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7 April 2016

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(54) Title: SRM ASSAYS TO CHEMOTHERAPY TARGETS

(57) Abstract: Quantitative analysis of proteins that are targets of chemotherapy by Selected reaction monitoring (SRM)/Multiple Reaction Monitoring (MRM) mass spectrometry, by measuring modified forms of disclosed specific peptides from the ENT1, ERCC1, FOLR1, RRM1, TUBB3, TOP01, and/or TOP02A proteins. These peptides can be quantified if a modified form thereof comprises a phosphorylated, tyrosine, threonine, serine, and/or other amino acid residues within their amino acid sequence. Biological samples that have been fixed in formalin are used for the analysis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/38874

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48, G01N 33/50, G01N 31/00, H01J 49/00 (2015.01)
CPC - G06F 19/18, G06F 19/24, G06F 19/20, G06F 19/22, G06F 19/16, A61B 5/14532, G01N 27/3271
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, G01N 33/50, G01N 31/00, H01J 49/00 (2015.01)
CPC- G06F 19/18, G06F 19/24, G06F 19/20, G06F 19/22, G06F 19/16, A61B 5/14532, G01N 27/3271

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- G01N 21/274, G01N 33/6848, G01N 33/6851, G01N 33/57407, G01N 2400/00, H01J 49/0036, H01J 49/0031, H01J 49/004, G01J 3/28, C07K 14/4732, C07K 14/77; USPC- 702/19, 702/23, 250/281, 435/4, 530/327, 530/326, 530/387.9, 530/344

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: Selected reaction monitoring, SRM, MRM, Multiple reaction monitoring,SLC29A1, ENT1, Equilibrative NBMPR-sensitive nucleoside transporter, Equilibrative nitrobenzylmercaptopurine riboside-sensitive nucleoside transporter, Equilibrative nucleoside transporter 1, MGC1465, MGC3778.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Reyes, et al. The Equilibrative Nucleoside Transporter (ENT1) can be phosphorylated at multiple sites by PKC and PKA. Mol Membr Biol. 2011, 28(6):412-26; Abstract, pg 415, col 2; pg 416, col 2; pg 422, Fig 6 and its legend	1, 2, 4, 5
X – Y	US 2010/0029005 A1 (Kamiie, et al.) 04 February 2010 (04.02.2010) claims 1, 8, 10; para [0082], [0014], [0157], SEQ ID NO:319	1, 2, 4, 5, (6-11)/(1, 2,4,5) 3, (6-11)/3, 12-16
A	NCBI Reference Sequence: NP_001071643.1. equilibrative nucleoside transporter 1 [Homo sapiens]. 20 April 2014 [Retrieved fromn the Internet 24 September 2015: <http://www.ncbi.nlm.nih.gov/protein/118582262?sat=18&satkey=18620465>]; pg 1, 2	1-16
Y	Expression Pathology. Liquid Tissue MS Protein Prep Kit for the isolation of proteins and peptides from formalin fixed tissue in preparation for mass spectrometry analysis. Instruction Manual (2009) [Retrieved from the Internet 20 November 2015: <http://www.expressionpathology.com/pdf/LiquidTissueManualV146.pdf>]; pg 5, 6	3, (6-16)/3
Y	Murata, et al. Human equilibrative nucleoside transporter 1 expression is a strong independent prognostic factor in UICC T3-T4 pancreatic cancer patients treated with preoperative gemcitabine-based chemoradiotherapy. J Hepatobiliary Pancreat Sci. 2012, 19(4):413-425; Abstract; pg 414, col 2	12-16

☐ 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
20 November 2015 (20.11.2015)	21 DEC 2015
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: Lee W. Young 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/US 15/38874

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:

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.

********** See Supplemental Sheet to continue **********

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.:
1-16, restricted to ENT1

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 15/38874

********** Supplemental Sheet **********

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

Group I+: claims 1-16, directed to a method for measuring the level of a protein in a biological sample, comprising detecting and/or quantifying the amount of one or more modified/unmodified protein fragment peptides in a protein digest prepared from said biological sample using mass spectrometry; and calculating the level of the protein in the sample. The method will be searched to the extent that the protein encompasses ENT1. It is believed that claims 1-16 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a mass spectrometry method for measuring the level of an ENT1 protein. Additional protein(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected protein(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. An exemplary election would be a mass spectrometry method for measuring the level of TOPO2A protein in a sample, i.e., claims 1-16.

Group II+: claim 17, directed to a composition comprising at least one peptide of a specified amino acid sequence. Group II+ will be searched upon payment of additional fees. The composition may be searched, for example, to the extent that it encompasses peptide of SEQ ID NO:1, i.e., claim 17. Additional peptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected peptide(s). 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. Another exemplary election would be a composition comprising peptides SEQ ID NO: 8-16, i.e., claim 17.

The inventions listed as Groups I+ and II+ 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:

Special Technical Features
The special technical feature of each invention of Group I+ is a specific protein recited therein, because no significant structural similarities can readily be ascertained among the proteins, and further because ENT1 protein was known in the art at the time of the invention (please see NCBI Reference NP_001071643.1 titled "Equilibrative nucleoside transporter 1 [Homo sapiens]" (20 April 2014) [Retrieved from the Internet 24 September 2015: <http://www.ncbi.nlm.nih.gov/protein/118582262?sat=18&satkey=18620465>] (hereinafter "NP_001071643.1").

The special technical feature of each invention of Group II+ is a peptide of a specific amino acid sequence, because no significant structural similarities can readily be ascertained among the amino acid sequences, and further because said sequences were known in the at at the time of the invention (please see NP_001071643.1, wherein fragments flanked by amino acids 59-73, 106-111, 112-131, 240-249, 250-255, 321-326, 360-368 correspond to SEQ ID NO:1, 2, 3, 4, 5, 6, 7, respectively, 100% identity).

The inventions of Group I+ do not include the shared or common technical feature of a composition comprising a peptide(s) of a specific amino acid sequence(s), as required by Group II+.

The inventions of Group II+ do not include the shared or common technical feature of a method for measuring the level of a protein in a biological sample, as required by Group I+.

Common Technical Features
The inventions of Group I+ share the technical feature of a method for measuring the level of a protein in a biological sample, comprising detecting and/or quantifying the amount of a modified/unmodified protein fragment peptides in a protein digest prepared from said biological sample using mass spectrometry; and calculating the level of the protein in the sample. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2013/0302334 A1 to Krizman, et al. (hereinafter "Krizman").

Krizman discloses "[a] method for measuring the level of the Bcl-2-like protein 11 (BIM) in a biological sample, comprising detecting and/or quantifying the level of one or more modified or unmodified BIM fragment peptides in a protein digest prepared from the biological sample using mass spectrometry; and calculating the level of modified or unmodified BIM protein in the sample; and wherein the level is a relative level or an absolute level" (claim 1; see also claim 33, "[a] method of determining the resistance of a cancer to one or more EGFR, HER2, and/or PI3K inhibitor(s), comprising determining the presence or level of BIM proteins in a cancer tissue or cancerous cells thereof using an SRM/MRM assay; wherein the presence of biologically active BIM protein(s) capable of mediating apoptosis of cells in said cancer tissue or cancerous cells thereof is indicative of the susceptibility of said cancer to one or more of said inhibitor(s)"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions of Group I+.

The inventions of Group II+ share the technical feature of a composition comprising at least one peptide of a specific amino acid sequence. However, this shared technical feature does not represent a contribution over prior art as being anticipated by Krizman, as above (claim 30, "[a] composition comprising one or more, two or more, or all three of the peptides in Table 1..."). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions of Group II+.

Groups I+ and II+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.