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(71) **Applicant**: **ERASMUS UNIVERSITY MEDICAL CENTER ROTTERDAM** [NL/NL]; Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL).

(72) **Inventors**: **VAN DONGEN, Jacobus Johannes Maria**; c/o Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL). **ORFAO DE MATOS CORREIA E VALE, José Alberto**; c/o University of Salamanca, Paseo de la Universidad de Coimbra s/n, Campus Miguel de Unameno, E-37007 Salamanca (ES). **FLORES MONTERO, Juan Alejandro**; c/o University of Salamanca, Paseo de la Universidad de Coimbra s/n, Campus Miguel de Unameno, E-37007 Salamanca (ES). **ALMEIDA PARRA, Julia Maria**; c/o University of Salamanca, Paseo de la Universidad de Coimbra s/n, Campus Miguel de Unameno, E-37007 Salamanca (ES). **VAN DER VELDEN, Vincent Henricus Johannes**; c/o Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL). **BÖTTCHER, Sebastian**; c/o University Hospital of Schleswig-Holstein, Arnold Heller Strasse 3, Haus 11, 24105 Kiel (DE). **LANGERAK, Anthonie Willem**; c/o Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL). **MEJSTRÍKOVÁ, Ester**; c/o Charles University, Vuvalu 84, 3015 GE 15006 Prague 5 (CZ). **SZCZEPANSKI, Tomasz**; c/o Medical University of Silesia, Ul. 3 Maja 13/15, PL-41-800 Zabrze (PL). **RITGEN, Matthias**; c/o University Hospital of Schleswig-Holstein, Arnold Heller Strasse 3, Haus 11, 24105 Kiel (DE). **MONTEIRO DA SILVA LUCIO, Paulo Jorge**; c/o Instituto Portugués de Oncologia, 4°Andar / Pavilhao de Medicina, R. Prof. Lima Basto, P-1093 Lisboa Codex (PT).

(74) **Agent**: **JANSEN, C.M.**; Johan de Wittlaan 7, NL-2517 JR Den Haag (NL).

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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**:
24 April 2014

WO 2013/187765 A3

(54) **Title**: METHODS, REAGENTS AND KITS FOR DETECTING MINIMAL RESIDUAL DISEASE

(57) **Abstract**: The invention relates to the field of minimal residual disease (MRD) diagnostics, which is progressively more applied for the evaluation of treatment effectiveness in patients with a hematological malignancy, such as B-cell precursor acute lymphoblastic leukemia (BCP-ALL), B-cell chronic lymphocytic leukemia (B-CLL), and multiple myeloma (MM). Provided are unique reagent compositions with carefully selected and thoroughly tested combinations of antibodies, for ≥ 8-color flow cytometric stainings as well as for 10-color and 12-color flow cytometric stainings, which can reach sensitivities of at least 10^{-4}, even down to 10^{-5}. Also provided are diagnostic kits and methods for detecting MRD.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2013/050420

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50 G01N33/574
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)
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, WPI Data, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LUCIO P ET AL: "Flow cytometric analysis of normal B cell differentiation: A frame of reference for the detection of minimal residual disease in precursor-B-ALL", LEUKEMIA, MACMILLAN PRESS LTD, US, vol. 13, no. 3, 1 January 1999 (1999-01-01), pages 419-427, XP002364964, ISSN: 0887-6924, DOI: 10.1038/SJ/LEU/2401279 abstract page 420, right-hand column; table 1 ----- -/--	1,2, 14-16

[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 September 2013	11/03/2014

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	Moreno de Vega, C

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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.
X	BRAYLAN R C ET AL: "OPTIMAL NUMBER OF REAGENTS REQUIRED TO EVALUATE HEMATOLYMPHOID NEOPLASIAS: RESULTS OF AN INTERNATIONAL CONSENSUS MEETING", CYTOMETRY, ALAN LISS, NEW YORK, US, vol. 46, no. 1, 15 February 2001 (2001-02-15), pages 23-27, XP009050615, ISSN: 0196-4763, DOI: 10.1002/1097-0320(20010215)46:1<23::AID-CYTO1033>3.0.CO;2-Z abstract page 26, left-hand column, paragraph 2; figure 1	1,2
Y	OWAIDAH TAREK M ET AL: "Expression of CD66c and CD25 in acute lymphoblastic leukemia as a predictor of the presence of BCR/ABL rearrangement.", HEMATOLOGY/ONCOLOGY AND STEM CELL THERAPY 2008 JAN-MAR, vol. 1, no. 1, January 2008 (2008-01), pages 34-37, XP009172900, ISSN: 1658-3876 abstract	1-7, 14-16
X	US 2004/224371 A1 (DE MATOS CORREIA E VALLE ALBER [ES] ET AL) 11 November 2004 (2004-11-11) the whole document	1,2, 14-16
Y	POPOV ALEXANDER ET AL: "Immunological Shift On Day 15 of Remission Induction in Children with CD10-Positive B-Cell Precursor ALL Treated by ALL-MB 2008 Protocol", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 114, no. 22, 20 November 2009 (2009-11-20), XP009172816, ISSN: 0006-4971 the whole document	1-7, 14-16

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN DONGEN J J M ET AL: "EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophentyping of normal, reactive and malignant leukocytes", INTERNET CITATION, 4 June 2009 (2009-06-04), pages 1-18, XP002555901, Retrieved from the Internet: URL:http://www.euroflow.org/imagenes/news/euroflow_handout_on_antibody_panels.pdf [retrieved on 2009-11-17] pages 2, 3, 8	1-4, 14-16
X	EP 2 259 065 A1 (UNIV ERASMUS MEDICAL CT [NL]) 8 December 2010 (2010-12-08) claims 1-13; table 7	1-4, 14-16

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2013/050420

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.:

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.:

1-7(completely); 14-16(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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-7(completely); 14-16(partially)

   Reagent composition for flow cytometric detection of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) comprising a panel of at least eight distinct fluorochrome-conjugated antibodies, the panel comprising at least antibodies against the core markers CD10, CD19, CD20, CD34 and CD45. Diagnostic kit comprising said composition. Multi-color flow cytometric method for detecting minimal residual disease (MRD) in a sample, comprising staining the sample with said reagent composition.

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2. claims: 8, 9(completely); 14-16(partially)

   Reagent composition for flow cytometric detection of chronic lymphocytic leukemia (CLL) comprising a panel of at least eight distinct fluorochrome-conjugated antibodies, the panel comprising at least antibodies against the core markers CD5, CD27, CD79b, CD3, CD200, CD81 and CD19. Diagnostic kit comprising said composition. Multi-color flow cytometric method for detecting minimal residual disease (MRD) in a sample, comprising staining the sample with said reagent composition.

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3. claims: 10-13(completely); 14-16(partially)

   Reagent composition for flow cytometric detection of multiple myeloma (MM) or related plasma cell disorders (PCD) comprising a panel of at least eight distinct fluorochrome-conjugated antibodies, the panel comprising at least antibodies against the core markers CD138, CD38, CD56 and CD19, supplemented with at least four additional markers selected from the group consisting of CD27, CD117, CD81, CD229, CD45, CyIg? and CyIg?. Diagnostic kit comprising said composition. Multi-color flow cytometric method for detecting minimal residual disease (MRD) in a sample, comprising staining the sample with said reagent composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2013/050420

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004224371 A1	11-11-2004	NONE	
EP 2259065 A1	08-12-2010	AU 2010254680 A1	12-01-2012
		CA 2764670 A1	09-12-2010
		EP 2259065 A1	08-12-2010
		EP 2438446 A1	11-04-2012
		JP 2012529046 A	15-11-2012
		US 2012165213 A1	28-06-2012
		WO 2010140885 A1	09-12-2010