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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR DETECTING PROTEASE ACTIVITY IN BIOLOGICAL SYSTEMS

(57) Abstract: The invention relates generally to compositions and methods for detecting protease activity in a subject or a biological sample using activatable antibodies, and the use of these compositions and methods in a variety of diagnostic indications.

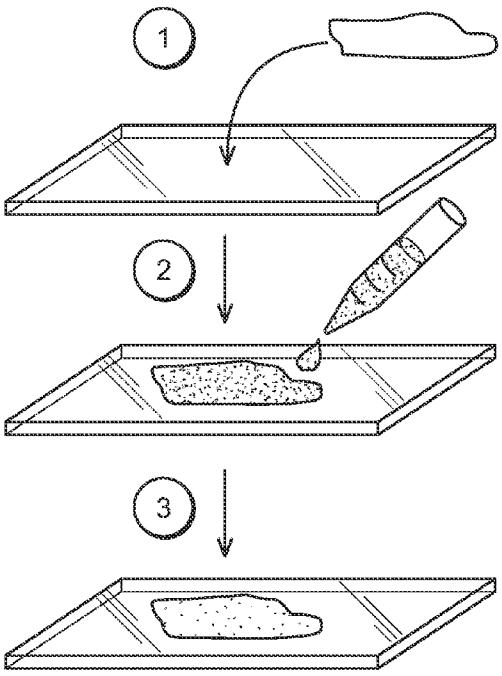

FIG. 1

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HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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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:
4 September 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/10216

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48; C12P 21/08; A61K 39/395 (2014.01)
USPC - 435/7.72; 530/387.3; 424/134.1
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 33/48, 33/53, 33/563, 33/531, 33/532, 33/533, 33/534, 33/535; C12P 21/08; A61K 39/395; C07K 16/46, 19/00 (2014.01)
USPC: 435/7.72, 7.1, 4, 23, 24; 530/387.3, 391.3, 391.1, 388.1, 387.1, 386, 380, 350; 424/134.1,133.1, 130.1, 9.1, 192.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Proquest Dialog; PubMed; PubMed Central; Google/Google Scholar: antibody, immunoglobulin, mask, block, inhibit, moiety, 'functional group,' linker, 'linking peptide,' 'ADAM8,' substrate, protease, activatable, cleavable

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2010/081173 A2 (STAGLIANO, NE et al.) July 15, 2010; paragraphs [00195]-[00197], [00349]-[00353]	1-3, 4/2, 4/3, 5-10, 12-18, 20-26, 28 ------ 11, 19
Y	WO 2009/025846 A2 (DAUGHERTY, PS et al.) February 26, 2009; paragraph [00150]; Table 2	11
Y	US 2010/0150939 A1 (BLANCHETOT, C et al.) June 17, 2010; paragraphs [0005]-[0008], [0026]-[0029]	19
A	US 2010/0189727 A1 (RODECK, U et al.) July 29, 2010; entire document	1-3, 4/2, 4/3, 5-26, 28
A	DONALDSON, J et al. Design And Development Of Masked Therapeutic Antibodies To Limit Off-Target Effects. Cancer Biol Ther. 07 November 2009, Vol. 8, No. 22; pages 2147-2152; entire document; DOI: 10.4161/cbt.8.22.9765.	1-3, 4/2, 4/3, 5-26, 28

☐ 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
11 June 2014 (11.06.2014)	08 JUL 2014
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. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/10216

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 filed or furnished:

   a. (means)

   ☒ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/10216

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.:

Groups I+: Claims 1-9, 10 (in-part), 11 (in-part), 12-18, 19 (in-part), 20-25, 26 (in-part), 28, a VEGF target, an Avastin (bevacizumab) antibody, protease ADAM8, SEQ ID NO: 33 (chemically synthesized linker amino acid sequence)

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/US14/10216

-***-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 1-28 are directed toward a method of detecting presence or absence of a cleaving agent and a target in a subject or a sample, the method comprising: (a) contacting a subject or biological sample with an activatable antibody comprising an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample, and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent or not present at a detectable level in the subject or biological sample.

The method of detecting presence or absence of a cleaving agent and a target in a subject or a sample will be searched to the extent that it encompasses a VEGF target, an Avastin (bevacizumab) antibody, protease ADAM8, and a linker comprising SEQ ID NO: 33 (chemically synthesized linker amino acid sequence. It is believed that claims 1-9, 10 (in-part), 11 (in-part), 12-18, 19 (in-part), 20-25, 26 (in-part) and 28 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a VEGF target, an Avastin (bevacizumab) antibody, protease ADAM8 and SEQ ID NO: 33 (chemically synthesized linker amino acid sequence). 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: a target EGFR, the antibody Erbitux (cetuximab), protease BACE, and SEQ ID NO: 34 (chemically synthesized linker amino acid sequence).

Groups I+ share the technical features including a method of detecting presence or absence of a cleaving agent and a target in a subject or a sample, the method comprising: (a) contacting a subject or biological sample with an activatable antibody comprising an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample, and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent or not present at a detectable level in the subject or biological sample; and wherein the activatable antibody comprises a first linking peptide (LPI) and a second linking peptide (LP2), and wherein the activatable antibody in an uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.

However, these shared technical features are previously disclosed by WO 2010/081173 A2 to Stagliano, et al. (hereinafter 'Stagliano'). Stagliano discloses a method of detecting presence or absence of a cleaving agent (a method of detecting presence or absence of a cleaving agent; paragraphs [00196], [00349]) and a target in a subject or a sample (and a target in a subject or sample; paragraph [00349]), the method comprising: (a) contacting a subject or biological sample with an activatable antibody comprising an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) measuring a level of activated activatable antibody in the subject or biological sample (contacting a subject or biological sample with an activatable antibody comprising an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) measuring a level of activated activatable antibody in the subject or biological sample (paragraphs [00349], [00350]), wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample (wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample; paragraph [00350]), and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent or not present at a detectable level in the subject or biological sample (wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent or not present at a detectable level in the subject or biological sample; paragraph [00350]); and wherein the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in an uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM (wherein the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in an uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM; paragraph [00410]).

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