases long, 17 to 50 bases long, 17 to 45 bases long, 17 to 40 bases long, 17 to 35 bases long, 17 to 30 bases long, 17 to 25 bases long, 18 to 60 bases long, 18 to 55 bases long, 18 to 50 bases long, 18 to 45 bases long, 18 to 40 bases long, 18 to 35 bases long, 18 to 30 bases long, 18 to 25 bases long, 19 to 60 bases long, 19 to 55 bases long, 19 to 50 bases long, 19 to 45 bases long, 19 to 40 bases long, 19 to 35 bases long, 19 to 30 bases long, 19 to 25 bases long, 20 to 60 bases long, 20 to 55 bases long, 20 to 50 bases long, 20 to 45 bases long, 20 to 40 bases long, 20 to 35 bases long, 20 to 30 bases

long, 20 to 25 bases long, 15 to 30 bases long, 15 to 29 bases long, 15 to 28 bases long, 15 to 27 bases long, 15 to 26 bases long, 15 to 25 bases long, 15 to 24 bases long, 15 to 23 bases long, 15 to 22 bases long, 15 to 21 bases long, 15 to 20 bases long, 15 to 19 bases long, 15 to 18 bases long, 16 to 30 bases long, 16 to 29 bases long, 16 to 28 bases long, 16 to 27 bases long, 16 to 26 bases long, 16 to 25 bases long, 16 to 24 bases long, 16 to 23 bases long, 16 to 22 bases long, 16 to 21 bases long, 16 to 20 bases long, 16 to 19 bases long, 16 to 18 bases long, 17 to 30 bases long, 17 to 29 bases long, 17 to 28 bases long, 17 to 27 bases long, 17 to 26 bases long, 17 to 25 bases long, 17 to 24 bases long, 17 to 23 bases long, 17 to 22 bases long, 17 to 21 bases long, 17 to 20 bases long, 17 to 19 bases long, 17 to 18 bases long, 18 to 30 bases long, 18 to 29 bases long, 18 to 28 bases long, 18 to 27 bases long, 18 to 26 bases long, 18 to 25 bases long, 18 to 24 bases long, 18 to 23 bases long, 18 to 22 bases long, 18 to 21 bases long, 18 to 20 bases long, 18 to 19 bases long, 19 to 30 bases long, 19 to 29 bases long, 19 to 28 bases long, 19 to 27 bases long, 19 to 26 bases long, 19 to 25 bases long, 19 to 24 bases long, 19 to 23 bases long, 19 to 22 bases long, 19 to 21 bases long, 19 to 20 bases long, 20 to 30 bases long, 20 to 29 bases long, 20 to 28 bases long, 20 to 27 bases long, 20 to 26 bases long, 20 to 25 bases long, 20 to 24 bases long, 20 to 23 bases long, 20 to 22 bases

long, 20 to 21 bases long, 5 to 25 bases long, 5 to 24
bases long, 5 to 23 bases long, 5 to 22 bases long, 5 to
21 bases long, 5 to 20 bases long, 5 to 19 bases long, 5
to 18 bases long, 5 to 17 bases long, 5 to 16 bases long,
5 to 15 bases long, 5 to 14 bases long, 5 to 13 bases
long, 5 to 12 bases long, 7 to 25 bases long, 7 to 24
bases long, 7 to 23 bases long, 7 to 22 bases long, 7 to
21 bases long, 7 to 20 bases long, 7 to 19 bases long, 7
to 18 bases long, 7 to 17 bases long, 7 to 16 bases long,
7 to 15 bases long, 7 to 14 bases long, 7 to 13 bases
long, 7 to 12 bases long, 9 to 25 bases long, 9 to 24
bases long, 9 to 23 bases long, 9 to 22 bases long, 9 to
21 bases long, 9 to 20 bases long, 9 to 19 bases long, 9
to 18 bases long, 9 to 17 bases long, 9 to 16 bases long,
9 to 15 bases long, 9 to 14 bases long, 9 to 13 bases
long, 9 to 12 bases long, 10 to 25 bases long, 10 to 24
bases long, 10 to 23 bases long, 10 to 22 bases long, 10
to 21 bases long, 10 to 20 bases long, 10 to 19 bases
long, 10 to 18 bases long, 10 to 17 bases long, 10 to 16
bases long, 10 to 15 bases long, 10 to 14 bases long, 10
to 13 bases long, 10 to 12 bases long, 60 bases long, 59
bases long, 58 bases long, 57 bases long, 56 bases long,
55 bases long, 54 bases long, 53 bases long, 52 bases
long, 51 bases long, 50 bases long, 49 bases long, 48
bases long, 47 bases long, 46 bases long, 45 bases long,
44 bases long, 43 bases long, 42 bases long, 41 bases
long, 40 bases long, 39 bases long, 38 bases long, 37

bases long, 36 bases long, 35 bases long, 34 bases long, 33 bases long, 32 bases long, 31 bases long, 30 bases long, 29 bases long, 28 bases long, 27 bases long, 26 bases long, 25 bases long, 24 bases long, 23 bases long, 22 bases long, 21 bases long, 20 bases long, 19 bases long, 18 bases long, 17 bases long, 16 bases long, 15 bases long, 14 bases long, 13 bases long, 12 bases long, 11 bases long, 10 bases long, 9 bases long, 8 bases long, 7 bases long, 6 bases long, or 5 bases long, but not limited thereto. These lengths may be increased or decreased by 1, 2, or 3 bases.

[0052]

The antisense oligomer of the present invention has an activity to cause simultaneous skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA. As used herein, such skipping of two or more numerically consecutive exons from pre-mRNA of interest is referred to as "multi-exon skipping" or "multi-skipping", and this activity is referred to as "multi-exon skipping activity" or "multi-skipping activity".

[0053]

Herein, the any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon mean a plurality of exons that increase one by one in exon number among 11 exons from the 45th

exon to the 55th exon included in pre-mRNA. The exon number means a number assigned to exons in order from the 5' end to the 3' end with an exon at the most upstream position of pre-mRNA defined as the first exon, followed by the second, the third, ..., and the 79th exons among 79 exons included in human dystrophin pre-mRNA. An intron is numbered as the same number as that of an exon positioned on the 5' side thereof. Specifically, the 45th intron is flanked by the 45th exon positioned on the 5' side thereof and the 46th exon positioned on the 3' side thereof. As used herein, the "nth" exon or intron means the nth exon or intron counted from the 5' end toward the 3' end in pre-mRNA.

[0054]

Table 4 shows combinations of exons included in the any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon.

[0055]

[Table 4]

Combination	Exons included	Combination	Exons included
Combination 1	45, 46	Combination 29	48-50
Combination 2	45-47	Combination 30	48-51
Combination 3	45-48	Combination 31	48-52
Combination 4	45-49	Combination 32	48-53
Combination 5	45-50	Combination 33	48-54
Combination 6	45-51	Combination 34	48-55
Combination 7	45-52	Combination 35	49, 50
Combination 8	45-53	Combination 36	49-51

Combination 9	45-54	Combination 37	49-52
Combination 10	45-55	Combination 38	49-53
Combination 11	46, 47	Combination 39	49-54
Combination 12	46-48	Combination 40	49-55
Combination 13	46-49	Combination 41	50, 51
Combination 14	46-50	Combination 42	50-52
Combination 15	46-51	Combination 43	50-53
Combination 16	46-52	Combination 44	50-54
Combination 17	46-53	Combination 45	50-55
Combination 18	46-54	Combination 46	51, 52
Combination 19	46-55	Combination 47	51-53
Combination 20	47, 48	Combination 48	51-54
Combination 21	47-49	Combination 49	51-55
Combination 22	47-50	Combination 50	52, 53
Combination 23	47-51	Combination 51	52-54
Combination 24	47-52	Combination 52	52-55
Combination 25	47-53	Combination 53	53, 54
Combination 26	47-54	Combination 54	53-55
Combination 27	47-55	Combination 55	54, 55
Combination 28	48, 49		

[0056]

Among the combinations of exons described in Table 4, for example, the combination 1, 2, 3, 4, 6, 8, 10, 18, 20, 21, 23, 25, 27, 28, 30, 32, 34, 36, 38, 40, 41, 43, 45, 46, 50, 52, or 55 is a skipping pattern expected to exert higher therapeutic effects on DMD. Multi-exon skipping in such a combination is expected to exert therapeutic effects on more patients with DMD.

[0057]

The any two or more numerically consecutive exons selected from the group consisting of the 45th exon to

the 55th exon may include a plurality of groups of consecutive exons and may be, for example, but not limited to, (example 1) exons 45 and 46 (first exon group) and exons 48 to 53 (second exon group), or (example 2) exons 46 and 47 (first exon group), exons 49 and 50 (second exon group), and exons 52 to 54 (third exon group).

[0058]

In the present invention, the term "activity to cause skipping" (i.e., multi-skipping activity) means, when human dystrophin pre-mRNA is taken as an example, an activity to produce human dystrophin mRNA having deletion of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in the human dystrophin pre-mRNA.

In other words, this activity means that by binding of the antisense oligomer of the present invention to a target site in human dystrophin pre-mRNA, the 5'-terminal nucleotide of an exon immediately downstream of the exons to be deleted is linked to the 3'-terminal nucleotide of an exon immediately upstream of the exons to be deleted when the pre-mRNA undergoes splicing, thus resulting in formation of mature mRNA which is free of codon frame shift (i.e., mature mRNA having deletion of the exons without frame shift).

[0059]

The antisense oligomer of the present invention exhibits a multi-skipping activity under physiological conditions. The term "under physiological conditions" refers to conditions set to mimic the *in vivo* environment in terms of pH, salt composition and temperature. The conditions are, for example, 25 to 40°C, preferably 37°C, pH 5 to 8, preferably pH 7.4 and 150 mM of sodium chloride concentration. When multi-skipping is induced, one or more, for example, 2, 3, 4, 5, 6, 7, 8, 9 or 10 antisense oligomers, can be used in combination as the antisense oligomer of the present invention.

[0060]

Whether multi-skipping is caused or not can be confirmed by introducing the antisense oligomer of the present invention into a dystrophin expression cell (e.g., human rhabdomyosarcoma cells), amplifying the region surrounding exons 45 to 55 of mRNA of the human dystrophin gene from the total RNA of the dystrophin expression cell by RT-PCR and performing nested PCR or sequence analysis on the PCR amplified product. The multi-skipping efficiency can be determined as follows. The mRNA for the human dystrophin gene is collected from test cells; in the mRNA, the polynucleotide level "A" of the band where any two or more numerically consecutive exons among exons 45 to 55 are skipped, the polynucleotide level "B" of the band where any one exon among exons 45 to 55 is skipped, and the polynucleotide

level "C" of the band where no skipping is caused are measured. Using these measurement values of "A", "B", and "C", the efficiency is calculated by the following equation.

$$\text{Skipping efficiency (\%)} = A / (A + B + C) \times 100$$

[0061]

For example, the multi-skipping efficiency of exons 45 to 55 can be determined by using a forward primer for exon 44 and a reverse primer for exon 56 to measure the polynucleotide level "A" of the band where exons 45 to 55 are multi-skipped, using the forward primer for exon 44 and a reverse primer for exon 46 to measure the polynucleotide level "B" of the band where exon 45 is single-skipped, and using the forward primer for exon 44 and the reverse primer for exon 46 to measure the polynucleotide level "C" of the band where no skipping is caused, followed by calculation by the equation using these measurement values of "A", "B", and "C".

[0062]

The number of exons to be deleted in human dystrophin mRNA by the antisense oligomer of the present invention is 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. This is referred to as a deletion pattern, and various deletion patterns may exist in admixture in results obtained in one skipping experiment or skipping treatment. For example, mRNA admixture having deletion of 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 exons is obtained by introducing the

antisense oligomer of the present invention to cells expressing human dystrophin pre-mRNA, and collecting its mRNA.

[0063]

In a certain embodiment, the term "activity to cause skipping" can be defined as (C1) to (C10) below.

[0064]

(C1) Any two numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the two numerically consecutive exons may be the 45th and the 46th exons, the 46th and the 47th exons, the 47th and the 48th exons, the 48th and the 49th exons, the 49th and the 50th exons, the 50th and the 51st exons, the 51st and the 52nd exons, the 52nd and the 53rd exons, the 53rd and the 54th exons, or the 54th and the 55th exons.

[0065]

(C2) Any three numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped

with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the three numerically consecutive exons may be the 45th to the 47th exons, the 46th to the 48th exons, the 47th to the 49th exons, the 48th to the 50th exons, the 49th to the 51st exons, the 50th to the 52nd exons, the 51st to the 53rd exons, the 52nd to the 54th exons, or the 53rd to the 55th exons.

[0066]

(C3) Any four numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the four numerically consecutive exons may be the 45th to the 48th exons, the 46th to the 49th exons, the 47th to the 50th exons, the 48th to the 51st exons, the 49th to the 52nd exons, the 50th to the 53rd

exons, the 51st to the 54th exons, or the 52nd to the 55th exons.

[0067]

(C4) Any five numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the five numerically consecutive exons may be the 45th to the 49th exons, the 46th to the 50th exons, the 47th to the 51st exons, the 48th to the 52nd exons, the 49th to the 53rd exons, the 50th to the 54th exons, or the 51st to the 55th exons.

[0068]

(C5) Any six numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the six numerically consecutive exons may be the 45th to the 50th exons, the 46th to the 51st exons, the 47th to the 52nd exons, the 48th to the 53rd exons, the 49th to the 54th exons, or the 50th to the 55th exons.

[0069]

(C6) Any seven numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the seven numerically consecutive exons may be the 45th to the 51st exons, the 46th to the 52nd exons, the 47th to the 53rd exons, the 48th to the 54th exons, or the 49th to the 55th exons.

[0070]

(C7) Any eight numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70%

or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the eight numerically consecutive exons may be the 45th to the 52nd exons, the 46th to the 53rd exons, the 47th to the 54th exons, or the 48th to the 55th exons.

[0071]

(C8) Any nine numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the nine numerically consecutive exons may be the 45th to the 53rd exons, the 46th to the 54th exons, or the 47th to the 55th exons.

[0072]

(C9) Any ten numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher,

75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the ten numerically consecutive exons may be the 45th to the 54th exons, or the 46th to the 55th exons.

[0073]

(C10) Eleven numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA are skipped with the efficiency of 5% or higher, 10% or higher, 15% or higher, 20% or higher, 25% or higher, 30% or higher, 35% or higher, 40% or higher, 45% or higher, 50% or higher, 55% or higher, 60% or higher, 65% or higher, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, or 95% or higher.

Herein, the eleven numerically consecutive exons may be the 45th to the 55th exons.

[0074]

The antisense oligomer of the present invention may be 10 to 60 bases long, 10 to 55 bases long, 10 to 50 bases long, 10 to 45 bases long, 10 to 40 bases long, 10 to 35 bases long, 10 to 30 bases long, 10 to 25 bases long, 15 to 60 bases long, 15 to 55 bases long, 15 to 50 bases long, 15 to 45 bases long, 15 to 40 bases long, 15 to 35 bases long, 15 to 30 bases long, 15 to 25 bases long, 16 to 60 bases long, 16 to 55 bases long, 16 to 50 bases long, 16 to 45 bases long, 16 to 40 bases long, 16

to 35 bases long, 16 to 30 bases long, 16 to 25 bases long, 17 to 60 bases long, 17 to 55 bases long, 17 to 50 bases long, 17 to 45 bases long, 17 to 40 bases long, 17 to 35 bases long, 17 to 30 bases long, 17 to 25 bases long, 18 to 60 bases long, 18 to 55 bases long, 18 to 50 bases long, 18 to 45 bases long, 18 to 40 bases long, 18 to 35 bases long, 18 to 30 bases long, 18 to 25 bases long, 19 to 60 bases long, 19 to 55 bases long, 19 to 50 bases long, 19 to 45 bases long, 19 to 40 bases long, 19 to 35 bases long, 19 to 30 bases long, 19 to 25 bases long, 20 to 60 bases long, 20 to 55 bases long, 20 to 50 bases long, 20 to 45 bases long, 20 to 40 bases long, 20 to 35 bases long, 20 to 30 bases long, 20 to 25 bases long, 15 to 30 bases long, 15 to 29 bases long, 15 to 28 bases long, 15 to 27 bases long, 15 to 26 bases long, 15 to 25 bases long, 15 to 24 bases long, 15 to 23 bases long, 15 to 22 bases long, 15 to 21 bases long, 15 to 20 bases long, 15 to 19 bases long, 15 to 18 bases long, 16 to 30 bases long, 16 to 29 bases long, 16 to 28 bases long, 16 to 27 bases long, 16 to 26 bases long, 16 to 25 bases long, 16 to 24 bases long, 16 to 23 bases long, 16 to 22 bases long, 16 to 21 bases long, 16 to 20 bases long, 16 to 19 bases long, 16 to 18 bases long, 17 to 30 bases long, 17 to 29 bases long, 17 to 28 bases long, 17 to 27 bases long, 17 to 26 bases long, 17 to 25 bases long, 17 to 24 bases long, 17 to 23 bases long, 17 to 22 bases long, 17 to 21 bases long, 17 to 20 bases long, 17

to 19 bases long, 17 to 18 bases long, 18 to 30 bases long, 18 to 29 bases long, 18 to 28 bases long, 18 to 27 bases long, 18 to 26 bases long, 18 to 25 bases long, 18 to 24 bases long, 18 to 23 bases long, 18 to 22 bases long, 18 to 21 bases long, 18 to 20 bases long, 18 to 19 bases long, 19 to 30 bases long, 19 to 29 bases long, 19 to 28 bases long, 19 to 27 bases long, 19 to 26 bases long, 19 to 25 bases long, 19 to 24 bases long, 19 to 23 bases long, 19 to 22 bases long, 19 to 21 bases long, 19 to 20 bases long, 20 to 30 bases long, 20 to 29 bases long, 20 to 28 bases long, 20 to 27 bases long, 20 to 26 bases long, 20 to 25 bases long, 20 to 24 bases long, 20 to 23 bases long, 20 to 22 bases long, 20 to 21 bases long, 60 bases long, 59 bases long, 58 bases long, 57 bases long, 56 bases long, 55 bases long, 54 bases long, 53 bases long, 52 bases long, 51 bases long, 50 bases long, 49 bases long, 48 bases long, 47 bases long, 46 bases long, 45 bases long, 44 bases long, 43 bases long, 42 bases long, 41 bases long, 40 bases long, 39 bases long, 38 bases long, 37 bases long, 36 bases long, 35 bases long, 34 bases long, 33 bases long, 32 bases long, 31 bases long, 30 bases long, 29 bases long, 28 bases long, 27 bases long, 26 bases long, 25 bases long, 24 bases long, 23 bases long, 22 bases long, 21 bases long, 20 bases long, 19 bases long, 18 bases long, 17 bases long, 16 bases long, 15 bases long, 14 bases long, 13 bases long, 12 bases long, 11 bases long, or 10 bases

long, but not limited thereto. These lengths may be increased or decreased by 1, 2, or 3 bases.

[0075]

One embodiment of the antisense oligomer of the present invention is a linked-type antisense oligomer configured to comprise a plurality of unit oligomers linked to each other, a pharmaceutically acceptable salt thereof, or hydrate thereof (hereinafter, referred to as the "linked-type antisense oligomer of the present invention"). The unit oligomers mean respective oligomers constituting the linked-type antisense oligomer of the present invention. Specifically, the unit oligomers mean moieties (units) comprising base sequences that hybridize with target base sequences having consecutive base sequences when the linked-type antisense oligomer of the present invention binds to the target base sequences in human dystrophin pre-mRNA.

[0076]

Specifically, the linked-type antisense oligomer of the present invention is an antisense oligomer comprising two or more unit oligomers linked to each other, or a pharmaceutically acceptable salt thereof, or hydrate thereof, wherein

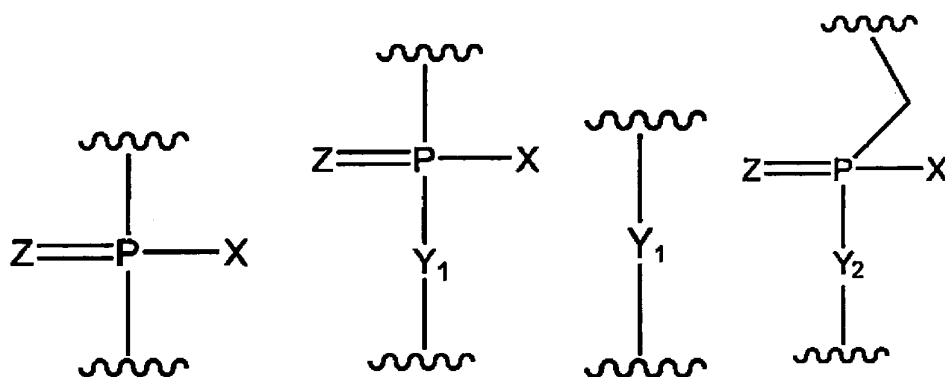
each of the unit oligomers comprises a base sequence complementary to a base sequence of any one region selected from the group consisting of the regions R1 to R24 (which may include the wild-type regions represented

by SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389 and their mutant types), or a partial base sequence thereof, and the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[0077]

The unit oligomers may be linked via a linker that does not contribute to hybridization, or may be linked directly without the mediation of a linker. When the unit oligomers are linked directly to each other, the 3' end of the unit positioned on the 5' side and the 5' end of the unit positioned on the 3' side form a phosphate bond or any one of the following groups.

[Formula 3]



wherein X represents -OH, -CH₂R¹, -O-CH₂R¹, -S-CH₂R¹, -NR²R³ or F;

R¹ represents H or an alkyl;

R² and R³, which may be the same or different, each represents H, an alkyl, a cycloalkyl or an aryl;

Y₁ represents O, S, CH₂, or NR¹;

Y₂ represents O, S, or NR¹;

Z represents O or S.

[0078]

In the linked-type antisense oligomer of the present invention, the unit oligomers may respectively target base sequences included in separate regions among the regions R1 to R24, or may respectively target base sequences included in the same region. In the linked-type antisense oligomer of the present invention, the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other. The respective base sequences of the unit oligomers that are not consecutive mean that the respective target base sequences of the unit oligomers constituting the linked-type antisense oligomer of the present invention are not consecutive as base sequences in human dystrophin pre-mRNA. Thus, when the respective target base sequences of the unit oligomers are compared to the base sequence of human dystrophin pre-mRNA, at least one base intervenes between the target base sequences as the base sequence of human dystrophin pre-mRNA. On the other hand, the respective base sequences of the unit oligomers that do not overlapped with each other mean that the respective target base sequences of the unit oligomers do not overlapped with each other as base sequences in human dystrophin pre-mRNA. The accidental presence of a match of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 bases between

the respective base sequences of the unit oligomers is accepted.

[0079]

The linked-type antisense oligomer of the present invention may be configured such that each of the unit oligomers comprises a base sequence complementary to (D1) any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(D2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(D3) a base sequence that hybridizes under stringent conditions to a base sequence complementary to any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, or

(D4) a partial base sequence of any one base sequence selected from the group consisting of the base sequences (D1), (D2), and (D3), and the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[0080]

The linked-type antisense oligomer of the present invention may comprise, as described above, a base sequence complementary to any one base sequence selected

from the group consisting of (D1), (D2), (D3), and (D4). The base sequence (D2) is a mutant of the base sequence (D1), and examples thereof also include

(D2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(D2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(D2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(D2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(D2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385

to 389, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(D2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(D2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(D2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(D2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(D2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(D2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(D2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(D2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(D2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(D2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(D2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0081]

Thus, each of the unit oligomers constituting the linked-type antisense oligomer of the present invention may comprise a base sequence complementary to any one base sequence selected from the group consisting of the base sequences (D1), (D2-1) to (D2-16), (D3), and (D4).

[0082]

The linked-type antisense oligomer of the present invention may comprise, as described above, a base sequence complementary to any one base sequence selected from the group consisting of (D1), (D2), (D3), and (D4). The base sequence (D4) relates to a partial base sequence of any one base sequence selected from the group consisting of the base sequences (D1), (D2), and (D3). In relation to (D4), the term "partial" means a partial region of consecutive bases, except for the full length, of (D1), (D2), or (D3). The partial region may be 5 to 25 bases long, 5 to 24 bases long, 5 to 23 bases long, 5 to 22 bases long, 5 to 21 bases long, 5 to 20 bases long, 5 to 19 bases long, 5 to 18 bases long, 5 to 17 bases long, 5 to 16 bases long, 5 to 15 bases long, 5 to 14 bases long, 5 to 13 bases long, 5 to 12 bases long, 7 to 25 bases long, 7 to 24 bases long, 7 to 23 bases long, 7 to 22 bases long, 7 to 21 bases long, 7 to 20 bases long,

7 to 19 bases long, 7 to 18 bases long, 7 to 17 bases long, 7 to 16 bases long, 7 to 15 bases long, 7 to 14 bases long, 7 to 13 bases long, 7 to 12 bases long, 9 to 25 bases long, 9 to 24 bases long, 9 to 23 bases long, 9 to 22 bases long, 9 to 21 bases long, 9 to 20 bases long, 9 to 19 bases long, 9 to 18 bases long, 9 to 17 bases long, 9 to 16 bases long, 9 to 15 bases long, 9 to 14 bases long, 9 to 13 bases long, 9 to 12 bases long, 10 to 25 bases long, 10 to 24 bases long, 10 to 23 bases long, 10 to 22 bases long, 10 to 21 bases long, 10 to 20 bases long, 10 to 19 bases long, 10 to 18 bases long, 10 to 17 bases long, 10 to 16 bases long, 10 to 15 bases long, 10 to 14 bases long, 10 to 13 bases long, 10 to 12 bases long, 25 bases long, 24 bases long, 23 bases long, 22 bases long, 21 bases long, 20 bases long, 19 bases long, 18 bases long, 17 bases long, 16 bases long, 15 bases long, 14 bases long, 13 bases long, 12 bases long, 11 bases long, 10 bases long, 9 bases long, 8 bases long, 7 bases long, 6 bases long, or 5 bases long, but not limited thereto. These lengths may be increased or decreased by 1, 2, or 3 bases.

[0083]

The size of each unit oligomer may be 5 to 30 bases long, 5 to 29 bases long, 5 to 28 bases long, 5 to 27 bases long, 5 to 26 bases long, 5 to 25 bases long, 5 to 24 bases long, 5 to 23 bases long, 5 to 22 bases long, 5 to 21 bases long, 5 to 20 bases long, 5 to 19 bases long,

5 to 18 bases long, 5 to 17 bases long, 5 to 16 bases long, 5 to 15 bases long, 5 to 14 bases long, 5 to 13 bases long, 5 to 12 bases long, 7 to 30 bases long, 7 to 29 bases long, 7 to 28 bases long, 7 to 27 bases long, 7 to 26 bases long, 7 to 25 bases long, 7 to 24 bases long, 7 to 23 bases long, 7 to 22 bases long, 7 to 21 bases long, 7 to 20 bases long, 7 to 19 bases long, 7 to 18 bases long, 7 to 17 bases long, 7 to 16 bases long, 7 to 15 bases long, 7 to 14 bases long, 7 to 13 bases long, 7 to 12 bases long, 9 to 30 bases long, 9 to 29 bases long, 9 to 28 bases long, 9 to 27 bases long, 9 to 26 bases long, 9 to 25 bases long, 9 to 24 bases long, 9 to 23 bases long, 9 to 22 bases long, 9 to 21 bases long, 9 to 20 bases long, 9 to 19 bases long, 9 to 18 bases long, 9 to 17 bases long, 9 to 16 bases long, 9 to 15 bases long, 9 to 14 bases long, 9 to 13 bases long, 9 to 12 bases long, 10 to 30 bases long, 10 to 29 bases long, 10 to 28 bases long, 10 to 27 bases long, 10 to 26 bases long, 10 to 25 bases long, 10 to 24 bases long, 10 to 23 bases long, 10 to 22 bases long, 10 to 21 bases long, 10 to 20 bases long, 10 to 19 bases long, 10 to 18 bases long, 10 to 17 bases long, 10 to 16 bases long, 10 to 15 bases long, 10 to 14 bases long, 10 to 13 bases long, 10 to 12 bases long, 30 bases long, 29 bases long, 28 bases long, 27 bases long, 26 bases long, 25 bases long, 24 bases long, 23 bases long, 22 bases long, 21 bases long, 20 bases long, 19 bases long, 18 bases long, 17 bases long,

16 bases long, 15 bases long, 14 bases long, 13 bases long, 12 bases long, 11 bases long, 10 bases long, 9 bases long, 8 bases long, 7 bases long, 6 bases long, 5 bases long, but not limited thereto. These lengths may be increased or decreased by 1, 2, or 3 bases. The unit oligomers may have the same size or different sizes.

[0084]

In a certain embodiment, the linked-type antisense oligomer of the present invention may be an antisense oligomer consisting of

(E1) any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, or

(E2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or
a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0085]

Herein, the base sequence (E2) is a mutant type of the base sequence (E1), and examples of such a mutant type also include

(E2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to

232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(E2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(E2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(E2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(E2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(E2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(E2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(E2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(E2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(E2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(E2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(E2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(E2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(E2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(E2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(E2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 18 to 21, 80 to 111, and 225 to 232, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0086]

Thus, the linked-type antisense oligomer of the present invention may consist of any one base sequence

selected from the group consisting of the base sequences (E1) and (E2-1) to (E2-16).

[0087]

Preferably, the linked-type antisense oligomer of the present invention comprises any one base sequence selected from the group consisting of SEQ ID NOS: 18 to 21, 80 to 111, and 225 to 232. In a certain embodiment, the linked-type antisense oligomer of the present invention consists of any one base sequence selected from the group consisting of SEQ ID NOS: 18 to 21, 80 to 111, and 225 to 232.

[0088]

Further, the antisense oligomer of the present invention may be an antisense oligomer consisting of (E'1) any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, or

(E'2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0089]

Herein, the base sequence (E'2) is a mutant type of the base sequence (E'1), and examples of such a mutant type also include

(E'2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(E'2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(E'2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(E'2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(E'2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(E'2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(E'2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(E'2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(E'2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180,

182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(E'2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(E'2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(E'2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(E'2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(E'2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(E'2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(E'2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0090]

Thus, the antisense oligomer of the present invention may consist of any one base sequence selected from the group consisting of the base sequences (E'1) and (E'2-1) to (E'2-16).

[0091]

Preferably, the antisense oligomer of the present invention comprises any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177,

179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232. In a certain embodiment, the antisense oligomer of the present invention consists of any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232.

[0092]

In a certain embodiment, the antisense oligomer of the present invention may be an antisense oligomer consisting of

(E''1) any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, or

(E''2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and

has a length within $\pm 15\%$ of the length of the any one base sequence selected,
or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0093]

Herein, the base sequence (E''2) is a mutant type of the base sequence (E''1), and examples of such a mutant type also include

(E''2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(E''2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157,

158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(E''2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(E''2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(E''2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(E''2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(E''2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142,

144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(E''2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(E''2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(E''2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(E''2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(E''2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75,

76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(E''2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(E''2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and

has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(E''2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(E''2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0094]

Thus, the antisense oligomer of the present invention may consist of any one base sequence selected

from the group consisting of the base sequences (E''1) and (E''2-1) to (E''2-16).

[0095]

In a certain embodiment, the antisense oligomer of the present invention may be an antisense oligomer consisting of

(E'''1) any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, or

(E'''2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0096]

Herein, the base sequence (E'''2) is a mutant type of the base sequence (E'''1), and examples of such a mutant type also include

(E'''2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(E''2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(E''2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(E''2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132,

139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(E''2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(E''2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(E''2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(E''2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(E''2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(E''2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(E''2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41,

43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(E''2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(E''2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(E''2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length

within $\pm 2\%$ of the length of the any one base sequence selected,

(E''2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(E''2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0097]

Thus, the antisense oligomer of the present invention may consist of any one base sequence selected from the group consisting of the base sequences (E''1) and (E''2-1) to (E''2-16).

[0098]

Preferably, the antisense oligomer of the present invention is an antisense oligomer comprising any one base sequence selected from the group consisting of SEQ

ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, or a pharmaceutically acceptable salt thereof, or hydrate thereof. In a certain embodiment, the antisense oligomer of the present invention is an antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0099]

More preferably, the antisense oligomer of the present invention is an antisense oligomer comprising any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162,

163, 225, and 228, or a pharmaceutically acceptable salt thereof, or hydrate thereof. In a certain embodiment, the antisense oligomer of the present invention is an antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0100]

In a certain embodiment, the linked-type antisense oligomer of the present invention is an antisense oligomer comprising any one base sequence selected from the group consisting of SEQ ID NOs: 80, 82, 86, 92, 97, 98, 100, 102, 225, 228, 231, and 232, or a pharmaceutically acceptable salt thereof, or hydrate thereof. In a certain embodiment, the linked-type antisense oligomer of the present invention consists of any one base sequence selected from the group consisting of SEQ ID NOs: 80, 82, 86, 92, 97, 98, 100, 102, 225, 228, 231, and 232. Further preferably, the linked-type antisense oligomer of the present invention comprises any one base sequence selected from the group consisting of SEQ ID NOs: 102, 225, and 228. In a certain embodiment, the linked-type antisense oligomer of the present invention is an antisense oligomer consisting of any one

base sequence selected from the group consisting of SEQ ID NOs: 102, 225, and 228, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0101]

The antisense oligomer of the present invention comprises a base sequence complementary to a base sequence of at least any one region selected from the group consisting of the regions R1 to R24, or a partial base sequence thereof. Any base sequence selected from the group consisting of (E1) and (E2-1) to (E2-16), the group consisting of (E'1) and (E'2-1) to (E'2-16), the group consisting of (E''1) and (E''2-1) to (E''2-16), or the group consisting of (E'''1) and (E'''2-1) to (E'''2-16) may be selected as the base sequence complementary to the base sequence of each region included in the regions R1 to R24, or a partial base sequence thereof. For example, as the base sequences complementary to the base sequences of the regions R1 to R24, or partial base sequences thereof, a base sequence complementary to the base sequence of each region, or a partial base sequence thereof may be used as to the regions R4 to R24, and a base sequence that is selected from the group consisting of (E1) and (E2-1) to (E2-16) and is included in each of the regions R1 to R3 may be used as to the regions R1 to R3.

[0102]

The antisense oligomer of the present invention (including the linked-type antisense oligomer of the present invention) may be an oligonucleotide, morpholino oligomer or peptide nucleic acid (PNA) oligomer (hereinafter, also referred to as the "antisense oligonucleotide of the present invention", the "antisense morpholino oligomer of the present invention", or the "antisense peptide nucleic acid oligomer of the present invention").

[0103]

The antisense oligonucleotide of the present invention is an antisense oligomer composed of nucleotides as constituent units. Such nucleotides may be any of ribonucleotides, deoxyribonucleotides and modified nucleotides.

[0104]

The modified nucleotide refers to one having fully or partly modified nucleobases, sugar moieties and/or phosphate bond moieties, which constitute the ribonucleotide or deoxyribonucleotide.

[0105]

The nucleobase includes, for example, adenine, guanine, hypoxanthine, cytosine, thymine, uracil, and modified bases thereof. Examples of such modified bases include, but not limited to, pseudouracil, 3-methyluracil, dihydrouracil, 5-alkylcytosines (e.g., 5-methylcytosine), 5-alkyluracils (e.g., 5-ethyluracil), 5-

halouracils (e.g., 5-bromouracil), 6-azapyrimidine, 6-alkylpyrimidines (e.g., 6-methyluracil), 2-thiouracil, 4-thiouracil, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5'-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, 1-methyladenine, 1-methylhypoxanthine, 2,2-dimethylguanine, 3-methylcytosine, 2-methyladenine, 2-methylguanine, N6-methyladenine, 7-methylguanine, 5-methoxyaminomethyl-2-thiouracil, 5-methylaminomethyluracil, 5-methylcarbonylmethyluracil, 5-methyloxyuracil, 5-methyl-2-thiouracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid, 2-thiocytosine, purine, 2,6-diaminopurine, 2-aminopurine, isoguanine, indole, imidazole, xanthine, etc.

[0106]

Modification of the sugar moiety may include, for example, modifications at the 2'-position of ribose and modifications of the other positions of the sugar. The modification at the 2'-position of ribose includes a modification of replacing the 2'-OH of ribose with -OR, -R, -R'OR, -SH, -SR, -NH₂, -NHR, -NR₂, -N₃, -CN, -F, -Cl, -Br or -I, wherein R represents an alkyl or an aryl and R' represents an alkylene.

The modification for the other positions of the sugar includes, for example, replacement of O at the 4' position of ribose or deoxyribose with S, bridging between 2' and 4' positions of the sugar, e.g., LNA

(locked nucleic acid) or ENA (2'-O,4'-C-ethylene-bridged nucleic acids), but is not limited thereto.

[0107]

A modification of the phosphate bond moiety includes, for example, a modification of replacing phosphodiester bond with phosphorothioate bond, phosphorodithioate bond, alkyl phosphonate bond, phosphoramidate bond or boranophosphate bond (cf., e.g., Enya et al: Bioorganic & Medicinal Chemistry, 2008, 18, 9154-9160) (cf., e.g., Japan Domestic Re-Publications of PCT Application Nos. 2006/129594 and 2006/038608).

[0108]

As used herein, the alkyl is preferably a straight or branched alkyl having 1 to 6 carbon atoms. Specific examples include methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, isopentyl, neopentyl, *tert*-pentyl, *n*-hexyl and isohexyl. The alkyl may optionally be substituted. Examples of such substituents are a halogen, an alkoxy, cyano and nitro. The alkyl may be substituted with 1 to 3 substituents.

[0109]

As used herein, the cycloalkyl is preferably a cycloalkyl having 3 to 12 carbon atoms. Specific examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl and cyclododecyl.

[0110]

As used herein, the halogen includes fluorine, chlorine, bromine and iodine.

[0111]

As used herein, the alkoxy is a straight or branched alkoxy having 1 to 6 carbon atoms such as methoxy, ethoxy, *n*-propoxy, isopropoxy, *n*-butoxy, isobutoxy, *sec*-butoxy, *tert*-butoxy, *n*-pentyloxy, isopentyloxy, *n*-hexyloxy, isohexyloxy, etc. Among others, an alkoxy having 1 to 3 carbon atoms is preferred.

[0112]

As used herein, the aryl is preferably an aryl having 6 to 10 carbon atoms. Specific examples include phenyl, α -naphthyl and β -naphthyl. Among others, phenyl is preferred. The aryl may optionally be substituted. Examples of such substituents are an alkyl, a halogen, an alkoxy, cyano and nitro. The aryl may be substituted with one to three of such substituents.

[0113]

As used herein, the alkylene is preferably a straight or branched alkylene having 1 to 6 carbon atoms. Specific examples include methylene, ethylene, trimethylene, tetramethylene, pentamethylene, hexamethylene, 2-(ethyl) trimethylene and 1-(methyl) tetramethylene.

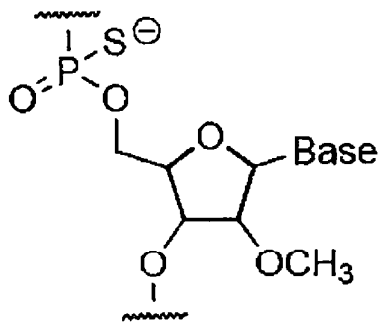
[0114]

As used herein, the acyl includes a straight or branched alkanoyl or aroyl. Examples of the alkanoyl include formyl, acetyl, 2-methylacetyl, 2,2-dimethylacetyl, propionyl, butyryl, isobutyryl, pentanoyl, 2,2-dimethylpropionyl, hexanoyl, etc. Examples of the aroyl include benzoyl, toluoyl and naphthoyl. The aroyl may optionally be substituted at substitutable positions and may be substituted with an alkyl(s).

[0115]

Preferably, the antisense oligonucleotide of the present invention is the antisense oligomer of the present invention having a group represented by general formula below as a constituent unit wherein the -OH group at position 2' of ribose is substituted with methoxy and the phosphate bond moiety is a phosphorothioate bond:

[Formula 4]



wherein Base represents a nucleobase.

[0116]

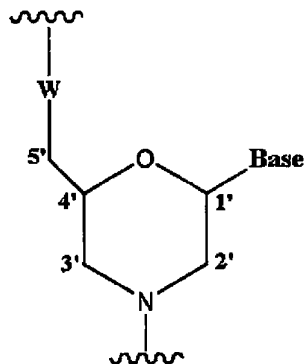
The antisense oligonucleotide of the present invention may be easily synthesized using various

automated synthesizer (e.g., AKTA oligopilot plus 10/100 (GE Healthcare)). Alternatively, the synthesis may also be entrusted to a third-party organization (e.g., Promega Corp. or Takara Co.), etc.

[0117]

The antisense morpholino oligomer of the present invention is an antisense oligomer comprising the constituent unit represented by general formula below:

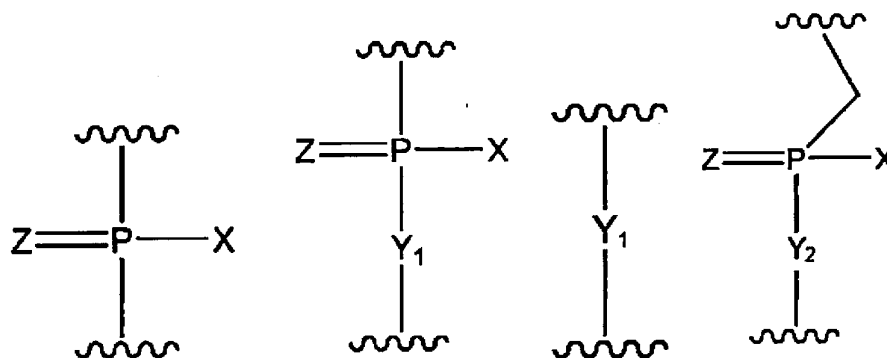
[Formula 5]



wherein Base has the same significance as defined above,
and,

W represents a group shown by any one of the following groups:

[Formula 6]



wherein X represents $-\text{CH}_2\text{R}^1$, $-\text{O}-\text{CH}_2\text{R}^1$, $-\text{S}-\text{CH}_2\text{R}^1$, $-\text{NR}^2\text{R}^3$, or F;

R^1 represents H or an alkyl;

R^2 and R^3 , which may be the same or different, each represents H, an alkyl, a cycloalkyl, or an aryl;

Y_1 represents O, S, CH_2 , or NR^1 ;

Y_2 represents O, S, or NR^1 ;

Z represents O or S.

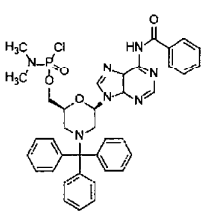
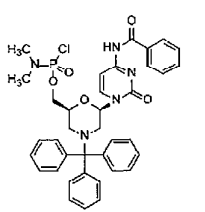
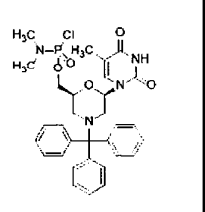
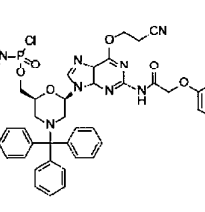
[0118]

Examples of morpholino monomer compounds that are used in synthesis of the antisense morpholino oligomer of the present invention include, but not limited to, the following morpholino monomer compound (A), morpholino monomer compound (C), morpholino monomer compound (T), and morpholino monomer compound (G) shown in Table 5.

[0119]

[Table 5]

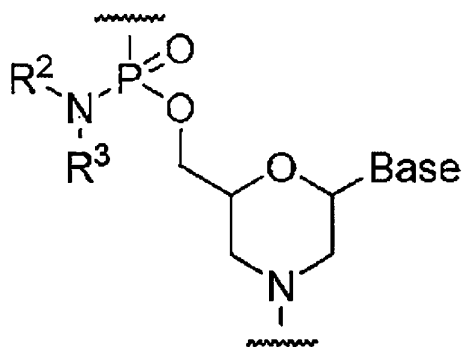
Table 5

Morpholino monomer compound (A)	Morpholino monomer compound (C)	Morpholino monomer compound (T)	Morpholino monomer compound (G)
			

[0120]

In the present invention, preferably, the morpholino oligomer is an oligomer having a group represented by general formula below as a constituent unit (phosphorodiamidate morpholino oligomer (hereinafter referred to as "PMO")).

[Formula 7]



wherein Base, R² and R³ have the same significance as defined above.

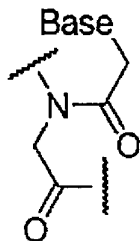
[0121]

The morpholino oligomer may be produced by the procedure described in, e.g., WO 1991/009033 or WO 2009/064471. In particular, PMO can be produced by the procedure described in WO 2009/064471 or WO2013/100190.

[0122]

The antisense peptide nucleic acid oligomer of the present invention is an antisense oligomer having a group represented by general formula below as a constituent unit:

[Formula 8]



wherein Base has the same significance as defined above.

The peptide nucleic acid oligomer can be produced in accordance with, e.g., the following literatures:

- 1) P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, Science, 254, 1497 (1991)
- 2) M. Egholm, O. Buchardt, P. E. Nielsen, R. H. Berg, JACS, 114, 1895 (1992)
- 3) K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen, O. Buchardt, J. Org. Chem., 59, 5767 (1994)
- 4) L. Christensen, R. Fitzpatrick, B. Gildea, K. H. Petersen, H. F. Hansen, T. Koch, M. Egholm, O. Buchardt, P. E. Nielsen, J. Coull, R. H. Berg, J. Pept. Sci., 1, 175 (1995)
- 5) T. Koch, H. F. Hansen, P. Andersen, T. Larsen, H. G. Batz, K. Otteson, H. Orum, J. Pept. Res., 49, 80 (1997) [0123]

The antisense oligomer of the present invention (including the linked-type antisense oligomer of the present invention) may be in the form of a pharmaceutically acceptable salt thereof, in the form of

a hydrate thereof, or in the form of a hydrate of the pharmaceutically acceptable salt.

[0124]

Examples of the pharmaceutically acceptable salt of the antisense oligomer of the present invention are alkali metal salts such as salts of sodium, potassium and lithium; alkaline earth metal salts such as salts of calcium and magnesium; metal salts such as salts of aluminum, iron, zinc, copper, nickel, cobalt, etc.; ammonium salts; organic amine salts such as salts of t-octylamine, dibenzylamine, morpholine, glucosamine, phenylglycine alkyl ester, ethylenediamine, N-methylglucamine, guanidine, diethylamine, triethylamine, dicyclohexylamine, N,N'-dibenzylethylenediamine, chloroprocaine, procaine, diethanolamine, N-benzylphenethylamine, piperazine, tetramethylammonium, tris(hydroxymethyl)aminomethane; hydrohalide salts such as salts of hydrofluorides, hydrochlorides, hydrobromides and hydroiodides; inorganic acid salts such as nitrates, perchlorates, sulfates, phosphates, etc.; lower alkane sulfonates such as methanesulfonates, trifluoromethanesulfonates and ethanesulfonates; arylsulfonates such as benzenesulfonates and p-toluenesulfonates; organic acid salts such as acetates, malates, fumarates, succinates, citrates, tartarates, oxalates, maleates, etc.; and, amino acid salts such as salts of glycine, lysine, arginine, ornithine, glutamic

acid and aspartic acid. These salts may be produced by known methods. Alternatively, the antisense oligomer of the present invention may be in the form of a hydrate thereof.

[0125]

2. Suppressor antisense oligomer

Another embodiment of the present invention provides a suppressor antisense oligomer which suppresses single exon skipping (hereinafter, referred to as "single skipping"). The suppressor antisense oligomer can suppress single skipping and thereby enhance an effect of multi-exon skipping by an antisense oligomer. The single skipping relates to skipping of only one exon, not simultaneous skipping of a plurality of exons.

[0126]

Specifically, the present invention provides a suppressor antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which suppresses single skipping of any one exon selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA. Hereinafter, the suppressor antisense oligomer and the pharmaceutically acceptable salt thereof and the hydrate of the antisense oligomer or the salt are also collectively referred to as the "suppressor antisense oligomer of the present invention". The suppressor antisense oligomer of the present invention may refer to each of the suppressor antisense

oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof.

[0127]

The suppressor antisense oligomer of the present invention suppresses single skipping by targeting the site of a splicing silencer sequence, a branch site sequence, or a splice site sequence in human dystrophin pre-mRNA and inhibiting splicing. The suppressor antisense oligomer of the present invention reduces the efficiency of single skipping of an intended exon as compared to a control.

[0128]

For example, the suppressor antisense oligomer that suppresses single skipping of any one exon selected from the group consisting of the 45th exon to the 55th exon targets a splicing silencer sequence of any one of the 44th to 56th exons, or a splicing silencer sequence, a branch site sequence, or a splice site sequence of any one of the 44th to 55th introns.

[0129]

As used herein, the splicing silencer sequence is a base sequence element that functions to suppress recognition of an exon in pre-mRNA. Examples of the splicing silencer sequence include recognition sequences of proteins or protein complexes, such as heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), hnRNP A2/B1,

DAZAP1, hnRNP I, Fox-1, Fox-2, hnRNP H1, hnRNP H2, hnRNP H3, hnRNP L, Sam68, and SRp40.

[0130]

As used herein, the splice site is the boundary between an exon and an intron. The splice site can be predicted from the 5' end of the intron starting at GU, AU, or the like, and the 3' end of the intron ending at AG, AC, or the like.

[0131]

As used herein, the branch site is a sequence within an intron that initiates an attack on a 5' splice site during splicing reaction and forms a covalent bond. The branch site sequence refers to a base sequence in an intron containing the branch site.

[0132]

Examples of the splicing silencer sequence, the branch site sequence, or the splice site sequence which is the target (hereinafter, referred to as a "suppressor sequence") of the suppressor antisense oligomer of the present invention include base sequences given below (SEQ ID NOs: 370 to 384 and 390). The positions of the suppressor sequence, the branch site sequence, and the splice site sequence can be detected using software such as SpliceAid (Francesco Piva et al., Bioinformatics, 25 (9), 1211-1213, 2009), SpliceAid-2 (Francesco Piva et al., Human Mutation, 33 (1), 81-85, 2012), SpliceAid-F (Matteo Giulietti et al., Nucleic Acids Res., 41, D125-

D131, 2012), ATtRACT-a (Girolamo Giudice et al., Database (Oxford), baw035, 2016), SROOGLE (Schrage Schwartz et al., Nucleic Acids Res., 37, W189-W192, 2009), Reg RNA (Hsi-Yuan Huang et al., Nucleic Acids Res., 34, W429-W434, 2006), Reg RNA 2.0 (Tzu-Hao Chang et al., BMC bioinformatics, 14 (Suppl 2), S4, 2013), Human Splicing Finder (Francois-Olivier Desmet et al., Nucleic Acids Res., 37, 9, e67, 2009), or SVM-BPfinder (Andre Corvelo et al., PLoS Comput Biol., 6, 11, e1001016, 2010).

[0133]

[Table 6]

Table 6

SEQ ID NO	Name of protein or protein complex or sequence site to be bound	Base sequence
370	hnRNP A1	UAGGA
371		UAGGAU
372		UAGGAA
373		UAGGCA
374		UAGGCU
375		UAGGGA
376		UAGGGC
377		UAGGGU
378		GGUAGGGC
379		AGAAC
380	Fox-1	AGCAUG
381		UGACUG
382		UGCAUG
383	Branch site	yUnAy
384	Splice site	GU
390		AG

y: A or U, n: A, G, C, U, or T

[0134]

Herein, specific examples of the splicing silencer sequence include SEQ ID NOs: 370 to 382. Examples of mutants of SEQ ID NOs: 370 to 382 also include

(F1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(F2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(F3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(F4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(F5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(F6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(F7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(F8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(F9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(F10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(F11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(F12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(F13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting

of SEQ ID NOs: 370 to 382, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(F14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(F15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(F16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 382, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0135]

In a certain embodiment of the present invention, the splicing silencer sequence may be a recognition sequence of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1). Examples of the recognition sequence of human hnRNP A1 are shown in SEQ ID NOs: 370 to 379.

[0136]

Herein, specific examples of the splice site sequence include SEQ ID NOs: 384 and 390. Examples of mutants of SEQ ID NOs: 384 and 390 also include

(G1) a base sequence that has at least 80% identity with the base sequence of SEQ ID NO: 384 or 390, and has a

length within $\pm 20\%$ of the length of the any one base sequence selected.

[0137]

Herein, specific examples of the branch site sequence include SEQ ID NO: 383. Examples of a mutant of SEQ ID NO: 383 also include

(H1) a base sequence that has at least 80% identity with the base sequence of SEQ ID NO: 383, and has a length within $\pm 20\%$ of the length of the any one base sequence selected.

[0138]

As for a mutant type of the suppressor sequence, the following target regions are also included in the target base sequence of the suppressor antisense oligomer of the present invention:

(I1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(I2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(I3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting

of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(I4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(I5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(I6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(I7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(I8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length

within $\pm 8\%$ of the length of the any one base sequence selected,

(I9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(I10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(I11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(I12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(I13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(I14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(I15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(I16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 370 to 384 and 390, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0139]

In one embodiment, the suppressor antisense oligomer of the present invention comprises a base sequence complementary to any one base sequence of a base sequence selected from the group consisting of the base sequences represented by SEQ ID NOs: 370 to 384 and 390 and the mutant type base sequences shown in (I1) to (I16) above, or a partial base sequence thereof. In another embodiment, the suppressor antisense oligomer of the present invention consists of a base sequence complementary to any one base sequence of a base sequence selected from the group consisting of the base sequences

represented by SEQ ID NOS: 370 to 384 and 390, or a partial base sequence thereof.

Herein, the term "partial" is as defined in the section about the antisense oligomer of the present invention.

[0140]

As a further embodiment, the suppressor antisense oligomer of the present invention is a suppressor antisense oligomer which consists of

(J1) any one base sequence selected from the group consisting of SEQ ID NOS: 257 to 275, or

(J2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 257 to 275, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0141]

Herein, the base sequence (J2) is a mutant type of the base sequence (J1). Such a mutant type also includes

(J2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 257 to 275, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(J2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(J2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(J2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(J2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(J2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(J2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length

within $\pm 9\%$ of the length of the any one base sequence selected,

(J2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(J2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(J2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(J2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(J2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(J2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(J2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(J2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(J2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0142]

Thus, a further embodiment of the suppressor antisense oligomer of the present invention is a suppressor antisense oligomer consisting of any one base sequence selected from the group consisting of (J1) and (J2-1) to (J2-16) or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0143]

A further embodiment of the suppressor antisense oligomer of the present invention is a suppressor antisense oligomer consisting of

(J'1) any one base sequence selected from the group consisting of SEQ ID NO: 260, 261, and 263, or

(J'2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 260, 261, and 263, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or

a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0144]

Herein, the base sequence (J'2) is a mutant type of the base sequence (J'1). Such a mutant type also includes

(J'2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 260, 261, and 263, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(J'2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 260, 261, and 263, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(J'2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(J'2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(J'2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(J'2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(J'2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(J'2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(J'2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(J'2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(J'2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(J'2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(J'2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263, and has a

length within $\pm 3\%$ of the length of the any one base sequence selected,

(J'2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 260, 261, and 263, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(J'2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 260, 261, and 263, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(J'2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 260, 261, and 263, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0145]

Thus, a further embodiment of the suppressor antisense oligomer of the present invention is a suppressor antisense oligomer consisting of any one base sequence selected from the group consisting of (J'1) and (J'2-1) to (J'2-16) or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0146]

The suppressor antisense oligomer of the present invention enhances a multi-skipping effect under

physiological conditions. The term "under physiological conditions" refers to conditions set to mimic the *in vivo* environment in terms of pH, salt composition and temperature. The conditions are, for example, 25 to 40°C, preferably 37°C, pH 5 to 8, preferably pH 7.4 and 150 mM of sodium chloride concentration.

[0147]

Whether the suppressor antisense oligomer of the present invention enhances a multi-exon skipping effect or not can be confirmed by providing (i) an experimental system for multi-exon skipping using only the antisense oligomer of the present invention alone and (ii) an experimental system for multi-exon skipping using the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention such that the other conditions are the same therebetween, and observing the difference between a multi-exon skipping effect obtained in the experimental system (ii) and a multi-exon skipping effect obtained in the experimental system (i).

[0148]

The multi-skipping effect is measured as mentioned in the section "1. Antisense oligomer" about the antisense oligomer of the present invention.

[0149]

The suppressor antisense oligomer of the present invention is 10 to 60 bases long and may be 10 to 55

bases long, 10 to 50 bases long, 10 to 45 bases long, 10 to 40 bases long, 10 to 35 bases long, 10 to 30 bases long, 10 to 25 bases long, 15 to 60 bases long, 15 to 55 bases long, 15 to 50 bases long, 15 to 45 bases long, 15 to 40 bases long, 15 to 35 bases long, 15 to 30 bases long, 15 to 25 bases long, 16 to 60 bases long, 16 to 55 bases long, 16 to 50 bases long, 16 to 45 bases long, 16 to 40 bases long, 16 to 35 bases long, 16 to 30 bases long, 16 to 25 bases long, 17 to 60 bases long, 17 to 55 bases long, 17 to 50 bases long, 17 to 45 bases long, 17 to 40 bases long, 17 to 35 bases long, 17 to 30 bases long, 17 to 25 bases long, 18 to 60 bases long, 18 to 55 bases long, 18 to 50 bases long, 18 to 45 bases long, 18 to 40 bases long, 18 to 35 bases long, 18 to 30 bases long, 18 to 25 bases long, 19 to 60 bases long, 19 to 55 bases long, 19 to 50 bases long, 19 to 45 bases long, 19 to 40 bases long, 19 to 35 bases long, 19 to 30 bases long, 19 to 25 bases long, 20 to 60 bases long, 20 to 55 bases long, 20 to 50 bases long, 20 to 45 bases long, 20 to 40 bases long, 20 to 35 bases long, 20 to 30 bases long, 20 to 25 bases long, 15 to 30 bases long, 15 to 29 bases long, 15 to 28 bases long, 15 to 27 bases long, 15 to 26 bases long, 15 to 25 bases long, 15 to 24 bases long, 15 to 23 bases long, 15 to 22 bases long, 15 to 21 bases long, 15 to 20 bases long, 15 to 19 bases long, 15 to 18 bases long, 16 to 30 bases long, 16 to 29 bases long, 16 to 28 bases long, 16 to 27 bases long, 16 to 26

bases long, 16 to 25 bases long, 16 to 24 bases long, 16 to 23 bases long, 16 to 22 bases long, 16 to 21 bases long, 16 to 20 bases long, 16 to 19 bases long, 16 to 18 bases long, 17 to 30 bases long, 17 to 29 bases long, 17 to 28 bases long, 17 to 27 bases long, 17 to 26 bases long, 17 to 25 bases long, 17 to 24 bases long, 17 to 23 bases long, 17 to 22 bases long, 17 to 21 bases long, 17 to 20 bases long, 17 to 19 bases long, 17 to 18 bases long, 18 to 30 bases long, 18 to 29 bases long, 18 to 28 bases long, 18 to 27 bases long, 18 to 26 bases long, 18 to 25 bases long, 18 to 24 bases long, 18 to 23 bases long, 18 to 22 bases long, 18 to 21 bases long, 18 to 20 bases long, 18 to 19 bases long, 19 to 30 bases long, 19 to 29 bases long, 19 to 28 bases long, 19 to 27 bases long, 19 to 26 bases long, 19 to 25 bases long, 19 to 24 bases long, 19 to 23 bases long, 19 to 22 bases long, 19 to 21 bases long, 19 to 20 bases long, 20 to 30 bases long, 20 to 29 bases long, 20 to 28 bases long, 20 to 27 bases long, 20 to 26 bases long, 20 to 25 bases long, 20 to 24 bases long, 20 to 23 bases long, 20 to 22 bases long, 20 to 21 bases long, 60 bases long, 59 bases long, 58 bases long, 57 bases long, 56 bases long, 55 bases long, 54 bases long, 53 bases long, 52 bases long, 51 bases long, 50 bases long, 49 bases long, 48 bases long, 47 bases long, 46 bases long, 45 bases long, 44 bases long, 43 bases long, 42 bases long, 41 bases long, 40 bases long, 39 bases long, 38 bases long, 37 bases long,

36 bases long, 35 bases long, 34 bases long, 33 bases long, 32 bases long, 31 bases long, 30 bases long, 29 bases long, 28 bases long, 27 bases long, 26 bases long, 25 bases long, 24 bases long, 23 bases long, 22 bases long, 21 bases long, 20 bases long, 19 bases long, 18 bases long, 17 bases long, 16 bases long, 15 bases long, 14 bases long, 13 bases long, 12 bases long, 11 bases long, or 10 bases long, but not limited thereto. These lengths may be increased or decreased by 1, 2, or 3 bases.

[0150]

The suppressor antisense oligomer of the present invention may be a linked-type antisense oligomer configured to comprise a plurality of unit oligomers linked to each other, or a pharmaceutically acceptable salt thereof, or hydrate thereof. The unit oligomers may be linked via a linker that does not contribute to hybridization, or may be linked directly without the mediation of a linker, as mentioned above. When the suppressor antisense oligomer of the present invention is a linked type, the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[0151]

The suppressor antisense oligomer of the present invention may be an oligonucleotide, morpholino oligomer, or peptide nucleic acid (PNA) oligomer.

[0152]

When the suppressor antisense oligomer of the present invention is an oligonucleotide (hereinafter, referred to as the "suppressor antisense oligonucleotide of the present invention"), the suppressor antisense oligonucleotide of the present invention is a suppressor antisense oligomer composed of nucleotides as constituent units. Such nucleotides may be any of ribonucleotides, deoxyribonucleotides and modified nucleotides.

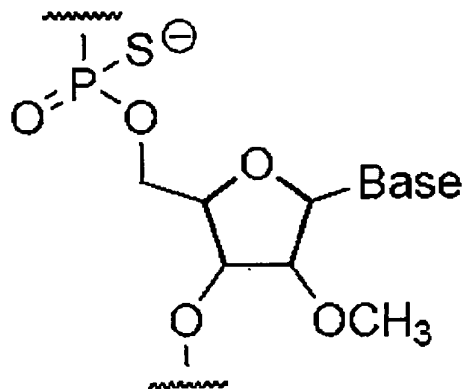
[0153]

The modified nucleotide is as mentioned above.

[0154]

Preferably, the suppressor antisense oligonucleotide of the present invention is the suppressor antisense oligomer of the present invention having a group represented by general formula below as a constituent unit wherein the -OH group at position 2' of ribose is substituted with methoxy and the phosphate bond moiety is a phosphorothioate bond:

[Formula 9]



wherein Base has the same significance as defined above.

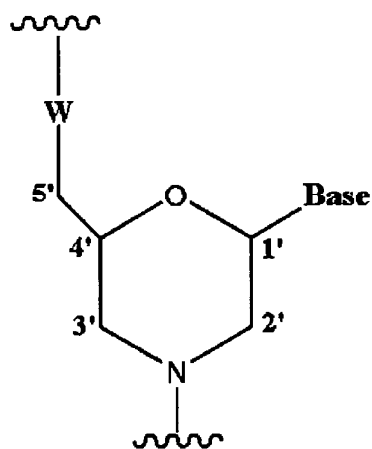
[0155]

The suppressor antisense oligonucleotide of the present invention may be easily synthesized using various automated synthesizer (e.g., AKTA oligopilot plus 10/100 (GE Healthcare)). Alternatively, the synthesis may also be entrusted to a third-party organization (e.g., Promega Corp. or Takara Co.), etc.

[0156]

When the suppressor antisense oligomer of the present invention is a morpholino oligomer (hereinafter, referred to as the "suppressor antisense morpholino oligomer of the present invention"), the suppressor antisense morpholino oligomer of the present invention is a suppressor antisense oligomer having a group represented by general formula below as a constituent unit:

[Formula 10]



wherein Base and W have the same significance as defined above.

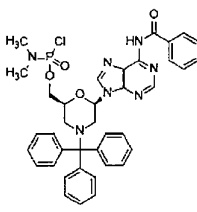
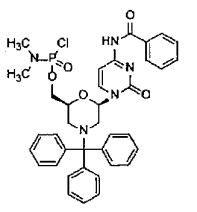
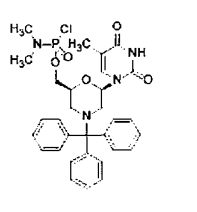
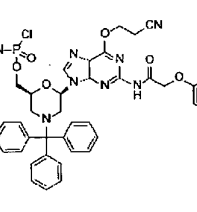
[0157]

Examples of morpholino monomer compounds that are used in synthesis of the suppressor antisense morpholino oligomer of the present invention include, but not limited to, the following morpholino monomer compound (A), morpholino monomer compound (C), morpholino monomer compound (T), and morpholino monomer compound (G) shown in Table 7.

[0158]

[Table 7]

Table 7

Morpholino monomer compound (A)	Morpholino monomer compound (C)	Morpholino monomer compound (T)	Morpholino monomer compound (G)
			

[0159]

In the suppressor antisense morpholino oligomer of the present invention, preferably the morpholino oligomer is PMO.

[0160]

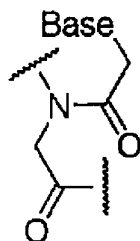
The morpholino oligomer may be produced by the procedure described in, e.g., WO 1991/009033 or WO

2009/064471. In particular, PMO can be produced by the procedure described in WO 2009/064471 or WO2013/100190.

[0161]

When the suppressor antisense oligomer of the present invention is a peptide nucleic acid oligomer (hereinafter, referred to as the "suppressor antisense peptide nucleic acid oligomer of the present invention"), the suppressor antisense peptide nucleic acid oligomer of the present invention is a suppressor antisense oligomer having a group represented by general formula (I) below as a constituent unit:

[Formula 11]



wherein Base has the same significance as defined above.

The peptide nucleic acid oligomer can be produced as mentioned above.

[0162]

The suppressor antisense oligomer of the present invention may be in the form of a pharmaceutically acceptable salt thereof, in the form of a hydrate thereof, or in the form of a hydrate of the pharmaceutically acceptable salt.

[0163]

Examples of the pharmaceutically acceptable salt of the suppressor antisense oligomer of the present invention are alkali metal salts such as salts of sodium, potassium and lithium; alkaline earth metal salts such as salts of calcium and magnesium; metal salts such as salts of aluminum, iron, zinc, copper, nickel, cobalt, etc.; ammonium salts; organic amine salts such as salts of t-octylamine, dibenzylamine, morpholine, glucosamine, phenylglycine alkyl ester, ethylenediamine, N-methylglucamine, guanidine, diethylamine, triethylamine, dicyclohexylamine, N,N'-dibenzylethylenediamine, chloroprocaine, procaine, diethanolamine, N-benzylphenethylamine, piperazine, tetramethylammonium, tris(hydroxymethyl)aminomethane; hydrohalide salts such as salts of hydrofluorides, hydrochlorides, hydrobromides and hydroiodides; inorganic acid salts such as nitrates, perchlorates, sulfates, phosphates, etc.; lower alkane sulfonates such as methanesulfonates, trifluoromethanesulfonates and ethanesulfonates; arylsulfonates such as benzenesulfonates and p-toluenesulfonates; organic acid salts such as acetates, malates, fumarates, succinates, citrates, tartarates, oxalates, maleates, etc.; and, amino acid salts such as salts of glycine, lysine, arginine, ornithine, glutamic acid and aspartic acid. These salts may be produced by known methods. Alternatively, the suppressor antisense

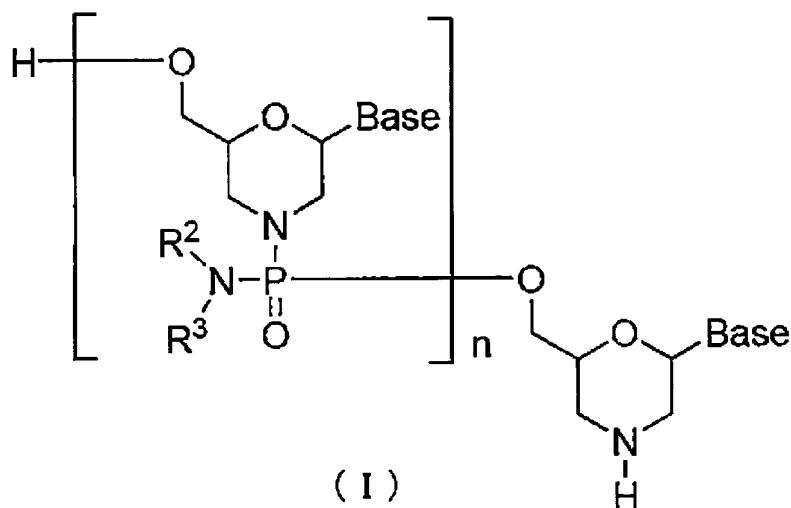
oligomer of the present invention may be in the form of a hydrate thereof.

[0164]

[Method for producing PMO]

Each of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention may be PMO. An embodiment of PMO is, for example, the compound represented by general formula (I) below (hereinafter, referred to as PMO (I)).

[Formula 12]



wherein Base, R² and R³ have the same significance as defined above; and,

n is a given integer of 1 to 99, preferably a given integer of 18 to 28.

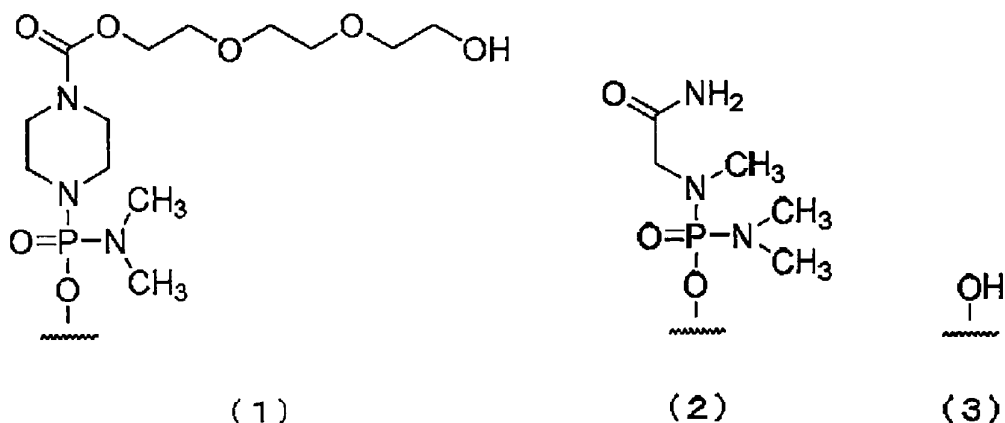
[0165]

PMO (I) can be produced in accordance with a known method (cf., e.g., WO2009/064471 or WO2013/100190).

[0166]

In the antisense oligomer of the present invention or the suppressor antisense oligomer of the present invention, the 5' end may be a group represented by any of chemical structures (1) to (3) below, and preferably is (3)-OH.

[Formula 13]



Hereinafter, the groups shown by (1), (2) and (3) above are referred to as "Group (1)," "Group (2)" and "Group (3)," respectively.

[0167]

3. Medical application

The present invention provides a pharmaceutical composition comprising the antisense oligomer of the present invention (also including the pharmaceutically acceptable salt thereof, or hydrate thereof) (hereinafter, referred to as the "pharmaceutical composition of the present invention"). The pharmaceutical composition of the present invention may further comprise the suppressor antisense oligomer of the

present invention (also including the pharmaceutically acceptable salt thereof, or hydrate thereof) and/or a pharmaceutically acceptable carrier.

[0168]

The present invention also provides a pharmaceutical composition comprising the antisense oligomer of the present invention (also including the pharmaceutically acceptable salt thereof, or hydrate thereof) and the suppressor antisense oligomer of the present invention (also including the pharmaceutically acceptable salt thereof, or hydrate thereof) (hereinafter, referred to as the "pharmaceutical combination of the present invention").

[0169]

When the pharmaceutical composition of the present invention comprises the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention, any combination of these oligomers is included. In the pharmaceutical combination of the present invention, any combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention is included. The antisense oligomer contained in the pharmaceutical composition of the present invention or the pharmaceutical combination of the present invention can be any antisense oligomer of the present invention and is, but not particularly limited to, preferably an

antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOS: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, more preferably an antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232, and further preferably an antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228.

The suppressor antisense oligomer contained in the pharmaceutical composition of the present invention or the pharmaceutical combination of the present invention can be any suppressor antisense oligomer of the present invention and is, but not particularly limited to, preferably a suppressor antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOS: 257 to 275, more preferably SEQ ID NO: 1,

5, 6, 7, 8, 10, 11, 14, 26, or 27, and further preferably a suppressor antisense oligomer consisting of any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263.

When the pharmaceutical composition of the present invention comprises the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention, preferably, the pharmaceutical composition comprises a combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention described above. Preferably, the pharmaceutical combination of the present invention comprises a combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention described above.

[0170]

In the pharmaceutical combination of the present invention, examples of the combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention include

(K1) a combination in which the antisense oligomer is an oligomer consisting of SEQ ID NO: 75 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 260,

(K2) a combination in which the antisense oligomer is an oligomer consisting of SEQ ID NO: 75 and the suppressor

antisense oligomer is an oligomer consisting of SEQ ID NO: 261,

(K3) a combination in which the antisense oligomer is an oligomer consisting of SEQ ID NO: 75 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 263,

(K4) a combination in which the antisense oligomer is an oligomer consisting of SEQ ID NO: 65 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 260,

(K5) a combination in which the antisense oligomer is an oligomer consisting of SEQ ID NO: 65 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 261,

(K6) a combination in which the antisense oligomer is an oligomer consisting of SEQ ID NO: 65 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 263,

(K7) a combination in which the antisense oligomer is a combination of oligomers consisting of SEQ ID NOs: 55 and 59 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 260,

(K8) a combination in which the antisense oligomer is a combination of oligomers consisting of SEQ ID NOs: 55 and 59 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 261, and

(K9) a combination in which the antisense oligomer is a combination of oligomers consisting of SEQ ID NOs: 55 and 59 and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 263.

The pharmaceutical composition of the present invention may comprise the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention in these combinations.

[0171]

The pharmaceutical composition of the present invention and the pharmaceutical combination of the present invention can each be used for the treatment of, for example, Duchenne muscular dystrophy, Becker muscular dystrophy, limb-girdle muscular dystrophy (LGMD), congenital muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, oculopharyngeal muscular dystrophy, cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL), and Alport's syndrome. The pharmaceutical combination of the present invention and the pharmaceutical composition of the present invention can each be administered to a human patient and in particular, a human patient with muscular dystrophy. The patient to receive the pharmaceutical combination of the present invention or the pharmaceutical composition of the present invention may be a human patient having a mutation that is amenable to skipping of two or more

exons selected from the group consisting of exons 45 to 55 in the dystrophin gene.

[0172]

One embodiment of the present invention provides a method for treatment of muscular dystrophy, which comprises administering to a patient with muscular dystrophy the antisense oligomer of the present invention or a combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention.

Another embodiment of the present invention provides a method for treatment of muscular dystrophy, which comprises administering to a patient with muscular dystrophy the pharmaceutical composition of the present invention or the pharmaceutical combination of the present invention.

The method for treatment may involve performing skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA. In the method for treatment, the patient with muscular dystrophy may be a patient having a mutation that is amenable to exon 45 to 55 skipping in the dystrophin gene. The patient may be a human and may be a human patient having a mutation that is amenable to exon 45 to 55 skipping in the dystrophin gene.

[0173]

The present invention further provides use of the antisense oligomer of the present invention or a combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention, or the pharmaceutical composition of the present invention or the pharmaceutical combination of the present invention in manufacturing of a medicament for the treatment of muscular dystrophy.

[0174]

The present invention further provides the antisense oligomer of the present invention or a combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention, or the pharmaceutical composition of the present invention or the pharmaceutical combination of the present invention for use in the treatment of muscular dystrophy. The treatment may involve performing skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA. In the treatment, the patient with muscular dystrophy may be a patient having a mutation that is amenable to exon 45 to 55 skipping in the dystrophin gene. The patient may be a human and may be a human patient having a mutation that is amenable to exon 45 to 55 skipping in the dystrophin gene.

[0175]

Administration route for the antisense oligomer of the present invention or the combination of the antisense oligomer of the present invention and the suppressor antisense oligomer of the present invention, or the pharmaceutical composition of the present invention or the pharmaceutical combination of the present invention is not particularly limited so long as it is pharmaceutically acceptable route for administration, and can be chosen depending upon method of treatment. In view of easiness in delivery to muscle tissues, preferred are intravenous administration, intraarterial administration, intramuscular administration, subcutaneous administration, oral administration, tissue administration, transdermal administration, etc. Also, dosage forms which are available for the composition of the present invention are not particularly limited, and include, for example, various injections, oral agents, drips, inhalations, ointments, lotions, etc.

[0176]

In administration of the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention to patients with muscular dystrophy, preferably, the composition of the present invention contains a carrier to promote delivery of the oligomer to muscle tissues. Such a carrier is not particularly limited as far as it is pharmaceutically acceptable, and examples include cationic carriers such

as cationic liposomes, cationic polymers, etc., or carriers using viral envelope. The cationic liposomes are, for example, liposomes composed of 2-O-(2-diethylaminoethyl)carbamoyl-1,3-O-dioleoylglycerol and phospholipids as the essential constituents (hereinafter referred to as "liposome A"), Oligofectamine (registered trademark) (manufactured by Invitrogen Corp.), Lipofectin (registered trademark) (manufactured by Invitrogen Corp.), Lipofectamine (registered trademark) (manufactured by Invitrogen Corp.), Lipofectamine 2000 (registered trademark) (manufactured by Invitrogen Corp.), DMRIE-C (registered trademark) (manufactured by Invitrogen Corp.), GeneSilencer (registered trademark) (manufactured by Gene Therapy Systems), TransMessenger (registered trademark) (manufactured by QIAGEN, Inc.), TransIT TKO (registered trademark) (manufactured by Mirus) and Nucleofector II (Lonza). Among others, liposome A is preferred. Examples of cationic polymers are JetSI (registered trademark) (manufactured by Qbiogene, Inc.) and Jet-PEI (registered trademark) (polyethylenimine, manufactured by Qbiogene, Inc.). An example of carriers using viral envelop is GenomeOne (registered trademark) (HVJ-E liposome, manufactured by Ishihara Sangyo). Alternatively, the medical devices described in Japanese Patent Nos. 2924179 and the cationic carriers described in Japanese Domestic Re-

Publication PCT Nos. 2006/129594 and 2008/096690 may be used as well.

[0177]

A concentration of the antisense oligomer of the present invention contained in the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may vary depending on kind of the carrier, etc., and is appropriately in a range of 0.1 nM to 100 μ M, preferably in a range of 1 nM to 10 μ M, and more preferably in a range of 10 nM to 1 μ M. A weight ratio of the antisense oligomer of the present invention contained in the composition of the present invention and the carrier (carrier/antisense oligomer of the present invention) may vary depending on property of the oligomer, type of the carrier, etc., and is appropriately in a range of 0.1 to 100, preferably in a range of 1 to 50, and more preferably in a range of 10 to 20.

A concentration of the suppressor antisense oligomer of the present invention contained in the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may vary depending on kind of the carrier, etc., and is appropriately in a range of 0.1 nM to 100 μ M, preferably in a range of 1 nM to 10 μ M, and more preferably in a range of 10 nM to 1 μ M. A weight ratio of the suppressor antisense oligomer of the present invention contained in

the composition of the present invention and the carrier (carrier/suppressor antisense oligomer of the present invention) may vary depending on property of the oligomer, type of the carrier, etc., and is appropriately in a range of 0.1 to 100, preferably in a range of 1 to 50, and more preferably in a range of 10 to 20.

[0178]

The pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may be in the form of an aqueous solution. In this case, the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may comprise the antisense oligomer of the present invention in a concentration of 2.5 to 500 mg/mL, 5 to 450 mg/mL, 10 to 400 mg/mL, 15 to 350 mg/mL, 20 to 300 mg/mL, 20 to 250 mg/mL, 20 to 200 mg/mL, 20 to 150 mg/mL, 20 to 100 mg/mL, 20 to 50 mg/mL, 20 to 40 mg/mL, 20 to 30 mg/mL, 23 to 27 mg/mL, 24 to 26 mg/mL, or 25 mg/mL. Alternatively, the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may comprise the antisense oligomer of the present invention in a concentration of 10 to 100 mg/mL, 15 to 95 mg/mL, 20 to 80 mg/mL, 25 to 75 mg/mL, 30 to 70 mg/mL, 35 to 65 mg/mL, 40 to 60 mg/mL, 45 to 55 mg/mL, 47 to 53 mg/mL, 48 to 52 mg/mL, 49 to 51 mg/mL, or 50 mg/mL.

[0179]

The pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention in the form of an aqueous solution may comprise the suppressor oligomer of the present invention in a concentration of 2.5 to 500 mg/mL, 5 to 450 mg/mL, 10 to 400 mg/mL, 15 to 350 mg/mL, 20 to 300 mg/mL, 20 to 250 mg/mL, 20 to 200 mg/mL, 20 to 150 mg/mL, 20 to 100 mg/mL, 20 to 50 mg/mL, 20 to 40 mg/mL, 20 to 30 mg/mL, 23 to 27 mg/mL, 24 to 26 mg/mL, or 25 mg/mL. Alternatively, the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may comprise the suppressor oligomer of the present invention in a concentration of 10 to 100 mg/mL, 15 to 95 mg/mL, 20 to 80 mg/mL, 25 to 75 mg/mL, 30 to 70 mg/mL, 35 to 65 mg/mL, 40 to 60 mg/mL, 45 to 55 mg/mL, 47 to 53 mg/mL, 48 to 52 mg/mL, 49 to 51 mg/mL, or 50 mg/mL.

[0180]

The pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may be in a dry form. In this case, in order to prepare the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention in an aqueous solution form, for example, 125 mg or 250 mg of the antisense oligomer of the present invention in a dry form may be mixed with 0.5 mL to 100 mL of water (which corresponds to the antisense oligomer of the present invention in a concentration of

1.25 mg/mL to 250 mg/mL or 2.5 mg/mL to 500 mg/mL), preferably with 1 mL to 50 mL of water (which corresponds to the antisense oligomer of the present invention in a concentration of 2.5 mg/mL to 125 mg/mL or 5 mg/mL to 250 mg/mL), more preferably with 5 mL to 10 mL of water (which correspond to the antisense oligomer of the present invention in a concentration of 12.5 mg/mL to 25 mg/mL or 25 mg/mL to 50 mg/mL) and used.

[0181]

When the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention is in a dry form, in order to prepare the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention in an aqueous solution form, for example, 125 mg or 250 mg of the suppressor oligomer of the present invention in a dry form may be mixed with 0.5 mL to 100 mL of water (which corresponds to the suppressor oligomer of the present invention in a concentration of 1.25 mg/mL to 250 mg/mL or 2.5 mg/mL to 500 mg/mL), preferably with 1 mL to 50 mL of water (which corresponds to the suppressor oligomer of the present invention in a concentration of 2.5 mg/mL to 125 mg/mL or 5 mg/mL to 250 mg/mL), more preferably with 5 mL to 10 mL of water (which correspond to the suppressor oligomer of the present invention in a concentration of 12.5 mg/mL to 25 mg/mL or 25 mg/mL to 50 mg/mL) and used.

[0182]

The concentrations of the antisense oligomer of the present invention and the suppressor oligomer of the present invention contained in the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may be the respective concentrations of the antisense oligomer of the present invention and the suppressor oligomer of the present invention or may be the total concentration of the antisense oligomer of the present invention and the suppressor oligomer of the present invention.

[0183]

In addition to the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention and the carrier described above, pharmaceutically acceptable additives may also be optionally formulated in the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention. Examples of such additives are emulsification aids (e.g., fatty acids having 6 to 22 carbon atoms and their pharmaceutically acceptable salts, albumin and dextran), stabilizers (e.g., cholesterol, phosphatidic acid, mannitol, and sorbitol), isotonizing agents (e.g., sodium chloride, glucose, maltose, lactose, sucrose, and trehalose), and pH controlling agents (e.g., hydrochloric acid, sulfuric

acid, phosphoric acid, acetic acid, sodium hydroxide, potassium hydroxide and triethanolamine). One or more of these additives can be used. The content of the additive in the composition of the present invention is appropriately 90 wt% or less, preferably 70 wt% or less and more preferably, 50 wt% or less.

[0184]

The pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention can be prepared by adding the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention to a carrier dispersion and adequately stirring the mixture. Additives may be added at an appropriate step either before or after addition of the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention. An aqueous solvent that can be used in adding the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention is not particularly limited as far as it is pharmaceutically acceptable, and examples are injectable water or injectable distilled water, electrolyte fluid such as physiological saline, etc., and sugar fluid such as glucose fluid, maltose fluid, etc. A person skilled in the art can appropriately choose conditions for pH and temperature for such matter.

[0185]

The pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention may be prepared into, e.g., a liquid form and its lyophilized preparation. The lyophilized preparation can be prepared by lyophilizing the composition of the present invention in a liquid form in a conventional manner. The lyophilization can be performed, for example, by appropriately sterilizing the composition of the present invention in a liquid form, dispensing an aliquot into a vial container, performing preliminary freezing for 2 hours at conditions in a range of about -40°C to -20°C, performing a primary drying in a range of about 0°C to 10°C under reduced pressure, and then performing a secondary drying in a range of about 15°C to 25°C under reduced pressure. In general, the lyophilized preparation of the composition of the present invention can be obtained by replacing the content of the vial with nitrogen gas and capping.

[0186]

The lyophilized preparation of the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention can be used in general upon reconstitution by adding an optional suitable solution (reconstitution liquid) and redissolving the preparation. Such a reconstitution liquid includes injectable water, physiological saline

and other infusion fluids. A volume of the reconstitution liquid may vary depending on the intended use, etc., is not particularly limited, and is suitably 0.5-fold to 2-fold greater than the volume prior to lyophilization or no more than 500 mL.

[0187]

It is desired to control a dose of the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention to be administered, by taking the following factors into account: the type and dosage form of the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention contained; patients' conditions including age, body weight, etc.; administration route; and the characteristics and extent of the disease. A single dose calculated as the amount of the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention can be 0.1 mg to 1 g per kg body weight, preferably 1 mg to 100 mg per kg body weight, more preferably 1 mg to 90 mg per kg body weight, and further preferably 1 mg to 80 mg per kg body weight. The frequency of administration may be once per 1 to 3 days, once per week, or once per 2 to 3 weeks. This numerical range may vary occasionally depending on type of the target disease, administration route and target molecule. Therefore, a dose or frequency of

administration lower than the range may be sufficient in some occasion and conversely, a dose or frequency of administration higher than the range may be required occasionally.

[0188]

In still another embodiment of the pharmaceutical composition of the present invention and/or the pharmaceutical combination of the present invention, there is provided a pharmaceutical composition comprising a vector capable of expressing the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention and the carrier described above. Such an expression vector may be a vector capable of expressing a plurality of the antisense oligomers of the present invention and/or the suppressor antisense oligomers of the present invention. The composition may be formulated with pharmaceutically acceptable additives as in the case with the composition of the present invention containing the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention. A concentration of the expression vector contained in the composition may vary depending upon type of the carrier, etc., and is appropriately in a range of 0.1 nM to 100 μ M, preferably in a range of 1 nM to 10 μ M, and more preferably in a range of 10 nM to 1 μ M. A weight ratio of the expression vector contained in the composition and

the carrier (carrier/expression vector) may vary depending on property of the expression vector, type of the carrier, etc., and is appropriately in a range of 0.1 to 100, preferably in a range of 1 to 50, and more preferably in a range of 10 to 20. The content of the carrier contained in the composition is the same as in the case with the composition of the present invention containing the antisense oligomer of the present invention and/or the suppressor antisense oligomer of the present invention, and a method for producing the same is also the same as in the case with the composition of the present invention.

[0189]

4. pre-mRNA of interest other than human dystrophin pre-mRNA

As used herein, the case where the pre-mRNA of interest is human dystrophin pre-mRNA is specifically described, but is not limited thereto. As for pre-mRNA of interest other than human dystrophin pre-mRNA, methods for designing, producing, and using an antisense oligomer and a suppressor antisense oligomer can be carried out in accordance with techniques and methods disclosed herein or known in the art. Those skilled in the art can also design, produce and use an antisense oligomer or a suppressor antisense oligomer for pre-mRNA of interest other than human dystrophin pre-mRNA on the basis of techniques and methods disclosed herein or known in the

art. Likewise, those skilled in the art can perform multi-exon skipping of the pre-mRNA of interest using the antisense oligomer and enhance the efficiency of multi-exon skipping using the suppressor antisense oligomer on the basis of techniques and methods disclosed herein or known in the art.

[0190]

As used herein, examples of the pre-mRNA of interest include human γ -sarcoglycan (SGCG) pre-mRNA, human Notch3 pre-mRNA, and human dystrophin pre-mRNA. In a certain embodiment of the present invention, the pre-mRNA of interest may be human dystrophin pre-mRNA. In another embodiment of the present invention, the pre-mRNA of interest may be SGCG or human Notch3 pre-mRNA.

[0191]

The base sequences of target regions of the antisense oligomer for use in multi-exon skipping in the pre-mRNA of interest of human SGCG or human Notch3 are as given below.

[0192]

In the present invention, the target regions in human SGCG pre-mRNA are shown as SEQ ID NOS: 283 and 284 below.

[0193]

[Table 8]

Table 8

Target region	Base sequence of target region	SEQ ID NO
Region in vicinity of acceptor of intron 3 (region indicated by range of -400 bases to +50 bases with 3' end of intron 3 defined as basing point 0)	ATTACAAAAATATGGAAATAGAGAGTAGCAGGAAAAAGTATATGGCATT TCTATTACCTAGAACCCCACTGTTGTAGGTGTCTTTCCCTTGACATAGCT GTGACCATATAGCTCTGTAAATTGCATATTTGTCTAAATAATTCATAT TTTTTCTATCCCTATCTTGCGTTTGGAGCTCATTTTATGATTGTTATT CTTTTATCTTTCAATAAAATACTCAAAATGTTAAAAAATATGATTGAGG AATTTTAAATTGTCTTAGCCACAAATTTATAGGATTTCCAGGATCTGTA ACAATGGATAAATAATTTTATAAAAAATCCTAAATTTACACAGAATTATA AAGATATAATCAATTTTAAACAGCACCTATTTTGCAAATTTTATAAATCT CTTTCTAGGACTCATCTCTGCTTCTACAATCAACCCAGAATGTGACTGT AAATGCGCG	283
Region in vicinity of donor of intron 4 (region indicated by range of -20 bases to +400 bases with 5' end of intron 4 defined as basing point 0)	CACAGGCAGGTTAAAAAGTCGGTGAGTCCAGCTTCATCATGGTGCTTTGC ATGCATGTTGTCCATGAATAGTGCTAAATGAATGCATTGTTTTTCTTC TAAAGAAATCAAAGCTACTTATGAACAAAATATGAATTTCTAAATATC ATGCTGTGTTGACCACAGACTAGCACCACAGAGTGGGGTGGGGGTGAG GGGACAGCCTGAAGTGCTTTGATTAGAGTTATTTTGTCCAAGAAATA GAGGACAATTAAAGCTAGTACAAGAAACAAAATGTTTATTGTGGGTAC CAAAGACTCTTGCAGCAACCAAAGGGAAGGAACCAAGTAGCTGGCCTC AAAGAGCACAGAGACCAGTGGCTGAGAGCCACGAATACATTTCAGTTCCC TCCCCGTTCTTTCTTCTTTTCTCTCC	284

[0194]

In the present invention, the target regions in human Notch3 pre-mRNA are shown below.

[0195]

[Table 9]

Table 9

Target region	Base sequence of target region	SEQ ID NO
Region in vicinity of acceptor of intron 3 (region indicated by range of -400 bases to +50 bases with 3' end of intron 3 defined as basing point 0)	AAGTGATCTGCCCCGCCGAGCCTCCCAAAGTTCTGGGATTACAGGCGTG AGCCACTGCTCCCAGCAAATGTGTGTTTGCTGCTGTGTTTCCTGCGTG TTTCTTGCTGTCTTGTGTGTATCTTTGTGTCTGGGGCCATCCTGCCCT GTGCTGCCCAACCAAGCCATCTCTGCCACAGGTGCCCGCTGGCTGGG TGGGTGAGCGGTGTCTGAGGAGGACCCCTGTCACTCAGGCCCCGTGTC TGGCCGTGGTGTCTGCCAGAGTTTCACTGGTGGCTGGCACC GCCGATTTC TCATGCCGCTGCCCGCTGGCTTCCGAGGTGAGAGGGGAAGAGTCTGGA GGGGAGGTAGTCGGGGGTGTGGTCAGTCCCTAACTCACCCTGTCTGGT CCCTCCAGGCCCTGACTGCTCCCTGCCAGATCCCTGCCTCAGCAGCCCT GTGCCCCAC	285
Region in vicinity of donor of intron 4 (region indicated by range of -20 bases to +400 bases with 5' end of intron 4 defined as basing point 0)	CGACTGTGCCTGTCTTCTGGTGAGTGAGCCCTACTCAGGAGAGTCAGA GGGGTGGCGTGGGGACAGCAGGCCAGCCCGGCGGTGACCATCCTTGCC CCCTTCCCTGCTAGGGTTTGAGGGTCAGAATTGTGAAGTGAACGTGGAC GACTGTCCAGGACACCGATGTCTCAATGGGGGACATGCGTGGATGGCG TCAACACCTATAACTGCCAGTGCCCTCCTGAGTGGACAGGTGGGCACTG CGGCCAGAGGGAGCGGGAGGCAGGCCCTCGGGTGGACATGCGCCAGGTG GCTGGACTGCTGCATCTGTGTGCCACAGGCCAGTTCTGCACGGAGGACG TGGATGAGTGTGAGTGCAGCCCAACGCCCTGCCACAATGGGGGTACCTG CTTCAACACGCTGGGTGGCCACAGCTGC	286

[0196]

The antisense oligomer for human SGCG that induces multi-exon skipping from human SGCG pre-mRNA targets, for example, the range of -400 to +50 bases with the 3' end of intron 3 defined as a basing point, and as a specific example, is an antisense oligomer for human SGCG consisting of

(L1) any one base sequence selected from the group

consisting of SEQ ID NOs: 287 to 308, or

(L2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0197]

Herein, the base sequence (L2) is a mutant type of the base sequence (L1). Such a mutant type also includes (L2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 287 to 308, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(L2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 287 to 308, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(L2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 287 to 308, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(L2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 287 to 308, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(L2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 287 to 308, and has a length

within $\pm 11\%$ of the length of the any one base sequence selected,

(L2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(L2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(L2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(L2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(L2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(L2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(L2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(L2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(L2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(L2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(L2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 287 to 308, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0198]

Thus, a further embodiment of the antisense oligomer for human SGCG is an antisense oligomer for human SGCG consisting of any one base sequence selected from the group consisting of (L1) and (L2-1) to (L2-16), or a pharmaceutically acceptable salt thereof, or hydrate thereof. The antisense oligomer for human SGCG is included in the present invention.

[0199]

The suppressor antisense oligomer that suppresses single skipping from human SGCG pre-mRNA (suppressor antisense oligomer for human SGCG) targets, for example, a splicing silencer sequence of intron 4 or a splice site of single skipping, and as a specific example, is a suppressor antisense oligomer for human SGCG consisting of

(M1) any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, or

(M2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0200]

Herein, the base sequence (M2) is a mutant type of the base sequence (M1). Such a mutant type also includes (M2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(M2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(M2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(M2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 12\%$ of the length of the any one base sequence selected,

(M2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length

within $\pm 11\%$ of the length of the any one base sequence selected,

(M2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(M2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(M2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(M2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(M2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(M2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(M2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(M2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(M2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(M2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(M2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 331 to 335, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0201]

Thus, a further embodiment of the suppressor antisense oligomer for human SGCG is a suppressor antisense oligomer for human SGCG consisting of any one base sequence selected from the group consisting of (M1) and (M2-1) to (M2-16), or a pharmaceutically acceptable salt thereof, or hydrate thereof. The suppressor antisense oligomer for human SGCG is included in the present invention. The suppressor antisense oligomer for human SGCG is included in the present invention. The suppressor antisense oligomer for human SGCG is capable of enhancing the efficiency of multi-skipping from SGCG pre-mRNA.

[0202]

The antisense oligomer for human Notch3 that induces multi-exon skipping from human Notch3 pre-mRNA targets, for example, a region in the vicinity of an acceptor of intron 3, i.e., the range of -400 to +50 bases with the 3' end of intron 3 defined as a basing point, and is, for example, an antisense oligomer for human Notch3 consisting of

(N1) any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, or

(N2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 309 to 330, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0203]

Herein, the base sequence (N2) is a mutant type of the base sequence (N1). Such a mutant type also includes (N2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 309 to 330, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(N2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 309 to 330, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(N2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 309 to 330, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(N2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 309 to 330, and has a length

within $\pm 12\%$ of the length of the any one base sequence selected,

(N2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(N2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(N2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(N2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(N2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(N2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(N2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(N2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(N2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(N2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(N2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(N2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 309 to 330, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0204]

Thus, a further embodiment of the antisense oligomer for human Notch3 is an antisense oligomer for human Notch3 consisting of any one base sequence selected from the group consisting of (N1) and (N2-1) to (N2-16), or a pharmaceutically acceptable salt thereof, or hydrate thereof. The antisense oligomer for human Notch3 is included in the present invention.

[0205]

The suppressor antisense oligomer that suppresses single skipping from human Notch3 pre-mRNA (suppressor antisense oligomer for human Notch3) targets, for example, a splicing silencer sequence of intron 4 or a splice site of single skipping, and as a specific example, is a suppressor antisense oligomer for human Notch3 consisting of

(O1) any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, or

(O2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 336 to 340, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0206]

Herein, the base sequence (O2) is a mutant type of the base sequence (O1). Such a mutant type also includes (O2-1) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 336 to 340, and has a length within $\pm 15\%$ of the length of the any one base sequence selected,

(O2-2) a base sequence that has at least 86% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 336 to 340, and has a length within $\pm 14\%$ of the length of the any one base sequence selected,

(O2-3) a base sequence that has at least 87% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 336 to 340, and has a length within $\pm 13\%$ of the length of the any one base sequence selected,

(O2-4) a base sequence that has at least 88% identity with any one base sequence selected from the group consisting of SEQ ID NOS: 336 to 340, and has a length

within $\pm 12\%$ of the length of the any one base sequence selected,

(O2-5) a base sequence that has at least 89% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 11\%$ of the length of the any one base sequence selected,

(O2-6) a base sequence that has at least 90% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 10\%$ of the length of the any one base sequence selected,

(O2-7) a base sequence that has at least 91% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 9\%$ of the length of the any one base sequence selected,

(O2-8) a base sequence that has at least 92% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 8\%$ of the length of the any one base sequence selected,

(O2-9) a base sequence that has at least 93% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 7\%$ of the length of the any one base sequence selected,

(O2-10) a base sequence that has at least 94% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 6\%$ of the length of the any one base sequence selected,

(O2-11) a base sequence that has at least 95% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 5\%$ of the length of the any one base sequence selected,

(O2-12) a base sequence that has at least 96% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 4\%$ of the length of the any one base sequence selected,

(O2-13) a base sequence that has at least 97% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 3\%$ of the length of the any one base sequence selected,

(O2-14) a base sequence that has at least 98% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 2\%$ of the length of the any one base sequence selected,

(O2-15) a base sequence that has at least 99% identity with any one base sequence selected from the group

consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 1\%$ of the length of the any one base sequence selected, and

(O2-16) a base sequence that has at least 99.5% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 336 to 340, and has a length within $\pm 0.5\%$ of the length of the any one base sequence selected.

[0207]

Thus, a further embodiment of the suppressor antisense oligomer for human Notch3 is a suppressor antisense oligomer for human Notch3 consisting of any one base sequence selected from the group consisting of (O1) and (O2-1) to (O2-16), or a pharmaceutically acceptable salt thereof, or hydrate thereof. The suppressor antisense oligomer for human Notch3 is included in the present invention. The suppressor antisense oligomer for human Notch3 is capable of enhancing the efficiency of multi-skipping from Notch3 pre-mRNA.

[0208]

The base sequences of SEQ ID NOs: 287 to 340 will be shown below.

SEQ ID NO	Target sequence	Base sequence
287	SCGCex4_(-400)-(-371)	TGCTACTCTCTATTTCCATATTTTGTAAT
288	SCGCex4_(-380)-(-351)	AAATGCCATATACTTTTCTGCTACTCTC
289	SCGCex4_(-360)-(-331)	AGTGGGGTTCTAGGTAATAGAAATGCCATA
290	SCGCex4_(-340)-(-311)	CAAGGAAAGACACCTACAACAGTGGGGTTC
291	SCGCex4_(-320)-(-291)	CTATATGGTCACAGCTATGTCAAGGAAAGA
292	SCGCex4_(-300)-(-271)	AAATATGCAATTTAACAGAGCTATATGGTC
293	SCGCex4_(-280)-(-251)	AAAATATGAATTATTTAGACAAATATGCAA
294	SCGCex4_(-260)-(-231)	ACGCAAGATAGGGATAGAAAAAATATGAA
295	SCGCex4_(-240)-(-211)	AATCATAAAATGAGCTCCAAACGCAAGATA
296	SCGCex4_(-220)-(-191)	TTGAAAGATAAAAGAATAACAATCATAAAA
297	SCGCex4_(-200)-(-171)	TTTAACATTTTGAGTATTTATTGAAAGATA
298	SCGCex4_(-180)-(-151)	AAATTCCTGAATCATATTTTAAACATTT
299	SCGCex4_(-160)-(-131)	TTTGTGGCTAAGACAATTAAAAATTCCTGA
300	SCGCex4_(-140)-(-111)	GATCCTGGAATCCTATAAATTTGTGGCTA
301	SCGCex4_(-120)-(-91)	ATTATTTATCCATTGTTACAGATCCTGGAA
302	SCGCex4_(-100)-(-71)	AAATTAGGATTTTATAAAATTTATTATC
303	SCGCex4_(-80)-(-51)	ATATCTTTATAATTCTGTGTAAATTAGGA
304	SCGCex4_(-60)-(-31)	AGGTGCTGTTTAAAAATGATTATATCTTTAT
305	SCGCex4_(-40)-(-11)	ATTTATAAAATTTGCAAAATAGGTGCTGTT
306	SCGCex4_(-20)-10	GAGATGAGTCCTAGAAAGAGATTTATAAAA
307	SCGCex4_1-30	CTGGGTTGATTGTAGAAGCAGAGATGAGTC
308	SCGCex4_21-50	CGCGCATTTACAGTCACATTCTGGGTTGAT
309	Notch3ex4_(-400)-(-371)	CTTTGGGAGGCTGCGGCGGGCAGATCACTT
310	Notch3ex4_(-380)-(-351)	TCACGCCTGTAATCCCAGAACTTTGGGAGG
311	Notch3ex4_(-360)-(-331)	CATTTGCTGGGAGCAGTGGCTCACGCCTGT
312	Notch3ex4_(-340)-(-311)	GAAACAGAGCAGCAACACACATTTGCTGG
313	Notch3ex4_(-320)-(-291)	ACAGGCAAGAAACACGCAGGGAACAGAGC
314	Notch3ex4_(-300)-(-271)	GACACAAAGATACACACAAGACAGGCAAGA
315	Notch3ex4_(-280)-(-251)	CACAGGGCAGGATGGCCCCAGACACAAGA
316	Notch3ex4_(-260)-(-231)	GAGATGGCTTGGTTGGGCAGCACAGGGCAG
317	Notch3ex4_(-240)-(-211)	CAGGCGGGCACCTGTGGGCAGAGATGGCTT
318	Notch3ex4_(-220)-(-191)	ACACCGCTCACCCACCCAGCCAGGCGGGCA
319	Notch3ex4_(-200)-(-171)	TGACAGGGGTCCTCCAGCTGACACCGCTCA
320	Notch3ex4_(-180)-(-151)	GGCCAGCACAGGGGCTGAGTGACAGGGGT
321	Notch3ex4_(-160)-(-131)	TGAACTCTGGCAGACACCAGGCCAGCACA
322	Notch3ex4_(-140)-(-111)	CGGGCGGTGCCAGCCACCACTGAACTCTGG
323	Notch3ex4_(-120)-(-91)	GGGGGCACCGGCATGAGAAATCGGGCGGTGC
324	Notch3ex4_(-100)-(-71)	CCTCTCACTCGGAAGCCACGGGGGCACCG
325	Notch3ex4_(-80)-(-51)	CCTCCCTCCAGACTCTTCCCTCTCACCT
326	Notch3ex4_(-60)-(-31)	ACTGACCACACCCCGACTACCTCCCTCC
327	Notch3ex4_(-40)-(-11)	CAGGACAGGGTGAGTTTAGGACTGACCACA
328	Notch3ex4_(-20)-10	CAGTCAGGGCCTGGAGGGACAGGACAGGG
329	Notch3ex4_1-30	GGCAGGGATCTGGCAGGGAGCAGTCAGGGC
330	Notch3ex4_21-50	GTGGGCACAAGGGCTGCTGAGGCAGGGATC
331	SCGCex4_216-246	TGTGGTGCTAGTCTGTGGTCAACACAGCAT
332	SCGCex4_226-256	CACCCCACTCTGTGGTGTAGTCTGTGGTC
333	SCGCex4_236-266	CCTCACCCCCCACTCTGTGGTGCTA

334	SGCGex4_246-276	CAGGCTGTCCCTCACCCCCACCCACTC
335	SGCGex4_256-286	CAAAGCACTTCAGGCTGTCCCTCACCCC
336	Notch3ex4_397-426	AGGGAAGGGGGCAAGGATGGTCACCGCCGG
337	Notch3ex4_407-436	CAAACCCTAGCAGGGAAGGGGGCAAGGATG
338	Notch3ex4_417-446	TCTGACCCTCAAACCCTAGCAGGGAAGGGG
339	Notch3ex4_427-456	ACTTCACAATTCTGACCCTCAAACCCTAGC
340	Notch3ex4_437-466	GTCCACGTTCCTTCAACAATTCTGACCCTC

[0209]

5. Method for enhancing efficiency of multi-exon skipping

The present invention provides a method for enhancing the efficiency of skipping of two or more numerically consecutive exons, which comprises

inhibiting a splicing silencer sequence, a splice site sequence, or a branch site sequence of pre-mRNA of interest when the two or more numerically consecutive exons are skipped from the pre-mRNA of interest (hereinafter, referred to as the "enhancement method of the present invention").

[0210]

In the enhancement method of the present invention, the skipping of two or more numerically consecutive exons in the pre-mRNA of interest can be performed using an antisense oligomer that induces multi-exon skipping, for example, the antisense oligomer of the present invention or the pharmaceutically acceptable salt thereof, or hydrate thereof.

[0211]

The enhancement method of the present invention can enhance the efficiency of multi-exon skipping by

inhibiting a splicing silencer sequence, a splice site sequence, or a branch site sequence in pre-mRNA of interest, and thereby suppressing single skipping. The enhancement method of the present invention comprises inhibiting a function of the splicing silencer sequence, the splice site sequence, or the branch site sequence in the pre-mRNA of interest, and more specifically, comprises suppressing single exon skipping by targeting these sequences using a suppressor antisense oligomer.

[0212]

In the enhancement method of the present invention, the inhibition of the splicing silencer sequence, the splice site, or the branch site sequence can be performed using a suppressor antisense oligomer such as the suppressor antisense oligomer of the present invention, or a pharmaceutically acceptable salt thereof, or hydrate thereof.

[0213]

In the enhancement method of the present invention, an oligomer comprising a base sequence complementary to the splicing silencer sequence, the branch site sequence, or the splice site sequence in the pre-mRNA of interest can be used as the suppressor antisense oligomer in the inhibition of the splicing silencer sequence, the branch site sequence, or the splice site sequence.

[0214]

The splicing silencer sequence, the branch site sequence, and the splice site sequence are as mentioned above. In a certain embodiment of the enhancement method of the present invention, the splicing silencer sequence may be a recognition sequence of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1). Examples of the recognition sequence of human hnRNP A1 are shown in SEQ ID NOs: 370 to 379.

[0215]

The term "using" or "used" in relation to the antisense oligomer and the suppressor antisense oligomer in the enhancement method of the present invention means that cells expressing the pre-mRNA of interest are allowed to incorporate the antisense oligomer and the suppressor antisense oligomer so that exon skipping is caused in the pre-mRNA of interest. Examples of the method for allowing the cells to incorporate the antisense oligomer and the suppressor antisense oligomer include introduction methods using cationic carriers such as cationic liposomes, cationic polymers, etc., or carriers using viral envelope. The cationic liposomes are, for example, liposomes composed of 2-O-(2-diethylaminoethyl)carbamoyl-1,3-O-dioleoylglycerol and phospholipids as the essential constituents (hereinafter referred to as "liposome A"), Oligofectamine (registered trademark) (manufactured by Invitrogen Corp.), Lipofectin (registered trademark) (manufactured by Invitrogen

Corp.), Lipofectamine (registered trademark) (manufactured by Invitrogen Corp.), Lipofectamine 2000 (registered trademark) (manufactured by Invitrogen Corp.), DMRIE-C (registered trademark) (manufactured by Invitrogen Corp.), GeneSilencer (registered trademark) (manufactured by Gene Therapy Systems), TransMessenger (registered trademark) (manufactured by QIAGEN, Inc.), TransIT TKO (registered trademark) (manufactured by Mirus) and Nucleofector II (Lonza). Among others, liposome A is preferred. Examples of cationic polymers are JetSI (registered trademark) (manufactured by Qbiogene, Inc.) and Jet-PEI (registered trademark) (polyethylenimine, manufactured by Qbiogene, Inc.). An example of carriers using viral envelop is GenomeOne (registered trademark) (HVJ-E liposome, manufactured by Ishihara Sangyo). Alternatively, the medical devices described in Japanese Patent Nos. 2924179 and the cationic carriers described in Japanese Domestic Re-Publication PCT Nos. 2006/129594 and 2008/096690 may be used as well.

[0216]

For the method for allowing cells expressing pre-mRNA of interest in the body of a patient to incorporate the antisense oligomer and the suppressor antisense oligomer, the administration method described in the section "3. Medical application" can be referred.

[0217]

In the enhancement method of the present invention, the inhibition of the splicing silencer sequence, the splice site sequence, or the branch site sequence in the pre-mRNA of interest enhances the efficiency of multi-exon skipping by 2% or higher, 4% or higher, 6% or higher, 8% or higher, 10% or higher, 12% or higher, 14% or higher, 16% or higher, 18% or higher, 20% or higher, 22% or higher, 24% or higher, 26% or higher, 28% or higher, 30% or higher, 32% or higher, 34% or higher, 36% or higher, 38% or higher, 40% or higher, 42% or higher, 44% or higher, 46% or higher, 48% or higher, 50% or higher, 52% or higher, 54% or higher, 56% or higher, 58% or higher, 60% or higher, 62% or higher, 64% or higher, 66% or higher, 68% or higher, 70% or higher, 72% or higher, 74% or higher, 76% or higher, 78% or higher, 80% or higher, 82% or higher, 84% or higher, 86% or higher, 88% or higher, 90% or higher, 92% or higher, 94% or higher, 96% or higher, 98% or higher, or 100% or higher when the efficiency obtained without the inhibition is defined as 100.

[0218]

In the enhancement method of the present invention, examples of the pre-mRNA of interest include human γ -sarcoglycan (SGCG) pre-mRNA, human Notch3 pre-mRNA, and human dystrophin pre-mRNA. In a certain embodiment of the present invention, the pre-mRNA of interest is human dystrophin pre-mRNA. When the pre-mRNA of interest is

human dystrophin pre-mRNA, the two or more numerically consecutive exons are exons selected from the group consisting of the 45th exon to the 55th exon in the human dystrophin pre-mRNA.

Examples

[0219]

Hereinafter, the present invention will be described in more detail with reference to Examples and Test Examples below, but is not deemed to be limited thereto.

[0220]

[Example 1: Production of antisense oligomer]

Production of 4-{[(2S,6R)-6-(4-benzamido-2-oxopyrimidin-1-yl)-4-tritylmorpholin-2-yl]methoxy}-4-oxobutanoic acid supported on amino polystyrene resin (Compound 1)

Step 1: Production of 4-{[(2S,6R)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl]methoxy}-4-oxobutanoic acid

Under argon atmosphere, 3.44 g of N-{1-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}benzamide and 1.1 g of 4-dimethylaminopyridine (4-DMAP) were suspended in 50 mL of dichloromethane, and 0.90 g of succinic anhydride was added to the suspension, followed by stirring at room temperature for 3 hours. To the reaction mixture, 10 mL of methanol was added, and the mixture was concentrated under reduced pressure. The residue was extracted using

ethyl acetate and 0.5 M aqueous potassium dihydrogenphosphate solution. The resulting organic layer was washed sequentially with 0.5 M aqueous potassium dihydrogenphosphate solution, water and brine in the order mentioned. The resulting organic layer was dried over sodium sulfate and concentrated under reduced pressure to give 4.0 g of the product.

Step 2; Production of 4-{[(2S,6R)-6-(4-benzamido-2-oxopyrimidin-1-yl)-4-tritylmorpholin-2-yl]methoxy}-4-oxobutanoic acid supported on amino polystyrene resin

After 4.0 g of 4-{[(2S,6R)-6-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-tritylmorpholin-2-yl]methoxy}-4-oxobutanoic acid was dissolved in 200 mL of pyridine (dehydrated), 0.73 g of 4-DMAP and 11.5 g of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride were added to the solution. Then, 25.0 g of amino polystyrene resin Primer support 200 amino (manufactured by GE Healthcare Japan Co., Ltd., 17-5214-97) and 8.5 mL of triethylamine were added to the mixture, followed by shaking at room temperature for 4 days. After completion of the reaction, the resin was taken out by filtration. The resulting resin was washed sequentially with pyridine, methanol and dichloromethane in the order mentioned, and dried under reduced pressure. To the resulting resin, 200 mL of tetrahydrofuran (dehydrate), 15 mL of acetic anhydride and 15 mL of 2,6-lutidine were added, and the mixture was shaken at room temperature for

2 hours. The resin was taken out by filtration, washed sequentially with pyridine, methanol and dichloromethane in the order mentioned and dried under reduced pressure to give 26.7 g of the product of interest.

The loading amount of the product was determined from the molar amount of the trityl per g resin by measuring UV absorbance at 409 nm using a known method. The loading amount of the resin was 129.2 $\mu\text{mol/g}$.

[0221]

Conditions of UV measurement

Apparatus: U-2910 (Hitachi, Ltd.)

Solvent: methanesulfonic acid

Wavelength: 409 nm

ϵ value: 45000

[0222]

Production of 4-{[(2S,6R)-6-(5-methyl-2,4--
dioxypyrimidin-1-yl)-4-tritylmorpholin-2-yl]methoxy}-4-
oxobutanoic acid supported on amino polystyrene resin
(Compound 2)

The title compound was produced in the same manner as in Compound 1 except that 1-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]-5-methylpyrimidine-2,4(1H,3H)-dione was used in this step instead of N-{1-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}benzamide used in Step 1 in the production of Compound 1.

The loading amount of the product was determined from the molar amount of the trityl per g resin by measuring UV absorbance at 409 nm using a known method. The loading amount of the resin was 164.0 $\mu\text{mol/g}$.

[0223]

Production of 4-{\[(2S,6R)-6-(6-benzamidopurin-9-yl)-4-tritylmorpholin-2-yl]methoxy}-4-oxobutanoic acid supported on amino polystyrene resin (Compound 3)

The title compound was produced in the same manner as in Compound 1 except that N-{9-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]purin-6-yl}benzamide was used in this step instead of N-{1-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}benzamide used in Step 1 in the production of Compound 1.

The loading amount of the product was determined from the molar amount of the trityl per g resin by measuring UV absorbance at 409 nm using a known method. The loading amount of the resin was 185.7 $\mu\text{mol/g}$.

[0224]

Production of 4-{\{(2S,6R)-6-\{6-(2-cyanoethoxy)-2-[(2-phenoxyacetyl)amino]purin-9-yl}\}-4-tritylmorpholin-2-yl}methoxy}-4-oxobutanoic acid supported on amino polystyrene resin (Compound 4)

The title compound was produced in the same manner as in Compound 1 except that N-{6-(2-cyanoethoxy)-9-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]purin-

2-yl}-2-phenoxyacetamide was used in this step instead of N-{1-[(2R,6S)-6-(hydroxymethyl)-4-tritylmorpholin-2-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}benzamide used in Step 1 in the production of Compound 1.

The loading amount of the product was determined from the molar amount of the trityl per g resin by measuring UV absorbance at 409 nm using a known method. The loading amount of the resin was 164.8 $\mu\text{mol/g}$.

[0225]

Production of antisense PMO targeting region in vicinity of donor or acceptor of intron in human dystrophin gene

According to the description given below, PMOs having the base sequences of PMO Nos. 1 to 232 (SEQ ID NOs: 1 to 232) shown in Table 11 which targeted regions in the vicinity of donors or acceptors of introns 44 to 55 in human dystrophin pre-mRNA shown in Table 10 were synthesized. The 5' end of each PMO is Group (3) below. The synthesized PMO was dissolved in water for injection (manufactured by Otsuka Pharmaceutical Factory, Inc.).

[Formula 14]



(3)

[0226]

The target base sequence of the antisense oligomer of the present invention or the suppressor antisense

oligomer of the present invention was described as "Ha₁_b₁-c₁", "Ha₂_b₂-c₂_Ha₃_b₃-c₃", or "Ha₄_b₄-c₄_Ha₅_b₅-c₅_Ha₆_b₆-c₆".

"Ha₁_b₁-c₁" is the target base sequence of the antisense oligomer of the first embodiment used herein. "Ha₁" represents the ath exon of the human dystrophin gene, "b₁" represents the 5'-terminal base of the target base sequence, and "c₁" represents the 3'-terminal base of the target base sequence. "Ha₁_b₁-c₁" may have one additional base at its 3' end, and such a target base sequence is represented by "Ha₁_b₁-c₁_N" ("N" represents a given base).

When "b₁" and "c₁" are positive integers, "b₁" and "c₁" each represent a base number in the downstream direction when the 5'-terminal base of the ath exon is counted as the 1st base. On the other hand, when "b₁" and "c₁" are negative numbers, "b₁" and "c₁" each represent a base number in the upstream direction when the 3'-terminal base of the (a - 1)th intron is counted as the 1st base.

For example, "H45_(-10)-15" means a base sequence in which the 5' end of the target base sequence is the 10th base in the upstream direction from the 3' end of the 44th intron and the 3' end of the target base sequence is the 15th base in the downstream direction from the 5' end of the 45th exon.

"Ha₂_b₂-c₂_Ha₃_b₃-c₃" is the target base sequence of the antisense oligomer of the second embodiment used herein. "Ha₂_b₂-c₂" which is the first part of "Ha₂_b₂-c₂_Ha₃_b₃-c₃" means the target base sequence of a 3' unit oligomer constituting the antisense oligomer, and the second part "Ha₃_b₃-c₃" means the target base sequence of a 5' unit oligomer constituting the antisense oligomer. "Ha₃_b₃-c₃" may have one additional base at its 3' end, and such a target base sequence is represented by "Ha₂_b₂-c₂_Ha₃_b₃-c₃_N" ("N" represents a given base). Each of "Ha₂_b₂-c₂" and "Ha₃_b₃-c₃" abides by the same notation as that of "Ha₁_b₁-c₁".

When "Ha₂" and "Ha₃" are the same, the "_Ha₃" part may be omitted.

For example, "H45_(-5)-5_25-35" or "H45_(-5)-5_H45_25-35" means a base sequence in which the target base sequence of the 3' unit oligomer constituting the antisense oligomer is "H45_(-5)-5" and the target base sequence of the 5' unit oligomer constituting the antisense oligomer is "H45_25-35".

"Ha₄_b₄-c₄_Ha₅_b₅-c₅_Ha₆_b₆-c₆" is the target base sequence of the antisense oligomer of the third embodiment used herein. "Ha₄_b₄-c₄" which is the first part of "Ha₄_b₄-c₄_Ha₅_b₅-c₅_Ha₆_b₆-c₆" means the target base sequence of a 3' unit oligomer constituting the antisense oligomer, the second part "Ha₅_b₅-c₅" means the target base sequence of an intermediate unit oligomer

constituting the antisense oligomer, and the third part "Ha₆_b₆-c₆" means the target base sequence of a 5' unit oligomer constituting the antisense oligomer. "Ha₆_b₆-c₆" may have one additional base at its 3' end, and such a target base sequence is represented by "Ha₄_b₄-c₄_Ha₅_b₅-c₅_Ha₆_b₆-c₆_N" ("N" represents a given base). Each of "Ha₄_b₄-c₄", "Ha₅_b₅-c₅", and "Ha₆_b₆-c₆" abides by the same notation as that of "Ha₁_b₁-c₁".

When "Ha₄" is the same with "Ha₅" and "Ha₆", the "_Ha₅" and "_Ha₆" parts may be omitted.

For example, "H45_(-5)-5_25-35_60-70" or "H45_(-5)-5_H45_25-35_H45_60-70" means a base sequence in which the target base sequence of the 3' unit oligomer constituting the antisense oligomer is "H45_(-5)-5", the target base sequence of the intermediate unit oligomer is "H45_25-35", and the target base sequence of the 5' unit oligomer is "H45_60-70".

[0227]

[Table 10]

Table 10 Target region in human dystrophin pre-mRNA

Target region	Range of target region	SEQ ID NO
(R1) Region in vicinity of donor of intron 44	Region indicated by range of -20 bases to +400 bases with 5' end of intron 44 defined as basing point 0	233
(R2) Region in vicinity of acceptor of intron 44	Region indicated by range of -600 bases to +50 bases with 3' end of intron 44 defined as basing point 0	234

(R3) Region in vicinity of donor of intron 45	Region indicated by range of -20 bases to +400 bases with 5' end of intron 45 defined as basing point 0	235
(R4) Region in vicinity of acceptor of intron 45	Region indicated by range of -400 bases to +50 bases with 3' end of intron 45 defined as basing point 0	236
(R5) Region in vicinity of donor of intron 46	Region indicated by range of -20 bases to +400 bases with 5' end of intron 46 defined as basing point 0	237
(R6) Region in vicinity of acceptor of intron 46	Region indicated by range of -400 bases to +50 bases with 3' end of intron 46 defined as basing point 0	238
(R7) Region in vicinity of donor of intron 47	Region indicated by range of -20 bases to +400 bases with 5' end of intron 47 defined as basing point 0	239
(R8) Region in vicinity of acceptor of intron 47	Region indicated by range of -400 bases to +50 bases with 3' end of intron 47 defined as basing point 0	240
(R9) Region in vicinity of donor of intron 48	Region indicated by range of -20 bases to +400 bases with 5' end of intron 48 defined as basing point 0	241
(R10) Region in vicinity of acceptor of intron 48	Region indicated by range of -400 bases to +50 bases with 3' end of intron 48 defined as basing point 0	242
(R11) Region in vicinity of donor of intron 49	Region indicated by range of -20 bases to +400 bases with 5' end of intron 49 defined as basing point 0	243
(R12) Region in vicinity of acceptor of intron 49	Region indicated by range of -400 bases to +50 bases with 3' end of intron 49 defined as basing point 0	244
(R13) Region in vicinity of donor of intron 50	Region indicated by range of -20 bases to +400 bases with 5' end of intron 50 defined as basing point 0	245

(R14) Region in vicinity of acceptor of intron 50	Region indicated by range of -400 bases to +50 bases with 3' end of intron 50 defined as basing point 0	246
(R15) Region in vicinity of donor of intron 51	Region indicated by range of -20 bases to +400 bases with 5' end of intron 51 defined as basing point 0	247
(R16) Region in vicinity of acceptor of intron 51	Region indicated by range of -400 bases to +50 bases with 3' end of intron 51 defined as basing point 0	248
(R17) Region in vicinity of donor of intron 52	Region indicated by range of -20 bases to +400 bases with 5' end of intron 52 defined as basing point 0	249
(R18) Region in vicinity of acceptor of intron 52	Region indicated by range of -400 bases to +50 bases with 3' end of intron 52 defined as basing point 0	250
(R19) Region in vicinity of donor of intron 53	Region indicated by range of -20 bases to +400 bases with 5' end of intron 53 defined as basing point 0	251
(R20) Region in vicinity of acceptor of intron 53	Region indicated by range of -400 bases to +50 bases with 3' end of intron 53 defined as basing point 0	252
(R21) Region in vicinity of donor of intron 54	Region indicated by range of -20 bases to +400 bases with 5' end of intron 54 defined as basing point 0	253
(R22) Region in vicinity of acceptor of intron 54	Region indicated by range of -400 bases to +50 bases with 3' end of intron 54 defined as basing point 0	254
(R23) Region in vicinity of donor of intron 55	Region indicated by range of -20 bases to +400 bases with 5' end of intron 55 defined as basing point 0	255
(R24) Region in vicinity of acceptor of intron 55	Region indicated by range of -400 bases to +50 bases with 3' end of intron 55 defined as basing point 0	256

[Table 11]

Table 11

PMO No.	Target region	Target base sequence	Base sequence of PMO	SEQ ID NO
1	R1	H44_150-175	TTTCGAAAAACAAATCAAAGACTTA	1
2		H44_157-196	ATGTGCTGAAGATAAATACAATTTGAAAAA AACAAATCAA	2
3		H44_159-193	TGCTGAAGATAAATACAATTCGAAAAAAC AAATC	3
4		H44_170-199	CAGATGTGCTGAAGATAAATACAATTTCGA	4
5		H44_179-206	AAGAGTCCAGATGTGCTGAAGATAAATA	5
6		H44_209-236	ACCCCTTCAGAACCCTGATCTTTAAGAAGT	6
7		H44_239-266	TGACAACAACAGTCAAAAGTAATTTCCA	7
8		H44_269-296	ATGATAATTTTCTTTCTAGTAATATAAT	8
9		H44_299-326	TCCATAGCACCCTGCTCTAATATTATCA	9
10		H44_330-355	GGCAAACTCTCTCATCCTGACACAAA	10
11		H44_354-381	TTTATCAGATAAACCAGCTCCGTCCAGG	11
12		H44_389-416	CTTCCCTCTGTCTACAGATTCAATTATAT	12
13		H44_413-438	AAAACACCTTGCTGTTACGATGCTTC	13
14		H44_423-452	TGCCCCAAAGCCACAAAACACCTTGCTGTT	14
15		H44_455-480	GGTCCAACATAAAGCCGAAATACAC	15
16		H44_479-506	TATGCCACAAGTTCTCCTTCTGGAAGG	16
17		H44_509-536	GATATTTCTAGCAACTTCATTTTAGCTA	17
18		H44_150-164_179-193	TGCTGAAGATAAATACAAATCAAAGACTTA	18
19		H44_150-164_192-206	AAGAGTCCAGATGTGCAAAATCAAAGACTTA	19
20		H44_161-175_179-193	TGCTGAAGATAAATATTTGAAAAAACAAA	20
21		H44_161-175_192-206	AAGAGTCCAGATGTGTTTCGAAAAAACAAA	21
22	R2	H45_(-598)-(-571)	TTAATTGCTTTTCAGGAGCATCCCATCAA	22
23		H45_(-568)-(-541)	TTTGATTAGAAGCCACAAAAAACTGAG	23
24		H45_(-538)-(-511)	TCATTTCAAATTCGTCTGCGTCAATGT	24
25		H45_(-518)-(-491)	TTGCTATATTAGAAGAAAATTCATTTCA	25
26		H45_(-515)-(-488)	TAATTGCTATATTAGAAGAAAATTCATT	26
27		H45_(-508)-(-479)	AAATAAAATTAATTGCTATATTAGAAGAAA	27
28		H45_(-478)-(-449)	CAGTATTAATAAAAAAACTCTAGAGATATTT	28
29		H45_(-470)-(-443)	TAGTCACAGTATTAATAAAAAAACTCTAG	29
30		H45_(-448)-(-421)	GTGAAAAAGAACAAACATAGGTTAGTCA	30
31		H45_(-439)-(-412)	ATACGAGAGGTGAAAAAGAACAAACATA	31
32		H45_(-436)-(-409)	TGGATACGAGAGGTGAAAAAGAACAAAC	32
33		H45_(-422)-(-395)	TTTCTTAGTGATCGTGGATACGAGAGGT	33
34		H45_(-418)-(-394)	GTTTCTTAGTGATCGTGGATACGAG	34
35		H45_(-418)-(-391)	TGGGTTTCTTAGTGATCGTGGATACGAG	35
36		H45_(-415)-(-391)	TGGGTTTCTTAGTGATCGTGGATAC	36
37		H45_(-413)-(-386)	GTATTTGGGTTTCTTAGTGATCGTGGAT	37
38		H45_(-405)-(-378)	TGAACAAAGTATTTGGGTTTCTTAGTGA	38
39		H45_(-389)-(-362)	TTGTAATAATTTAAACATGAACAAAGTAT	39
40		H45_(-349)-(-322)	TCCCCACAAGGATGTTCCATGTTTAATA	40
41		H45_(-320)-(-293)	ACCTTTTCAAGAGCAAATTCGATTTCTT	41
42		H45_(-305)-(-280)	CAATTAGTTGGAACCTTTTCAAGAG	42
43		H45_(-290)-(-263)	TATAATGTCTACAAATCAATTAGTTGG	43
44		H45_(-275)-(-250)	AGCTAGAGGATGTTATAATGTCTTAC	44

45	H45_(-260)-(-233)	ATTTTGTAGCTTGTGCTAGAGGAT	45
46	H45_(-230)-(-203)	AAAGCACCTCTCGGTTAGCTCCAGTTT	46
47	H45_(-200)-(-173)	CAGAAAGACACCTTTTATGTGTGAGGA	47
48	H45_(-190)-(-163)	GGATACAAGACAGAAAGACACCTTTTAT	48
49	H45_(-185)-(-160)	AAAGGATACAAGACAGAAAGACACCT	49
50	H45_(-171)-(-144)	TGACATGCCCATATCCAAAGGATACAAG	50
51	H45_(-168)-(-141)	AACTGACATGCCCATATCCAAAGGATAC	51
52	H45_(-142)-(-115)	AGCTCCATGTGAAAATTTCCCTATGAAA	52
53	H45_(-110)-(-83)	TGCAGTTGTACTGCCAAAGAAAGAAATA	53
54	H45_(-80)-(-53)	TGAGAAAAGATTAAACAGTGTGCTACCA	54
55	H45_(-78)-(-51)	TTTGAGAAAAGATTAAACAGTGTGCTAC	55
56	H45_(-70)-(-43)	TCTTTTATTTGAGAAAAGATTAAACAG	56
57	H45_(-60)-(-33)	AAGCCCCATGTCTTTTATTTGAGAAAA	57
58	H45_(-50)-(-23)	AACAAAAATGAAGCCCCATGTCTTTTTA	58
59	H45_(-44)-(-17)	AGCAGAAAACAAAAATGAAGCCCCATGTC	59
60	H45_(-25)-3	TTCTGTAAAGATACCAAAAAGGCAAAAC	60
61	H45_(-23)-5	AGTTCCTGTAAAGATACCAAAAAGGCAA	61
62	H45_(-22)-3	TTCTGTAAAGATACCAAAAAGGCAA	62
63	H45_(-21)-7	GGAGTTCCTGTAAAGATACCAAAAAGGCA	63
64	H45_(-20)-5	AGTTCCTGTAAAGATACCAAAAAGGC	64
65	H45_(-20)-8	TGGAGTTCCTGTAAAGATACCAAAAAGGC	65
66	H45_(-18)-10	CCTGGAGTTCCTGTAAAGATACCAAAAAG	66
67	H45_(-17)-8	TGGAGTTCCTGTAAAGATACCAAAA	67
68	H45_(-16)-12	ATCCTGGAGTTCCTGTAAAGATACCAAAA	68
69	H45_(-15)-10	CCTGGAGTTCCTGTAAAGATACCAAAA	69
70	H45_(-14)-14	CCATCCTGGAGTTCCTGTAAAGATACCAA	70
71	H45_(-10)-16	TGCCATCCTGGAGTTCCTGTAAAGATA	71
72	H45_(-5)-20	CCAATCCATCCTGGAGTTCCTGTAA	72
73	H45_1-25	GCTGCCCAATGCCATCCTGGAGTTC	73
74	H45_6-30	TTGCCGCTGCCCAATGCCATCCTGG	74
75	H45_11-35	ACAGTTTGCCGCTGCCCAATGCCAT	75
76	H45_11-40	TGACAACAGTTTGCCGCTGCCCAATGCCAT	76
77	H45_16-35	ACAGTTTGCCGCTGCCCAAT	77
78	H45_16-40	TGACAACAGTTTGCCGCTGCCCAAT	78
79	H45_21-45	TGTTCTGACAACAGTTTGCCGCTGC	79
80	H45_(-423)-(-412)_16-33	AGTTTGCCGCTGCCCAATATACGAGAGGTG	80
81	H45_(-416)-(-402)_16-30	TTGCCGCTGCCCAATGTGATCGTGGATACG	81
82	H45_(-411)-(-400)_16-33	AGTTTGCCGCTGCCCAATTAGTGATCGTGG	82
83	H45_(-405)-(-391)_16-30	TTGCCGCTGCCCAATTGGGTTTCTTAGTGA	83
84	H45_(-402)-(-391)_16-33	AGTTTGCCGCTGCCCAATTGGGTTTCTTAG	84
85	H45_(-156)-(-143)-(-141)-(-128)	AATTTCCCTATGAACTGACATGCCCATATA	85
86	H45_(-78)-(-65)_(-44)-(-31)	TGAAGCCCCATGTCAAACAGTGTGCTAC	86
87	H45_(-78)-(-65)_(-34)-(-21)	AAAACAAAAATGAAGAACAGTGTGCTAC	87

88		H45_(-78)-(-65)_16-29	TGCCGCTGCCCAATAAACAGTGTGCTAC	88
89		H45_(-78)-(-65)_21-34	CAGTTTGCCGCTGCAAAACAGTGTGCTAC	89
90		H45_(-78)-(-65)_27-40	TGACAACAGTTTGCAAAACAGTGTGCTAC	90
91		H45_(-74)-(-62)-(-40)-(-28)	AAATGAAGCCCCAATTAAACAGTGTG	91
92		H45_(-72)-(-58)-(-38)-(-26)	AAAAATGAAGCCCCAAAGATTAAACAGTG	92
93		H45_(-71)-(-58)-(-38)-(-25)	CAAAAATGAAGCCCCAAAGATTAAACAGT	93
94		H45_(-71)-(-58)_16-29	TGCCGCTGCCCAATAAAGATTAAACAGT	94
95		H45_(-71)-(-58)_21-34	CAGTTTGCCGCTGCAAAAGATTAAACAGT	95
96		H45_(-71)-(-58)_27-40	TGACAACAGTTTGCAAAAGATTAAACAGT	96
97		H45_(-68)-(-60)-(-36)-(-28)_19-28	GCCGCTGCCC_AAATGAAGCAGATTAAAC	97
98		H45_(-64)-(-51)-(-44)-(-31)	TGAAGCCCCATGTCTTTGAGAAAAGATT	98
99		H45_(-64)-(-51)-(-35)-(-22)	AAACAAAAATGAAGTTTGAGAAAAGATT	99
100		H45_(-64)-(-51)_16-29	TGCCGCTGCCCAATTTTGAGAAAAGATT	100
101		H45_(-64)-(-51)_21-34	CAGTTTGCCGCTGCTTTTGAGAAAAGATT	101
102		H45_(-64)-(-51)_27-40	TGACAACAGTTTGCTTTTGAGAAAAGATT	102
103		H45_(-44)-(-31)_16-29	TGCCGCTGCCCAATTGAAGCCCCATGTC	103
104		H45_(-44)-(-31)_27-40	TGACAACAGTTTGCTGAAGCCCCATGTC	104
105		H45_(-44)-(-31)_21-33_T	TAGTTTGCCGCTGCTGAAGCCCCATGTC	105
106		H45_(-38)-(-25)_16-29	TGCCGCTGCCCAATCAAAAATGAAGCCC	106
107		H45_(-38)-(-25)_21-34	CAGTTTGCCGCTGCCAAAAATGAAGCCC	107
108		H45_(-38)-(-25)_27-40	TGACAACAGTTTGCCAAAAATGAAGCCC	108
109		H45_(-34)-(-21)_16-29	TGCCGCTGCCCAATAAAACAAAAATGAA	109
110		H45_(-34)-(-21)_21-34	CAGTTTGCCGCTGCAAAACAAAAATGAA	110
111		H45_(-34)-(-21)_27-40	TGACAACAGTTTGCAAAACAAAAATGAA	111
112		H45_1-21	CCCAATGCCATCCTGGAGTTC	112
144	R3	H45_167-194	TTAGATCTGTCGCCCTACCTCTTTTTTC	144
145		H45_227-254	TCTCATGAAATATCTTCTAAAGAAAGC	145
146		H45_257-284	ATGTTAGTGCCCTTCACCCTGCTTATAA	146
147		H45_287-314	GCTGTTGATTAATGGTTGATAGGTTCTT	147
148		H45_317-344	TGAAAAAAGAAATAAAAAATTTCTTTA	148
149		H45_347-374	TAAGTAGCCACAAGTATATATTTTAGTA	149
150	R4	H46_(-200)-(-173)	TCAAGAATCTCTAAATGATAAGAGATTA	150
151		H46_(-171)-(-144)	TTCACTTTGAACAAAGTAATTTCAATAT	151
152		H46_(-146)-(-119)	ACCATACATAATTTAAGAAAATTCATTC	152
153		H46_(-141)-(-114)	TGTTAACCATACATAATTTAAGAAAATTC	153
154		H46_(-136)-(-109)	AAAGATGTTAACCATACATAATTTAAGA	154
155		H46_(-126)-(-99)	AAGCAATTTAAAGATGTTAACCATACA	155
156		H46_(-116)-(-89)	ATTTAAAAATAAGCAATTTAAAGATGT	156
157		H46_(-111)-(-84)	TGCCAATTTAAAAATAAGCAATTTAAAA	157
158		H46_(-106)-(-79)	AAACATGGCAATTTAAAAATAAGCAATT	158
159		H46_(-81)-(-54)	TATTTGTTAATGCAAACTGGGACACAAA	159
160		H46_(-51)-(-24)	TTATTTTTTTTTTCCAACATAGTTCTCAA	160
161		H46_(-18)-6	TCTAGCCTGGAGAAAGAATAA	161
162		H46_(-15)-9	TCTTCTAGCCTGGAGAAAGAAGAA	162

163		H46_(-9)-15	TTTTGTTCTTCTAGCCTGGAGAAA	163
164		H46_139-166	TTGAGAAAATAAAATTACCTTGACTTGC	164
165		H46_169-196	ACTTCTTTATGCAAGCAGGCCCTGGGGG	165
166		H46_199-226	CAATGATTGAATTAATAATAGATTCAT	166
167	R5	H46_229-256	GAACATGAATAACCTAATGGGCAGAAA	167
168		H46_259-286	ATAAAGTTGTGAGAAAAACACTTTAGCA	168
169		H46_289-316	ACTGGTTCAGAACTGCAGGGTTAAGAAG	169
170		H46_319-346	ACACACATATATACATATGTTCTTATGT	170
171		H47_(-200)-(-173)	CTCACCCCTCAGTAGATAAAATCTCTGT	171
172		H47_(-171)-(-144)	TCTGAGCACAGAGCTGATTGACTGAAAC	172
173		H47_(-141)-(-114)	AGTGGTACCTCAAAATACCAACAGTTTTT	173
174	R6	H47_(-111)-(-84)	ATCAAAATGAAGCGACTTGACCGAGGGC	174
175		H47_(-81)-(-54)	TACCTTGTCTTTGCTTCTATTGATTAGT	175
176		H47_(-51)-(-24)	GTTTAAATGAATTACAGCACAATTCCA	176
177		H47_(-16)-12	TTCCACCAGTAACTGAAACAGACAAATG	177
178		H47_1-29	TGGCGCAGGGGCAACTCTTCCACCAGTAA	178
179	R7	H47_135-162	TTAATGTCTAACCTTTATCCACTGGAGA	179
180		H48_(-21)-7	TGGAACCTGAAAGGAAAATACATTTTA	180
181	R8	H48_1-28	CTTGTCTTCAGGTAAAGCTCTGGAAC	181
182	R9	H48_177-204	AAGCAAAAAGTCCCTACCTGAACGTCA	182
183		H49_(-21)-7	CAGTTTCCTGGGAAAAGAACCCATATA	183
184	R10	H49_22-47	ATCTCTTCCACATCCGGTTGTTAGC	184
185	R11	H49_93-120	TAGAGTTGCTTCATTACCTTCACTGGC	185
186		H50_(-200)-(-173)	ACCCTACAAATATTTATCAATTGCTCCA	186
187		H50_(-171)-(-144)	TAAAGGAATTATAATTATTTAGCCAAC	187
188		H50_(-141)-(-114)	TAAATTAACTTTAGTGGGTAGAATTTCT	188
189	R12	H50_(-111)-(-84)	TATTATTGGATTCTATTATATTTTACT	189
190		H50_(-81)-(-54)	TCATGAACATCTTAATCCATTGGTGAA	190
191		H50_(-51)-(-24)	TACTTATTCGATTAACACTTTGAAGATA	191
192		H50_(-21)-7	ACTTCCTCTTTAACAGAAAAGCATAAC	192
193		H50_100-127	GGGATCCAGTATACTTACAGGCTCCAAT	193
194	R13	H50_90-114	CTTACAGGCTCCAATAGTGGTCAGT	194
195	R14	H51_(-18)-10	TGAGTAGGAGCTAAAATATTTTGGGTTT	195
196	R15	H51_224-251	TATCATTTTTTCTCATACCTTCTGCTTG	196
197		H52_(-47)-(-20)	TTAGTATCAGGGTCTTTCAGCGTTGTGT	197
198	R16	H52_(-18)-10	GCATTGTTGCCGTGAAGAACAATATCC	198
199		H52_9-38	AACTGGGGACGCCCTCTGTTCCAAATCCTGC	199
200	R17	H52_109-136	GCTTGTTAAAAAACTTAETTCGATCCGT	200
201		H53_(-197)-(-170)	AATATTAGTTTCTGTAAATTATTTTCC	201
202		H53_(-167)-(-140)	ACAACAGGATTCTTTGCTTTTTTGATGG	202
203		H53_(-137)-(-110)	TTATTCATTGTGTATGGCTAGGATGAT	203
204	R18	H53_(-107)-(-80)	ATCTCACATTTATGTTGCTTATTTAAAA	204
205		H53_(-77)-(-50)	GAGACATTTTAAATGTAACCTCCAAACG	205
206		H53_(-47)-(-20)	AAATATATAGTAGTAAATGCTAGTCTGG	206
207		H53_(-18)-10	ATTCTTTCAACTAGAATAAAAGGAAAAA	207
208	R19	H53_203-230	GGTATCTTTGATACTAACCTTGGTTTCT	208
209	R20	H54_(-18)-10	TGGCCAACTGCTATAGATTTTATGAGA	209
210		H54_146-173	AAATAATGTAATTCATACCTTTTATGAA	210
211	R21	H54_176-203	CCCCATTATTACAGCCAACAGTAGTTTT	211
212		H54_206-233	CAAATCCTCATGGTCCATCCAGTTTCAC	212
213		H54_236-263	CCAAGCTCCAGTTTAGCTGGATTGGAAA	213

214		H54_266-293	TTTAGTTGGTATTTATCGTCTTGAACCC	214
215		H54_296-323	TAAATAGAACTCTGAGCCAAGTCCGTG	215
216		H54_326-353	GGGCAAATGAGATCTTATGTTCTCTGTT	216
217	R22	H55_(-197) - (-170)	TTTACTTTTCATTTTATTTAACTTAAAG	217
218		H55_(-167) - (-140)	TAAGCAACAACATAATATTGTGCAGTC	218
219		H55_(-137) - (-110)	TGGGGTGAGTTGTTGCTACAGCTCTTCC	219
220		H55_(-107) - (-80)	TGGAGGAACTAAATTGTAATATACCAAC	220
221		H55_(-77) - (-50)	CACCTAGTGAACCTCCATAAAAAGAGAAA	221
222		H55_(-47) - (-20)	CAGATGCAATTATTAATATCAGAATGG	222
223		H55_(-18) - 10	CTCACTCACCTGCAAAGGACCAATGT	223
224		H55_12-34	AGTTTCTTCCAAAGCAGCCTCTC	224
113	R23	H55_177-204	AATGECTGACTTACTTGCCATTGTTTCA	113
114		H55_191-218	AGTGCTAAAGCGGAAATGCCTGACTTAC	114
115		H55_221-248	TGCTGAGAATTGTTCAATTGCATCCACA	115
116		H55_251-278	TGTCCTTGGCTGTCTCAGTTACAAGTACA	116
117		H55_281-308	TCAGGCTGTATAAAAGCAACTATTTTGT	117
118		H55_311-338	CTCTCCTCCTTGCCAAATACCGAAATA	118
119		H55_334-361	GATGTTTCTTCTCCTCTGCCTCTCTC	119
120		H55_341-368	TATAAATGATGTTTCTTCTCCTCTGCT	120
121		H55_371-398	TTTTCTAAGACGAGGGTGTTAAGTGA	121
122		H55_401-428	TGCAAATGTTTCTCTGCTCAGAGCATGT	122
123		H55_431-458	TTTCTCCTTGACCGAAGCTCTGGTTTTA	123
124		H55_461-488	CCAATCCCTAAGTTATTTCTCTGAGCAA	124
125		H55_491-518	CAAAAATGTCAACTTTTAAATTTAATA	125
126		H55_521-548	GCTTTCCTGTAAAATATTAATAAACA	126
127		H55_548-575	TAAATAAACAGACAATTCATACAGATG	127
128	R24	H56_(-400) - (-373)	AATAATTTTCACTATATGGATCAAGT	128
129		H56_(-380) - (-355)	AAAGTGTAACCCAGTGCCAATAATTT	129
130		H56_(-345) - (-318)	TATTTTATGGAAGTGATAGGCAATAAAA	130
131		H56_(-315) - (-288)	TATATCTTCTAGTCTATGGACAAAATGT	131
132		H56_(-285) - (-258)	TGTATTTATAACTTTATAAAGTTCACAA	132
133		H56_(-255) - (-228)	TATTCCTTGCCATTATGAGTTGGAAGT	133
134		H56_(-225) - (-198)	AAATGGGCATCTTATTAGTTGTAATAGA	134
135		H56_(-195) - (-168)	ATTGCCTTCTCCTGTTATTATGTAGATT	135
136		H56_(-165) - (-138)	AGATACATCTCAAAATCCCTTTTCTTGGC	136
137		H56_(-135) - (-108)	AGACATTTCAATCAGGCTAAACTAACAA	137
138		H56_(-105) - (-78)	TTGCAAAATATAAATAATTATTAGTTCA	138
139		H56_(-76) - (-51)	ACAAGCGATGAATGTGAATTTGGAGA	139
140		H56_(-59) - (-34)	ATTACCAAAACAAAAGAAACAAGCGAT	140
141		H56_(-39) - (-14)	GGAAGAAGAATATGTGCAGAAATTACC	141
142		H56_(-31) - (-6)	GGACAGCAGGAAGAAGAATATGTGCA	142
143		H56_(-14) - 13	TCACCTTGGAGGTCCTACAGGACAGCAG	143
225	R1 + R24	H44_188-202_H56_(-156) - (-142)	ACATCTCAAATCCCTGTCCAGATGTGCTGA	225
226		H44_188-202_H56_(-124) - (-110)	ACATTTCAATCAGGCGTCCAGATGTGCTGA	226
227		H44_188-202_H56_(-85) - (-71)	TGGAGAATTGCAAAAGTCCAGATGTGCTGA	227
228		H56_(-156) - (-142)_H44_188-202	GTCCAGATGTGCTGAACATCTCAAAATCCCT	228

229		H56_(-124)-(-110)_H44_188-202	GTCCAGATGTGCTGAACATTTC AATCAGGC	229
230		H56_(-85)-(-71)_H44_188-202	GTCCAGATGTGCTGATGGAGAATTGCAAAA	230
231	R2 +	H45_16-35_H46_(-134)-(-115)	GTTAACCATACATAATTTAAACAGTTTGCC GCTGCCCAAT	231
232	R4	H45_16-35_H46_(-103)-(-84)	TGGCAATTTAAAAATAAGCAACAGTTTGCC GCTGCCCAAT	232

[0229]

In the PMO of Table 11,

PMO for the target region R2 includes PMO Nos. 22 to 71, 91 to 93, 98 and 99 in another embodiment, and PMO Nos. 22 to 59, 91 to 93, 98 and 99 in still another embodiment;

PMO for the target region R4 includes PMO Nos. 150 to 163 in another embodiment, and PMO Nos. 150 to 160 in still another embodiment;

PMO for the target region R6 includes PMO Nos. 171 to 177 in another embodiment, and PMO Nos. 171 to 176 in still another embodiment;

PMO for the target region R8 includes PMO No. 180 in another embodiment; PMO for the target region R10 includes PMO No. 183 in another embodiment; PMO for the target region R12 includes PMO Nos. 186 to 191 in another embodiment

PMO for the target region R16 includes PMO Nos. 197 and 198 in another embodiment, and PMO No. 197 in still another embodiment;

PMO for the target region R18 includes PMO Nos. 201 to 206 in another embodiment;

PMO for the target region R22 includes PMO Nos. 217 to 223 in another embodiment, and PMO Nos. 217 to 222 in still another embodiment; and

PMO for the target region R24 includes PMO Nos. 128 to 142 in another embodiment.

[0230]

0.2 g of 4- $\{[(2S,6R)-6-(4\text{-benzamide-2-oxopyrimidin-1-yl})-4\text{-tritylmorpholin-2-yl}]methoxy\}$ -4-oxobutanoic acid supported on an aminopolystyrene resin (Compound 1), 4- $\{[(2S,6R)-6-(5\text{-methyl-2,4--dioxopyrimidin-1-yl})-4\text{-tritylmorpholin-2-yl}]methoxy\}$ -4-oxobutanoic acid supported on amino polystyrene resin (Compound 2), 4- $\{[(2S,6R)-6-(6\text{-benzamidopurin-9-yl})-4\text{-tritylmorpholin-2-yl}]methoxy\}$ -4-oxobutanoic acid supported on amino polystyrene resin (Compound 3), or 4- $\{(2S,6R)-6-\{6-(2\text{-cyanoethoxy})-2-[(2\text{-phenoxyacetyl})amino]purin-9-yl\}-4\text{-tritylmorpholin-2-yl}\}methoxy\}$ -4-oxobutanoic acid supported on amino polystyrene resin (Compound 4) appropriate for the 5'-terminal base was filled in a column with a filter tip. Then, the synthetic cycle shown in Table 12 was started using a nucleic acid synthesizing machine (AKTA Oligopilot 10 plus). The desired morpholino monomer compound was added in each coupling cycle to give the base sequence of each PMO described in Table 11 (see Table 12).

[0231]

[Table 12]

Table 12

Step	Reagent	Volume (mL)	Time (min)
1	deblocking solution	18-32	1.8-3.2
2	neutralizing and washing solution	30	1.5
3	coupling solution B	5	0.5
4	coupling solution A	1.3	0.25
5	coupling reaction by the reagents added in the steps 3 and 4		120-300
6	acetonitrile	20	1.0
7	capping solution	9	2.0
8	acetonitrile	30	2.0

Note that only for 3'-terminal acetylation, only the steps 1, 2, 7, and 8 were carried out again after the final cycle.

[0232]

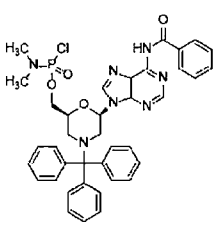
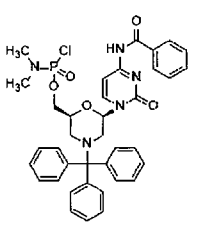
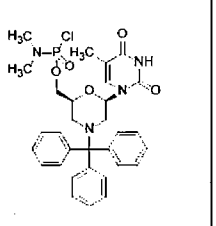
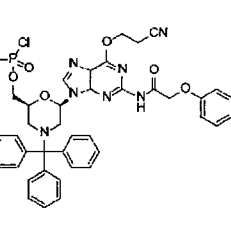
The deblocking solution used was dichloromethane solution containing 3% (w/v) trifluoroacetic acid. The neutralizing and washing solution used was a solution obtained by dissolving N,N-diisopropylethylamine to be 10% (v/v) and tetrahydrofuran to be 5% (v/v) in dichloromethane containing 35% (v/v) acetonitrile. The coupling solution A used was a solution obtained by dissolving the morpholino monomer compound in tetrahydrofuran to be 0.10 M. The coupling solution B used was a solution obtained by dissolving N,N-diisopropylethylamine to be 20% (v/v) and tetrahydrofuran to be 10% (v/v) in acetonitrile. The capping solution used was a solution obtained by dissolving 20% (v/v) acetic anhydride and 30% (v/v) 2,6-lutidine in acetonitrile.

The morpholino monomer compound (A), the morpholino monomer compound (C), the morpholino monomer compound (T), and the morpholino monomer compound (G) described in Table 13 were used as the morpholino monomer compound.

[0233]

[Table 13]

Table 13

Morpholino monomer compound (A)	Morpholino monomer compound (C)	Morpholino monomer compound (T)	Morpholino monomer compound (G)
			

[0234]

The aminopolystyrene resin loaded with the PMO synthesized above was recovered from the reaction vessel and dried at room temperature for at least 2 hours under reduced pressure. The dried PMO loaded onto aminopolystyrene resin was charged in a reaction vessel, and 5 mL of 28% ammonia water-ethanol (1/4) was added thereto. The mixture was stirred at 55°C for 15 hours. The aminopolystyrene resin was separated by filtration and washed with 1 mL of water-ethanol (1/4). The resulting filtrate was concentrated under reduced pressure. The resulting residue was dissolved in 10 mL of a solvent mixture of 20 mM of acetic acid-

triethylamine buffer (TEAA buffer) and acetonitrile (4/1) and filtered through a membrane filter. The filtrate obtained was purified by reversed phase HPLC. The conditions used are shown in Table 14.

[0235]

[Table 14]

Table 14

Column	XBridge 5 μ m C18 (Waters, ϕ 19 $\times$ 50 mm, 1 CV = 14 mL)
Flow rate	10 mL/min
Column temperature	room temperature
Solution A	20 mM TEAA buffer
Solution B	acetonitrile (CH ₃ CN)
Gradient	Solution B: (10% $\rightarrow$ 70%)/15 CV

CV: column volume

[0236]

Each fraction was analyzed, and the product of interest was recovered and concentrated under reduced pressure. To the concentrated residue, 0.5 mL of 2 M phosphoric acid aqueous solution was added, and the mixture was stirred for 15 minutes. Furthermore, 2 mL of 2 M sodium hydroxide aqueous solution was added to make the mixture alkaline, followed by filtration through a membrane filter (0.45 μ m).

The resulting aqueous solution containing the product of interest was purified by an anionic exchange resin column. The conditions used are shown in Table 15.

[0237]

[Table 15]

Table 15

Column	Source 15Q (GE Healthcare, $\phi 10 \times 108$ mm, 1 CV = 8.5 mL)
Flow rate	8.5 mL/min
Column temperature	room temperature
Solution A	10 mM sodium hydroxide aqueous solution
Solution B	10 mM sodium hydroxide aqueous solution, 1 M sodium chloride aqueous solution
Gradient	Solution B: (1% $\rightarrow$ 50%)/ 40 CV

[0238]

Each fraction was analyzed (on HPLC) and the product of interest was obtained as an aqueous solution. To the resulting aqueous solution was added 0.1 M phosphate buffer (pH 6.0) for neutralization. Next, the mixture obtained was demineralized by reversed phase HPLC under the conditions described in Table 16.

[0239]

[Table 16]

Table 16

Column	XBridge 5 μ m C18 (Waters, $\phi 10 \times 50$ mm, 1 CV = 4 mL)
Flow rate	4 mL/min
Column temperature	60°C
Solution A	water
Solution B	CH ₃ CN
Gradient	Solution B: (0% $\rightarrow$ 50%)/20 CV

[0240]

The product of interest was recovered and the mixture was concentrated under reduced pressure. The resulting residue was dissolved in water. The aqueous solution obtained was freeze-dried to give each PMO as the compound of interest as a white cotton-like solid. The calculated and measured values of ESI-TOF-MS are shown in Table 17 below.

[0241]

[Table 17]

Table 17

PMO No.	Target base sequence	Calculated	Measured
1	H44_150-175	8610.02	8610.48
2	H44_157-196	13354.66	13354.45
3	H44_159-193	11652.07	11652.09
4	H44_170-199	10012.48	10013.39
5	H44_179-206	9392.27	9392.54
6	H44_209-236	9230.21	9230.92
7	H44_239-266	9256.24	9256.17
8	H44_269-296	9264.2	9263.99
9	H44_299-326	9181.19	9181.46
10	H44_330-355	8514.98	8514.36
11	H44_354-381	9231.21	9231.24
12	H44_389-416	9147.17	9147.4
13	H44_413-438	8551.97	8552.71
14	H44_423-452	9830.43	9830.2
15	H44_455-480	8588.01	8587.53
16	H44_479-506	9262.21	9262.15
17	H44_509-536	9226.19	9225.7
18	H44_150-164_179-193	9989.49	9989.23
19	H44_150-164_192-206	10006.49	10006.81
20	H44_161-175_179-193	10013.5	10013.95
21	H44_161-175_192-206	10030.5	10031.48
22	H45_(-598) - (-571)	9221.2	9221.27
23	H45_(-568) - (-541)	9312.25	9311.93
24	H45_(-538) - (-511)	9218.18	9218.33
25	H45_(-518) - (-491)	9283.22	9283.26
26	H45_(-515) - (-488)	9307.23	9307.34
27	H45_(-508) - (-479)	10027.5	10027.98
28	H45_(-478) - (-449)	9979.48	9980.37
29	H45_(-470) - (-443)	9295.25	9296.02
30	H45_(-448) - (-421)	9385.28	9385.45
31	H45_(-439) - (-412)	9403.3	9403.98
32	H45_(-436) - (-409)	9435.3	9435.78
33	H45_(-422) - (-395)	9388.23	9388.34
34	H45_(-418) - (-394)	8363.88	8363.44
35	H45_(-418) - (-391)	9404.23	9404.6
36	H45_(-415) - (-391)	8354.87	8354.48
37	H45_(-413) - (-386)	9385.21	9385.07
38	H45_(-405) - (-378)	9371.23	9371.33
39	H45_(-389) - (-362)	9325.25	9325.6
40	H45_(-349) - (-322)	9221.2	9221.14
41	H45_(-320) - (-293)	9211.19	9211.14
42	H45_(-305) - (-280)	8640	8640.11
43	H45_(-290) - (-263)	9284.22	9284.05
44	H45_(-275) - (-250)	8656	8656.36
45	H45_(-260) - (-233)	9347.22	9347
46	H45_(-230) - (-203)	9198.19	9198.43
47	H45_(-200) - (-173)	9335.24	9335.21

48	H45_(-190)-(-163)	9312.25	9312.03
49	H45_(-185)-(-160)	8670.05	8670.14
50	H45_(-171)-(-144)	9273.24	9273.53
51	H45_(-168)-(-141)	9233.23	9233.36
52	H45_(-142)-(-115)	9254.22	9254.39
53	H45_(-110)-(-83)	9392.27	9392.55
54	H45_(-80)-(-53)	9352.26	9352.54
55	H45_(-78)-(-51)	9358.25	9358.63
56	H45_(-70)-(-43)	9323.23	9323.51
57	H45_(-60)-(-33)	9260.21	9260.39
58	H45_(-50)-(-23)	9238.22	9238.79
59	H45_(-44)-(-17)	9291.26	9291.37
60	H45_(-25)-3	9281.25	9281.54
61	H45_(-23)-5	9321.26	9321.92
62	H45_(-22)-3	8287.9	8287.54
63	H45_(-21)-7	9353.26	9353.35
64	H45_(-20)-5	8303.9	8303.52
65	H45_(-20)-8	9344.25	9344.57
66	H45_(-18)-10	9304.24	9304.44
67	H45_(-17)-8	8318.9	8318.93
68	H45_(-16)-12	9279.23	9278.95
69	H45_(-15)-10	8270.88	8270.96
70	H45_(-14)-14	9231.21	9231.28
71	H45_(-10)-16	8607.98	8607.59
72	H45_(-5)-20	8213.85	8213.45
73	H45_1-25	8214.85	8214.52
74	H45_6-30	8190.84	8190.04
75	H45_11-35	8198.85	8198.45
76	H45_11-40	9877.43	9877.12
77	H45_16-35	6544.28	6544.07
78	H45_16-40	8222.86	8222.85
79	H45_21-45	8244.85	8245.01
80	H45_(-423)-(-412)_16-33	9997.46	9997.49
81	H45_(-416)-(-402)_16-30	9964.44	9964.38
82	H45_(-411)-(-400)_16-33	9979.44	9979.18
83	H45_(-405)-(-391)_16-30	9945.42	9945.04
84	H45_(-402)-(-391)_16-33	9945.42	9945.07
85	H45_(-156)-(-143)-(-141)-(-128)	9190.2	9189.84
86	H45_(-78)-(-65)_(-44)-(-31)	9256.22	9256.49
87	H45_(-78)-(-65)_(-34)-(-21)	9354.28	9354.26
88	H45_(-78)-(-65)_16-29	9232.21	9233.24
89	H45_(-78)-(-65)_21-34	9263.21	9263.82
90	H45_(-78)-(-65)_27-40	9295.23	9295.5
91	H45_(-74)-(-62)_(-40)-(-28)	8643.02	8643.41
92	H45_(-72)-(-58)_(-38)-(-26)	9354.28	9354.28

93	H45_(-71)-(-58)_(-38)-(-25)	9314.27	9314.5
94	H45_(-71)-(-58)_16-29	9264.23	9262.81
95	H45_(-71)-(-58)_21-34	9295.23	9295.83
96	H45_(-71)-(-58)_27-40	9327.25	9327.61
97	H45_(-68)-(-60)_(-36)-(-28)_19-28	9274.24	9274.8
98	H45_(-64)-(-51)_(-44)-(-31)	9310.23	9310.1
99	H45_(-64)-(-51)_(-35)-(-22)	9408.29	9408.3
100	H45_(-64)-(-51)_16-29	9286.22	9286.47
101	H45_(-64)-(-51)_21-34	9317.22	9317.12
102	H45_(-64)-(-51)_27-40	9349.24	9349.37
103	H45_(-44)-(-31)_16-29	9184.19	9183.98
104	H45_(-44)-(-31)_27-40	9247.21	9247.14
105	H45_(-44)-(-31)_21-33_T	9230.19	9230.7
106	H45_(-38)-(-25)_16-29	9210.22	9210.11
107	H45_(-38)-(-25)_21-34	9241.22	9241.87
108	H45_(-38)-(-25)_27-40	9273.24	9273.24
109	H45_(-34)-(-21)_16-29	9266.25	9266.72
110	H45_(-34)-(-21)_21-34	9297.25	9297.85
111	H45_(-34)-(-21)_27-40	9329.27	9329.78
112	H45_1-21	6859.387	6859.02
144	H45_167-194	9121.14	9121.27
145	H45_227-254	9253.22	9253.57
146	H45_257-284	9203.18	9203.09
147	H45_287-314	9369.21	9369.79
148	H45_317-344	9342.27	9342.18
149	H45_347-374	9268.22	9268.72
150	H46_(-200)-(-173)	9326.25	9325.78
151	H46_(-171)-(-144)	9243.21	9243.17
152	H46_(-146)-(-119)	9221.22	9221.07
153	H46_(-141)-(-114)	9276.23	9275.87
154	H46_(-136)-(-109)	9310.25	9310.07
155	H46_(-126)-(-99)	9295.25	9295.56
156	H46_(-116)-(-89)	9349.26	9349.16
157	H46_(-111)-(-84)	9334.26	9333.8
158	H46_(-106)-(-79)	9319.26	9319.07
159	H46_(-81)-(-54)	9318.24	9317.86
160	H46_(-51)-(-24)	9167.16	9167.63
161	H46_(-18)-6	8037.81	8037.34
162	H46_(-15)-9	8004.79	8004.24
163	H46_(-9)-15	7943.74	7944.19
164	H46_139-166	9293.23	9292.5
165	H46_169-196	9319.22	9319.52
166	H46_199-226	9325.25	9325.91
167	H46_229-256	9361.27	9361.58
168	H46_259-286	9351.26	9351.88
169	H46_289-316	9400.26	9400.38
170	H46_319-346	9219.2	9219.51

171	H47_(-200) - (-173)	9142.18	9142.19
172	H47_(-171) - (-144)	9320.24	9320.43
173	H47_(-141) - (-114)	9230.21	9230.7
174	H47_(-111) - (-84)	9354.26	9354.05
175	H47_(-81) - (-54)	9215.16	9215.57
176	H47_(-51) - (-24)	9262.23	9262.28
177	H47_(-16) - 12	9257.24	9257.23
178	H47_1-29	9612.33	9613.79
179	H47_135-162	9236.2	9236.32
180	H48_(-21) - 7	9351.26	9350.96
181	H48_1-28	9277.21	9277.18
182	H48_177-204	9233.23	9232.72
183	H49_(-21) - 7	9304.24	9304.25
184	H49_22-47	8509.94	8509.66
185	H49_93-120	9244.19	9244.51
186	H50_(-200) - (-173)	9149.19	9148.87
187	H50_(-171) - (-144)	9292.23	9291.93
188	H50_(-141) - (-114)	9330.22	9330.33
189	H50_(-111) - (-84)	9237.17	9236.8
190	H50_(-81) - (-54)	9260.21	9259.85
191	H50_(-51) - (-24)	9259.21	9258.85
192	H50_(-21) - 7	9183.21	9183.31
193	H50_100-127	9271.22	9271.25
194	H50_90-114	8277.87	8278.28
195	H51_(-18) - 10	9411.24	9411.6
196	H51_224-251	9135.14	9135.51
197	H52_(-47) - (-20)	9330.2	9330.03
198	H52_(-18) - 10	9270.22	9270.37
199	H52_9-38	9893.42	9893.2
200	H52_109-136	9236.2	9235.9
201	H53_(-197) - (-170)	9231.18	9231.18
202	H53_(-167) - (-140)	9298.2	9298.58
203	H53_(-137) - (-110)	9353.21	9353.6
204	H53_(-107) - (-80)	9225.19	9224.55
205	H53_(-77) - (-50)	9278.23	9278.35
206	H53_(-47) - (-20)	9373.25	9373.48
207	H53_(-18) - 10	9319.26	9319
208	H53_203-230	9249.18	9249.06
209	H54_(-18) - 10	9316.22	9316.33
210	H54_146-173	9267.22	9267.45
211	H54_176-203	9181.19	9181.18
212	H54_206-233	9142.18	9142.41
213	H54_236-263	9311.23	9311.57
214	H54_266-293	9249.18	9248.66
215	H54_296-323	9344.25	9344.57
216	H54_326-353	9308.21	9307.9
217	H55_(-197) - (-170)	9206.17	9206.95
218	H55_(-167) - (-140)	9278.23	9278.65
219	H55_(-137) - (-110)	9316.2	9316.05
220	H55_(-107) - (-80)	9327.25	9326.87
221	H55_(-77) - (-50)	9290.26	9290.43

222	H55_(-47)-(-20)	9342.25	9341.86
223	H55_(-18)-10	9185.21	9185.78
224	H55_12-34	6858.387	6858.97
113	H55_177-204	9203.18	9203.35
114	H55_191-218	9320.24	9320.46
115	H55_221-248	9301.22	9301.7
116	H55_251-278	9253.2	9253.12
117	H55_281-308	9300.22	9300.57
118	H55_311-338	9126.18	9126.01
119	H55_334-361	9058.12	9057.77
120	H55_341-368	9154.16	9154.22
121	H55_371-398	9412.24	9412.07
122	H55_401-428	9308.21	9308.06
123	H55_431-458	9210.17	9209.71
124	H55_461-488	9181.19	9181.31
125	H55_491-518	9260.23	9260.72
126	H55_521-548	9237.22	9236.93
127	H55_548-575	9304.26	9304.44
128	H56_(-400)-(-373)	9283.22	9282.84
129	H56_(-380)-(-355)	8584.99	8585.11
130	H56_(-345)-(-318)	9397.26	9397.16
131	H56_(-315)-(-288)	9275.21	9275.43
132	H56_(-285)-(-258)	9258.21	9257.72
133	H56_(-255)-(-228)	9307.21	9307.98
134	H56_(-225)-(-198)	9364.24	9364.79
135	H56_(-195)-(-168)	9224.17	9224.08
136	H56_(-165)-(-138)	9172.18	9172.28
137	H56_(-135)-(-108)	9256.24	9256.9
138	H56_(-105)-(-78)	9291.23	9291.37
139	H56_(-76)-(-51)	8754.04	8754.09
140	H56_(-59)-(-34)	8629.04	8629.59
141	H56_(-39)-(-14)	8723.04	8723.84
142	H56_(-31)-(-6)	8773.06	8773.68
143	H56_(-14)-13	9297.23	9296.6
225	H44_188-202_H56_(-156)-(-142)	9876.43	9876.87
226	H44_188-202_H56_(-124)-(-110)	9956.45	9956.24
227	H44_188-202_H56_(-85)-(-71)	10069.49	10070.12
228	H56_(-156)-(-142)_H44_188-202	9876.43	9876.63
229	H56_(-124)-(-110)_H44_188-202	9956.45	9956.72
230	H56_(-85)-(-71)_H44_188-202	10069.49	10069.93
231	H45_16-35_H46_(-134)-(-115)	13207.58	13207.56
232	H45_16-35_H46_(-103)-(-84)	13281.61	13281.25

[0242]

Production of antisense PMO targeting splicing silencer
in intron of human dystrophin gene or splice site
involved in single skipping of human dystrophin gene

(i) PMO having the base sequence of PMO No. 233 (SEQ ID NO: 257) targeting a splicing silencer sequence of intron 44 or a splice site involved in single skipping, (ii) PMOs having the base sequences of PMO Nos. 234 to 244 (SEQ ID NOs: 258 to 268) targeting a splicing silencer sequence of intron 45 or a splice site involved in single skipping, (iii) PMOs (linked type) having the base sequences of PMO Nos. 245 to 249 (SEQ ID NOs: 269 to 273) targeting a region (R2) in the vicinity of an acceptor of intron 44 and a splicing silencer sequence of intron 45 or a splice site involved in single skipping, and (iv) PMOs having the base sequences of PMO Nos. 250 to 251 (SEQ ID NOs: 274 to 275) targeting a splicing silencer sequence of exon 45 in the human dystrophin gene were synthesized in the same manner as in the PMO targeting a region in the vicinity of a donor or an acceptor of an intron. The 5' end of each PMO is Group (3), as in the PMO targeting a region in the vicinity of a donor or an acceptor of an intron. Each PMO is shown in Table 18, and the calculated (Clcd) and measured (Found) values of ESI-TOF-MS are shown in Table 19.

[0243]

[Table 18]

Table 18

PMO No.	Target	Target base sequence	Base sequence of PMO	SEQ ID NO
233	(i)	H45_(-93) - (-70)	GTGTGCTACCACATGCAGTTGTAC	257
234		H45_166-189	TCTGTGCGCCCTACCTCTTTTTTCT	258
235		H45_178-201	ATTCCTATTAGATCTGTGCGCCCTA	259
236		H45_183-206	TTTTCATTCCTATTAGATCTGTGCG	260
237		H45_188-211	AAATGTTTTTCATTCCTATTAGATC	261
238		H45_191-214	CTAAAATGTTTTTCATTCCTATTAG	262
239	(ii)	H45_197-220	AGTCTGCTAAAATGTTTTTCATTC	263
240		H46_(-35) - (-12)	AGAATAAAATTGTTATTTTTTTTTT	264
241		H46_(-22)_2	GCCTGGAGAAAGAAGAATAAAATT	265
242		H46_(-12)_12	TGTTCTTCTAGCCTGGAGAAAGAA	266
243		H46_(-2)_22	GATATTCTTTTGTCTTCTAGCCT	267
244		H45_20236-20259	ATCCATGCATTCCTCCATGCACAG	268
245		H45_16-30_191-205	TTTCATTCCTATTAGTTGCCGCTGCCCAAT	269
246		H45_16-30_197-211	AAATGTTTTTCATTCCTTGCCGCTGCCCAAT	270
247	(iii)	H45_16-33_197-208	TGTTTTCATTCAGTTTGCCGCTGCCCAAT	271
248		H45_16-35_183-192	AGATCTGTCGACAGTTTGCCGCTGCCCAAT	272
249		H45_18-35_191-202	CATTCCTATTAGACAGTTTGCCGCTGCCCA	273
250	(iv)	H45_31-54	TGCATTCAATGTTCTGACAACAGT	274
251		H45_131-154	GTTTGCAGACCTCCTGCCACCGCA	275

A base sequence presumed to correspond to the splicing silencer sequence in the intron or the splice site involved in single skipping is underlined.

A base sequence presumed to correspond to the splicing silencer sequence in the intron or the splice site involved in single skipping is underlined.

[0244]

[Table 19]

Table 19

PMO No.	Target base sequence	Calculated	Measured
233	H45_(-93) - (-70)	7938.75	7938.95
234	H45_166-189	7757.67	7757.89
235	H45_178-201	7848.72	7848.99
236	H45_183-206	7869.71	7869.94
237	H45_188-211	7886.73	7886.24
238	H45_191-214	7886.73	7886.37
239	H45_197-220	7887.73	7887.49
240	H46_(-35) - (-12)	7955.74	7955.61
241	H46_(-22)_2	8061.82	8062.19
242	H46_(-12)_12	7986.77	7986.42
243	H46_(-2)_22	7860.7	7860.83
244	H45_20236-20259	7842.73	7842.9
245	H45_16-30_191-205	9824.39	9824.66
246	H45_16-30_197-211	9833.4	9833.32
247	H45_16-33_197-208	9840.39	9840.1
248	H45_16-35_183-192	9908.43	9908.14
249	H45_18-35_191-202	9843.41	9843.08
250	H45_31-54	7848.72	7848.99
251	H45_131-154	7844.73	7844.85

[0245]

[Example 2: Test on multi-exon skipping activity of antisense oligomer]

<Test Example 1>

In vitro assay of multi-exon skipping in human dystrophin gene - (1): induction of multi-exon skipping

Procedures

Each antisense oligomer (PMO Nos. 55, 59, 78, 65, 75, 77, 102, 35, 52, 47, 50, 46, 43, 40, 139, 119, 142, 113, 51, 11, 45, 54, 41, 74, 66, 64, 63, 68, 76, 28, 27, 225, 228, 1, 5, 132, 131, 128, 163, 162, and 161 (SEQ ID NO: 55, 59, 78, 65, 75, 77, 102, 35, 52, 47, 50, 46, 43, 40, 139, 119, 142, 113, 51, 11, 45, 54, 41, 74, 66, 64, 63, 68, 76, 28, 27, 225, 228, 1, 5, 132, 131, 128, 163,

162, and 161)) of Table 11 was transfected in a concentration of 25 to 50 μ M either singly or in combination of two or three oligomers with 3.5×10^5 of RD cells (human rhabdomyosarcoma cell line, CCL-136, purchased from ATCC). The reagent used for the transfection was an Amaxa Cell Line Nucleofector Kit L on Nucleofector II (Lonza). The Program T-030 was used.

After transfection, the cells were cultured for three nights in 2 mL of Eagle's minimal essential medium (EMEM) (manufactured by Sigma, hereinafter the same) containing 10% fetal bovine serum (FBS) (manufactured by Invitrogen) under conditions of 37°C and 5% CO₂.

The RD cells were washed once with PBS (manufactured by Nissui, hereinafter the same) and 350 μ L of Buffer RLT (manufactured by Qiagen) containing 1% 2-mercaptoethanol (manufactured by Nacalai Tesque, Inc.) was added to the cells. After the cells were allowed to stand at room temperature for a few minutes to lyse the cells, the lysate was collected into a QIAshredder homogenizer (manufactured by Qiagen). A homogenate was produced by centrifugation at 15,000 rpm for 2 minutes. The total RNA was extracted according to the protocol attached to RNeasy Mini Kit (manufactured by Qiagen). The concentration of the total RNA extracted was determined using a NanoDrop ND-1000 (manufactured by LMS Co., Ltd.).

[0246]

One-Step RT-PCR was performed with 800 ng of the extracted total RNA using a QIAGEN One Step RT-PCR Kit (manufactured by Qiagen). A reaction solution was prepared in accordance with the protocol attached to the kit. TaKaRa PCR Thermal Cycler Dice Touch (manufactured by Takara Bio Inc.) was used as the thermal cycler. The RT-PCR program used is as follows.

50°C, 30 mins: reverse transcription reaction
95°C, 15 mins: polymerase activation, reverse transcriptase inactivation, cDNA thermal denaturation
[94°C, 10 seconds; 57°C, 30 seconds; 72°C, 1 minute]
× 33 cycles: PCR amplification
72°C, 10 mins: final extension
[0247]

The base sequences of the forward primer and reverse primer used for RT-PCR are given below.

Forward primer: 5'-ATTTGACAGATCTGTTGAGAAATGG-3' (SEQ ID NO: 276)

Reverse primer 1: 5'-GGCTGTTTTTCATCCAGGTTGTG-3' (SEQ ID NO: 277)

Reverse primer 2: 5'-AGTTGCTGCTCTTTTCCAGGT-3' (SEQ ID NO: 278)

[0248]

Transcripts having multi-exon skipping of exons 45 to 55 can be detected by a combination of the forward primer and the reverse primer 2. Transcripts having no skipping and transcripts having single exon skipping of

exon 45 can be detected by a combination of the forward primer and the reverse primer 1.

The reaction product of the PCR above was analyzed using a Bioanalyzer (manufactured by Agilent Technologies, Inc.) and MultiNA (manufactured by Shimadzu Corp.).

[0249]

The polynucleotide level "A" of the band with skipping of any two or more numerically consecutive exons among exons 45 to 55, the polynucleotide level "B" of the band with skipping of any one exon among exons 45 to 55, and the polynucleotide level "C" of the band without skipping were measured. Based on these measurement values of "A", "B", and "C", the skipping efficiency of multi-exon skipping was determined by the following equation.

$$\text{Skipping efficiency (\%)} = A / (A + B + C) \times 100$$

[0250]

Results

The results are shown in Figures 1 to 16. The introduction of PMO Nos. 55, 59, 78, 65, 75, 77, 102, 35, 52, 47, 50, 46, 43, 40, 139, 119, 142, 113, 51, 11, 45, 54, 41, 74, 66, 64, 63, 68, 76, 28, 27, 225, 228, 1, 5, 132, 131, 128, 163, 162, and 161 (SEQ ID NO: 55, 59, 78, 65, 75, 77, 102, 35, 52, 47, 50, 46, 43, 40, 139, 119, 142, 113, 51, 11, 45, 54, 41, 74, 66, 64, 63, 68, 76, 28, 27, 225, 228, 1, 5, 132, 131, 128, 163, 162, and 161)

singly or in combination induced multi-exon skipping of exons 45 to 55.

These results revealed that the antisense oligomers of the present invention described in Table 11 effectively induce or would effectively induce multi-exon skipping when used singly or in combination.

It was also confirmed that the introduction of PMO Nos. 6, 7, 8, 10, 14, 26, 29, 38, 39, 53, 58, 67, 80, 82, 86, 92, 97, 98, 100, 121, 122, 124, 125, 126, 130, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 231, 232 (SEQ ID NO: 6, 7, 8, 10, 14, 26, 29, 38, 39, 53, 58, 67, 80, 82, 86, 92, 97, 98, 100, 121, 122, 124, 125, 126, 130, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 231, 232) also induce multi-exon skipping of exons 45 to 55.

[0251]

<Test Example 2>

In vitro assay of multi-exon skipping in human dystrophin gene - (2): promotion of multi-exon skipping

Procedures

The antisense oligomer (PMO No. 75) of Table 11 was added in a concentration of 25 to 50 μM to 3.5×10^5 of RD cells (human rhabdomyosarcoma cell line, CCL-136, purchased from ATCC), and each antisense oligomer (PMO No. 236, 237, or 239) of Table 18 was introduced in combination therewith in a concentration of 25 to 50 μM .

The assay was conducted by the same procedures as in Test Example 1.

[0252]

Results

The results are shown in Figures 17 and 18. The introduction of PMO No. 75 (SEQ ID NO: 75) in combination with PMO No. 236, 237, or 239 (SEQ ID NO: 260, 261, or 263) enhanced the skipping efficiency of multi-exon skipping of exons 45 to 55 as compared to the case of PMO No. 75 singly. On the other hand, this approach decreased the skipping efficiency of single exon skipping of exon 45.

These results revealed that the antisense oligomer of the present invention described in Table 11 promote or would promote multi-exon skipping when used in combination with the suppressor antisense oligomer of the present invention described in Table 18.

[0253]

<Test Example 3>

In vitro assay of multi-exon skipping in human dystrophin gene - (3): promotion of overall multi-exon skipping

Procedures

Test Example 3 was conducted by the same procedures as in Test Example 2 except that One-Step RT-PCR was performed as follows.

One-Step RT-PCR was performed with 800 ng of the extracted total RNA using a QIAGEN One Step RT-PCR Kit

(manufactured by Qiagen). A reaction solution was prepared in accordance with the protocol attached to the kit. TaKaRa PCR Thermal Cycler Dice Touch (manufactured by Takara Bio Inc.) was used as the thermal cycler. The RT-PCR program used is as follows.

50°C, 30 mins: reverse transcription reaction

95°C, 15 mins: polymerase activation, reverse transcriptase inactivation, cDNA thermal denaturation

[94°C, 10 seconds; 60°C, 30 seconds; 72°C, 2 min] × 40 cycles: PCR amplification

72°C, 10 mins: final extension

[0254]

The base sequences of the forward primer and reverse primer used for RT-PCR are given below.

Forward primer 2: 5'-ATTTGACAGATCTGTTGAGAAATGG-3' (SEQ ID NO: 279)

Reverse primer 3: 5'-GGCTCCAATAGTGGTCAGTCC-3' (SEQ ID NO: 280)

Forward primer 3: 5'-CCTGAGAATTGGGAACATGC-3' (SEQ ID NO: 281)

Reverse primer 4: 5'-CCTCCTTCCATGACTCAAGC-3' (SEQ ID NO: 282)

[0255]

Transcripts having multi-exon skipping of exons 45 and 46, exons 45 to 47, exons 45 to 48, or exons 45 to 49 in the human dystrophin gene can be detected by a combination of the forward primer 2 and the reverse

primer 3. On the other hand, transcripts having multi-exon skipping of exons 45 and 46, exons 45 to 47, exons 45 to 48, exons 45 to 49, exons 45 to 50, exons 45 to 51, or exons 45 to 52 are detected by a combination of the forward primer 3 and the reverse primer 4.

The reaction product of the PCR above was analyzed using a Bioanalyzer (manufactured by Agilent Technologies, Inc.) and MultiNA (manufactured by Shimadzu Corp.) to quantify the amount of the PCR product of the transcript having each exon skipping.

[0256]

Results

The results are shown in Figures 19 to 22. The introduction of PMO No. 75 (SEQ ID NO: 75) in combination with PMO No. 236 or 239 (SEQ ID NO: 260 or 263) enhanced the skipping efficiency of multi-exon skipping of exons 45 to 55 (Test Example 2) as well as multi-exon skipping of exons 45 and 46, exons 45 to 47, exons 45 to 48, exons 45 to 49, exons 45 to 50, exons 45 to 51, and exons 45 to 52 as compared to the case of PMO No. 75 singly.

These results revealed that the antisense oligomer of the present invention described in Table 11 promote or would promote overall multi-exon skipping when used in combination with the suppressor antisense oligomer of the present invention described in Table 18.

Industrial Applicability

[0257]

The present invention provides an antisense oligomer and a suppressor antisense oligomer that cause multi-exon skipping in human dystrophin. Use of the antisense oligomer and the suppressor antisense oligomer of the present invention provides a novel therapeutic agent for DMD and method for treatment of DMD.

Claims

[Claim 1]

An antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which causes simultaneous skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA,

the antisense oligomer comprising a base sequence complementary to a base sequence of at least one region selected from the group consisting of regions R1 to R24 represented by

region R_n (wherein n is an odd number of 1 to 23) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the N^Ath exon and a base sequence of NY bases in the downstream direction from the 5' end of the N^Bth intron in the human dystrophin pre-mRNA, and

region R_n (wherein n is an even number of 2 to 24) which consists of a base sequence of NX bases in the upstream direction from the 3' end of the N^Ath intron and a base sequence of NY bases in the downstream direction from the 5' end of the N^Bth exon in the human dystrophin pre-mRNA,
or a partial base sequence thereof, wherein

when n is 1, NA = 44, NB = 44, NX = 20, and NY =
400,
when n is 2, NA = 44, NB = 45, NX = 600, and NY =
50,
when n is 3, NA = 45, NB = 45, NX = 20, and NY =
400,
when n is 4, NA = 45, NB = 46, NX = 400, and NY =
50,
when n is 5, NA = 46, NB = 46, NX = 20, and NY =
400,
when n is 6, NA = 46, NB = 47, NX = 400, and NY =
50,
when n is 7, NA = 47, NB = 47, NX = 20, and NY =
400,
when n is 8, NA = 47, NB = 48, NX = 400, and NY =
50,
when n is 9, NA = 48, NB = 48, NX = 20, and NY =
400,
when n is 10, NA = 48, NB = 49, NX = 400, and NY =
50,
when n is 11, NA = 49, NB = 49, NX = 20, and NY =
400,
when n is 12, NA = 49, NB = 50, NX = 400, and NY =
50,
when n is 13, NA = 50, NB = 50, NX = 20, and NY =
400,
when n is 14, NA = 50, NB = 51, NX = 400, and NY =

50,
when n is 15, NA = 51, NB = 51, NX = 20, and NY =
400,
when n is 16, NA = 51, NB = 52, NX = 400, and NY =
50,
when n is 17, NA = 52, NB = 52, NX = 20, and NY =
400,
when n is 18, NA = 52, NB = 53, NX = 400, and NY =
50,
when n is 19, NA = 53, NB = 53, NX = 20, and NY =
400,
when n is 20, NA = 53, NB = 54, NX = 400, and NY =
50,
when n is 21, NA = 54, NB = 54, NX = 20, and NY =
400,
when n is 22, NA = 54, NB = 55, NX = 400, and NY =
50,
when n is 23, NA = 55, NB = 55, NX = 20, and NY =
400, or
when n is 24, NA = 55, NB = 56, NX = 400, and NY =
50.

[Claim 2]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 1, wherein

the region R1 is a region that consists of a base sequence of 20 bases in the upstream direction from the

3' end of the 44th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 44th intron in the human dystrophin pre-mRNA,

the region R2 is a region that consists of a base sequence of 600 bases in the upstream direction from the 3' end of the 44th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 45th exon in the human dystrophin pre-mRNA,

the region R3 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 45th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 45th intron in the human dystrophin pre-mRNA,

the region R4 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 45th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 46th exon in the human dystrophin pre-mRNA,

the region R5 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 46th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 46th intron in the human dystrophin pre-mRNA,

the region R6 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 46th intron and a base sequence of 50 bases

in the downstream direction from the 5' end of the 47th exon in the human dystrophin pre-mRNA,

the region R7 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 47th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 47th intron in the human dystrophin pre-mRNA,

the region R8 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 47th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 48th exon in the human dystrophin pre-mRNA,

the region R9 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 48th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 48th intron in the human dystrophin pre-mRNA,

the region R10 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 48th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 49th exon in the human dystrophin pre-mRNA,

the region R11 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 49th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 49th intron in the human dystrophin pre-mRNA,

the region R12 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 49th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 50th exon in the human dystrophin pre-mRNA,

the region R13 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 50th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 50th intron in the human dystrophin pre-mRNA,

the region R14 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 50th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 51st exon in the human dystrophin pre-mRNA,

the region R15 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 51st exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 51st intron in the human dystrophin pre-mRNA,

the region R16 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 51st intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 52nd exon in the human dystrophin pre-mRNA,

the region R17 is a region that consists of a base sequence of 20 bases in the upstream direction from the

3' end of the 52nd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 52nd intron in the human dystrophin pre-mRNA,

the region R18 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 52nd intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 53rd exon in the human dystrophin pre-mRNA,

the region R19 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 53rd exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 53rd intron in the human dystrophin pre-mRNA,

the region R20 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 53rd intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 54th exon in the human dystrophin pre-mRNA,

the region R21 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 54th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 54th intron in the human dystrophin pre-mRNA,

the region R22 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 54th intron and a base sequence of 50 bases

in the downstream direction from the 5' end of the 55th exon in the human dystrophin pre-mRNA,

the region R23 is a region that consists of a base sequence of 20 bases in the upstream direction from the 3' end of the 55th exon and a base sequence of 400 bases in the downstream direction from the 5' end of the 55th intron in the human dystrophin pre-mRNA, or

the region R24 is a region that consists of a base sequence of 400 bases in the upstream direction from the 3' end of the 55th intron and a base sequence of 50 bases in the downstream direction from the 5' end of the 56th exon in the human dystrophin pre-mRNA.

[Claim 3]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 1 or 2, wherein

the antisense oligomer comprises a base sequence complementary to

(a) any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(b) a base sequence that hybridizes under stringent conditions to a base sequence complementary to any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(c) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting

of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or

(d) a partial base sequence of any one base sequence selected from the group consisting of the base sequences (a), (b), and (c).

[Claim 4]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 3, wherein

the antisense oligomer is an antisense oligomer comprising two or more unit oligomers linked to each other, wherein

each of the unit oligomers comprises a base sequence complementary to a base sequence of any one region selected from the group consisting of the regions R1 to R24, or a partial base sequence thereof, and the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[Claim 5]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 3, wherein

the antisense oligomer is an antisense oligomer comprising two or more unit oligomers linked to each other, wherein

each of the unit oligomers comprises a base sequence complementary to

(a) any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(b) a base sequence that hybridizes under stringent conditions to a base sequence complementary to any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389,

(c) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 233 to 256, 341 to 369, and 385 to 389, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or

(d) a partial base sequence of any one base sequence selected from the group consisting of the base sequences (a), (b), and (c),

and the respective base sequences of the unit oligomers are neither consecutive nor overlapped with each other.

[Claim 6]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 4 or 5, wherein each of the unit oligomers comprises a base sequence complementary to a consecutive base sequence of 5- to 20-base length in the region.

[Claim 7]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 3, wherein the antisense oligomer consists of

(1) any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, or

(2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 1 to 111, 113 to 177, 179, 180, 182, 183, 185 to 193, 195 to 198, 200 to 223, and 225 to 232, and has a length within $\pm 15\%$ of the length of the any one base sequence selected.

[Claim 8]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 3 and 7, wherein the antisense oligomer consists of any one base sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, 7, 8, 10, 11, 14, 26, 27, 28, 29, 35, 38, 39, 40, 41, 43, 45, 46, 47, 50, 51, 52, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 80, 82, 86, 92, 97, 98, 100, 102, 113, 119, 121, 122, 124, 125, 126, 128, 130, 131, 132, 139, 142, 144, 146, 147, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 225, 228, 231, and 232.

[Claim 9]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 3, 7 and 8, wherein the antisense oligomer consists of any one base sequence selected from the group consisting of SEQ ID NOS: 1, 5, 11, 27, 28, 35, 40, 41, 43, 45, 46, 47, 50, 51, 52, 54, 55, 59, 63, 64, 65, 66, 68, 74, 75, 76, 77, 78, 102, 113, 119, 128, 131, 132, 139, 142, 161, 162, 163, 225, and 228.

[Claim 10]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 9, wherein the antisense oligomer is an oligonucleotide.

[Claim 11]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 10, wherein the sugar moiety and/or the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is modified.

[Claim 12]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 10 or 11, wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected

from the group consisting of -OR, -R, -R'OR, -SH, -SR, -NH₂, -NHR, -NR₂, -N₃, -CN, -F, -Cl, -Br, and -I

(wherein R is an alkyl or an aryl and R' is an alkylene).

[Claim 13]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 10 to 12, wherein the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is any one selected from the group consisting of a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond and a boranophosphate bond.

[Claim 14]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 9, wherein the antisense oligomer is a morpholino oligomer.

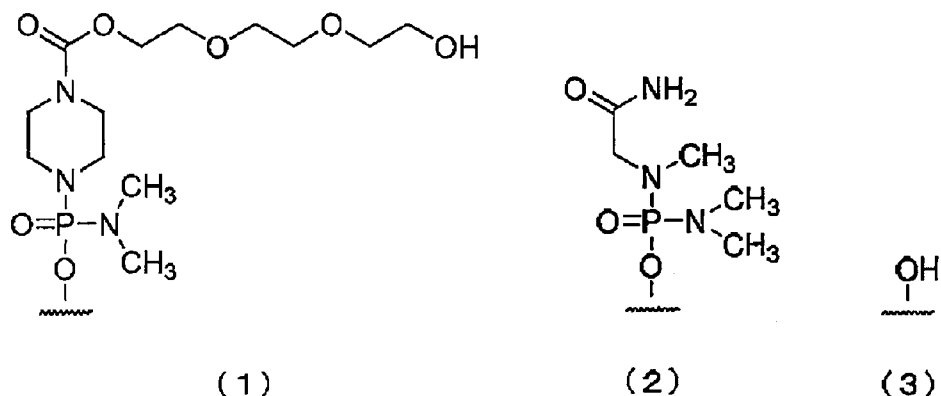
[Claim 15]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 14, wherein the antisense oligomer is a phosphorodiamidate morpholino oligomer.

[Claim 16]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 14 or 15, wherein the 5' end is any one of chemical formulae (1) to (3) below:

[Formula 15]



[Claim 17]

A suppressor antisense oligomer or a pharmaceutically acceptable salt thereof, or hydrate thereof which suppresses single skipping of any one exon selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA,

the suppressor antisense oligomer comprising a base sequence complementary to

- (a) any one base sequence of a base sequence selected from the group consisting of SEQ ID NOs: 370 to 384,
- (b) a base sequence that has at least 85% identity with any one base sequence of a base sequence selected from the group consisting of SEQ ID NOs: 370 to 384, and has a length within $\pm 15\%$ of the length of the any one base sequence selected, or
- (c) a partial base sequence of the base sequence (a) or (b).

[Claim 18]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 17, wherein the suppressor antisense oligomer consists of

(1) any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, or (2) a base sequence that has at least 85% identity with any one base sequence selected from the group consisting of SEQ ID NOs: 257 to 275, and has a length within $\pm 15\%$ of the length of the any one base sequence selected.

[Claim 19]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 17 or 18, wherein the suppressor antisense oligomer consists of any one base sequence selected from the group consisting of SEQ ID NOs: 260, 261, and 263.

[Claim 20]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 17 to 19, wherein the suppressor antisense oligomer is an oligonucleotide.

[Claim 21]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 20, wherein the sugar moiety

and/or the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is modified.

[Claim 22]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 20 or 21, wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected from the group consisting of -OR, -R, -R'OR, -SH, -SR, -NH₂, -NHR, -NR₂, -N₃, -CN, -F, -Cl, -Br, and -I

(wherein R is an alkyl or an aryl and R' is an alkylene).

[Claim 23]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 20 to 22, wherein the phosphate bond moiety of at least one nucleotide constituting the oligonucleotide is any one selected from the group consisting of a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond and a boranophosphate bond.

[Claim 24]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 17 to 19, wherein the suppressor antisense oligomer is a morpholino oligomer.

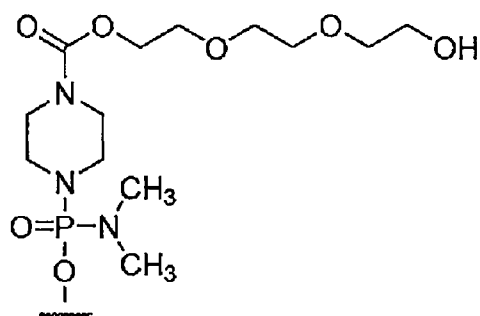
[Claim 25]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 24, wherein the suppressor antisense oligomer is a phosphorodiamidate morpholino oligomer.

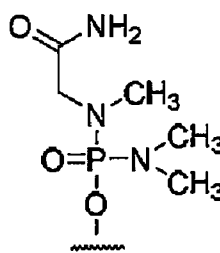
[Claim 26]

The suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to claim 24 or 25, wherein the 5' end is any one of chemical formulae (1) to (3) below:

[Formula 16]



(1)



(2)



(3)

[Claim 27]

A pharmaceutical composition comprising the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 16.

[Claim 28]

The pharmaceutical composition according to claim 27, further comprising the suppressor antisense oligomer

or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 17 to 26.

[Claim 29]

A pharmaceutical composition comprising the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 16, and the suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 17 to 26.

[Claim 30]

The pharmaceutical composition according to claim 28 or 29, wherein

(1) the antisense oligomer is an oligomer consisting of SEQ ID NO: 75, and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 260,

(2) the antisense oligomer is an oligomer consisting of SEQ ID NO: 75, and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 261, or

(3) the antisense oligomer is an oligomer consisting of SEQ ID NO: 75, and the suppressor antisense oligomer is an oligomer consisting of SEQ ID NO: 263.

[Claim 31]

The pharmaceutical composition according to any one of claims 27 to 30, further comprising a pharmaceutically acceptable carrier.

[Claim 32]

The pharmaceutical composition according to any one of claims 27 to 31 for treatment of muscular dystrophy.

[Claim 33]

The pharmaceutical composition according to any one of claims 27 to 32 for being administered to a human patient.

[Claim 34]

A method for treatment of muscular dystrophy, which comprises administering to a patient with muscular dystrophy the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 16, or the pharmaceutical composition according to any one of claims 27 to 33.

[Claim 35]

The method for treatment according to claim 34, wherein the patient with muscular dystrophy is a patient with a mutation that is amenable to exon 45 to 55 skipping in the dystrophin gene.

[Claim 36]

The method for treatment according to claim 34 or 35, wherein the patient is a human.

[Claim 37]

Use of the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 16, or the pharmaceutical composition according to any one of claims

27 to 33 in manufacturing of a medicament for the treatment of muscular dystrophy.

[Claim 38]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 16, or the pharmaceutical composition according to any one of claims 27 to 33 for use in the treatment of muscular dystrophy.

[Claim 39]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof, or the pharmaceutical composition according to claim 38, wherein the treatment involves performing skipping of any two or more numerically consecutive exons selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA.

[Claim 40]

The antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof, or the pharmaceutical composition according to claim 38 or 39, wherein in the treatment, a patient with muscular dystrophy is a human.

[Claim 41]

A method for enhancing the efficiency of skipping of two or more numerically consecutive exons, which comprises

inhibiting a splicing silencer sequence, a splice site sequence, or a branch site sequence of pre-mRNA of interest when the two or more numerically consecutive exons are skipped from the pre-mRNA of interest.

[Claim 42]

The method according to claim 41, wherein the splicing silencer sequence is a recognition sequence of heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1).

[Claim 43]

The method according to claim 41 or 42, wherein the pre-mRNA of interest is human dystrophin pre-mRNA.

[Claim 44]

The method according to any one of claims 41 to 43, wherein the two or more numerically consecutive exons are selected from the group consisting of the 45th exon to the 55th exon in human dystrophin pre-mRNA.

[Claim 45]

The method according to claim 44, wherein the skipping of the two or more numerically consecutive exons of the pre-mRNA of interest is performed using the antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 16.

[Claim 46]

The method according to claim 44 or 45, wherein specific inhibition of the splicing silencer sequence, the splice site sequence, or the branch site sequence is

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performed using the suppressor antisense oligomer or the pharmaceutically acceptable salt thereof, or hydrate thereof according to any one of claims 17 to 26.