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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, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, 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:

27 November 2014

(54) Title: SPECIFIC SITES FOR MODIFYING ANTIBODIES TO MAKE IMMUNOCONJUGATES

(57) Abstract: The present invention provides specific sites for modifying antibodies or antibody fragments by replacing at least one native amino acid in the constant region of a parental antibody or antibody fragment with cysteine, which can be used as a site of attachment for a payload or linker-payload combination.



WO 2014/124316 A3

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/015393

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07K16/30 A61P35/00 A61K47/48
 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)

C07K A61K

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)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Anonymous: "www.bioinf.org.uk : Dr. Andrew C.R. Martin's Group at UCL", 25 February 2014 (2014-02-25), XP055122195, Retrieved from the Internet: URL:http://www.bioinf.org.uk/abs/ [retrieved on 2014-06-06] the whole document ----- -/--	1



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

13 June 2014

Date of mailing of the international search report

29/09/2014

Name and mailing address of the ISA/

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Bettio, Andrea

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/015393

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 and necessary to the claimed invention, the international search was carried out on the basis of:
- 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 purpose of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto 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/US2014/015393

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.: 7, 8, 10, 15, 40, 45, 46
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:
see FURTHER INFORMATION sheet PCT/ISA/210
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 additional sheet

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 an 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.:
1-3, 9, 11-13, 16-38, 41, 42, 47-52(completely); 56, 57(partially)

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/US2014/015393

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/034488 A2 (GENENTECH INC [US]; EIGENBROT CHARLES W [US]; JUNUTULA JAGATH REDDY [U] 30 March 2006 (2006-03-30)	1,2,13, 24-28, 30-32, 34,36, 41,47, 48,56,57
Y	Page 98, line 8-page 97, line 22; figures 20-25	2,3,9, 11-13, 16-23, 29, 32-37, 41,42, 47-49, 56,57
X	----- WO 2009/092011 A1 (MEDIMMUNE LLC [US]; DIMASI NAZZARENO [US]; GAO CHANGSHOU [US]; WU HERR) 23 July 2009 (2009-07-23)	13, 24-27, 34,38, 47,48
Y	Paragraphs 220-227, 260-270	2,3,9, 11-13, 16-23, 29, 32-37, 41,42, 47-49, 56,57
X	----- VOYNOV VLADIMIR ET AL: "Design and application of antibody cysteine variants", BIOCONJUGATE CHEMISTRY, ACS, WASHINGTON, DC, US, vol. 21, no. 2, 17 February 2010 (2010-02-17), pages 385-392, XP002598497, ISSN: 1043-1802, DOI: 10.1021/BC900509S [retrieved on 2010-01-21] cited in the application Abstract; page 386, first paragraph, page 390, figure 4.	38
X	----- WO 2010/141902 A2 (NOVARTIS AG [CH]; MASSACHUSETTS INST TECHNOLOGY [US]; CHENNAMSETTY NAR) 9 December 2010 (2010-12-09) Claim 1; paragraphs 189-191, 206 ----- -/--	38

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/015393

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JAGATH R JUNUTULA1 ET AL: "Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index", INTERNET CITATION, 2008, pages 1-8, XP007905289, ISSN: 1087-0156 Retrieved from the Internet: URL: http://dx.doi.org/10.1038/nbt.1480 [retrieved on 2008-07-24] cited in the application Abstract, page 3, first paragraph.	1-3,9, 11-13, 16-38, 41,42, 47-52, 56,57
Y	STEPHEN C. ALLEY ET AL: "Contribution of Linker Stability to the Activities of Anticancer Immunoconjugates", BIOCONJUGATE CHEMISTRY, vol. 19, no. 3, 1 March 2008 (2008-03-01), pages 759-765, XP055037546, ISSN: 1043-1802, DOI: 10.1021/bc7004329 cited in the application Abstract; page 761, figure 1	24-31
Y	W. OU ET AL: "Site-specific protein modifications through pyrroline-carboxy-lysine residues", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 108, no. 26, 28 June 2011 (2011-06-28), pages 10437-10442, XP055122289, ISSN: 0027-8424, DOI: 10.1073/pnas.1105197108 Abstract; page 10438	49-52
A	LYONS A ET AL: "SITE-SPECIFIC ATTACHMENT TO RECOMBINANT ANTIBODIES VIA INTRODUCED SURFACE CYSTEINE RESIDUES", PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 3, no. 8, 1 January 1990 (1990-01-01), pages 703-708, XP001000052, ISSN: 0269-2139 cited in the application Abstract, page 707, table 1	1-3,9, 11-13, 16-38, 41,42, 47-52, 56,57
A	US 2012/009621 A1 (YAMASAKI MOTOO [JP] ET AL) 12 January 2012 (2012-01-12) Paragraphs 338, 407, 444	1-3,9, 11-13, 16-38, 41,42, 47-52, 56,57
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/015393

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO 2013/093809 A1 (PFIZER [US]) 27 June 2013 (2013-06-27) Page 125, SEQID 52; page 137, SEQID 17, 29, 30, 38. -----	1,3,38
Y,P	WO 2013/185115 A1 (SUTRO BIOPHARMA INC [US]) 12 December 2013 (2013-12-12) Paragraphs 380-347 -----	1,38

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/015393

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006034488	A2	30-03-2006	
		AU 2005286607 A1	30-03-2006
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		US 2012009621 A1	12-01-2012
		WO 2011118739 A1	29-09-2011
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WO 2013185115	A1	12-12-2013	
		US 2014046030 A1	13-02-2014
		WO 2013185115 A1	12-12-2013

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3, 9, 11-13, 16-38, 41, 42, 47-52(completely); 56, 57(partially)

An immunoconjugate comprising a modified antibody or antibody fragment thereof wherein said modified antibody or antibody fragment comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 117, 119, 121, 124, 139, 152, 153, 155, 157, 164, 169, 171, 174, 189, 205, 207, 246, 258, 269, 274, 286, 288, 290, 292, 293, 320, 322, 326, 333, 334, 335, 337, 344, 355, 360, 375, 382, 390, 392, 398, 400 and 422 of the heavy chain of said antibody or antibody fragment, and wherein said positions are numbered according to the EU system

2. claims: 4-6, 14, 39, 43, 44(completely); 56, 57(partially)

An immunoconjugate comprising a modified antibody or antibody fragment thereof wherein said modified antibody or antibody fragment comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 107, 108, 109, 114, 129, 142, 143, 145, 152, 154, 156, 159, 161, 165, 168, 169, 170, 182, 183, 197, 199, and 203 of a light chain of said antibody or antibody fragment, wherein said positions are numbered according to the EU system, and wherein said light chain is a human kappa light chain, and wherein the antibody does not comprise a substitution of one or more amino acids with cysteine on its constant region selected from positions 117, 119, 121, 124, 139, 152, 153, 155, 157, 164, 169, 171, 174, 189, 205, 207, 246, 258, 269, 274, 286, 288, 290, 292, 293, 320, 322, 326, 333, 334, 335, 337, 344, 355, 360, 375, 382, 390, 392, 398, 400 and 422 of the heavy chain of said antibody or antibody fragment, and wherein said positions are numbered according to the EU system

3. claims: 53-55

A method to select an amino acid of an antibody that is suitable for replacement by cysteine to provide a suitable site for conjugation according to present claim 53

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 7, 8, 10, 15, 40, 45, 46

Present claims 7, 8, 10, 15, 40, 45, 46 refers to an immunoconjugate wherein a TAG encoded amino acid has been introduced at a position chosen from 143, 145, 147, 156, 159, 163 and 168 of a light chain of the antibody wherein said positions are numbered according to the Kabat system.

"www.bioinf.org.uk : Dr. Andrew C.R. Martin's Group at UCL", 25 February 2014 (2014-02-25), Retrieved from the Internet:
URL:<http://www.bioinf.org.uk/abs/> (page 1) discloses the Kabat numbering scheme for antibodies: insertions are numbered between residue H100 and H101 with letters up to K. If there are more residues than that, there is no standard way of numbering them. It is therefore not possible to identify positions 43, 145, 147, 156, 159, 163 and 168.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.