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- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM,

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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))

- (88) **Date of publication of the international search report:**
14 March 2013



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(54) **Title:** PHENOCOPY MODEL OF DISEASE

(57) **Abstract:** Methods and compositions for generating nonhuman disease models through splicing modulation.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/043894

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/7125 A61P25/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C12N A61K
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. R. BECKER ET AL: "Human cardiomyopathy mutations induce myocyte hyperplasia and activate hypertrophic pathways during cardiogenesis in zebrafish", DISEASE MODELS & MECHANISMS, vol. 4, no. 3, 18 January 2011 (2011-01-18), pages 400-410, XP55038437, ISSN: 1754-8403, DOI: 10.1242/dmm.006148	1,2, 31-36
Y	page 400, right-hand column - page 401, left-hand column; figures 1,5 ----- -/--	3-21,29, 30,42-44

Further documents are listed in the continuation of Box C.

See patent family annex.

* 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 10 January 2013	Date of mailing of the international search report 18/01/2013
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Bucka, Alexander
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/043894

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 and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)
 - on paper
 - in electronic form
 - b. (time)
 - in the international application as filed
 - together with the international application in electronic form
 - subsequently to this Authority for the purpose of search
2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
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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.:
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:

see additional sheet

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 an 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.:

1-21, 29-36, 42-48

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.

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/043894

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARND HEUSER ET AL: "Mutant Desmocollin-2 Causes Arrhythmogenic Right Ventricular Cardiomyopathy", AM. J. HUM. GENET., vol. 79, no. 6, 1 December 2006 (2006-12-01), pages 1081-1088, XP55039052, page 1082, left-hand column; figure 1 page 1083, right-hand column figure 4	1,2, 31-36
Y	----- SCHMID ALOICIA ET AL: "Animal models of spinal muscular atrophy", JOURNAL OF CHILD NEUROLOGY, DECKER PERIODICALS, HAMILTON, CA, vol. 22, no. 8, 1 August 2007 (2007-08-01), pages 1004-1012, XP002550020, ISSN: 0883-0738, DOI: 10.1177/0883073807305667 the whole document	3-21,29, 30,42-44
X	----- WO 2007/002390 A2 (ISIS PHARMACEUTICALS INC [US]; COLD SPRING HARBOR LAB [US]; BAKER BREN) 4 January 2007 (2007-01-04)	45-48
A	examples 6-9; table 6; sequences 50,51	1-21, 29-36, 42-44
A	----- SINGH NIRMAL K ET AL: "Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron", MOLECULAR AND CELLULAR BIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 26, no. 4, 1 February 2006 (2006-02-01), pages 1333-1346, XP002596112, ISSN: 0270-7306 abstract; figures 1,4	1-21, 29-36, 42-44
A	----- MOULTON JON D ET AL: "Gene knockdowns in adult animals: PPMOs and vivo-morpholinos", MOLECULES, MOLECULAR DIVERSITY PRESERVATION INTERNATIONAL, BASEL, CH, vol. 14, no. 3, 25 March 2009 (2009-03-25), pages 1304-1323, XP002547034, ISSN: 1420-3049, DOI: 10.3390/MOLECULES14031304 page 1309; table 2	1-21, 29-36, 42-44
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/043894

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BENOIT KANZLER ET AL: "Morpholino oligonucleotide-triggered knockdown reveals a role for maternal E-cadherin during early mouse development", MECHANISMS OF DEVELOPMENT, vol. 120, no. 12, 1 December 2003 (2003-12-01), pages 1423-1432, XP55038479, ISSN: 0925-4773, DOI: 10.1016/j.mod.2003.09.008 the whole document	1-21, 29-36, 42-44
X	Y. HUA ET AL: "Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model", GENES & DEVELOPMENT, vol. 24, no. 15, 1 August 2010 (2010-08-01), pages 1634-1644, XP55038360, ISSN: 0890-9369, DOI: 10.1101/gad.1941310 the whole document	45-48
A		1-21, 29-36, 42-44
A	SUZAN M. HAMMOND ET AL: "Mouse Survival Motor Neuron Alleles That Mimic SMN2 Splicing and Are Inducible Rescue Embryonic Lethality Early in Development but Not Late", PLOS ONE, vol. 5, no. 12, 1 January 2010 (2010-01-01), page e15887, XP55038361, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0015887 the whole document	1-21, 29-36, 42-44
A	M. D. MCCAULEY ET AL: "Animal models of arrhythmogenic cardiomyopathy", DISEASE MODELS & MECHANISMS, vol. 2, no. 11-12, 1 November 2009 (2009-11-01), pages 563-570, XP55038483, ISSN: 1754-8403, DOI: 10.1242/dmm.002840 table 1	1-21, 29-36, 42-44
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/043894

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ROBINSON BLAINE W ET AL: "Zebrafish Mll Depletion Model Phenocopies Mammalian Mll Depletion and implicates MLL as Master Regulator of Novel Developmental Hematopoietic Targets", BLOOD; 51ST ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY, AMERICAN SOCIETY OF HEMATOLOGY, US; NEW ORLEANS, LA, USA, vol. 114, no. 22, 20 November 2009 (2009-11-20), pages 1409-1410, XP008156358, ISSN: 0006-4971 the whole document</p>	1-21, 29-36, 42-44
T	<p>-----</p> <p>AHASHI KENTARO ET AL: "TSUNAMI: an antisense method to phenocopy splicing-associated diseases in animals", YEAST MPH1 HELICASE DISSOCIATES RAD51-MADE D-LOOPS: IMPLICATIONS FOR CROSSOVER CONTROL IN MITOTIC RECOMBINATION,, vol. 26, no. 16, 15 August 2012 (2012-08-15), pages 1874-1884, XP008156416, ISSN: 1549-5477 the whole document</p>	1-21, 29-36, 42-44
X	<p>-----</p> <p>PASSINI MARCO A ET AL: "Antisense Oligonucleotides Delivered to the Mouse CNS Ameliorate Symptoms of Svere Spinal Muscular Atrophy", SCIENCE / SCIENCE TRANSLATIONAL MEDICINE, WASHINGTON, DC : AAAS, US, vol. 3, no. 72, 2 March 2011 (2011-03-02), pages 58-68, XP009161512, ISSN: 1946-6242 page 59, right-hand column; figures 2,3,5 page 65, right-hand column</p>	45-48
X	<p>-----</p> <p>J. H. WILLIAMS ET AL: "Oligonucleotide-Mediated Survival of Motor Neuron Protein Expression in CNS Improves Phenotype in a Mouse Model of Spinal Muscular Atrophy", JOURNAL OF NEUROSCIENCE, vol. 29, no. 24, 17 June 2009 (2009-06-17), pages 7633-7638, XP55044377, ISSN: 0270-6474, DOI: 10.1523/JNEUROSCI.0950-09.2009 page 7634, left-hand column; figures 2,3</p>	45-48
X	<p>-----</p> <p>US 2007/292408 A1 (SINGH RAVINDRA N [US] ET AL) 20 December 2007 (2007-12-20) example 7</p> <p>-----</p> <p style="text-align: center;">-/--</p>	45-48

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/043894

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>KHOO BERNARD ET AL: "Splicing therapeutics in SMN2 and APOB", CURRENT OPINION IN MOLECULAR THERAPEUTICS, THOMSON REUTERS (SCIENTIFIC) LTD, vol. 11, no. 2, 1 April 2009 (2009-04-01), pages 108-115, XP008158072, ISSN: 2040-3445 the whole document</p>	45-48
A	<p>A. H. M. BURGHEES ET AL: "Antisense oligonucleotides and spinal muscular atrophy: skipping along", GENES & DEVELOPMENT, vol. 24, no. 15, 1 August 2010 (2010-08-01), pages 1574-1579, XP55044388, ISSN: 0890-9369, DOI: 10.1101/gad.1961710 figure 7</p>	45-48
A	<p>CLARIBEL D WEE ET AL: "The genetics of spinal muscular atrophies", CURRENT OPINION IN NEUROLOGY, vol. 23, no. 5, 1 October 2010 (2010-10-01), pages 450-458, XP55038503, ISSN: 1350-7540, DOI: 10.1097/WCO.0b013e32833e1765 the whole document</p>	45-48

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/043894

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007002390	A2	04-01-2007	
		EP 1910395 A2	16-04-2008
		EP 2548560 A1	23-01-2013
		US 2010216238 A1	26-08-2010
		WO 2007002390 A2	04-01-2007

US 2007292408	A1	20-12-2007	
		US 2007292408 A1	20-12-2007
		US 2010087511 A1	08-04-2010
		US 2012165394 A1	28-06-2012

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-21, 29-36, 42-44

A method of producing a non-human animal that phenocopies a human disease caused by altered splicing of a human gene, referred to as a human disease gene, comprising administering splicing modulating antisense oligonucleotides (ASOs) that exacerbate mis- splicing of the human disease gene to a non-human animal comprising (a) the human disease gene or (b) an endogenous gene corresponding to the human disease gene, wherein the synthetic ASOs are administered in sufficient quantity and via a route effective to produce an animal that phenocopies the human disease.

2. claims: 22-28(partially)

A synthetic antisense oligonucleotide (ASO) that is complementary to and hybridizes to a mis-splicing site on human SMN2 pre-mRNA and inhibits splicing of exon 7 of SMN2 gene when injected intracerebroventricularly in a mild "type III" SMA nonhuman neonate, wherein the ASO comprises 5'-GTGAGCACCTTCCTTCTT-3' (SEQ ID NO. 10).

3. claims: 22-28(partially)

A synthetic antisense oligonucleotide (ASO) that is complementary to and hybridizes to a mis-splicing site on human SMN2 pre-mRNA and inhibits splicing of exon 7 of SMN2 gene when injected intracerebroventricularly in a mild "type III" SMA nonhuman neonate, wherein the ASO comprises 5'-GGAATGTGAGCACCTTCCTT-3' (SEQ ID NO.: 11).

4. claims: 37-41

A synthetic ASO that (a) base pairs with high specificity to a cis - element on a pre-mRNA of a gene that encodes a protein that promotes small nuclear ribonucleoprotein particle (snRNP) assembly and (b) interferes with binding of a splicing modulator which is a splicing activator or a splicing repressor.

5. claims: 45-48

A method of treating spinal muscular atrophy (SMA) in a mammal, comprising introducing into the mammal, via intracerebroventricular (ICV) injection, MOE ASO that correct mis-splicing of SMN2, in an amount sufficient to restore correct splicing of SMN2 and produce sufficient functional SMN2 protein to treat SMA in the mammal.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210
