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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 February 2022 (24.02.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/040106 A3

(51) International Patent Classification:
C12Q 1/6886 (2018.01) *C12Q 1/6876* (2018.01)
C12Q 1/6883 (2018.01) *C12Q 1/6806* (2018.01)

(21) International Application Number:
PCT/US2021/046186

(22) International Filing Date:
16 August 2021 (16.08.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/066,269 16 August 2020 (16.08.2020) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US).

(72) Inventor: **GOODARZI, Hani**; 600 16th Street, GH S312D, San Francisco, CA 94158 (US).

(74) Agent: **ZURAWSKI, John A.** et al.; Ballard Spahr LLP, 1735 Market Street, 51st Floor, Philadelphia, PA 19103 (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, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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, KM, 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))*

(88) Date of publication of the international search report:
31 March 2022 (31.03.2022)

(54) Title: SYSTEM AND METHODS OF DETECTION OF ONCRNAS FOR CANCER DIAGNOSIS

(57) Abstract: The present disclosure relates generally to detection of non-coding RNA molecules in a sample or diagnosis of a subject based upon detection or quantification of non-coding RNA molecules in a sample of the subject, specifically to identify and use of molecular biomarkers for cancer diagnosis.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/6886; C12Q 1/6883; C12Q 1/6876; C12Q 1/6806 (2021.01)

CPC - C12Q 1/6886; C12Q 1/6883; C12Q 1/6876; C12Q 1/6806; C12Q 2500/00; C12Q 2600/16; C12Q 2600/178

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2019/094780 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 16 May 2019; paragraphs [0069], [0092], [0144]; Claims 1, 21, 30, 40, 43, 45, 46	1, 19-21, 30-31, 41, 43/41, 46, 50, 54, 58-63 --- 2-3, 32, 42, 43/42, 47-48, 51-52, 55-56, 64
Y	(SHARMA, E et al.) The Characteristics and Trends in Adrenocortical Carcinoma: A United States Population Based Study. Journal of Clinical Medical Research. August 2018, Epub 27 June 2018, Vol. 10, No. 8; pages 636-640; abstract; DOI: 10.14740/jocmr3503w.	2-3, 48, 52, 56
Y	(LUO, SS et al.) Genome-wide analysis to identify a novel microRNA signature that predicts survival in patients with stomach adenocarcinoma. Journal of Cancer. 17 October 2019, Vol. 10, No. 25; pages 6298-6313; abstract; DOI: 10.7150/jca.33250	32, 42, 43/42, 47, 48/47, 51, 52/51, 55, 56/55, 64
T	(SIGMA-ALDRICH) MISSION(R) Synthetic microRNA Inhibitor, Human. Webpage (online). Merck KGaA. 15 August 2021 [retrieved on 4 February 2022]. Retrieved from the Internet: [URL: https://www.sigmaaldrich.com/US/en/product/sigma/hstud0987]; page 1	32, 42, 43/42, 47, 51, 55, 64

☐ 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
"D" document cited by the applicant in the international application
"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
04 February 2022 (04.02.2022)	FEB 23 2022
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. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of 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.
PCT/US21/46186

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.: 4-18,22-29,33-40,44-45,49,53,57
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:
-***-Please See Supplemental Page-***-

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.:
Group I+, Claims 1-3, 19-21, 30-32, 41-43, 46-48, 50-52, 54-56, 58-69; adrenal gland cancer (cancer); SEQ ID NO: 1 (non-coding RNA sequence)

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.

-***-Continued From Box No. 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 1-3, 19-21, 30-32, 41-43, 46-48, 50-52, 54-56, 58-69, adrenal gland cancer (cancer), and SEQ ID NO: 1 (non-coding RNA sequence) are directed towards methods and systems for detection of non-coding RNA molecules in a sample or diagnosis of cancer in a subject based upon detection or quantification of non-coding RNA molecules in a sample of the subject.

The method and systems of Claims 1, 2-3 (each in-part), 19-21, 30-31, 32 (in-part), 41, 42-43 (each in-part), 46, 47-48 (each in-part), 50, 51-52 (each in-part), 54, 55-56 (each in-part), 58-63, and 64 (in-part) are believed to encompass the first named invention of Group I+ and are the claims that will be searched without fee to the extent that they encompass adrenal gland cancer (first exemplary cancer) and SEQ ID NO: 1 (first exemplary non-coding RNA sequence).

Applicant is invited to elect additional cancer(s) and non-coding RNA sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and where available as an option within at least one searchable claim, to be searched. Additional cancer(s) and non-coding RNA sequence(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected cancer(s) and non-coding RNA sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be bile duct cancer (cancer) and SEQ ID NO: 2 (non-coding RNA sequence).

No technical features are shared between the cancers and non-coding RNA sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a method of diagnosing a subject with a benign, pre-malignant, or malignant hyperproliferative cell, said method comprising: detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject; a method of detecting a cancer cell in a subject comprising: detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample by contacting the sample with one or a plurality of probes comprising nucleotide sequences complementary to the at least one non-coding RNA sequences; a method of diagnosing a subject with a cancer, comprising: a) detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject by contacting the sample with one or a plurality of probes specific for the at least one non-coding RNA or functional fragment thereof; and b) diagnosing the subject as having the cancer if the presence or quantity of the at least one non-coding RNA or functional fragment thereof is detected in the sample; a method of treating a subject in need thereof diagnosed with or suspected of having a cancer, comprising: a) contacting one or a plurality of probes specific for at least one non-coding RNA or functional fragment thereof with a sample from the subject; b) quantifying the amount of the at least one non-coding RNA or functional fragment thereof in the sample; c) calculating one or more scores based upon the presence, absence, or quantity of the at least one non-coding RNA or functional fragment thereof; d) correlating the one or more scores to the presence, absence, or quantity of the at least one non-coding RNA or functional fragment thereof, such that, if the amount of the at least one non-coding RNA or functional fragment thereof is greater than the quantity of the at least one non-coding RNA or functional fragment thereof in a control sample; or, if the amount of the at least one non-coding RNA or functional fragment thereof is substantially equal to the quantity of the at least one non-coding RNA or functional fragment thereof in a sample taken from a subject known to have a cancer, then the subject is diagnosed as having the cancer; and e) administering to the subject a therapeutically effective amount of treatment for the cancer; a system comprising: a) a sample; b) one or a plurality of probes and/or stains that bind to at least one non-coding RNA or functional fragment thereof; and c) one or more devices capable of quantifying the presence, absence and/or amount of the at least one probe or stain that binds the at least one non-coding RNA and/or functional fragment thereof; a method for characterizing the stage of development or pathology of a sample comprising a hyperproliferative cell, said method comprising: a) contacting a plurality of probes specific for at least one non-coding RNA or functional fragment thereof with the sample; b) quantifying the amount of the at least one non-coding RNA or functional fragment thereof in the sample; c) calculating one or more normalized scores based upon the presence, absence, or quantity of the at least one non-coding RNA or functional fragment thereof; and d) correlating the one or more scores to the quantity of the at least one noncoding RNA or functional fragment thereof, such that if the amount of the at least one non-coding RNA or functional fragment thereof is greater than the quantity of the at least one non-coding RNA or functional fragment thereof in a control sample, the correlating step comprises characterizing the sample as comprising a hyperproliferative cell; a method of determining whether a subject has a malignant growth, said method comprising detecting the presence, absence, or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject by contacting the sample with: a) a probe specific for the at least one non-coding RNA or functional fragment thereof; or b) a substrate specific for the at least one non-coding RNA or functional fragment thereof; a method of processing RNA from a sample of a subject comprising: (i) separating small noncoding RNA in the total RNA from total mRNA (ii) analyzing the small noncoding RNA; a composition comprising any one or plurality of cDNAs; these shared technical features are previously disclosed by WO 2019/094780 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) (hereinafter 'California').

California discloses a method of diagnosing a subject with a benign, pre-malignant, or malignant hyperproliferative cell (a method of diagnosing a subject with a benign, pre-malignant, or malignant hyperproliferative cell; claim 1), said method comprising: detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject (said method comprising: detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject; claim 1); a method of detecting a cancer cell in a subject comprising (a method of detecting a cancer cell in a subject comprising; claim 21): detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample by contacting the sample with one or a plurality of probes comprising nucleotide sequences complementary to the at least one non-coding RNA sequences (detecting the presence of at least one non-coding RNA or functional fragment thereof in a sample by contacting the sample with one or a plurality of probes comprising nucleotide sequences complementary to the at least one non-coding RNA sequences; claim 21); a method of diagnosing a subject with a cancer (a method of diagnosing a subject with a cancer; claim 30), comprising: a) detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from previous Supplemental Box-***-

in a sample of the subject by contacting the sample with one or a plurality of probes specific for the at least one non-coding RNA or functional fragment thereof (detecting the presence, absence, and/or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject by contacting the sample with one or a plurality of probes specific for the at least one non-coding RNA or functional fragment thereof; claims 30, 39); and b) diagnosing the subject as having the cancer if the presence or quantity of the at least one non-coding RNA or functional fragment thereof is detected in the sample (diagnosing the subject as having the cancer if the presence or quantity of the at least one non-coding RNA or functional fragment thereof is detected in the sample; claim 30); a method of treating a subject in need thereof diagnosed with or suspected of having a cancer (a method of treating a subject in need thereof diagnosed with or suspected of having a cancer; claim 39), comprising: a) contacting one or a plurality of probes specific for at least one non-coding RNA or functional fragment thereof with a sample from the subject (comprising: a) contacting one or a plurality of probes specific for at least one non-coding RNA with a sample from the subject; claim 39); b) quantifying the amount of the at least one non-coding RNA or functional fragment thereof in the sample (quantifying the amount of the at least one non-coding RNA in the sample; claim 39); c) calculating one or more scores based upon the presence, absence, or quantity of the at least one non-coding RNA or functional fragment thereof (calculating one or more scores based upon the presence, absence, or quantity of the at least one non-coding RNA; claim 39); d) correlating the one or more scores to the presence, absence, or quantity of the at least one non-coding RNA or functional fragment thereof (correlating the one or more scores to the presence, absence, or quantity of the at least one non-coding RNA; claim 39), such that, if the amount of the at least one non-coding RNA or functional fragment thereof is greater than the quantity of the at least one non-coding RNA or functional fragment thereof in a control sample (such that, if the amount of the at least one non-coding RNA is greater than the quantity of the at least one non-coding RNA in a control sample; claim 39); or, if the amount of the at least one non-coding RNA or functional fragment thereof is substantially equal to the quantity of the at least one non-coding RNA or functional fragment thereof in a sample taken from a subject known to have a cancer, then the subject is diagnosed as having the cancer (then the subject is diagnosed as having the cancer; claim 39); and e) administering to the subject a therapeutically effective amount of treatment for the cancer (administering to the subject a therapeutically effective amount of treatment for the cancer; claim 39); a system comprising (claim 43): a) a sample (claim 43); b) one or a plurality of probes and/or stains that bind to at least one non-coding RNA (one or a plurality of probes and/or stains that bind to at least one non-coding RNA; claim 43) or functional fragment thereof; and c) one or more devices capable of quantifying the presence, absence and/or amount of the at least one probe or stain that binds the at least one non-coding RNA (one or more devices capable of quantifying the presence, absence and/or amount of the at least one probe or stain that binds the at least one non-coding RNA; claim 43) and/or functional fragment thereof; a method for characterizing the stage of development or pathology of a sample comprising a hyperproliferative cell (a method for characterizing the stage of development or pathology of a sample comprising a hyperproliferative cell; claim 45), said method comprising: a) contacting a plurality of probes specific for at least one non-coding RNA (said method comprising: a) contacting a plurality of probes specific for at least one non-coding RNA; claim 45) or functional fragment thereof with the sample; b) quantifying the amount of the at least one non-coding RNA or functional fragment thereof in the sample (quantifying the amount of the at least one non-coding RNA in the sample; claim 45); c) calculating one or more normalized scores based upon the presence, absence, or quantity of the at least one non-coding RNA (calculating one or more normalized scores based upon the presence, absence, or quantity of the at least one non-coding RNA; claim 45) or functional fragment thereof; and d) correlating the one or more scores to the quantity of the at least one noncoding RNA (correlating the one or more scores to the quantity of the at least one noncoding RNA; claim 45) or functional fragment thereof, such that if the amount of the at least one non-coding RNA or functional fragment thereof is greater than the quantity of the at least one non-coding RNA or functional fragment thereof in a control sample (such that if the amount of the at least one non-coding RNA is greater than the quantity of the at least one non-coding RNA in a control sample; claim 45), the correlating step comprises characterizing the sample as comprising a hyperproliferative cell (the correlating step comprises characterizing the sample as comprising a hyperproliferative cell; claim 45); a method of determining whether a subject has a malignant growth (a method of determining whether a subject has a malignant growth; claim 46), said method comprising detecting the presence, absence, or quantity of at least one non-coding RNA or functional fragment thereof in a sample of the subject (said method comprising detecting the presence, absence, or quantity of at least one non-coding RNA in a sample of the subject; claim 46) by contacting the sample with: a) a probe specific for the at least one non-coding RNA or functional fragment thereof (by contacting the sample with: a) a probe specific for the at least one non-coding RNA; claim 46); or b) a substrate specific for the at least one non-coding RNA (a substrate specific for the at least one non-coding RNA; claim 46) or functional fragment thereof; a method of processing RNA from a sample of a subject (a method of processing RNA from a sample of a subject; paragraph [0069]) comprising: (i) separating small noncoding RNA in the total RNA from total mRNA (separating small noncoding RNA in the total RNA from total mRNA; paragraph [0069]) (ii) analyzing the small noncoding RNA (analyzing the small noncoding RNA; paragraph [0069]); a composition comprising any one or plurality of cDNAs (reverse transcription reaction which produces a cDNA sequence (composition comprising cDNA); paragraph [0072]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the California reference, unity of invention is lacking.