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**Published**:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
27 October 2016

WO 2016/149659 A3

(54) Title: ANTISENSE-INDUCED EXON EXCLUSION IN MYOSTATIN

(57) Abstract: The present disclosure relates to antisense oligomers and related compositions and methods for decreasing the expression of functional human myostatin and methods for treating muscular dystrophy and related disorders and more specifically relates to inducing exclusion of myostatin exon 2 and thereby reducing the levels of myostatin protein.

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**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☒ on paper or in the form of an image file.

   b. ☒ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 77, 81
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-11, 13, 15-19, 21, 23, 25-29, 31-33, 35-37, 39-55, 58-76, (78,82)/(26-29,31-33,35-37,39,46-53), 80, 84

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/04, C07H 21/02, C07H 21/00, A61K 48/00, A61K 31/5377, C07D 295/108 (2016.01)
CPC - C07F 9/5727, C07F 9/598, C07F 9/65583, C07F 9/6561, C07F 9/65846, C07H 21/00, C07F 9/6544
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07H 21/04, C07H 21/02, C07H 21/00, A61K 48/00, A61K 31/5377, C07D 295/108 (2016.01)
CPC- C07F 9/5727, C07F 9/598, C07F 9/65583, C07F 9/6561, C07F 9/65846, C07H 21/00, C07F 9/6544

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C07D 265/30, A61K 31/496 (2016.01) CPC- A61K 31/5377, C07D 265/30, C07D 295/108, C07F 9/222, C07F 9/26, A61K 31/496, C12N 15/1136, C12N 15/113, C12N 15/1132, C12N 15/1138, C12N 15/1135, C07K 7/08, C12N 2310/11, C12N 2310/3145...

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: exonic splicing enhancer, ESE, Intron splicing enhancers ISE, intron-exon junction, splice site, myostatin, MSTN, arginine rich peptide, CPP, intron 1, exon 2, intron 2...
GenCore 6.4.1: SEQ ID NO: 1, 6, 7, 8, 16

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | US 2007/0122821 A1 (Iversen, et al.) 31 May 2007 (31.05.2007) claims 1, 7, 10-15, para [0009], [0010], [0052], [0068], [0069], [0101], SEQ ID NO: 2-5, 7, 13 | 25-29, 39-45, 58-76, (78,82)/(26-29,39), 79, (80,84)/(40-45,58-71,73-76)<br>---<br>1-11, 13, 15-19, 21, 23, 31-33, 35-37, 46-55, (78,82)/(31-33,35-37,46-53), (80,84)/(46-53) |
| Y | Bestas, et al. Design and application of bispecific splice-switching oligonucleotides. Nucleic Acid Ther. 2014, 24(1):13-24; pg 13, col 2 to pg 14, col 1; pg 16, col 2 and Fig 2 and its legend | 1-7, 54, 55 |
| Y | US 2015/0073140 A1 (Sarepta Therapeutics Inc.) 12 March 2015 (12.03.2015) para [0075], [0255], [0342], Fig 1A, 1B | 8-11, 13, 15-19, 21, 23, 31-33, 35-37, 46-53, (78,82)/(31-33,35-37,41-53), (80,84)/(46-53) |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 August 2016 | 09 SEP 2016 |

| Name and mailing address of the ISA/US | Authorized officer: |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

INTERNATIONAL SEARCH REPORT

International application No.
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In Continuation of Box III. Observations where unity of invention is lacking:

Group I+: claims 1-38, 54-57, 72, 79, (78,82)/(26-38), 83, directed to an antisense compound targeting an intron/exon junction of human myostatin pre-mRNA, and a pharmaceutical composition comprising thereof. The antisense compound and a pharmaceutical composition/medicament comprising thereof will be searched to the extent that the target region encompasses SEQ ID NO:6, the targeting sequence encompasses SEQ ID NO:1, a compound of Formula (I), wherein Y is O, R1 is -N(CH3)2, T is OH, R2 is H. It is believed that claims 1-11, 13, 15-19, 21, 23, 25-29, 31-33, 35-37, 54, 55, 72, 79, (78,82)/(26-29,31-33,35-37), encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass an antisense compound comprising the target region encompasses SEQ ID NO:6, the targeting sequence SEQ ID NO:1, a compound of Formula (I), wherein wherein Y is O, R1 is -N(CH3)2, T is OH, R2 is H, and a pharmaceutical composition comprising thereof. Additional target region(s), targeting sequence, and/or a specific compound of Formula (I) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected target region(s), targeting sequence, and/or a specific compound of Formula (I). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an antisense compound targeting an intron/exon junction of human myostatin pre-mRNA, and a pharmaceutical composition comprising thereof. The antisense compound and a pharmaceutical composition comprising thereof will be searched to the extent that the compound encompasses target region encompasses SEQ ID NO:7, the targeting sequence encompasses SEQ ID NO: NO:16, a compound of Formula (I), wherein Y is O, R1 is -N(CH3)2, T is OH, R2 is R2 is G/CPP, i.e. claims 1-14, 16-19, 21, 23-35, 37, 54-57, 72, (78,82)/(26-35,37), 79.

Group II: claims 39-53, 58-71, 73-76, (78,82)/(39,46-53), 79, (80,84)/(40-53,58-71,73-76), directed to a method comprising administering an antisense compound targeting an intron/exon junction of human myostatin pre-mRNA.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of some inventions of Group I+ is a specific target nucleic acid sequence recited therein, because no significant structural similarities can readily be ascertained among the nucleic acid sequences, and further because SEQ ID NO:6 was known in the art at the time of the invention (see US 2007/0122821 A1 to Iversen, et al. (31 May 2007) (hereinafter "Iversen '821"); SEQ ID NO:12, 100% identity).

The special technical feature of some inventions of Group I+ is a specific targeting nucleic acid sequence recited therein, because no significant structural similarities can readily be ascertained among the nucleic acid sequences, and further because SEQ ID NO:1 and SEQ ID NO:16, wherein each N is T were known in the art at the time of the invention (see Iversen '821, as above; SEQ ID NO 13, nucleic acids 26-2 and 44-22, respectively, 100% identity).

The special technical feature of some inventions of Group I+ is a specific embodiment of a compound of Formula (I), because the common structural core thereof was known in the art at the time of the invention (please see, US 2015/0073140 A1 to Hanson et al. (12 March 2015) (hereinafter "Hanson"); para [0075] "FIG. 1B shows a morpholino oligomer as in FIG. 1A, but where the backbone linkages comprise one piperazino phosphorodiamidate linkage"; please see the compound depicted in Fig 1B, wherein each Nu is a nucleobase which taken together form a targeting sequence (Fig 1A, B; Fig 1B, Base; para [0255], "Each morpholino ring structure supports a base pairing moiety (Pi), to form a sequence of base pairing moieties which is typically designed to hybridize to a selected antisense target in a cell or in a subject being treated"; para [0342], "Experiments in support of the invention and designed to target the MI/M2 segment of influenza A virus (H1N I strain PR8) using PMO with modified linkages of the invention were performed using oligomers based on SEQ ID NO:3, listed below in Table 4 and described in Example 29");
Z is 20 (para para [0342], "Experiments in support of the invention and designed to target the MI/M2 segment of influenza A virus (H1N I strain PR8) using PMO with modified linkages of the invention were performed using oligomers based on SEQ ID NO:3, listed below in Table 4", wherein SEQ ID NO:3 is 20 nucleobase-long);
Y is O (Fig 1B);
T is a moiety of the claimed formula, wherein A is -N(R7)2, wherein R7 is C1 alkyl (-NMe2);
R6 is a moiety of the claimed formula, wherein R10 is -(CO)-R11-OH, wherein R11 is -(O-C2 alkyl)2;
each instance of R1 is independently selected from -N(R13)2, wherein R13 is C1 alkyl and a moiety of Formula (II), wherein R15 and R17 are each H (Fig 1B);
R2 is H (Fig 1B)), and, therefore, no significant structural similarities can readily be ascertained among the claimed compounds.

The special technical feature of some inventions of Group I+ is a specific embodiment of a compound of Formula (VI), because the common structural core thereof was known in the art at the time of the invention (please see, Hanson, as above; para [0075] "FIG. 1B shows a morpholino oligomer as in FIG. 1A, but where the backbone linkages comprise one piperazino phosphorodiamidate linkage"; please see the compound depicted in Fig 1B, wherein
each Nu is a nucleobase which taken together form a targeting sequence (Fig 1A, B; Fig 1B, Base; para [0255], "Each morpholino ring structure supports a base pairing moiety (Pi), to form a sequence of base pairing moieties which is typically designed to hybridize to a selected antisense target in a cell or in a subject being treated"; para [0342], "Experiments in support of the invention and designed to target the MI/M2 segment of influenza A virus (HIN I strain PR8) using PMO with modified linkages of the invention were performed using oligomers based on SEQ ID NO:3, listed below in Table 4 and described in Example 29");
Z is 20 (para [0342], "Experiments in support of the invention and designed to target the MI/M2 segment of influenza A virus (H IN I strain PR8) using PMO with modified linkages of the invention were performed using oligomers based on SEQ ID NO:3, listed below in Table 4", wherein SEQ ID NO:3 is 20 nucleobase-long);
Y is O (Fig 1B);
each R1 is independently selected from -[piperazin] and -N(CH2)2 (Fig 1B)), and, therefore, no significant structural similarities can readily be ascertained among the claimed compounds.

********** See the Following Supplemental Sheet to continue **********

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In Continuation of Box III. Observations where unity of invention is lacking and the Preceding Supplemental Sheet:

The inventions of Group I+ do not include the shared or common technical feature of a method comprising administering an antisense compound targeting an intron/exon junction of human myostatin pre-mRNA, as required by Group II.

Common Technical Features
The inventions of Groups I+ and II share the technical feature of an antisense oligomer compound of 12 to 40 subunits, comprising: at least one subunit that is a nucleotide analog having (i) a modified intenucleoside linkage, (ii) a modified sugar moiety, or (iii) a combination of the foregoing; and a targeting sequence complementary to 12 or more contiguous nucleotides in a target region spanning an intron/exonjunction of human myostatin pre-mRNA, wherein the contiguous nucleotides include the intron/exon junction, and wherein said intron/exon junction is selected from intron I/exon 2 and exon 2/intron 2. Some inventions of Groups I+ and II share the technical feature of a pharmaceutical composition, comprising said antisense oligomer. However, this shared technical feature does not represent a contribution over prior art as being obvious over a paper titled "Design and application of bispecific splice-switching oligonucleotides" by Bestas, et al. (Nucleic Acid Ther. 2014, 24(1):13-24) (hereinafter "Bestas") in view of US 2014/0045916 A1 to Iversen, et al. (13 February 2014) (hereinafter "Iverson '916") as follows:

Bestas discloses an antisense oligomer compound of 12 to 40 subunits (pg 16, col 2, "...25mer AONs were designed to target either the consensus splice sites of exon 2 (AON1 and AON4)", comprising: a targeting sequence complementary to 12 or more contiguous nucleotides in a target region spanning an intron/exonjunction of human myostatin pre-mRNA (pg 16, col 2, "we designed SSOs that promoted skipping of exon 2 from the Mstn pre-mRNA to disrupt the mRNA reading frame so as to reduce levels of myostatin protein. Four 25mer AONs were designed to target either the consensus splice sites of exon 2 (AON1 and AON4)..."), wherein the contiguous nucleotides include the intron/exon junction, and wherein said intron/exon junction is selected from intron I/exon 2 and exon 2/intron 2 (pg 16, Fig 2 and its legend, AON1 and AON4, "Development of SSOs for Mstn exon 2 skipping... SSOs were developed to target consensus splice sites ... of themyostatin (Mstn)" gene as depicted in schematic").

Although Bestas discloses an antisense nucleotide analog having (i) a modified intenucleoside linkage, (ii) a modified sugar moiety is efficient in vivo (pg 13, 2nd col, last para to pg 14m 1st col, "The therapeutic potential of this strategy has recently been shown in two independent systemic clinical trials. In one of the studies the authors showed dose-dependent SSO efficacy, using the AON analog 2'-O-methyl phosphorothioateRNA(2'-OMePS RNA), in patients with DMD... Similarly, a phase 2 systemic trial using phosphorodiamidate morpholino oligomer (PMO) chemistry has also demonstrated dose dependent restoration of dystrophin"), Bestas does not specifically disclose that AON1 or AON4 comprises at least one subunit that is a nucleotide analog having (i) a modified intenucleoside linkage, (ii) a modified sugar moiety.

Iversen '916 discloses an antisense oligomer compound of 12 to 40 subunits, comprising: at least one subunit that is a nucleotide analog having (i) a modified intenucleoside linkage, (ii) a modified sugar moiety, or (iii) a combination of the foregoing; and a targeting sequence complementary to 12 or more contiguous nucleotides in a target region spanning an intron/exonjunction of human myostatin pre-mRNA (claim 1, "a myostatin-expression-inhibiting amount of a substantially uncharged antisense compound (i) composed of morpholino subunits and phosphorus-containing intersubunit linkages joining a morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent subunit.(ii) capable of uptake by target muscle cells in the subject, (iii) containing between 10-40 nucleotide bases, and (iv) having a base sequence effective to hybridize to an expression-sensitive region of processed or preprocessed human myostatin RNA transcript, identified, in its processed form, by SEQ ID NO:6").
It would have been obvious to one of ordinary skill in the art to combine, in the course of routine experimentation and with a reasonable expectation of success, Bestas and Iversen '916 by introducing morpholino subunits and phosphorus-containing inter-subunit linkages joining a morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent subunit, to increase their resistant to nucleases (Iversen '916, para [0004], "... Rnase-inactive [antisense oligomers] do not promote cleavage of bound RNA and are believed to act by sterically blocking the molecular machinery from transcribing, processing, or translating thetarget sequence. While these compounds tend to produce fewer side reactions, such as nonselective cleavage, thanphosphorothioate oligomers").

Bestas in view of Iversen '916 also discloses a pharmaceutical composition, comprising said antisense oligomer (Iversen '916, para [0154]-[0162], "IX. Formulations A morpholino antisense oligonucleotide composition may be administered in any convenient physiologically acceptable vehicle..."). As said technical features would have been obvious to one of ordinary skill in the art at the time of the invention, they cannot be considered special technical feature that would otherwise unify the groups.

Groups I+ and II lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.