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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2024 (21.03.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/059841 A3

(51) International Patent Classification:
G01N 27/327 (2006.01) *G01N 33/543* (2006.01)
G01N 33/52 (2006.01)

(21) International Application Number:
PCT/US2023/074376

(22) International Filing Date:
15 September 2023 (15.09.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/407,417 16 September 2022 (16.09.2022) US
63/448,134 24 February 2023 (24.02.2023) US

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(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,

(54) Title: ANALYTE DETECTION AND QUANTIFICATION

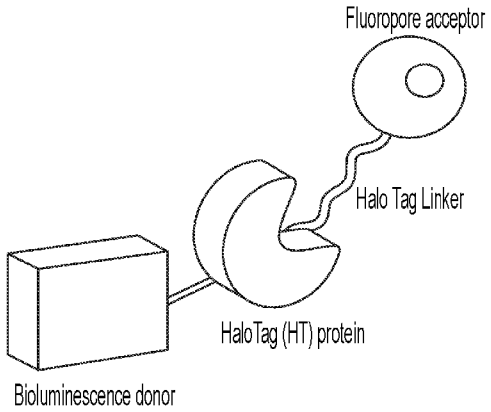

FIG. 1

(57) Abstract: The present features methods and components for analyte detection in general, methods and components for quantifying analytes associated with bladder cancer, and treatment of patients identified as having analyte levels predictive of bladder cancer. The general methods and components can be used to detect the presence of small amounts of analytes in sample.

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[Continued on next page]

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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:
30 May 2024 (30.05.2024)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/74376

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. G01N 27/327; G01N 33/52; G01N 33/543 (2023.01)
ADD.
CPC - INV. G01N 33/54388; G01N 27/3272; G01N 27/3274; G01N 33/52; G01N 33/54386; G01N 33/588
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)
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.
X --- Y	LIYANAGE. "A noninvasive multiplexed biomarker-based bladder cancer test to improve patient follow-up frequency and compliance" ACS. 22 August 2021; Retrieved from the Internet on 22 December 2023: <URL: https://acs.digitellinc.com/sessions/150229/view>; Abstract	35 --- 1-4, 43, 47, 49
X	US 2017/0319657 A1 (PURDUE RESEARCH FOUNDATION et. al) 09 November 2017; paragraph [0055]	48
Y	WO 2010/037395 A2 (DAKO DENMARK A/S et. al) 08 April 2010; claim 1593; pg754, lines 10-11	1-4
Y	US 2021/0140911 A1 (ANTMODITY KOREA INC. et. al) 13 May 2021; claims 14, 34; paragraphs [0051], [0107]-[0109], [0127], [0130]	3-4, 15, 42, 43
Y	SCHULZ. "The Development of Non-Invasive Diagnostic Tools in Bladder Cancer" pages 497-507. Onco Targets and Therapy. Vol. 15. May 2022; pg500, paragraph 5; DOI: 10.2147/OTT.S283891	15
Y	WO 2020/117952 A2 (GENENTECH INC. et al.) 11 June 2020; abstract; claim 27; paragraph [0588]	47, 49
Y	US 10,138,267 B2 (HAO, XIUJUAN) 27 November 2018; paragraph [0096]	43
Y	US 2021/0321957 A1 (ALACRITY PATIENT SERVICES INC.) 21 October 2021; paragraphs [0064], [0078], [0088], [0176], [0278], [0310], [0468], [0474], [0598], [0771], [0896]	49

☐ 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
20 January 2024 (20.01.2024)	MAR 28 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.
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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.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

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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.: 5-14, 19-34, 38-41
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.:
Groups I+, Claims 1-4, 15-18, 35-37, 42-49, and miR-205, miR-16-1, miR-143, UCA1 mRNA, IGF2 or IGF2 nucleic acid, ANXA10 or ANXA10 nucleic acid (bladder cancer biomarker panel).

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/US23/74376

-***-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.

Groups I+, Claims 1-4, 15-18, 35-37, 42-49, and miR-205, miR-16-1, miR-143, UCA1 mRNA, IGF2 or IGF2 nucleic acid, ANXA10 or ANXA10 nucleic acid (bladder cancer biomarker panel) are directed towards multi-analyte detection for bladder cancer and methods associated therewith.

The multi-analyte detection and methods of Claims 1-4, 15, 35, 42-43, 47-49 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass miR-205, miR-16-1, miR-143, UCA1 mRNA, IGF2 or IGF2 nucleic acid, ANXA10 or ANXA10 nucleic acid (first exemplary bladder cancer biomarker panel). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 15, 35, 43 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional bladder cancer biomarker panel(s) to be searched. Additional bladder cancer biomarker panel(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected bladder cancer biomarker panel(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. Exemplary elections would be miR-205, miR-16-1, miR-143, UCA1 mRNA, IGF2 or IGF2 nucleic acid, ANXA10 or ANXA10 nucleic acid, NMP-22 or NMP-22 nucleic acid, HCFHrp or HCFHrp nucleic acid, miR-200C, UPKB or UPKB nucleic acid, ABL1 or ABL1 nucleic acid, and CRH or CRH nucleic acid, NMP-22 or NMP-22 nucleic acid, HCFHrp or HCFHrp nucleic acid, miR-200C, UPKB or UPKB nucleic acid, ABL1 or ABL1 nucleic acid, and CRH or CRH nucleic acid (bladder cancer biomarker panel).

Groups I+ share the technical features including: an analyte detection signal amplifier comprising (a) an analyte binding molecule; (b) a localized surface plasmon resonance nanostructure and (c) either (i) a bioluminescence resonance energy transfer (BRET) assembly complex, wherein the BRET assembly complex comprises a luciferase donor conjugated to a fluorophore acceptor or (ii) a fluorescence resonance energy transfer (FRET) assembly complex, wherein the FRET assembly complex comprises a fluorescent donor conjugated to a fluorophore acceptor; a multi-analyte detection system comprising multiple localized surface plasmon nanostructure sensors comprising an analyte capture molecule for biomarkers; wherein each sensor type may be present on one or more platforms; a method of determining whether a human subject has bladder cancer comprising the steps of detecting the amount of biomarkers in a biological sample from the subject; a method of detecting or quantifying an analyte in a sample comprising the steps of: a) contacting a localized surface plasmon resonance sensor with the sample, wherein the sensor comprises an analyte capture molecule; b) providing an analyte detection signal amplifier to the sample, wherein the analyte detection signal amplifier comprises the BRET assembly complex and binds to the analyte; c) adding a luciferase substrate; and detecting fluorescence, bioluminescence, localized surface plasmon resonance or surface-enhanced Raman scattering; a method of treating a subject for bladder cancer comprising (a) detecting the amount of analytes associated with bladder cancer using the method; and (b) administering a therapeutically effective amount of a bladder cancer therapeutic to a subject determined to have bladder cancer based in whole or in part on (a); a method of treating a subject for bladder cancer comprising administering a therapeutically effective amount of a bladder cancer therapeutic to a subject determined to have bladder cancer, based in whole or in part, on the results of using the method. These shared technical features are previously disclosed by the publication entitled "A noninvasive multiplexed biomarker-based bladder cancer test to improve patient follow-up frequency and compliance" by Liyanage (hereinafter "Liyanage") in view of : US 2020/0103412 A1 to Commonwealth Scientific and Industrial Research Organisation (hereinafter "Commonwealth").

Liyanage discloses an analyte detection signal amplifier comprising (a) an analyte binding molecule; (b) a localized surface plasmon resonance nanostructure and a bioluminescence-based detection (a novel localized surface plasmon resonance enhanced bioluminescence based biomarker detection platform; abstract); a multi-analyte detection system comprising multiple localized surface plasmon nanostructure sensors comprising an analyte capture molecule for biomarkers; wherein each sensor type may be present on one or more platforms (a localized surface plasmon resonance enhanced bioluminescence based biomarker detection platform that simultaneously quantifies a panel of five different biomarkers from patient urine; abstract); a method of determining whether a human subject has bladder cancer comprising the steps of detecting the amount of biomarkers in a biological sample from the subject (a localized surface plasmon resonance enhanced bioluminescence based biomarker detection platform that simultaneously quantifies a panel of five different biomarkers from patient urine for diagnosing bladder cancer; abstract); a method of detecting or quantifying an analyte in a sample comprising the steps of: a) contacting a localized surface plasmon resonance sensor with the sample, wherein the sensor comprises an analyte capture molecule; b) providing an analyte detection signal amplifier to the sample; and detecting fluorescence, bioluminescence, localized surface plasmon resonance or surface-enhanced Raman scattering (a localized surface plasmon resonance enhanced bioluminescence based (detecting bioluminescence) biomarker detection platform that simultaneously quantifies a panel of five different biomarkers from patient urine for diagnosing bladder cancer; abstract); a method of treating a subject for bladder cancer comprising (a) detecting the amount of analytes associated with bladder cancer using the method; and (b) administering a therapeutically effective amount of a bladder cancer therapeutic to a subject determined to have bladder cancer based in whole or in part on (a) (a localized surface plasmon resonance enhanced bioluminescence based biomarker detection platform that simultaneously quantifies a panel of five different biomarkers from patient urine for diagnosing bladder cancer, wherein a positive diagnosis would intrinsically be followed by treatment which involves administration of a therapeutic to the subject; abstract); a method of treating a subject for bladder cancer comprising administering a therapeutically effective amount of a bladder cancer therapeutic to a subject determined to have bladder cancer, based in whole or in part, on the results of using the method (a localized surface plasmon resonance enhanced bioluminescence based biomarker detection platform that simultaneously quantifies a panel of five different biomarkers from patient urine for diagnosing bladder cancer, wherein a positive diagnosis would intrinsically be followed by treatment which involves administration of a therapeutic to the subject; abstract).

Liyanage does not disclose (c) either (i) a bioluminescence resonance energy transfer (BRET) assembly complex, wherein the BRET assembly complex comprises a luciferase donor conjugated to a fluorophore acceptor or (ii) a fluorescence resonance energy transfer (FRET) assembly complex, wherein the FRET assembly complex comprises a fluorescent donor conjugated to a fluorophore acceptor;

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

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

wherein the analyte detection signal amplifier comprises the BRET assembly complex and binds to the analyte; c) adding a luciferase substrate.

Commonwealth discloses (c) a bioluminescence resonance energy transfer (BRET) assembly complex, wherein the BRET assembly complex comprises a luciferase donor conjugated to a fluorophore acceptor (sensor molecule each comprising a domain that binds one or more analytes, a bioluminescent luciferase donor domain and a fluorescent acceptor domain; abstract; paragraphs [0040], [0043]); wherein the analyte detection signal amplifier comprises the BRET assembly complex and binds to the analyte; c) adding a luciferase substrate (sensor molecule each comprising a domain that binds one or more analytes, a bioluminescent luciferase donor domain and a fluorescent acceptor domain, and adding a luciferase substrate; abstract; paragraphs [0010], [0040], [0043]).

It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the analyte detection system of Liyanage, for the integration of a BRET assembly complex comprising a luciferase donor and fluorophore acceptor and a luciferase substrate, as taught by Commonwealth, as this combination would provide the capability of detecting bladder cancer biomarkers in patient samples using a surface plasmon nanosensor comprising a BRET detection system, thereby providing a diagnostic platform for bladder cancer with improved sensitivity and accuracy.

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