(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 August 2012 (02.08.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/103355 A3

(51) International Patent Classification:
G01N 33/48 (2006.01)

(21) International Application Number:
PCT/US2012/022756

(22) International Filing Date:
26 January 2012 (26.01.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/397,729	26 January 2011 (26.01.2011)	US
61/436,399	26 January 2011 (26.01.2011)	US
61/525,008	18 August 2011 (18.08.2011)	US

(71) Applicant *(for all designated States except US)*: **CEPHEID** [US/US]; 904 Caribbean Drive, Sunnyvale, California 94089 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MICHOT, Bernard** [FR/FR]; Cepheid Europe, S.A. Vira Solelh, F-81470 Maurens-Scopont (FR). **DELFOUR, Olivier** [FR/FR]; Cepheid Europe, S.A. Vira Solelh, F-81470 Maurens-Scopont (FR).

(74) Agent: **SCARR, Rebecca B.**; Casimir Jones, S.C., 2275 Deming Way, Ste 310, Middleton, Wisconsin 53562 (US).

(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.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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

(88) Date of publication of the international search report:
13 March 2014

WO 2012/103355 A3

(54) Title: METHODS OF DETECTING LUNG CANCER

(57) Abstract: Methods of detecting lung cancer, such as non-small cell lung cancer, including squamous cell carcinoma and adenocarcinoma, are provided. Methods of detecting changes in the levels of one or more small RNAs associated with lung cancer are also provided. Compositions and kits are also provided.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/22756

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2012.01)
USPC - 436/64; 435/6.1, 435/6.14
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): G01N 33/48 (2012.01)
USPC: 436/64; 435/6.1, 435/6.14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.11, 435/6.12; 436/94

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, Google Scholar: lung, cancer$2, RNA, small, U2, lung cancer, UGUCUUUCCUUGUUGGAGCAG, 13207, 13207-R, TGACCTGTCCTGTTCTGCCCCCAGCCCCTCACAGTGCT, GCCCTGACCTGTCCTGTTCT, GCCCTGACCTGTCCTGTTC, TGTAGAGCAGGGAGCAGGAAG, TGTAGAGCAGGGAGCAGGAAG, TGTAGAGCAGGGAGCAGGAA, TGTAGAGCAGGGAGCAGG, T

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	US 2010/0233704 A1 (MICHOT et al.) 16 September 2010 (16.09.2010) abstract, para [0008][0011]; [0050]; [0060]; [0057]	1-4, 14, 18-20, 34 and 35 --- 5, 8, 10, 15, 16, 36, 37 and 41
Y -- A	KOTAKE et al. "Splicing factor SF3b as a target of the antitumor natural product pladeinolide" Nture Chem. Biol.; September 2007; Vol. 3, No. 9; pg. 570-575. pg. 570, col 1, para 1; pg. 571, col 1, para 2	1-4, 14, 18-20, 34 and 35 --- 5, 8, 10, 15, 16, 36, 37 and 41
A	PERSSON et al., Identification of New MicroRNAs in Paired Normal and Tumor Breast Tissue Suggests a Dual Role for the ERBB2/Her2 Gene, Cancer Res, 2 January 2011, Vol. 71, pages 78-86, para [0013], SEQ ID NO: 35	5, 8, 10, 15, 16, 36, 37 and 41
A, P	WATAHIKI et al. "MicroRNAs Associated with Metastatic Prostate Cancer" PLOS One; 30 September, 2011; Vol. 6, No. 9; e24950; pg. 1-13 especially Table 4.	10

☐ 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
26 June 2012 (26.06.2012)	11 JUL 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/22756

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.: 21-33
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:
Claim 21 is unsearchable under Annex C of the administrative instructions. In particular, the applicant failed to submit a valid CRF in response to the ISA/225 of 01 February 2012. Therefore, no searchable sequence was available to the ISA in an acceptable format. Claims 22-33 are dependent from claim 21.

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. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: claims 1-20 and 34-38, directed to a method for detecting the presence of, facilitating diagnosis of, or monitoring therapy of lung cancer in a subject, comprising detecting the level of at least one RNA selected from small U2, miR-720, miR-451, 13207, and 13750, in a sample from the subject, wherein detection of a level of small U2 that is greater than a normal level of small U2, or detection of a level of at least one RNA selected from 13207, miR-720, miR-451, and 13750 that is less than a normal level of the respective RNA, indicates the presence of lung cancer in the subject; wherein the first invention is limited to the detection of an increase in small U2 relative to a normal level (claims 1-4, 14, 18-20, 34 and 35). (Applicants may opt for additional markers to be searched by specifying the marker and paying an additional invention search fee for each elected marker).

- Please see extra sheet for continuation -

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.:
1-20 and 34-38, directed to markers small U2, 13207 and 13750 - claims 1-5, 8, 10, 14-16, 18-20, and 34-37

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/US 12/22756

Continuation of Box III: Lack of Unity of Invention

Groups II+: claims 39 and 41, directed to compositions comprising an oligonucleotide that comprises at least eight contiguous nucleotides complementary to an RNA selected from small U2, miR-720, miR-451, 13207, and 13750; wherein the first invention is limited to small U2 (Applicants may opt for additional markers to be searched by specifying the marker and paying an additional invention search fee for each elected marker).

Groups III+: claims 40 and 41, directed to compositions comprising an oligonucleotide that comprises at least eight contiguous nucleotides complementary to a cDNA reverse transcribed from an RNA selected from small U2, miR-720, miR-451, 13207, and 13750; wherein the first invention is limited to small U2. (Applicants may opt for additional markers to be searched by specifying the marker and paying an additional invention search fee for each elected marker).

The inventions listed as Groups I+ - 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 special technical features of the claims of Groups I+ to III+ are disclosed above in the Group descriptions.

The only common technical elements shared by all of the above groups is that they are related to any of small U2, miR-720, miR-451, 13207 or 13750. The claims of Groups I+ share the further common technical element of being related to a method for detecting the presence of, facilitating diagnosis of, or monitoring therapy of lung cancer in a subject, comprising detecting the level of at least one RNA selected from small U2, miR-720, miR-451, 13207, and 13750, in a sample from the subject, wherein detection of a level of small U2 that is greater than a normal level of small U2, or detection of a level of at least one RNA selected from 13207, miR-720, miR-451, and 13750 that is less than a normal level of the respective RNA, indicates the presence of lung cancer in the subject; the special technical features of the Group II+ claims share a further common technical element of being related to compositions comprising an oligonucleotide that comprises at least eight contiguous nucleotides complementary to an RNA selected from small U2, miR-720, miR-451, 13207, and 13750; the claims of Groups III+ share the common technical element of being related to compositions comprising an oligonucleotide that comprises at least eight contiguous nucleotides complementary to a cDNA reverse transcribed from an RNA selected from small U2, miR-720, miR-451, 13207, and 13750. These common technical elements do not represent an improvement over the prior art of US 2010/0233704 A1 to Michot et al., which discloses methods of detecting lung cancer in a sample from a patient, wherein one or more target RNAs changes in expression in lung cancer (abstract), methods of detecting the presence of lung cancer in a subject are provided. In some embodiments, a method comprises detecting a level of at least one target RNA in a sample from the subject. (para [0008]), "a decreased level of one or more target RNAs...in a sample is indicative of the presence of lung cancer in an individual from whom the sample has been taken (para [0070]); further wherein the decreased RNA may be MiR-720 or MiR-451 (miR-451; Table 23). Michot further teaches wherein target RNAs can be measured in samples collected at one or more times from a patient to monitor the status or progress of lung cancer in the patient (para [0050]). Although Michot does not specifically recite wherein said collection at one or more times may be used in assessing the progress of therapy for the cancer, it would have been obvious to a person skilled in the art to perform said monitoring, as taught by Michot, during therapy, in order to determine whether a therapy should be continued, modified, or discontinued. Additionally, Michot teaches compositions comprising an oligonucleotide that is complementary to an RNA selected from miR-720, and miR-451 (para [0192]), or cDNA reverse transcribed therefrom (para [0193]). Michot further teaches wherein the probes comprise at lest 8 (15) contiguous nucleotides complementary to the target sequence (para [0194]).

Although Michot does not specifically disclose wherein miR-720 decreases in lung cancer, Michot teaches the assessment of the expression of said marker (Table 3), and thus further includes said marker in the compositions disclosed in paras [0192]-[0914], as above. Additionally, addressing the common technical element shared by Groups I+ to III+ regarding 13207, US 2005/0227917 A1 to Garcia et al. teaches polynucleotides differentially expressed in cancer cells (abstract), including 13207 (Table 89) thus the existence of 13207, and nucleic acid sequences relating thereto, do not represent an improvement over the prior art. Further, addressing the common technical element shared by Groups I+ to III+ regarding the existence of small U2, the article entitled "A Fragile site in the Human U2 Small Nuclear RNA Gene Cluster Is Revealed by Adenovirus Type 12 Infection" by Durnam et al. discloses the existence of small U2 (typically referred to in the prior art as "U2 small nuclear RNA"), as well as nucleic acid sequences related thereto (the U2 small nuclear RNA gene cluster mapped very close to and was frequently disrupted by the gaps and breaks induced specifically in human 17q21-q22 region by highly oncogenic adenovirus type 12; abstract). Therefore the existence of small U2, and nucleic acid sequences relating thereto, do not represent an improvement over the prior art. Finally, regarding 13750, the prior art does not specifically recite said number as an identifiable nomenclature for a nucleic acid sequence. However, the article entitled "Identification of New MicroRNAs in Paired Normal and Tumor Breast Tissues Suggests a Dual Role for the EBB2/Her2 Gene" by Persson et al. discloses a nucleic acid sequence that is 100% identical to the sequence indicated by the instant specification (para [0013], SEQ ID NO: 35)(miR-4732, see supplemental material S4). Thus said sequence also does not represent an improvement over the prior art. Although neither Garcia, Durnam, nor Persson specifically discloses oligonucleotides complementary to said sequences, nor cDNA reverse transcribed from said sequences, it would have been obvious to a person skilled in the art to combine the teaching of Michot regarding oligonucleotides complementary to and cDNA reverse transcribed from sequences associated with cancer with the teachings of Garcia, Durnam and Persson, each of which also individually indicates an association between the sequences and cancer, to provide nucleic acid sequences useful for probes to detect the levels of said nucleic acid in samples from patients to assess the potential for the association of each sequence with a particular cancer, such as lung cancer.

Therefore, the inventions of Groups I - III lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.