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(71) Applicant (for all designated States except US):  
RIKSHOSPITALET- RADIUMHOSPITALET HF  
[NO/NO]; Oslo, N-0027 (NO).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SKOTHEIM,  
Rolf I. [NO/NO]; Gaustadveien 12C, N-0372 Oslo (NO).  
LOTHE, Ragnhild A. [NO/NO]; Erling Skjalgssonsgt  
23E, N-0267 Oslo (NO). LIND, Guro E. [NO/NO];  
Pilotveien 10, N-0770 Oslo (NO). AHLQUIST, Terje C.  
[NO/NO]; Klaus Torgårs vei 12A, N-0372 Oslo (NO).

(74) Agent: PLOUGMANN & VINGTOFT A/S; Sundkrogs-  
gade 9, DK-COPENHAGEN 2100 (DK).

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(54) Title: NEW MARKERS FOR CANCER

(57) Abstract: The present invention relates to novel markers for hypermethylation of gene promoters in cancers. In particular the present invention relates to a method of determining whether a tumour is developing in the aero-digestive system, or whether a subject is relapsing after treatment of such a tumour. The method comprises determining the methylation level, the number of methylated CpG sites or the methylation state of CpG sites in a nucleic acid sequence in the promoter region, first exon or intron, of one or more genes selected from the group consisting of CNRIP1, MAL, FBN1, SPG20, SNCA, and INA. The method further relates to a diagnostic kit for detecting tumours in the aero-digestive tract.

WO 2008/102002 A3

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2008/052156

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C12Q1/68

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)  
C12Q

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, WPI Data, EMBASE, BIOSIS, MEDLINE, 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	WO 2004/110246 A (ILLUMINA INC [US]; FAN JIAN-BING [US]; BIBIKOVA MARINA [US]) 23 December 2004 (2004-12-23) crip1 = CNRIP1 page 58	1-19
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X	US 2007/015156 A1 (GOGGINS MICHAEL G [US]) 18 January 2007 (2007-01-18) page 23; claims 1-3; table 3	1-19
X	US 2006/003359 A1 (FEINBERG ANDREW P [US] ET AL) 5 January 2006 (2006-01-05) CRIP = CNRIP1claims 1-10	1-19
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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 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)
- \*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.
- \*Z\* document member of the same patent family

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European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl;  
Fax: (+31-70) 340-3016

Authorized officer

Gabriels, Jan

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	US 2003/113750 A1 (DISTLER JUERGEN [DE] ET AL) 19 June 2003 (2003-06-19) claim 1; table 1	1-19
X	WO 2006/135886 A (UNIV MICHIGAN [US]; CLARKE MICHAEL F [US]; WANG XINHAO [US]; LEWICKI J) 21 December 2006 (2006-12-21) discloses CRIP1 (=CNRIP1), FBN1, SNCA, AND INA claim 1; tables 5,7A,7C,17A,17C	1-19
X	WO 2005/005601 A (UNIV MICHIGAN [US]; CLARKE MICHAEL F [US]; LIU RUI [US] CLARKE MICHAEL) 20 January 2005 (2005-01-20) discloses CRIP1 (= CNRIP1), TAHCCP1 (= SPG20), FBN1, SNCA, and INA page 32, line 16 page 49, line 27 page 30, line 2 page 40, line 66 page 39, line 74	1-19
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X	WO 01/49879 A (AROS APPLIED BIOTECHNOLOGY APS [DK]; OERNTOFT TORBEN F [DK]; THYKJAER) 12 July 2001 (2001-07-12) NACP (=SNCA) page 65; claims 1-4	1-19

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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.
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X	US 2005/181375 A1 (AZIZ NATASHA [US] ET AL) 18 August 2005 (2005-08-18) MGC12702 (=INA) involved in lung metastases to the brain page 81, line 15; table 6a page 153, line 17; table 12a	1-19
A	MORI ET AL: "A Genome-Wide Search Identifies Epigenetic Silencing of Somatostatin, Tachykinin-1, and 5 Other Genes in Colon Cancer" GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, US, vol. 131, no. 3, September 2006 (2006-09), pages 797-808, XP005659138 ISSN: 0016-5085 cited in the application the whole document	1-19
A	WO 02/070742 A (EPIGENOMICS AG [DE]) 12 September 2002 (2002-09-12) the whole document	1-19
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; April 2007 (2007-04), MORI YURIKO ET AL: "The PKC binding protein gene NELL1 and the T-cell differentiation protein gene MAL are hypermethylated in gastric cancer." XP002452608 Database accession no. PREV200700303890 the whole document & PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 48, April 2007 (2007-04), page 271, 98TH ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-FOR-CANCER-RESEARCH; LOS ANGELES, CA, USA; APRIL 14 -18, 2007 ISSN: 0197-016X	1-19

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/052156

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/047787 A (EXACT SCIENCES CORP [US]; SHUBER ANTHONY P [US]) 4 May 2006 (2006-05-04) the whole document	1
A	"Abstracts" BRITISH JOURNAL OF ORAL AND MAXILLOFACIAL SURGERY, CHURCHILL LIVINGSTONE, EDINBURGH, GB, vol. 44, no. 5, October 2006 (2006-10), pages 428-474, XP005610720 ISSN: 0266-4356 page 442, left-hand column, last paragraph	1
A	SHAW R J ET AL: "Promoter methylation of P16, RAR beta, E-cadherin, cyclin A1 and cytoglobin in oral cancer: quantitative evaluation using pyrosequencing" BRITISH JOURNAL OF CANCER, vol. 94, no. 4, February 2006 (2006-02), pages 561-568, XP002452604 ISSN: 0007-0920 the whole document	1

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2008/052156

## 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 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.:

### 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:

**Invention 1: claims 1-19 (all partially)**

Diagnostic kits comprising nucleic acids derived from CNRIP1, primers thereto, antibodies specifically recognising methylated nucleic acid sequences thereof and their use in methods for determining whether a subject has developed, is developing or is predisposed for developing cancer, or whether a subject is relapsing after treatment of such a cancer by determining the methylation state of CpG sites in the nucleic acid sequence in the promoter region/sequence of said gene.

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**Invention 2: claims 1-19 (all partially)**

Diagnostic kits comprising nucleic acids derived from SPG20, primers thereto, antibodies specifically recognising methylated nucleic acid sequences thereof and their use in methods for determining whether a subject has developed, is developing or is predisposed for developing cancer, or whether a subject is relapsing after treatment of such a cancer by determining the methylation state of CpG sites in the nucleic acid sequence in the promoter region/sequence of said gene.

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**Invention 3: claims 1-19 (all partially)**

Diagnostic kits comprising nucleic acids derived from FBN1, primers thereto, antibodies specifically recognising methylated nucleic acid sequences thereof and their use in methods for determining whether a subject has developed, is developing or is predisposed for developing cancer, or whether a subject is relapsing after treatment of such a cancer by determining the methylation state of CpG sites in the nucleic acid sequence in the promoter region/sequence of said gene.

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**Invention 4: claims 1-19 (all partially)**

Diagnostic kits comprising nucleic acids derived from SNCA, primers thereto, antibodies specifically recognising methylated nucleic acid sequences thereof and their use in methods for determining whether a subject has developed, is developing or is predisposed for developing cancer, or whether a subject is relapsing after treatment of such a cancer by determining the methylation state of CpG sites in the nucleic acid sequence in the promoter region/sequence of said gene.

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**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Invention 5: claims 1-19 (all partially)

Diagnostic kits comprising primers for INA and methods using these for determining whether a subject has developed, is developing or is predisposed for developing a tumour in the aero-digestive system, or whether a subject is relapsing after treatment of such a tumour by determining the methylation state of CpG sites in a nucleic acid sequence in the promoter region/sequence of one or more genes.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2008/052156

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2008/052156

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