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Declarations under Rule 4.17:

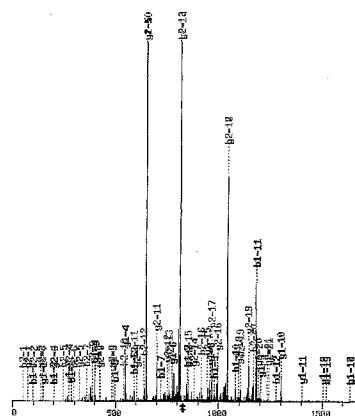
- as to applicant's entitlement to apply for and be granted
a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

[Continued on next page]

(54) Title: REAGENTS FOR THE DETECTION OF PROTEIN PHOSPHORYLATION IN LEUKEMIA SIGNALING PATH-
WAYS

PTSIPPKEETTPpYIAQVFQQK

Seq #	b	y	(+1)	Seq #	b	y	(+2)
P 1	98.1	2483.7	21	P 1	49.6	1242.4	21
T 2	199.2	2386.6	20	T 2	100.1	1193.8	20
S 3	286.3	2285.5	19	S 3	143.7	1143.3	19
I 4	399.5	2198.4	18	I 4	200.2	1099.7	18
P 5	496.6	2085.2	17	P 5	248.8	1043.1	17
P 6	593.7	1988.2	16	P 6	297.4	994.6	16
K 7	721.9	1891.0	15	K 7	361.4	946.0	15
E 8	851.0	1762.9	14	E 8	426.0	881.9	14
E 9	980.1	1633.8	13	E 9	490.6	817.4	13
T 10	1081.2	1504.6	12	T 10	541.1	752.8	12
T 11	1182.3	1403.5	11	T 11	591.7	702.3	11
P 12	1279.4	1302.4	10	P 12	640.2	651.7	10
Y* 13	1522.6	1205.3	9	Y* 13	761.8	603.2	9
I 14	1635.8	962.1	8	I 14	818.4	481.6	8
A 15	1706.8	849.0	7	A 15	853.9	425.0	7
Q 16	1835.0	777.9	6	Q 16	918.0	369.5	6
V 17	1934.0	649.8	5	V 17	967.6	325.4	5
F 18	2081.3	550.6	4	F 18	1041.1	275.8	4
Q 19	2209.4	403.5	3	Q 19	1105.2	202.2	3
Q 20	2337.5	275.3	2	Q 20	1169.3	138.2	2
K 21	2465.7	147.2	1	K 21	1233.4	74.1	1



(57) Abstract: The invention discloses nearly 288 novel phosphorylation sites identified in signal transduction proteins and pathways underlying human Leukemia, and provides phosphorylation-site specific antibodies and heavy-isotope labeled peptides (AQUA peptides) for the selective detection and quantification of these phosphorylated sites/proteins, as well as methods of using the reagents for such purpose. Among the phosphorylation sites identified are sites occurring in the following protein types: Adaptor/Scaffold proteins, Cytoskeletal proteins, Cellular Metabolism enzymes, G Protein/GTPase Activating/Guanine Nucleotide Exchange Factor proteins, Immunoglobulin Superfamily proteins, Inhibitor proteins, Lipid Kinases, Nuclear DNA Repair/RNA Binding/Transcription proteins, Serine/Threonine Protein Kinases, Tyrosine Kinases, Protein Phosphatases, and Translation/Transporter proteins.

**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:

18 November 2010

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 06/34050

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/53 (2010.01)

USPC - 435/7.1

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 33/53 (2010.01)

USPC- 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 IPC(8)- 435/7.8; 435/346; 435/7.21; 435/7.4; 436/536; 436/501; 530/387.7; 530/402; 530/387.9; 530/388.22; 530/388.1; 530/387.9; 530/389.1; 424/139.1; 424/143.1; 424/130.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PubWest, DialogPRO (Chemical Engineering and Biotechnology Abstracts, INSPEC, NTIS, PASCAL, Current Contents Search, MEDLINE): Leukemia pathway, ARYPHIGQKPGGSOFLR, tyrosine, phosphorylated, RNA binding, binding protein, phosphorylation profile. GenCore 6.3: SEQ ID NO:170

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/0190688 A1 (Crosby, et al.) 09 October 2003 (09.10.2003) para [0011], [0047], [0069]-[0070]	1
A	SHU, et al. Identification of Phosphoproteins and Their Phosphorylation Sites in the WEHI-231 B Lymphoma Cell Line, Molecular & Cellular Proteomics 2004, 3(3):279-286; pg 282	1
A	Dulubova, et al. ARPP-16/ARPP-19: a highly conserved family of cAMP-regulated phosphoproteins. J Neurochem. 2001, 77(1):229-238	1
A	US 2002/0142432 A1 (Roch, et al.) 03 October 2002 (03.10.2002) SEQ ID NO:11	1
A	US 2005/0003450 A1 (Rush, et al.) 01 June 2005 (01.06.2005) para [0167]	1
A	GenBank NP_006619. cyclic AMP phosphoprotein, 19 kD [Homo sapiens]. 23-APR-2005. [Retrieved from the Internet 07 September 2010: < http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?val=5729732&sat=NCBI&satkey=10025249 >]	1
A,T	US 2008/0248490 A1 (Polakiewicz, et al.) 09 October 2008 (09.10.2008) SEQ ID NO 170	1

☐ 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 06/34050

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:

Group I-: claim 1, drawn to a method of method for obtaining a phosphorylation profile of RNA binding proteins that are phosphorylated in Leukemia signaling pathways, said method comprising the step of utilizing one or more isolated antibody that specifically binds a RNA binding protein selected from Column A, Rows 171-226, of Table 1 only when phosphorylated at the tyrosine listed in corresponding Column D, Rows 171-226, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 171-226, of Table 1 (SEQ ID NOs: 170-183,185-203, and 205-225), to detect the phosphorylation of one or more of said RNA binding proteins, thereby obtaining a phosphorylation profile for said RNA binding proteins. The first invention encompasses an antibody that specifically binds to the RNA binding protein ARPP-19 (GenBank Accession Number NP_006619, listed in Column A, Row 171. Should an additional fee(s) be paid, Applicant is invited to elect an additional protein(s) to be searched.

***** See Supplemental Box 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, restricted to SEQ ID NO:170

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.

***** Supplemental Box *****

Continuation of Box III: LOU

Group II+, claims 2-15, drawn to a method for detecting or quantifying a signaling protein that is tyrosine phosphorylated in Leukemia signaling pathways, said method comprising the step of utilizing one or more of the following reagents to detect or quantify one or more Leukemia-related signaling protein(s) selected from Column A of Table 1 only when phosphorylated at the tyrosine listed in corresponding Column D of Table 1:

(i) an isolated phosphorylation site-specific antibody that specifically binds said protein only when phosphorylated at the tyrosine listed in corresponding Column D of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-3, 6-28, 30-42, 44-93, 95-168, 170-183, 185-203, 205-278, and 280-288), wherein said antibody does not bind said protein when not phosphorylated at said tyrosine; and/or

(ii) a heavy-isotope labeled peptide (AQUA peptide) for the quantification of said protein, said labeled peptide comprising the phosphorylation site peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-3, 6-28, 30-42, 44-93, 95-168, 170-183, 185-203, 205-278, and 280-288). The first invention encompasses an antibody that specifically binds to the Acetyltransferase CAS1 NP_075051.3 and SEQ ID NO: 1. Should an additional fee(s) be paid, Applicant is invited to elect an additional protein(s) and SEQ ID NO(s) to be searched. The exact claims searched will depend on the specifically elected protein(s) and SEQ ID NO(s):.

Group III+, claims 16-17, 21-23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49-50, drawn to an isolated phosphorylation site-specific antibody that specifically binds a human Leukemia-related signaling protein selected from Column A of Table 1 only when phosphorylated at the tyrosine listed in corresponding Column D of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-3, 6-28, 30-42, 44-93, 95-168, 170-183, 185-203, 205-278, and 280-288), wherein said antibody does not bind said signaling protein when not phosphorylated at said tyrosine. The first invention encompasses an antibody that specifically binds to the Acetyltransferase CAS1 NP_075051.3 and SEQ ID NO: 1. Should an additional fee(s) be paid, Applicant is invited to elect an additional protein(s) and SEQ ID NO(s) to be searched. The exact claims searched will depend on the specifically elected protein(s) and SEQ ID NO(s):.

Group IV+, claims 18-20, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 51-52, drawn to a heavy-isotope labeled peptide (AQUA peptide) for the quantification of a human Leukemia-related signaling protein selected from Column A of Table 1, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-3, 6-28, 30-42, 44-93, 95-168, 170-183, 185-203, 205-278, and 280-288), comprising the phosphorylatable tyrosine or serine listed in corresponding Column D, Rows 2-289, of Table 1. The first invention encompasses a peptide of SEQ ID NO: 1. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. The exact claims searched will depend on the specifically elected SEQ ID NO(s):.

The inventions listed as Groups I+ through IV+ 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:

Groups I+ do not include the inventive concept of a heavy-isotope labeled peptide (AQUA peptide) for the quantification of said protein, said labeled peptide comprising the phosphorylation site peptide sequence, as required by Groups II+ through IV+.

The inventions of Group I+ share the technical feature of a method for obtaining a phosphorylation profile of RNA binding proteins that are phosphorylated in Leukemia signaling pathways, said method comprising the step of utilizing one or more isolated antibody that specifically binds a RNA binding protein. However, this shared technical feature does not represent a contribution over the prior art. Specifically, US 2003/0190688 A1 to Crosby, et al. (hereinafter "Crosby") teaches a method for obtaining a phosphorylation profile of RNA binding proteins (para [0069]--markers may be examined to provide the most informative c-Abl signaling profile) that are phosphorylated in Leukemia signaling pathways, said method comprising the step of utilizing one or more isolated antibody that specifically binds a RNA binding protein to detect the phosphorylation of one or more of said RNA binding proteins, thereby obtaining a phosphorylation profile for said RNA binding proteins (para [0011]--phospho-specific antibodies that bind to proteins phosphorylated at specific tyrosines, serines or threonines and para [0070]--the cancer is chronic myelogenous leukemia (CML) or acute lymphocytic leukemia (ALL)) and only when phosphorylated at the tyrosine (para [0047]--the antibodies may also be tested by Western blotting against cell preparations containing the phosphorylated target protein to confirm reactivity with the desired phosphorylated target). As said method was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another technical feature of the inventions listed as Group I+ is the specific phosphorylation site sequence recited therein. The inventions do not share a special technical feature, because US 2002/0142432 A1 to Roch, et al. (hereinafter "Roch") teaches the claimed SEQ ID NO: 170 (100% identity to SEQ ID NO: 11--arYphlgqkpggsdfir). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Group II+ share the technical feature of a method for detecting or quantifying a signaling protein that is tyrosine phosphorylated in Leukemia signaling pathways, said method comprising the step of utilizing one or more of the following reagents to detect or quantify one or more Leukemia-related signaling protein(s). However, this shared technical feature does not represent a contribution over the prior art. Specifically, Crosby teaches a method for detecting or quantifying a signaling protein (para [0121]-- (b) detecting the level of at least one biomarker disclosed herein in said test tissue of step (a) using at least one phospho-specific antibody of the invention under conditions suitable for formation of an antibody-protein complexes) that is tyrosine phosphorylated in Leukemia signaling pathways (para [0011]--phospho-specific antibodies that bind to proteins phosphorylated at specific tyrosines, serines or threonines and para [0070]--the cancer is chronic myelogenous leukemia (CML) or acute lymphocytic leukemia (ALL)), said method comprising the step of utilizing one or more of the following reagents to detect or quantify one or more Leukemia-related signaling protein(s) and when phosphorylated at the tyrosine wherein said antibody does not bind said protein when not phosphorylated at said tyrosine (para [0047]--the antibodies may also be tested by Western blotting against cell preparations containing the phosphorylated target protein to confirm reactivity with the desired phosphorylated target). As said method was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

--Please see the following Supplemental Box --

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 06/34050

***** Supplemental Box *****

Continuation of Box III:

Another technical feature of the inventions listed as Group II+ is the specific phosphorylation site sequence recited therein. The inventions do not share a special technical feature, because US 2002/0142432 A1 to Roch, et al. (hereinafter "Roch") teaches the claimed SEQ ID NO: 170 (100% identity to SEQ ID NO: 11--arYphlgqkpggsdflr). Without a shared special technical feature, the inventions lack unity with one another.

Finally, another technical feature of the inventions listed as Group II+ is the specific (ii) a heavy-isotope labeled peptide (AQUA peptide) for the quantification of said protein, said labeled peptide comprising the phosphorylation site peptide sequence listed in corresponding Column E of Table 1 (SEQ 10 NOs: 1-3,6-28, 30-42, 44-93, 95-168, 170-183, 185-203, 205-278, and 280-288). The inventions do not share a special technical feature, because 1) Roch discloses the claimed SEQ ID NO: 170, comprising the phosphorylatable tyrosine or serine listed in corresponding Column D, Rows 171, of Table 1(100% identity to SEQ ID NO: 11--arYphlgqkpggsdflr), and 2) US 2005/0003450 to Rush, et al. (hereinafter "Rush") discloses a heavy-isotope labeled peptide (AQUA peptide) (para [0167]-- heavy-isotope in the analyzed peptides for quantification is Aqua (absolute quantification)). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Group III+ share the technical feature of an isolated phosphorylation site-specific antibody that specifically binds a human Leukemia-related signaling protein. However, this shared technical feature does not represent a contribution over the prior art. Specifically, Crosby teaches an isolated phosphorylation site-specific antibody that binds a human Leukemia-related signaling protein and (para [0011]--phospho-specific antibodies that bind to proteins phosphorylated at specific tyrosines, serines or threonines and para [0070]--the cancer is chronic myelogenous leukemia (CML) or acute lymphocytic leukemia (ALL)) and only when phosphorylated at the tyrosine wherein said antibody does not bind said signaling protein when not phosphorylated at said tyrosine (para [0046]-- specificity for the desired antigen and for reactivity only with the phosphorylated form of the antigen). As said antibody was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Another technical feature of the inventions listed as Group III+ is the specific phosphorylation site sequence recited therein. The inventions do not share a special technical feature, because Roch teaches the claimed SEQ ID NO: 170 (100% identity to SEQ ID NO: 11--arYphlgqkpggsdflr). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Group IV+ share the technical feature of a heavy-isotope labeled peptide (AQUA peptide) for the quantification of a human Leukemia-related signaling protein. However, this shared technical feature is obvious over the prior art. Specifically, 1) Roch teaches the claimed SEQ ID NO: 170, comprising the phosphorylatable tyrosine or serine listed in corresponding Column D, Rows 171, of Table 1(100% identity to SEQ ID NO: 11--arYphlgqkpggsdflr) but neither Crosby nor Roch teaches a heavy-isotope labeled peptide (AQUA peptide), and 2) Rush teaches a heavy-isotope labeled peptide (AQUA peptide) (para [0167]-- heavy-isotope in the analyzed peptides for quantification is Aqua (absolute quantification)). As said heavy-isotope labeled peptide would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

The inventions of Groups I+ through IV+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.