(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2018 (15.02.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/029350 A3

(51) International Patent Classification:
C07K 14/74 (2006.01)

(21) International Application Number:
PCT/EP2017/070460

(22) International Filing Date:
11 August 2017 (11.08.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/373,695 11 August 2016 (11.08.2016) US

(71) Applicant: **KITE PHARMA EU B.V.** [NL/NL]; Science Park 408, 1098 XH Amsterdam (NL).

(72) Inventor: **WEISSBRICH, Bianca**; Eerste Osterparkstraat 37-2, 1091GT Amsterdam (NL).

(74) Agent: **STOLMÁR & PARTNER PATENTANWÄLTE PARTG MBB**; Blumenstr. 17, 80331 München (DE).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, 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))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
11 May 2018 (11.05.2018)

(48) Date of publication of this corrected version:
05 April 2018 (05.04.2018)

(15) Information about Correction:
see Notice of 05 April 2018 (05.04.2018)

WO 2018/029350 A3

(54) Title: METHODS OF MAKING AND USING SOLUBLE MHC MOLECULES

(57) Abstract: Provided herein is a novel K_{on}-rate assay and an improved TCR ligand k_{off}-rate assay, which enables a broader application through a novel combination with UV peptide exchange technology. The disclosure enables K_{off}-rate MHC monomer preparation in a high throughput manner, which can then be used to screen TCR candidates for extended peptide libraries in assays such as the TCR ligand K_{off}-rate assay that was previously not feasible. Further, the UV peptide exchange with the K_{off}-rate MHC monomers allows the analysis of TCR candidates recognizing specific peptides carrying the amino acid cysteine, which previously could interfere with or even abolish the k_{off}-rate measurement.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/070460

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/74
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 G01N

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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Bianca Weißbrich: "T cell receptor binding avidity of antigen-specific CD8 + cytotoxic T cells in chronic infection", , 18 November 2015 (2015-11-18), pages 1-150, XP055414369, München Retrieved from the Internet: URL:https://mediatum.ub.tum.de/doc/1254464/1254464.pdf [retrieved on 2017-10-10] page 44, paragraph 1 - page 52, paragraph 1 page 53, paragraph 2 - page 68, last paragraph page 90 - page 91; figures 4-40,4-41	1-22

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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
25 January 2018	19/02/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Burkhardt, Peter

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Magdalena Nauerth: "Development of a novel TCR avidity assay for human CD8+ T cells", , 1 January 2012 (2012-01-01), XP055308572, Retrieved from the Internet: URL:http://mediatum.ub.tum.de/doc/1097838/1097838.pdf page 35, paragraph 1 - page 38, last paragraph	1-12
Y	RODENKO B ET AL: "Generation of peptide-MHC class I complexes through UV-mediated ligand exchange", NATURE PROTOCOLS, NATURE PUBLISHING GROUP, GB, vol. 1, no. 3, 1 January 2006 (2006-01-01), pages 1120-1131, XP003027415, ISSN: 1750-2799, DOI: 10.1038/NPROT.2006.121 page 1120, column 2, last paragraph - page 1122, column 2, paragraph 1; figures	1-22, 41-54
X	M. NAUERTH ET AL: "TCR-Ligand koff Rate Correlates with the Protective Capacity of Antigen-Specific CD8+ T Cells for Adoptive Transfer", SCIENCE TRANSLATIONAL MEDICINE, vol. 5, no. 192, 3 July 2013 (2013-07-03), pages 192ra87-192ra87, XP055414589, ISSN: 1946-6234, DOI: 10.1126/scitranslmed.3005958	13-22
Y	page 7, column 2, paragraph 3 page 2, column 1, paragraph 2 - page 4, column 1, paragraph 2	1-12
Y	BIANCA WEISSBRICH ET AL: "Adoptive immunotherapy", ONCOIMMUNOLOGY, vol. 2, no. 10, 1 October 2013 (2013-10-01), page e26199, XP055211662, DOI: 10.4161/onci.26199 page 1, column 2, paragraph 2 - column 3, paragraph 1; figure 1	1-12
A	WO 99/11775 A1 (HARVARD COLLEGE [US]; GEN HOSPITAL CORP [US]; WALKER JUERGEN B [US]; G) 11 March 1999 (1999-03-11) page 2, line 14 - page 4, line 28	1-11

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLEUN HOMBRINK ET AL: "Mixed functional characteristics correlating with TCR-ligand k off -rate of MHC-tetramer reactive T cells within the naive T-cell repertoire", EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 43, no. 11, 25 November 2013 (2013-11-25), pages 3038-3050, XP055211633, ISSN: 0014-2980, DOI: 10.1002/eji.201343397	13-22
Y	page 3038, column 1, last paragraph - page 3039, column 1, paragraph 3	13-22

X	MAGDALENA NAUERTH ET AL: "The clinical potential for k off -rate measurement in adoptive immunotherapy", EXPERT REVIEW OF CLINICAL IMMUNOLOGY, vol. 9, no. 12, 1 December 2013 (2013-12-01), pages 1151-1153, XP055211630, ISSN: 1744-666X, DOI: 10.1586/1744666X.2013.855609	13-22
Y	page 1152, column 1, paragraph 2 - column 2, last paragraph	13-22

X	BATARD P ET AL: "Dextramers: New generation of fluorescent MHC class I/peptide multimers for visualization of antigen-specific CD8<+> T cells", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 310, no. 1-2, 20 March 2006 (2006-03-20), pages 136-148, XP028017573, ISSN: 0022-1759, DOI: 10.1016/J.JIM.2006.01.006 [retrieved on 2006-03-20]	41-54
Y	page 137, column 2, paragraph 2 - page 138, column 1, paragraph 3	41-54

X	JUN HUANG ET AL: "Detection, phenotyping, and quantification of antigen-specific T cells using a peptide-MHC dodecamer", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, vol. 113, no. 13, 15 March 2016 (2016-03-15), pages E1890-E1897, XP055349051, US ISSN: 0027-8424, DOI: 10.1073/pnas.1602488113	41-54
Y	page E1896, column 1, paragraph 4 - page E1897, column 2, paragraph 1	41-54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2017/070460

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 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.:

1-22, 41-54

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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-12

   relating to a method of generating a detectably labelled, soluble MHC monomer loaded with a cleavable peptide the method inter alia comprising conjugating a MHC comprising a non naturally occuring cysteine residue with a detectable label

   ---

2. claims: 13-22

   relating to a method of identifying a TCR that associates with a MHC complex

   ---

3. claims: 23-28

   relating to a method of selecting a T cell suitable for adoptive transfer

   ---

4. claims: 29-40

   relating to a method of identifying member of a binding pair of interest

   ---

5. claims: 41-54

   relating to a method of identifying a TCR that associates with a MHC peptide complex

   ---

6. claims: 55-58

   relating to a method of determining dissociation constant KD between a TCR and a MHC peptide complex

   ---

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/070460

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9911775 A1	11-03-1999	AT 321131 T	15-04-2006
		DE 69833949 T2	22-02-2007
		EP 1017799 A1	12-07-2000
		US 6248564 B1	19-06-2001
		WO 9911775 A1	11-03-1999