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

(57) **Abstract:** The invention relates to the treatment and prevention of cancer by administering agents that inhibit the activity of microRNAs that modulate tumor suppressor genes, which can include PTEN, p53, and INPP4B, among others. Inhibitors can include oligonucleotides that are at least partially complementary to these miRNAs. In some embodiments, these inhibitors are chemically modified oligonucleotides, including locked nucleic acids (LNAs).



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/033359

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/113 A61K31/713
 ADD. C07H21/00 A61P35/00

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, BIOSIS, EMBASE, Sequence Search, EMBL, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/056737 A2 (MIRNA THERAPEUTICS INC [US]; PATRAWALA LUBNA [US]; TANG DEAN G; KELNAR) 20 May 2010 (2010-05-20) pages 85,94 - page 95; claim 1 -----	1-4,6-26
X	WO 2010/066384 A1 (MAX PLANCK GESELLSCHAFT [DE]; UNIV REGENSBURG [DE]; MEISTER GUNTER [DE]) 17 June 2010 (2010-06-17) claims 13,14; examples 3,4 -----	1-4,6-26
X	LI MA ET AL: "miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis", NATURE CELL BIOLOGY, 1 January 2010 (2010-01-01), XP055022319, ISSN: 1465-7392, DOI: 10.1038/ncb2024 page 253, right-hand column - page 254, left-hand column; figure 4 ----- -/-	1-4,6-26



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

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Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 243 833 A1 (UNIV ERASMUS MEDICAL CT [NL]) 27 October 2010 (2010-10-27) the whole document	1-4,6-26
A	----- ZHANG J G ET AL: "MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC)", CLINICA CHIMICA ACTA, ELSEVIER BV, AMSTERDAM, NL, vol. 411, no. 11-12, 3 June 2010 (2010-06-03), pages 846-852, XP027011490, ISSN: 0009-8981 [retrieved on 2010-03-16] abstract; figures 2-6	1-4,6-26
A	----- US 2009/239818 A1 (CHENG JIN Q [US]) 24 September 2009 (2009-09-24) figures 2-4; example 1	1-4,6-26
A	----- PARK JONG-KOOK ET AL: "Antisense inhibition of microRNA-21 or -221 arrests cell cycle, induces apoptosis, and sensitizes the effects of gemcitabine in pancreatic adenocarcinoma", PANCREAS, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 38, no. 7, 1 October 2009 (2009-10-01), pages e190-e199, XP008156538, ISSN: 1536-4828 abstract; figure 2; table 1	1-4,6-26
A	----- JASON T HUSE ET AL: "The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo", GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, PLAINVIEW, NY, US, vol. 23, no. 11, 1 June 2009 (2009-06-01), pages 1327-1337, XP002653515, ISSN: 0890-9369, DOI: 10.1101/GAD.1777409 [retrieved on 2011-07-26] figure 1	1-4,6-26
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/033359

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAURA POLISENO ET AL.: "Identification of the miR-106b similar to 25 MicroRNA Cluster as a Proto-Oncogenic PTEN-Targeting Intron That Cooperates with Its Host Gene MCM7 in Transformation", SCIENCE SIGNALING, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, US, vol. 3, no. 117, 13 April 2010 (2010-04-13), pages ra29-1, XP008156437, ISSN: 1937-9145, DOI: 10.1126/SCISIGNAL.2000594 figures 2,3 -----	1-4,6-26
A	TAN HAO-XIANG ET AL: "MicroRNA-9 reduces cell invasion and E-cadherin secretion in SK-Hep-1 cell", MEDICAL ONCOLOGY, vol. 27, no. 3, 1 September 2010 (2010-09-01), pages 654-660, XP055038553, ISSN: 1357-0560, DOI: 10.1007/s12032-009-9264-2 figure 3; table 1 -----	1-4,6-26
A	YANHUI LOU ET AL.: "MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein", INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE, vol. 26, no. 6, 21 October 2010 (2010-10-21), XP055038528, ISSN: 1107-3756, DOI: 10.3892/ijmm_00000530 abstract; figures 5-7 -----	1-4,6-26
A	WAN HAI-YING ET AL: "Regulation of the transcription factor NF-κB1 by microRNA-9 in human gastric adenocarcinoma", MOLECULAR CANCER, BIOMED CENTRAL, LONDON, GB, vol. 9, no. 1, 26 January 2010 (2010-01-26), page 16, XP021067965, ISSN: 1476-4598 the whole document ----- -/--	1-4,6-26

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/033359

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAIOS ALEXANDROS ET AL: "Potential role of miR-9 and miR-223 in recurrent ovarian cancer", MOLECULAR CANCER, BIOMED CENTRAL, LONDON, GB, vol. 7, no. 35, 28 April 2008 (2008-04-28) , pages 1-14, XP002526990, ISSN: 1476-4598, DOI: 10.1186/1476-4598-7-35 figure 2; example I -----	1-4,6-26
A	LAURA POLISENO ET AL: "A coding-independent function of gene and pseudogene mRNAs regulates tumour biology", NATURE, vol. 465, no. 7301, 24 June 2010 (2010-06-24), pages 1033-1038, XP055038449, ISSN: 0028-0836, DOI: 10.1038/nature09144 figure 1 -----	1-4,6-26
A	ACHIM AIGNER: "MicroRNAs (miRNAs) in cancer invasion and metastasis: therapeutic approaches based on metastasis-related miRNAs", JOURNAL OF MOLECULAR MEDICINE, vol. 89, no. 5, 14 January 2011 (2011-01-14), pages 445-457, XP055038556, ISSN: 0946-2716, DOI: 10.1007/s00109-010-0716-0 table 1 -----	1-4,6-26
A	NICK R LESLIE ET AL: "Non-genomic loss of PTEN function in cancer: not in my genes", TRENDS IN PHARMACOLOGICAL SCIENCES, vol. 32, no. 3, March 2011 (2011-03), pages 131-140, XP028170469, ISSN: 0165-6147, DOI: 10.1016/J.TIPS.2010.12.005 [retrieved on 2010-12-20] page 136, right-hand column - page 137, left-hand column -----	1-4,6-26

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.:
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.:

1-4, 6-26(all partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-4, 6-26(all partially)

A method of treating or preventing cancer in a subject in need thereof comprising administering to the subject an inhibitor of miRNA, wherein the miRNA is a PTEN regulator, wherein the miRNA is miR-9 (SEQ ID NO: 1); related pharmaceutical compositions.

2-67. claims: 1-26(partially)

A method of treating or preventing cancer in a subject in need thereof comprising administering to the subject an inhibitor of miRNA, wherein the miRNA is a PTEN regulator, wherein in each separate invention the miRNA is selected from a group consisting of miR-15b (SEQ ID NO: 2), miR-93 (SEQ ID NO: 3), miR-106a (SEQ ID NO: 4), miR-106b (SEQ ID NO: 5), miR-17-5p (SEQ ID NO: 6), miR-20a (SEQ ID NO: 7), miR-20b (SEQ ID NO: 8), miR-19a (SEQ ID NO: 9), miR-19b (SEQ ID NO: 10), miR-21 (SEQ ID NO: 11), miR-22 (SEQ ID NO: 12), miR-23a (SEQ ID NO: 13), miR-23b (SEQ ID NO: 14), miR-25 (SEQ ID NO: 15), miR-92a (SEQ ID NO: 16), miR-92b (SEQ ID NO: 17), miR-26a (SEQ ID NO: 18), miR-32 (SEQ ID NO: 19), miR-136 (SEQ ID NO: 20), miR-141 (SEQ ID NO: 21), miR-144 (SEQ ID NO: 22), miR-182 (SEQ ID NO: 23), miR-193b (SEQ ID NO: 24), miR-205 (SEQ ID NO: 25), miR-214 (SEQ ID NO: 26), miR-216a (SEQ ID NO: 27), miR-217 (SEQ ID NO: 28), miR-221 (SEQ ID NO: 29), miR-222 (SEQ ID NO: 30), miR-302a (SEQ ID NO: 31), miR-302b (SEQ ID NO: 32), miR-302c (SEQ ID NO: 33), miR-302d (SEQ ID NO: 34), miR-302c (SEQ ID NO: 35), miR-302f (SEQ ID NO: 36), miR-330-5p (SEQ ID NO: 37), miR-363 (SEQ ID NO: 38), miR-367 (SEQ ID NO: 39), miR-372 (SEQ ID NO: 40), miR-373 (SEQ ID NO: 41), miR-375 (SEQ ID NO: 42), miR-486 (SEQ ID NO: 43), miR-494 (SEQ ID NO: 44), miR-512-3p (SEQ ID NO: 45), miR-518c * (SEQ ID NO: 46), miR-518e (SEQ ID NO: 47), miR-519a (SEQ ID NO: 48), miR-519b-3p (SEQ ID NO: 49), miR-519c (SEQ ID NO: 50), miR-519d (SEQ ID NO: 51), miR-520a (SEQ ID NO: 52), miR-520b (SEQ ID NO: 53), miR-520c (SEQ ID NO: 54), miR-520d (SEQ ID NO: 55), miR-520e (SEQ ID NO: 56), miR-550a (SEQ ID NO: 57), miR-625 (SEQ ID NO: 58), miR-659 (SEQ ID NO: 59), miR-663 (SEQ ID NO: 60), miR-671-5p (SEQ ID NO: 61), miR-760 (SEQ ID NO: 62), miR-877 (SEQ ID NO: 63), miR-1225-5p (SEQ ID NO: 64), miR-1299 (SEQ ID NO: 65), miR-1913 (SEQ ID NO: 66) and miR-23* (SEQ ID NO: 67); related pharmaceutical compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/033359

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010056737 A2	20-05-2010	US 2010179213 A1 WO 2010056737 A2	15-07-2010 20-05-2010
WO 2010066384 A1	17-06-2010	EP 2358902 A1 US 2012053224 A1 WO 2010066384 A1	24-08-2011 01-03-2012 17-06-2010
EP 2243833 A1	27-10-2010	CA 2759597 A1 CN 102421899 A EA 201101531 A1 EP 2243833 A1 EP 2421968 A1 JP 2012524758 A KR 20110138414 A US 2012088811 A1 WO 2010122109 A1	28-10-2010 18-04-2012 30-05-2012 27-10-2010 29-02-2012 18-10-2012 27-12-2011 12-04-2012 28-10-2010
US 2009239818 A1	24-09-2009	NONE	