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[Continued on next page]

(54) Title: COLON CANCER GENE EXPRESSION SIGNATURES AND METHODS OF USE

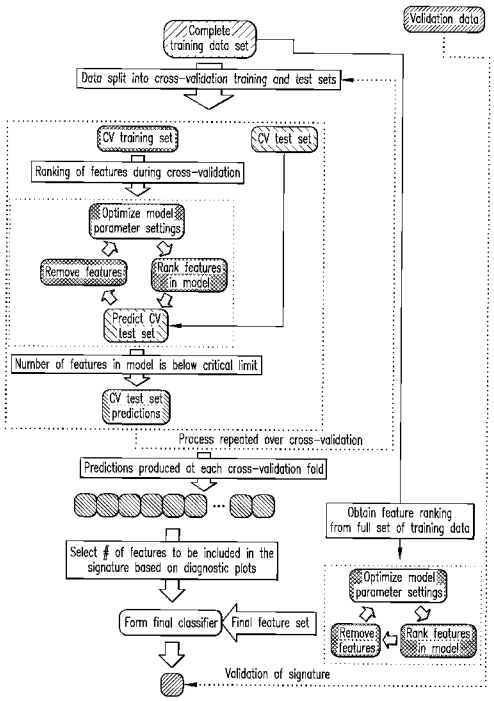

FIG. 1

(57) Abstract: A gene expression signature of colon cancer, microarrays including them and methods of using the colon gene expression signature are provided. The gene expression signature is especially useful for determining the prognosis of a patient diagnosed with colon cancer, such as stage II colon cancer. The gene signature described herein is also useful for determining effectiveness of surgical resection with or without adjuvant chemotherapy, and determining possibility of cancer recurrence in patients with colon cancer.

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Published:

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

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11 October 2012

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/22594

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48; C07H 21/00; C12Q 1/68 (2012.01)
USPC - 435/6.1, 6.11, 6.12
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; C07H 21/00; C12Q 1/68 (2012.01)
USPC - 435/6.1, 6.11, 6.12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 436/94; 536/24.3

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (PGPB,USPT,USOC,EPAB,JPAB); PubMed (MEDLINE)
colon cancer, expression, biomarker, diagnosis, classification

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7,695,913 B2 (COWENS et al.) 13 April 2010 (13.04.2010) col 1, ln 34-47; col 1, ln 51 - col 2, ln 8; col 3, ln 7-25; col 7, ln 26-37; col 11, ln 35-41; col 12, ln 38 - col 13, ln 3	1-5
A	US 2010/0261169 A1 (WALLACH et al.) 14 October 2010 (14.10.2010)	1-5
A	US 2006/0003359 A1 (FEINBERG et al.) 5 January 2006 (05.01.2006)	1-5

☐ 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
03 July 2012 (03.07.2012)	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 12/22594

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.: 6, 7, 11, 18-27, 36, 43-53 and 57-67
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 CONTINUATION 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.:

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.:
Claims 1 - 5

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 12/22594

Continuation of Box III: Observations where unity of invention is lacking

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.

Group I: claims 1-5, directed to a method of diagnosing or classifying colon cancer in a sample from a subject, comprising: i) detecting an expression level of at least 2 colon cancer-related nucleic acid molecules in Table 6 in a sample comprising nucleic acids obtained from a subject; and ii) comparing the expression of the at least 2 colon cancer-related nucleic acid molecules, or a decision score derived therefrom, to a control threshold indicative of a diagnosis of colon cancer, wherein the expression level, or a decision score derived therefrom, on the same side of the threshold indicates a diagnosis of colon cancer, thereby diagnosing colon cancer in the sample obtained from the subject.

Group II: claims 8-10, directed to a method for predicting a response to a treatment for colon cancer, comprising: a) detecting an expression level of at least 2 colon cancer-related nucleic acid molecules listed in Table 6 in a sample comprising nucleic acids obtained from a subject; and b) comparing the expression level of the at least 2 colon cancer-related nucleic acid molecules, or a decision score derived therefrom to a control threshold indicative of a known response to treatment, wherein the expression level, or a decision score derived therefrom, on the same side of the threshold indicates a similar response to treatment, thereby predicting response to treatment.

Group III: claims 12-14, directed to a method for predicting long term survival of a subject with colon cancer, comprising: 1) detecting an expression level of at least 2 colon cancer-related nucleic acid molecules listed in Table 6 in a sample comprising nucleic acids obtained from a subject; and 2) comparing the expression level of the at least 2 colon cancer-related nucleic acid molecules, or a decision score derived therefrom to a control threshold indicative of having a history of long term survival, wherein the expression level, or a decision score derived therefrom, on the same side of the threshold indicates long term survival of the subject, thereby predicting long term survival of a subject.

Group IV: claims 15 and 16, a method for predicting of recurrence of colon cancer in a subject, comprising: i) detecting an expression level of at least 2 colon cancer-related nucleic acid molecules listed in Table 6 in a sample comprising nucleic acids obtained from a subject; and ii) comparing the expression level of the at least 2 colon cancer-related nucleic acid molecules, or a decision score derived therefrom to a control threshold indicative of a history of recurrence, wherein the expression level, or a decision score derived therefrom, on the same side of the threshold indicates a recurrence in the subject.

Group V: claim 17, directed to a method of preparing a personalized colon cancer genomics profile for a subject, comprising: a) detecting an expression level of at least 2 colon cancer-related nucleic acid molecules listed in Table 6 in a sample comprising nucleic acids obtained from a subject; and b) creating a report summarizing the data obtained by the gene expression analysis.

Groups VI+: claims 28-35, directed to a probe or set thereof for detecting a gene expression signature for colon cancer, comprising or consisting substantially of a nucleic acid molecule between 20 and 40 nucleotides in length, capable of specifically hybridizing to one of the nucleic acid sequences set forth as SEQ ID NOs: 1-636 or its complement; wherein the first invention is limited to the first two probe sequences: SEQ ID NOs: 1 and 2 (claims 28-33)(applicants may opt for additional sequences to be searched by specifying the SEQ ID NO: and paying an additional invention search fee for each elected sequence).

Groups VII+: claims 37-39, directed to a pair of primers for the amplification of a gene expression signature for colon cancer nucleic acid, comprising: 1) a forward primer 15 to 40 nucleotides in length comprising a nucleic acid sequence that specifically hybridizes to any one of the nucleic acid sequences set forth as SEQ ID NOs: 1-636 or its complement; and 2) a reverse primer 15 to 40 nucleotides in length comprising a nucleic acid sequence that specifically hybridizes to any one of the nucleic acid sequences set forth as SEQ ID NOs: 1-636 or its complement, wherein the set of primers is capable of directing the amplification of the nucleic acid; wherein the first invention is limited to primer sets that hybridize and enable amplification of the first two SEQ ID NOs: SEQ ID NO: 1 and SEQ ID NO: 2 (applicants may opt for additional sequences to be searched by specifying the SEQ ID NO: and paying an additional invention search fee for each elected sequence).

Group VIII: claims 40-42 and 54-56, directed to a method for preparing a gene expression profile indicative of colon cancer prognosis, comprising: i) detecting the expression level of less than 1000 transcripts in a sample comprising RNA isolated from a colon cancer specimen, wherein at least 50 transcripts listed in Table 6 are detected.

The inventions listed as Groups I - VIII 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 feature of the claims of Groups I-VIII are indicated in the Group descriptions, above.

********** CONTINUED ON NEXT PAGE **********

INTERNATIONAL SEARCH REPORT

International application No.
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Continuation of Box III: Observations where unity of invention is lacking

The only common technical element shared by all of the above groups is that they are related to nucleic acids associated with colon cancer. Groups I-V and VIII share the further common technical element of being related to determination of the expression of at least two nucleic acid markers associated with colon cancer, wherein Groups I-IV also share the further common technical element wherein the markers are either both upregulated or both downregulated. Groups V and VIII also share the common technical element of being related to gene expression profiles. These common technical elements do not represent an improvement over the prior art of US 7,695,913 B2 to Cowens et al. (hereinafter 'Cowens'), which discloses a method for diagnosing colon cancer in a sample obtained from a subject (abstract), comprising: detecting an expression level of at least 2 colon cancer-related nucleic acid molecules in a sample (col 7, ln 26-37) comprising nucleic acids obtained from a subject (col 3, ln 7-25); and comparing the expression level of the at least 2 colon cancer-related nucleic acid molecules (col 7, ln 26-37) to a control threshold (col 12, ln 38 - col 13, ln 3) indicative of a diagnosis of colon cancer (col 12, ln 38 - col 13, ln 3), thereby diagnosing colon cancer in the sample obtained from the subject (col 3, ln 7-25). Although Cowens does not explicitly recite wherein the expression level, or a decision score derived therefrom, on the same side of the threshold indicates a diagnosis of colon cancer, Cowens teaches wherein "evidence of increased expression of one or more of the genes listed in Table 1.2A, 2.2A, 3.2A, 4.2A and/or 5.2A, or the corresponding expression product, indicates a decreased likelihood of a positive clinical outcome" (col 8, ln 9-38). It would have been obvious to a person skilled in the art to apply the requirement for two or more genes to be increased in expression, based on the teaching of Cowens. Further, Cowens teaches the use of Gene expression profiling (col 7, ln 16-20). Further, Groups VI+ and VII+ share the common technical elements of being related to oligonucleotide probes and primer pairs which hybridize to marker nucleic acids for the detection and amplification of marker nucleic acids. These common technical elements also do not improve upon the prior art of Cowens (col 17, ln 43 - col 18, ln 14). Although Cowens does not explicitly recite wherein the oligonucleotides comprise between 15 and 40 nucleotides in length, Cowens teaches wherein the oligonucleotides are short polynucleotides (col 12, ln 27-37). It would have been obvious to a person skilled in the art to use oligonucleotide primers and probes in the claimed size ranges based on the specific annealing temperatures and base compositions used in order to assure specific binding to the target sequences.

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