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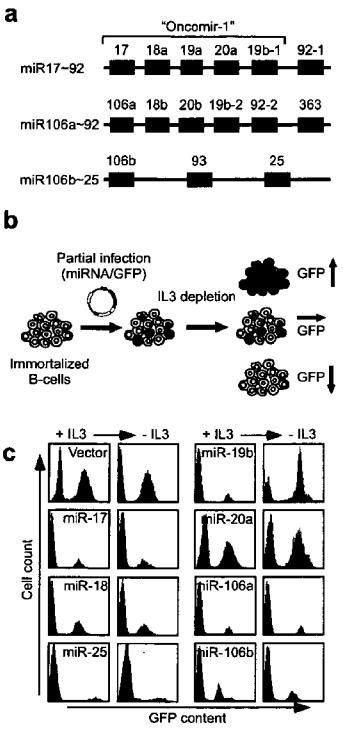
*[Continued on next page]*

(54) Title: METHODS AND COMPOSITIONS FOR THE DETECTION AND TREATMENT OF CANCER INVOLVING MIRNAS AND MIRNA INHIBITORS AND TARGETS

Figure 1




(57) Abstract: The present invention relates to microRNAs (miRNAs) which are associated with cancer, particularly including hematologic malignancies, and particularly T-cell acute lymphoblastic leukemia (T-ALL), and to the assessment and modulation thereof in the treatment and management of cancer. The present invention is directed to methods and compositions for diagnosing and treating cancer, particularly T-ALL, by modulating miRNAs, and the use of miRNAs and antagonists thereof, particularly antagomirs, for predicting and assessing response to treatment, in assays for isolating and selecting antagists, and as compositions for the treatment and management of cancer. Methods and compositions are provided for treatment or alleviation of cancer, particularly T-ALL, with antagonists/antagomirs of miRNAs, particularly one or more of miR-19b, miR-20a, miR26, miR92, miR148 and miR223.

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

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/00365

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/04; C12N 15/11 (2011.01)
USPC - 536/24.5; 514/44A
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; C12N 15/11 (2011.01)
USPC - 536/24.5; 514/44A

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)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: screening, inhibitor, antagonist, treating, prevent, hematological, cancer, tumor, Haematologica, malignancy, lymphoma, leukemia, antagonist, miR-19, miR-26, miR-17-92 cluster, T-ALL, Pten, phosphatase and tensin homolog deleted on chromosome ten, Bim, Bcl2L11, Bcl2-like11, Phf6, PHD finger protein 6

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 2007/0072204 A1 (HANNON et al.) 29 March 2007 (29.03.2007) Abstract, para [0009], [0015], [0022], [0035], [0037], [0040]-[0041], [[0044]-[0045], [0064]-[0065], [0069], [0075], [0097], and [0100] | 1-2, 15-16, 24-25<br>---<br>3-4 |
| X<br>---<br>Y | US 2009/0143326 A1 (OBAD et al.) 04 June 2009 (04.06.2009) para [0004], [0011], [0019], [0034], [0067], [0096], [0174], [0186], [0231], [0234], [0243], [0260], [0263], [0270], [0273], and [0410] | 14, 18, 20-21<br>---<br>17, 19 |
| X | US 2010/0029003 A1 (BARTEL et al.) 04 February 2010 (04.02.2010) para [0019], [0024], [1276], [1337], and [1530] | 22-23 |
| Y | US 2005/0261218 A1 (ESAU et al.) 24 November 2005 (24.11.2005) para [0839] | 3 |
| Y | US 2005/0256072 A1 (ARONIN et al.) 17 November 2005 (17.11.2005) para [0091], [0111], and Table I | 4 |
| Y | US 2009/0131356 A1 (BADER et al.) 21 May 2009 (21.05.2009) para [0011], [0018], [0061], [0126], and [0145] | 17, 19 |
| A | HUSE et al. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev. 2009, 23:1327-1337 | 1-4, 14-25 |

☒ 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 |
|---|---|
| 30 July 2011 (30.07.2011) | 05 AUG 2011 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 11/00365

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | OLIVE et al. miR-19 is a key oncogenic component of mir-17-92. Leukemia 2005, 19(11):2013-6 | 1-4, 14-25 |
| A, P | MAVRAKIS et al. Genome-wide RNAi screen identifies miR-19 targets in Notchinduced acute T-cell leukaemia (T-ALL). Nat Cell Biol. ePub 28 February 2010, 12(4): 372-379 | 1-4, 14-25 |
| A | PEZZOLESI et al. Differential Expression of PTEN-Targeting MicroRNAs miR-19a and miR-21 in Cowden Syndrome. Am J Hum Genet. 2008, 82(5):1141-9 | 1-4, 14-25 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/00365

**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:

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.

Group I+: claims 1-4, drawn to a method for identifying a compound for treatment or prevention of a hematological malignancy, wherein said compound antagonizes or inhibits the expression or activity of an miRNA associated with said malignancy. The first invention is restricted to miR-19. Should an additional fee(s) be paid, Applicant is invited to elect an additional miRNA(s) to be searched and/or binding moieties to be searched. The exact claims searched will depend on Applicant's election.

Group II, claims 5-13, drawn to a method for detecting or evaluating a hematological malignancy in a mammal.

**********Continued in the extra 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 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.:
1-4, 14-25, limited to miR-19 and miR-26

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

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********** Supplemental Box **********

Continuation of: Box No III (unity of invention is lacking)

Group III+, claims 14-25, drawn to a composition for use in treatment of a hematological malignancy comprising one or more antagomir which is complementary to one or more miRNA. The first invention is restricted to antagomir which is complementary to miR-19. Should an additional fee(s) be paid, Applicant is invited to elect an additional antagomir(s) to be searched and/or binding moieties to be searched. The exact claims searched will depend on Applicant's election.

The inventions listed as Groups I+ through III+ 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:

The inventions of Groups I+ and II do not include the inventive concept of a composition for use in treatment of a hematological malignancy comprising one or more antagomir which is complementary to one or more miRNA, as required by Group III+.

The inventions of Groups I+ and III+ do not include the inventive concept of a method for identifying a compound for treatment or prevention of a hematological malignancy, as required by Group II.

The inventions of Groups II-III+ do not include the inventive concept of a method for identifying a compound for treatment or prevention of a hematological malignancy, as required by Group I+.

The inventions of Group I+ share the technical feature of a method for identifying a compound for treatment or prevention of a hematological malignancy, wherein said compound antagonizes or inhibits the expression or activity of an miRNA associated with said malignancy comprising: (a) contacting a compound with a cell expressing one or more miRNA, and (b) determining the expression or activity of one or more of said miRNA; wherein the expression or activity of said miRNA is inhibited or reduced. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2007/0072204 A1 to HANNON et al. (hereinafter 'Hannon') that discloses a method for identifying a compound for treatment or prevention of a hematological malignancy (Abstract - 'screening methods to identify compounds and reagents useful in cancer ... prevention, and therapy'; para [0044] - 'Preferred cancers for treatment using methods provided herein include B-cell malignancies, lymphomas, ... or leukemia', wherein "B-cell malignancies, lymphomas, ... or leukemia" each is a hematological malignancy),
---wherein said compound antagonizes or inhibits the expression or activity of an miRNA associated with said malignancy (para [0009] - 'a method of identifying an inhibitor of the mir17-92 cistron'; para [0041] - 'The term "a mir17-92 cistron inhibitor", ... refers to an agent that decreases the expression and/or activity of one or more miRNAs encoded by the mir17-92 cistron'; para [0065] - 'A decrease in the level of one or more miRNA members of the mir17-92 cistron in the test level indicates that the candidate agent is an inhibitor of the mir17-92 cistron and a potential cancer therapeutic agent') comprising:
(a) contacting a compound with a cell expressing one or more miRNA selected from miR-19 and miR-20 (para [0009] - 'a) contacting a candidate agent with a cancer cell expressing one or more miRNAs encoded by the mir17-92 cistron'; para [0041] - 'The term "a mir17-92 cistron inhibitor", ... refers to an agent that decreases the expression and/or activity of one or more miRNAs encoded by the mir17-92 cistron, which include ...miR-19a, miR-20, miR-19b-1'); and
(b) determining the expression or activity of one or more of said miRNA (para [0009] - 'b) determining the activity of one or more miRNAs encoded by the mir17-92 cistron in the cell, thereby generating data for a test activity level');
---wherein the expression or activity of said miRNA is inhibited or reduced (para [0009] - c) comparing the test activity level of each miRNA encoded by the mir17-92 cistron ... wherein a decrease in the test activity level of one or more miRNAs encoded by the mir17-92 cistron indicates that the candidate agent is an inhibitor of the mir17-92 cistron'; para [0037] - 'Inhibition of the mir-19b miRNA level encoded by the mir17-92 cistron using an antisense oligonucleotide'; para [0040] - 'the cancer therapeutic and preventative methods of the present invention decrease the expression and/or activity of miR-19b-1'). As said method was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Group III+ share the technical feature of a composition for use in treatment of a hematological malignancy comprising one or more antagomir which is complementary to one or more miRNA. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2009/0143326 A1 to OBAD et al. (hereinafter 'Obad') that discloses a composition for use in treatment of a hematological malignancy (para [0231] - 'oligonucleotide ... for the manufacture of a medicament for the treatment of a disease associated with the expression of microRNAs selected from... lymphocytic leukemia', wherein leukemia is a hematological malignancy; para [0273] - 'the use of an oligonucleotide a... for the manufacture of a medicament for the treatment of a disease selected from ... lymphoma', wherein lymphoma is a hematological malignancy)
--- comprising one or more antagomir which is complementary to one or more miRNA selected from miR-19 and miR-20 (para [0096] - 'the oligomers ... may consist of or comprise a contiguous nucleotide sequence which is complementary to a corresponding microRNA sequence selected from the group consisting of ... miR-19a, miR-19b, miR-20', wherein " contiguous nucleotide sequence which is complementary to a corresponding microRNA sequence" are antagomir of microRNA; para [0034] - 'Antagonism of miR-19b by a fully LNA-modified 8-mer (SEQ ID #3222) LNA-antimiR and a 15-mer (SEQ ID #3229) mixmer antimiR'; para [0410] - 'transfection of the 15-mer LNA-antimiR SEQ ID #3229 into HeLa efficiently antagonizes miR-19a ...transfection of the 8-mer LNA-antimiR SEQ ID #3222 resulted in effective miR-19a antagonism'; Specification: para [0004] - 'miRNA function can transiently be antagonized by antagomirs-chemically modified oligonucleotides complementary to individual miRNAs'). As said composition was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Finally, another special technical feature of the inventions listed as Groups I+ and III+ is the miRNA recited therein. As no significant structural similarities can readily be ascertained among the miRNAs, the inventions do not share a special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

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