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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 November 2008 (27.11.2008)

PCT

(10) International Publication Number
WO 2008/143979 A3

(51) International Patent Classification:
*A01K 67/027* (2006.01) *C12N 15/85* (2006.01)
*C12N 15/11* (2006.01)

(21) International Application Number:
PCT/US2008/006293

(22) International Filing Date: 16 May 2008 (16.05.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/930,532 16 May 2007 (16.05.2007) US
61/065,139 8 February 2008 (08.02.2008) US

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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, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*

*[Continued on next page]*

(54) **Title:** TUMOR SUPPRESSOR GENE SCREENING USING RNA INTERFERENCE LIBRARIES AND METHOD OF TREATMENT

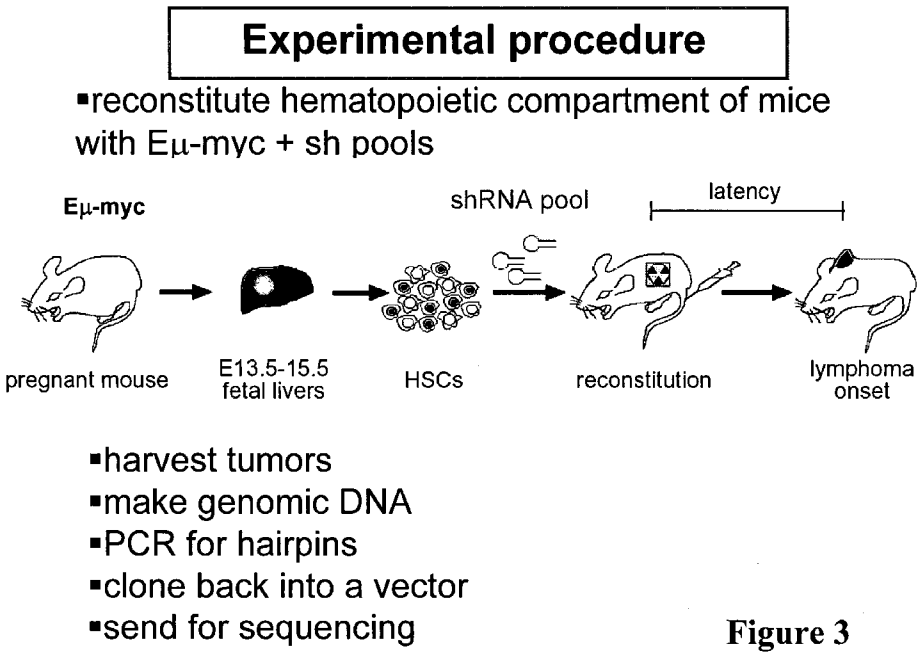


**Figure 3**

(57) **Abstract:** The present invention is directed to methods of identifying tumor suppressor genes in vivo, tumor suppressors thus found, methods of treatment taking advantage of the identified tumor suppressors, methods of and kits for diagnosis of cancer using the identified tumor suppressor, and pharmaceutical composition comprising an identified tumor suppressor or modulators thereof.

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

**(88) Date of publication of the international search report:**
19 March 2009

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/006293

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K67/027 C12N15/11 C12N15/85

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 practical, search terms used)
EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2004/029219 A (COLD SPRING HARBOR LAB [US]; FRIDMAN JORDAN [US]; HANNON GREGORY J [US) 8 April 2004 (2004-04-08) pages 18,30; figures 13,20; example 7 | 1-3 |
| X | WO 2007/053184 A (COLD SPRING HARBOR LAB [US]; DICKINS ROSS [US]; HANNON GREGORY J [US];) 10 May 2007 (2007-05-10) page 46, line 11 - line 20; claim 23 page 53, line 2 - line 5 page 3, line 28 - page 4, line 1 | 1-3 |

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[X] Further documents are listed in the continuation of Box C.

[X] 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 document 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)

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"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 |
|---|---|
| 6 August 2008 | 19/01/2009 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Rutz, Berthold |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/006293

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WENDEL HG: "In vivo RNAi library screen to identify mediators of disease progression and drug resistance in CML" REPORT FOR U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND, [Online] no. W81XWH-04-1-0803, September 2006 (2006-09), XP002491251 Retrieved from the Internet: URL:http://handle.dtic.mil/100.2/ADA469370 > [retrieved on 2008-08-06] page 5 pages 8-11 | 1-3 |
| A | HEMANN MICHAEL T ET AL: "An epi-allelic series of p53 hypomorphs created by stable RNAi produces distinct tumor phenotypes in vivo" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 33, no. 3, 1 March 2003 (2003-03-01), pages 396-400, XP002449244 ISSN: 1061-4036 figure 2 | 1-3 |
| A | DICKINS R A ET AL: "Probing tumor phenotypes using stable and regulated synthetic microRNA precursors" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 37, no. 11, 1 November 2005 (2005-11-01), pages 1289-1294, XP002430682 ISSN: 1061-4036 | |
| A | XUE WEN ET AL: "Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas." NATURE 8 FEB 2007, vol. 445, no. 7128, 8 February 2007 (2007-02-08), pages 656-660, XP002491252 ISSN: 1476-4687 | |
| A | STEGMEIER FRANK ET AL: "A lentiviral microRNA-based system for single-copy polymerase II-regulated RNA interference in mammalian cells" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 102, no. 37, September 2005 (2005-09), pages 13212-13217, XP002482035 ISSN: 0027-8424 | |

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# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/006293

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | BERNS KATRIEN ET AL: "A large-scale RNAi screen in human cells identifies new components of the p53 pathway." NATURE 25 MAR 2004, vol. 428, no. 6981, 25 March 2004 (2004-03-25), pages 431-437, XP003002475 ISSN: 1476-4687 | |
| A | SILVA J M ET AL: "Second-generation shRNA libraries covering the mouse and human genomes" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 37, no. 11, 2 October 2005 (2005-10-02), pages 1281-1288, XP002399751 ISSN: 1061-4036 | |
| A | PADDISON PATRICK J ET AL: "A resource for large-scale RNA-interference-based screens in mammals." NATURE 25 MAR 2004, vol. 428, no. 6981, 25 March 2004 (2004-03-25), pages 427-431, XP002992273 ISSN: 1476-4687 | |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/006293

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b 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. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] on paper

   [X] in electronic form

   c. time of filing/furnishing

   [X] contained in the international application as filed

   [X] filed together with the international application in electronic form

   [ ] furnished 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 allsearchable 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 reportcovers 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.:

see additional sheet(s)

**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: 1-3

method of identifying a novel tumor suppressor gene by identifying siRNAs from a library which knock-down tumor suppressor genes in a mouse tumor model (murine hematopoietic stem cells expressing Myc)

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Invention 2: claims 4-24 (all partially)

method of treating cancer by increasing the activity of the target tumor suppressor gene Mek1 (SEQ ID NO: 1) idenitified in a screen for tumor suppressor genes having decreased activity in cancerous tissue, by administering a therapeutic agent known to be effective in cancer associated with increased or decreased activity of Mek1, by administering a therapeutic gene known to not interfere with or antagonize decreased activities of Mek1, pharmaceutical compositions, method for identifying a therapeutic agent by contacting a mouse model which expresses an shRNA against the tumor suppressor gene Mek1 (SEQ ID NO:1) with a candidate agent and monitoring the mouse, method for identifying a candidate therapeutic agent in vitro by using cells expressing shRNA against the tumor suppressor gene Mek1 (SEQ ID NO:1) method of diagnosing a cancer by determining activity or expression of Mek1 in a sample

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Invention 3-47: claims 4-24 (all partially)

method of treating cancer by increasing the activity of the target tumor suppressor gene Rad17 (SEQ ID NO:2), Angpt2 (SEQ ID NO: 3), Numb (SEQ ID NO: 4),..., ATR (SEQ ID NO: 46) idenitified in a screen for tumor suppressor genes having decreased activity in cancerous tissue, by administering a therapeutic agent known to be effective in cancer associated with increased or decreased activity of said tumor suppressor gene, by administering a therapeutic gene known to not interfere with or antagonize decreased activities of said tumor suppressor gene, pharmaceutical compositions, method for identifying a therapeutic agent by contacting a mouse model which expresses an shRNA against said tumor suppressor gene with a candidate agent and monitoring the mouse, method for identifying a candidate therapeutic agent in vitro by using cells expressing shRNA against said tumor suppressor gene, method of diagnosing a cancer by determining activity or expression of said tumor suppressor gene in a sample

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/006293

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2004029219 A | 08-04-2004 | AU 2003283976 A1 | 19-04-2004 |
| | | CA 2499188 A1 | 08-04-2004 |
| | | EP 1546397 A2 | 29-06-2005 |
| | | US 2008226553 A1 | 18-09-2008 |
| | | US 2008025958 A1 | 31-01-2008 |
| WO 2007053184 A | 10-05-2007 | CA 2610265 A1 | 10-05-2007 |
| | | EP 1896587 A2 | 12-03-2008 |