



- (51) **International Patent Classification:**  
*C12Q 1/68* (2006.01)
- (21) **International Application Number:**  
PCT/EP2012/051663
- (22) **International Filing Date:**  
1 February 2012 (01.02.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
11152864.2 1 February 2011 (01.02.2011) EP
- (71) **Applicants** (for all designated States except US):  
**AZIENDA OSPEDALIERA UNIVERSITARIA SENESE** [IT/IT]; Strada delle Scotte 14, I-53100 Siena (IT). **CENTRO DI RIFERIMENTO ONCOLOGICO - ISTITUTO NAZIONALE TUMORI - AVIANO** [IT/IT]; Via Franco Gallini 2, I-33081 Aviano (PN) (IT).

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))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

- (88) **Date of publication of the international search report:**  
28 February 2013

- (72) **Inventors; and**
- (75) **Inventors/Applicants** (for US only): **SIGALOTTI, Luca** [IT/IT]; Via dei Meassi 55/C, I-33085 Maniago (PN) (IT). **MAIO, Michele** [IT/IT]; Via U. Foscolo 7, I-33080 Roveredo in Piano (PN) (IT).
- (74) **Agents:** **CAPASSO, Olga** et al.; Via Vincenzo Bellini, 20, I-00198 Rome (IT).
- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,



WO 2012/104340 A3

(54) **Title:** MARKERS OF MELANOMA AND USES THEREOF

(57) **Abstract:** The invention relates to a method for determining prognosis, predicting or monitoring response to therapy of patients affected by melanoma through the evaluation of the methylation of different genomic DNA sequences, including the Long Interspersed Nucleotide Element- 1 (LINE-1) repetitive DNA sequences and/or the concomitant methylation profile of specific groups of genes defined as methylation signature.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2012/051663

## 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, 8-10, 17-19, 27, 28(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.

INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2012/051663

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C12Q1/68  
ADD.  
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 practicable, search terms used)  
EPO-Internal, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y  A	WO 2008/134596 A2 (WAYNE JOHN CANCER INST [US]; HOON DAVE S B [US]; SUNAMI EIJI [US]) 6 November 2008 (2008-11-06) abstract claims 14,16 page 7; figures 30,31 page 4 - page 5; figures 2,3 page 1 page 5 page 6  -----  -/--	27,28  1,8-10, 17-19

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

Date of the actual completion of the international search  22 August 2012	Date of mailing of the international search report  10/01/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Celler, Jakub

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2012/051663

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LAHTZ CHRISTOPH ET AL: "Methylation of PTEN as a prognostic factor in malignant melanoma of the skin.", THE JOURNAL OF INVESTIGATIVE DERMATOLOGY FEB 2010 LNKD- PUBMED:19798057, vol. 130, no. 2, February 2010 (2010-02), pages 620-622, XP002636328, ISSN: 1523-1747 abstract	1,8-10, 17-19, 27,28
Y	----- TELLEZ CARMEN S ET AL: "CpG island methylation profiling in human melanoma cell lines", MELANOMA RESEARCH,, vol. 19, no. 3, 1 June 2009 (2009-06-01), pages 146-155, XP009148010, ISSN: 1473-5636, DOI: DOI:10.1097/CMR.0B013E32832B274E abstract page 153, column 2	1,8-10, 17-19, 27,28
Y	----- MORI TAKUJI ET AL: "Predictive utility of circulating methylated DNA in serum of melanoma patients receiving biochemotherapy.", JOURNAL OF CLINICAL ONCOLOGY : OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY 20 DEC 2005 LNKD- PUBMED:16361635, vol. 23, no. 36, 20 December 2005 (2005-12-20), pages 9351-9358, XP002636329, ISSN: 0732-183X abstract figure 1; table 2	1,8-10, 17-19, 27,28
A	----- JOHN THOMAS ET AL: "Predicting clinical outcome through molecular profiling in stage III melanoma.", CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 AUG 2008 LNKD- PUBMED:18698035, vol. 14, no. 16, 15 August 2008 (2008-08-15), pages 5173-5180, XP002636330, ISSN: 1078-0432 abstract	1,8-10, 17-19, 27,28
	----- -/--	

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2012/051663

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	E. MERHAVI ET AL: "Promoter Methylation Status of Multiple Genes in Uveal Melanoma", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 48, no. 10, 1 October 2007 (2007-10-01), pages 4403-4406, XP055023384, ISSN: 0146-0404, DOI: 10.1167/iovs.07-0272 abstract page 445, column 2	1,8-10, 17-19, 27,28
Y	WO 2009/086472 A2 (WAYNE JOHN CANCER INST [US]; HOON DAVE S B [US]; TANEMURA ATSUSHI [JP]) 9 July 2009 (2009-07-09) abstract; claim 1	1,8-10, 17-19, 27,28
Y	KORABIOWSKA MONIKA ET AL: "Analysis of adenomatous polyposis coli gene expression, APC locus-microsatellite instability and APC promoter methylation in the progression of melanocytic tumours", MODERN PATHOLOGY, NATURE PUBLISHING GROUP, US, vol. 17, no. 12, 1 December 2004 (2004-12-01), pages 1539-1544, XP002489097, ISSN: 0893-3952, DOI: 10.1038/MODPATHOL.3800238 [retrieved on 2004-07-16] abstract	1,8-10, 17-19, 27,28
Y	HUNTER W. RICHARDS ET AL: "Epigenetic marks in melanoma", PIGMENT CELL & MELANOMA RESEARCH, vol. 22, no. 1, 1 February 2009 (2009-02-01), pages 14-29, XP055023516, ISSN: 1755-1471, DOI: 10.1111/j.1755-148X.2008.00534.x abstract page 10, column 2 page 19, column 1	1,8-10, 17-19, 27,28
A	US 2003/129602 A1 (HUANG TIM HUI-MING [US]) 10 July 2003 (2003-07-10)  abstract page 16; table 1	1,8-10, 17-19, 27,28

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/051663

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO 2008134596	A2	06-11-2008	AU 2008245546 A1	06-11-2008
			CA 2685376 A1	06-11-2008
			EP 2147123 A2	27-01-2010
			EP 2339022 A2	29-06-2011
			US 2009068660 A1	12-03-2009
			US 2011151465 A1	23-06-2011
			US 2011159501 A1	30-06-2011
			US 2011159502 A1	30-06-2011
			US 2011159510 A1	30-06-2011
			WO 2008134596 A2	06-11-2008
-----				
WO 2009086472	A2	09-07-2009	AU 2008345036 A1	09-07-2009
			CA 2710520 A1	09-07-2009
			EP 2238265 A2	13-10-2010
			US 2009220980 A1	03-09-2009
			US 2011212444 A1	01-09-2011
			WO 2009086472 A2	09-07-2009
-----				
US 2003129602	A1	10-07-2003	US 6605432 B1	12-08-2003
			US 7807358 B1	05-10-2010
			US 2003129602 A1	10-07-2003
-----				

**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, 8-10, 17-19, 27, 28(all partially)

The subject matter of the above claim as characterised by the constellation of at least 16 genes comprising the gene first mentioned in claim 1, i. e. ABCA8.

---

2-206. claims: 1-28(partially)

The subject matter of the above claim as characterised by the constellation of at least 16 genes as characterised by that they comprise each of the genes mentioned in claim 1, starting with ABC4 through to ZNF558, respectively.

---