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(71) Applicant: **THE JOHNS HOPKINS UNIVERSITY** [US/US]; Technology Ventures, 100 N. Charles Street, 5th Floor, Baltimore, MD 21201 (US).

(72) Inventors: **LEUNG, Anthony, K.**; 10107 Carillon Court, Ellicott City, MD 21042 (US). **GABELLI, Sandra, B.**; 2933 Excelsior Springs Ct., Ellicott City, MD 21042 (US). **DANIELS, Casey, M.**; 17801 Bowie Mill Road, Derwood, MD 20855 (US).

(74) Agents: **CORLESS, Peter, F.** et al.; Mintz Levin Cohn Ferris Glovsky And Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR IDENTIFYING ADP-RIBOSYLATED SITES BY MASS SPECTROMETRY

(57) Abstract: The present disclosure relates to the use of a nudix hydrolase(RppH or NudT16) to cleave poly ADP-ribosylated proteins and leave a tag (phosphoribose) to be analyzed by mass spectrometry to identify post-translational modification (PTM) profiles of proteins. The disclosure relates to methods for identifying an ADP- ribosylation profile in hydrolyzed proteins using mass spectrometry.

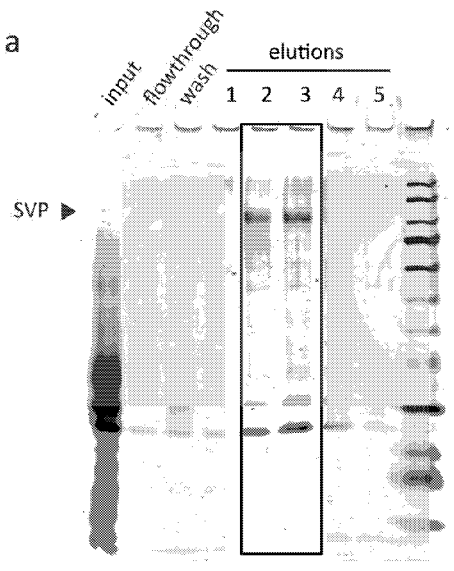

Figure 1A

WO 2016/201257 A3

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:
23 February 2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/036934

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00; A61K 38/16; A61K 38/17 (2016.01)
CPC - A61K 38/1709; C07K 14/4723; C12Q 1/48 (2016.11)
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 - A61K 38/00; A61K 38/16; A61K 38/17
CPC - A61K 38/1709; C07K 14/4723; C12Q 1/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/325; 435/375; 514/12; 514/21.3 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, PubMed, Google

Search terms used: PARP inhibitor ADP ribose tag sample digest hydrolase protease serine trypsin administer treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0155834 A1 (GUARENTE et al) 18 June 2009 (18.06.2009) entire document	1, 2, 7, 10-12, 16, 17, 21-23
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Y		3-6, 8, 9, 13-15, 18-20, 24-35
Y	PALAZZO et al. "Processing of protein ADP-ribosylation by Nudix hydrolases," Biochem J., 19 March 2015 (19.03.2015), Vol. 468, No. 2, Pgs. 293-301. entire document	3-6, 8, 9, 13-15, 18-20, 30-35
Y	WO 2009/077521 A1 (FONDAZIONE TELETHON et al) 25 June 2009 (25.06.2009) entire document	8, 9, 19, 20
Y	US 2010/0069372 A1 (KAZANTSEV) 18 March 2010 (18.03.2010) entire document	24-29
Y	US 2013/0252999 A1 (CHINA MEDICAL UNIVERSITY) 26 September 2013 (26.09.2013) entire document	35
P, X	DANIELS et al. "Nudix hydrolases degrade proteinconjugated ADP-ribose," Scientific Reports, 16 December 2015 (16.12.2015), Vol. 5, Pgs. 1-12. entire document	1-35
A	DOLLE et al. "NAD and ADP-ribose metabolism in mitochondria," FEBS Journal, 03 June 2013 (03.06.2013), Vol. 280, Iss. 15, Pgs. 3530-3541. entire document	1-35
A	WAHLBERG et al. "Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors," Nature Biotechnology, 19 February 2012 (19.02.2012), Vol. 30, No. 3, Pgs. 283-288 and Supplementary Information. entire document	1-35

☐ 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	Date of mailing of the international search report
30 November 2016	30 DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/036934

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:

ISA/225 was mailed on 08 July 2016. No approved electronic sequence listing was submitted in response to the ISA/225.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/036934

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 Extra Sheet(s).

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.:
1-35 restricted to a PARP inhibitor of compound 1.

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/US2016/036934

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 need to be paid.

Group I+: claims 1-35 are drawn to PARP inhibitors; and methods and kits comprising the same.

The first invention of Group I+ is restricted to a PARP inhibitor, methods and kits comprising the same, wherein the PARP inhibitor is selected to be compound 1 (see claim 35). It is believed that claims 1-35 read on this first named invention and thus these claims will be searched without fee to the extent that they read on a PARP inhibitor of compound 1.

Applicant is invited to elect additional PARP inhibitors to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a PARP inhibitor, methods and kits comprising the same, wherein the PARP inhibitor is selected to be compound 2. Additional PARP inhibitors will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element responsible for inhibiting an enzyme from the poly ADP ribose polymerase family, requiring the selection of alternatives for the PARP inhibitor, where "the PARP inhibitor is any one or more of the following PARP inhibitors (see table in claim 35 of the instant application)".

The Groups I+ share the technical features of a method of generating phosphoribose tags on an adenosine diphosphate (ADP)-ribosylated protein comprising: obtaining a sample including the ADP-ribosylated protein; incubating the sample with a hydrolase; and generating one or more phosphoribose tags on the ADP-ribosylated protein; a method of identifying sites of adenosine diphosphate (ADP)-ribosylation on a protein, comprising: obtaining a sample including one or more proteins having ADP-ribosylation; incubating the sample with a hydrolase; digesting the hydrolyzed sample; and detecting one or more ADP-ribosylation sites using a spectrometric technique; a method of detecting a disease associated with adenosine diphosphate (ADP)-ribosylation, comprising: obtaining a sample from a subject; incubating the sample with a hydrolase; digesting the hydrolyzed sample; detecting one or more ADP-ribosylation sites using a spectrometric technique; and identifying the disease associated with ADP-ribosylation; a kit for detecting ADP-ribosylation comprising a Nudix hydrolase, hydrolysis buffer, salts, PARP inhibitor, urea, and denaturing buffers. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2009/0155834 A1 to Guarente et al. discloses a method of generating phosphoribose tags on an adenosine diphosphate (ADP)-ribosylated protein (Methods of identifying agents which alter the NAD-dependent acetylation status and mono-ADP-ribosylation of nuclear proteins are disclosed, Abstract; to generate peptides with and without acetylated lysines, Para. [0046]; 32P-labeled ADP-ribose, Para. [0203]) comprising: obtaining a sample including the ADP-ribosylated protein (ADP ribosylated intact H3 protein, Para. [0244]); incubating the sample with a hydrolase (ProteinA-Sepharose beads was incubated in the reaction buffer containing 32P-labeled NAD and histone H1 or H2B, Para. [0261]; NAD hydrolase activity of both ySir2p and mSir2a was stimulated in the presence of the diacetylated peptide, Para. [0263]); and generating one or more phosphoribose tags on the ADP-ribosylated protein (biosynthetic step that transfers phosphoribose from nicotinate mononucleotide to dimethyl benzimidazole, Para. [0201]); a method of identifying sites of adenosine diphosphate (ADP)-ribosylation on a protein (Methods of identifying agents which alter the NAD-dependent acetylation status and mono-ADP-ribosylation of nuclear proteins are disclosed, Abstract; to generate peptides with and without acetylated lysines, Para. [0046]; 32P-labeled ADP-ribose, Para. [0203]), comprising: obtaining a sample including one or more proteins having ADP-ribosylation (determine unique nucleotide sequences for normal and altered proteins, and to determine nucleotide sequences to be used as target sites for antisense nucleic acids, Para. [0177]); incubating the sample with a hydrolase (ProteinA-Sepharose beads was incubated in the reaction buffer containing 32P-labeled NAD and histone H1 or H2B, Para. [0261]; NAD hydrolase activity of both ySir2p and mSir2a was stimulated in the presence of the diacetylated peptide, Para. [0263]); digesting the hydrolyzed sample (digests the phosphodiester bond in the ADP-ribose moiety, Para. [0203]); and detecting one or more ADP-ribosylation sites using a spectrometric technique (Electron-spray mass spectroscopy (also referred to herein as mass spectroscopy) was done on the PE Sciex Model API365 system, Para. [0244]); a method of detecting a disease associated with adenosine diphosphate (ADP)-ribosylation (Methods of identifying agents which alter the NAD-dependent acetylation status and mono-ADP-ribosylation of nuclear proteins are disclosed, Abstract; to generate peptides with and without acetylated lysines, Para. [0046]; 32P-labeled ADP-ribose, Para. [0203]), comprising: obtaining a sample from a subject (The cell samples thus treated are then examined for longer-lived, slower aged colonies, using any of the methods described herein or other appropriate methods, Para. [0139]); incubating the sample with a hydrolase (ProteinA-Sepharose beads was incubated in the reaction buffer containing 32P-labeled NAD and histone H1 or H2B, Para. [0261]; NAD hydrolase activity of both ySir2p and mSir2a was stimulated in the presence of the diacetylated peptide, Para. [0263]); digesting the hydrolyzed sample (digests the phosphodiester bond in the ADP-ribose moiety, Para. [0203]); detecting one or more ADP-ribosylation sites using a spectrometric technique; and identifying the disease associated with ADP-ribosylation (Electron-spray mass spectroscopy (also referred to herein as mass spectroscopy) was done on the PE Sciex Model API365 system, Para. [0244]); a kit (kit, Para. [0240]) for detecting ADP-ribosylation comprising a Nudix hydrolase (Methods of identifying agents which alter the NAD-dependent acetylation status and mono-ADP-ribosylation of nuclear proteins are disclosed, Abstract; to generate peptides with and without acetylated lysines, Para. [0046]; 32P-labeled ADP-ribose, Para. [0203]), hydrolysis buffer (incubated in the reaction buffer containing 32P-labeled NAD and histone H1 or H2B., Para. [0261]), salts (salts, Para. [0142]), PARP inhibitor (PARP inhibitor, Para. [0203]), urea, and denaturing buffers (denaturing conditions, Para. [0229]; buffer, Para. [0230]).

"NAD and ADP-ribose metabolism in mitochondria" to Dolle et al. discloses a nudix hydrolase and urea (ADPR can also be degraded into ribose-5-phosphate and AMP by another NUDIX hydrolase, Pg. 3536, left-hand column, first paragraph; Accumulation of ammonia is prevented by activation of the urea cycle via SIRT3-dependent ornithine carbamoyltransferase deacetylation, Pg. 3533, left-hand column, second paragraph).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.