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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 July 2024 (18.07.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/151667 A3

(51) International Patent Classification:
C12Q 1/6886 (2018.01) *C12Q 1/6869* (2018.01)
C12Q 1/6806 (2018.01) *G06N 20/00* (2019.01)
C12Q 1/6809 (2018.01) *G16B 20/00* (2019.01)

(21) International Application Number:
PCT/US2024/010932

(22) International Filing Date:
09 January 2024 (09.01.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/437,946 09 January 2023 (09.01.2023) US

(71) Applicant: **CLEARNOTE HEALTH, INC.** [US/US]; 10578 Science Center Drive, Suite 210, San Diego, California 92121 (US).

(72) Inventors: **LEVY, Samuel**; c/o CLEARNOTE HEALTH, INC., 10578 Science Center Drive, Suite 210, SAN DIEGO, California 92121 (US). **GULER, Gulfem Dilek**; c/o CLEARNOTE HEALTH, INC., 10578 Science Center Drive, Suite 210, San Diego, California 92121 (US). **MOGNOL, Giulana P.**; c/o CLEARNOTE HEALTH, INC., 10578 Science Center Drive, Suite 210, SAN DIEGO, California 92121 (US). **NING, Yuhong**; c/o CLEARNOTE HEALTH, INC., 10578 Science Center Drive, Suite 210, San Diego, California 92121 (US).

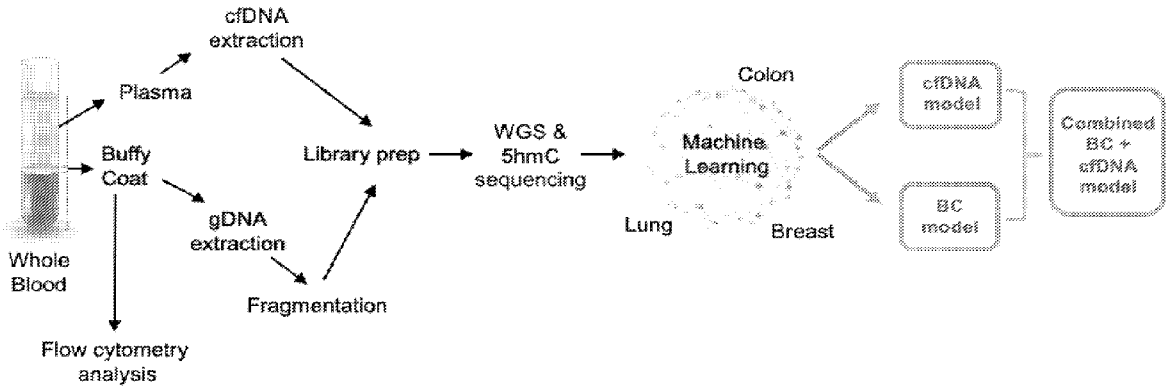
(74) Agent: **REED, Dianne E.**; VLP Law Group LLP, 555 Bryant Street, Suite 820, Palo Alto, California 94301 (US).

(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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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).

(54) Title: 5-HYDROXYMETHYLATION ANALYSIS OF BUFFY COAT GDNA IN CANCER DETECTION

FIG. 2



(57) Abstract: Methods for analyzing peripheral blood samples are provided, wherein buffy coat gDNA is analyzed to provide a buffy coat gDNA hydroxymethylation signature. The buffy coat gDNA hydroxymethylation signature is correlated with the presence of cancer in a patient. Combining the buffy coat gDNA hydroxymethylation signature with another feature value related to the patient increases the predictiveness of the process. In some embodiments, an additional feature value is the cell-free DNA (cfDNA) hydroxymethylation signature in a peripheral blood sample obtained from the same patient. The combination of feature values may be carried out using an ensemble-type analysis such as a stacked ensemble analysis.

WO 2024/151667 A3

[Continued on next page]

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:
22 August 2024 (22.08.2024)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US24/10932

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:
-***-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.:
1-9

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/US24/10932

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12Q 1/6886; C12Q 1/6806; C12Q 1/6809; C12Q 1/6869; G06N 20/00; G16B 20/00 (2023.01)
ADD.

CPC - INV. C12Q 1/6886; C12Q 1/6806; C12Q 1/6809; C12Q 1/6869; G06N 20/00; G16B 20/00; G16B 40/20; G16H 50/20

ADD. C12Q 2535/122; C12Q 2537/164; C12Q 2600/154; G01N 2800/7028

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 database consulted during the international search (name of database 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.
Y	LI ET AL. "5-Hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers" 1243-1257. SPRINGER NATURE | Cell Research | Vol 27 No 10 | October 2017. Web. 2017; abstract; pages 1245-1246, 1253; DOI: 10.1038/cr.2017.121	1-9
Y	STEMCELL TECHNOLOGIES. "How to Prepare a Buffy Coat from Whole Blood" STEMCELL TECHNOLOGIES. Website. March 2020; Retrieved from the Internet on 04/04/2024, URL:<https://www.stemcell.com/how-to-prepare-a-buffy-coat.html>; page 1, first paragraph	1-9
Y	MA ET AL. "Cell-Free DNA Provides a Good Representation of the Tumor Genome Despite Its Biased Fragmentation Patterns" PLOS ONE | DOI:10.1371/journal.pone.0169231 January 3, 2017. Web. 2017; abstract; page 2, last paragraph	1-9
Y	SIEGEL ET AL. "Multiple Regression" Practical Business Statistics (Eighth Edition). Web. 2022; Retrieved from the Internet 04/04/2024, URL:<https://www.sciencedirect.com/topics/mathematics/f-test> Abstract; page 11.	7
A	US 2021/0166813 A1 (GRAIL INC.) 03 June 2021; abstract; paragraphs [0009], [0040], [0138], [0303]; claim 1	1-9
A	US 2022/0127683 A1 (THE CHINESE UNIVERSITY OF HONG KONG) 28 April 2022; paragraphs [0064], [0125], [0136], [0140], [0277]; claim 7	1-9

☐ 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 April 2024 (04.04.2024)	JUN 10 2024
Name and mailing address of the ISA/ 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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US24/10932

-***-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 1-9, are directed toward methods analyzing buffy coat in a peripheral blood sample.

Group II, Claims 10-23 are directed toward methods for analyzing blood samples obtained from a patient.

Group III, Claim 24 is directed toward determining the likelihood that a patient has cancer.

The inventions listed as Groups I, II, and III]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 features of Group I include analyzing buffy coat, not present in Groups II or III; the special technical features of Group II include analyzing blood samples, not present in Groups I and III; the special technical features of Group III include determining the likelihood that a patient has cancer, not present in Groups I and II.

Groups I-IV share the technical features of: peripheral blood sample; and buffy coat.

Groups I-III share the technical features including: a method for analyzing; obtaining a buffy coat hydroxymethylation signature for the patient by extracting genomic DNA (gDNA) from the buffy coat; sequencing the gDNA in a manner that identifies 5-hydroxymethylcytosine (5hmC)-containing sites therein; determining the extent of hydroxymethylation of the sequenced gDNA at each of a plurality of hydroxymethylation biomarker loci in a reference data set for a population group of individuals who have at least one shared characteristic, wherein the biomarker loci are preselected as differentially hydroxy methylated with respect to the at least one shared characteristic hydroxymethylation signature.

However, these shared technical features are previously disclosed by the US 2017/0073774 A1 to The Chinese University of Hong Kong (hereinafter "Hong Kong") and The publication entitled "Identification of a 5-Hydroxymethylation Signature in Circulating Cell-Free DNA for the Noninvasive Detection of Colorectal Cancer" to Hongwei Liu, et al. (hereinafter "Liu").
Hong Kong discloses peripheral blood sample (peripheral blood sample; paragraph [0270]); and buffy coat (buffy coat; paragraph [0211]); a method for analyzing (analyzing sample from subject; claim 1); obtaining a buffy coat methylation signature for the patient by extracting genomic DNA (gDNA) from the buffy coat (obtaining a methylation signature from analyzed DNA fragments. Whereby DNA can be buffy coat, further wherein buffy coat includes genomic DNA; paragraphs [0211], [0276], [0428]); sequencing the gDNA (sequencing DNA, which can be gDNA; paragraph [0276]; claim 1).

Hong Kong does not disclose hydroxymethylation signature; in a manner that identifies 5-hydroxymethylcytosine (5hmC)-containing sites therein; determining the extent of hydroxymethylation of the sequenced gDNA at each of a plurality of hydroxymethylation biomarker loci in a reference data set for a population group of individuals who have at least one shared characteristic, wherein the biomarker loci are preselected as differentially hydroxy methylated with respect to the at least one shared characteristic hydroxymethylation signature.

Liu discloses hydroxymethylation signature (identification of hydroxymethylation signature; title); in a manner that identifies 5-hydroxymethylcytosine (5hmC)-containing sites therein. (identifying 5hmC sites; title; page 13, second column, first paragraph); determining the extent of hydroxymethylation of the sequenced DNA at each of a plurality of hydroxymethylation biomarker loci in a reference data set for a population group of individuals who have at least one shared characteristic, wherein the biomarker loci are preselected as differentially hydroxy methylated with respect to the at least one shared characteristic hydroxymethylation signature (TACSTD2 (locus gene) used to determine the extent of hydroxymethylation of sequenced DNA. Where subjects are suspected to have colorectal cancer. Further, whereby groups were analyzed by differentially expressed gene packages (preselected biomarkers and loci) in accordance to the difference in 5-hmC levels in DNA; title; abstract; page 10, second column, last paragraph).

It would have been obvious to one of ordinary skill in the art before the relevant date of the invention to modify the Hong Kong invention to provide hydroxymethylation signature; in a manner that identifies 5-hydroxymethylcytosine (5hmC)-containing sites therein; determining the extent of hydroxymethylation of the sequenced gDNA at each of a plurality of hydroxymethylation biomarker loci in a reference data set for a population group of individuals who have at least one shared characteristic, wherein the biomarker loci are preselected as differentially hydroxy methylated with respect to the at least one shared characteristic hydroxymethylation signature, as taught by Liu, in order to provide a method for detecting cancer using existing 5hmC methods.

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