

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
8 November 2007 (08.11.2007)

PCT

(10) International Publication Number
WO 2007/127219 A3

(51) International Patent Classification:

C12N 15/87 (2006.01) *C07K 16/28* (2006.01)
C12N 15/11 (2006.01)

(21) International Application Number:

PCT/US2007/009975

(22) International Filing Date: 25 April 2007 (25.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/794,817 25 April 2006 (25.04.2006) US

(71) Applicant (for all designated States except US): **THE CBR INSTITUTE FOR BIOMEDICAL RESEARCH, INC.** [US/US]; 800 Huntington Avenue, Boston, MA 02115 (US).

(72) Inventors; and

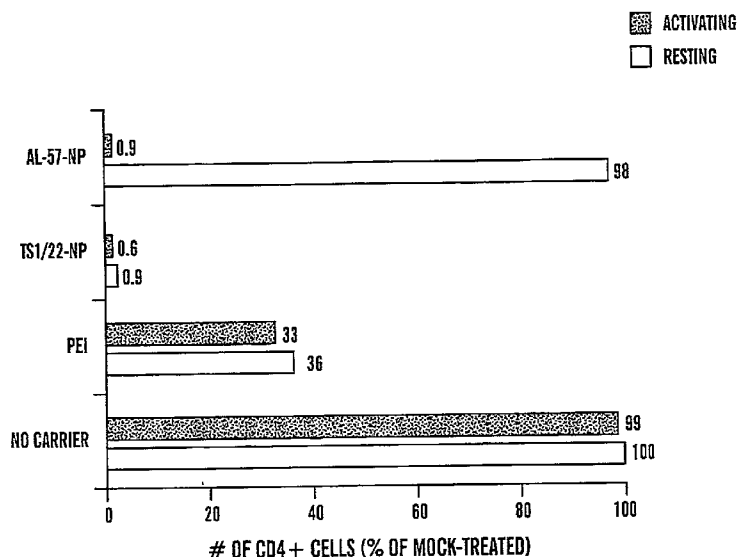
(75) Inventors/Applicants (for US only): **PEER, Daniel** [IL/US]; 57 Saint Paul Street, Brookline, MA 02446 (US).**SHIMAOKA, Motomu** [JP/US]; 73 Longwood Avenue Apt 2F, Brookline, MA 02446 (US). **LIEBERMAN, Judy** [US/US]; 63 Buckminster Road, Brookline, MA 02445 (US).(74) Agents: **RESNICK, David, S.** et al.; Nixon Peabody LLP, 100 Summer Street, Boston, MA 02110-2131 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,

[Continued on next page]

(54) Title: TARGETED DELIVERY TO LEUKOCYTES USING PROTEIN CARRIERS



(57) Abstract: Disclosed herein are a leukocyte-selective delivery agent comprising, a targeting moiety that selectively binds LFA-I, a protein carrier moiety covalently linked to the targeting moiety, and a therapeutic agent associated with the carrier moiety. The delivery agent may be further selective for activated leukocytes, wherein the targeting moiety selectively binds LFA-I in its activated conformation. The targeting moiety comprises an antibody or functional fragment thereof, such as an scFv. Examples of antibodies or fragments thereof which selectively bind LFA-I activated conformation bind to the locked open I domain of LFA-I, or binds to the leg domain of the $\beta 2$ subunit of LFA-I ((ILP2)- The antibody or functional fragment thereof may alternatively bind non-selectively to both low affinity and high affinity LFA-I. Examples

of a non-protein carrier are a basic polypeptide such as protamine or a functional fragment thereof. One such fragment is RSQSRSYRQRQSRRRRRRS. The therapeutic agent may comprise one or more of a nucleic acid, a small molecule, a polypeptide, and an antibody or functional fragment thereof. An example of a nucleic acid delivery agent comprises an RNA interference molecule. Examples of RNA interference molecules are siRNA, dsRNA, StRNA, shRNA, miRNA, and combinations thereof. Specific siRNAs are provided. Other examples of a nucleic acid delivery agent are a small RNA, an antagomir, an LNA, and an antisense oligonucleotide. Methods for leukocyte-selective delivery, or activated leukocyte-selective delivery in vivo, in vitro and ex vivo are also provided.



FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,
PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

Declaration under Rule 4.17:

- *of inventorship (Rule 4.17(iv))*

(88) Date of publication of the international search report:

27 March 2008

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/009975

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/87 C12N15/11 C07K16/28

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)

C12N C07K

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 practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>PEER DAN ET AL: "Selective gene silencing in activated leukocytes by targeting siRNAs to the integrin lymphocyte function-associated antigen-1"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 104, no. 10, March 2007 (2007-03), pages 4095-4100, XPO02461641</p> <p>ISSN: 0027-8424</p> <p>the whole document</p> <p style="text-align: center;">----- -/--</p>	1-34

☒ 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 document 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

31 January 2008

Date of mailing of the international search report

08/02/2008

Name and mailing address of the ISA/

European Patent Office, P.B. 5816 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

RENGGLI-ZULLIGER, N

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/009975

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SONG E ET AL: "Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 23, no. 6, 22 May 2005 (2005-05-22), pages 709-717, XP002390626 ISSN: 1087-0156 the whole document	1-34
Y	-& WO 2006/023491 A (CBR INST FOR BIOMEDICAL RES IN [US]; LIEBERMAN JUDY [US]; SONG ERWEI [] 2 March 2006 (2006-03-02) cited in the application	1-34
Y	----- WO 2005/079515 A (DYAX CORP [US]; COHEN EDWARD H [US]; RONDON ISAAC J [US]) 1 September 2005 (2005-09-01) cited in the application abstract and pages 98-99 abstract	1-34
Y	----- XIE CAN ET AL: "The integrin alpha-subunit leg extends at a Ca2+-dependent epitope in the thigh/genu interface upon activation" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 101, no. 43, 26 October 2004 (2004-10-26), pages 15422-15427, XP002461644 ISSN: 0027-8424 abstract; figures 6,7	1-34
Y	----- LU CHAFEN ET AL: "The binding sites for competitive antagonistic, allosteric antagonistic, and agonistic antibodies to the I domain of integrin LFA-1" JOURNAL OF IMMUNOLOGY, vol. 173, no. 6, 15 September 2004 (2004-09-15), pages 3972-3978, XP002461643 ISSN: 0022-1767 abstract; table 1 ----- -/--	1-34

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/009975

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>HUANG C ET AL: "STRUCTURAL AND FUNCTIONAL STUDIES WITH ANTIBODIES TO THE INTEGRIN BETA2 SUBUNIT"</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 28, no. 275, 14 July 2000 (2000-07-14), pages 21514-21524, XP002901973</p> <p>ISSN: 0021-9258</p> <p>abstract; figure 1</p> <p>-----</p>	1-34
Y	<p>LI X ET AL: "Single-chain antibody-mediated gene delivery into ErbB2-positive human breast cancer cells"</p> <p>CANCER GENE THERAPY, NORWALK, CT, US, vol. 8, no. 8, 2001, pages 555-565, XP002994670</p> <p>ISSN: 0929-1903</p> <p>abstract</p> <p>-----</p>	1-34
A	<p>WAGNER E ET AL: "TRANSFERRIN-POLYCATION CONJUGATED AS CARRIERS FOR DNA UPTAKE INTO CELLS"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 87, no. 9, May 1990 (1990-05), pages 3410-3414, XP002002759</p> <p>ISSN: 0027-8424</p> <p>abstract</p> <p>-----</p>	1-34
A	<p>VORNLOCHER ET AL: "Antibody-directed cell-type-specific delivery of siRNA"</p> <p>TRENDS IN MOLECULAR MEDICINE, ELSEVIER CURRENT TRENDS, vol. 12, no. 1, January 2006 (2006-01), pages 1-3, XP005250430</p> <p>ISSN: 1471-4914</p> <p>the whole document</p> <p>-----</p>	1-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/009975

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:

Although claims 16-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
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:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable 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 reportcovers 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.:

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

Information on patent family members

International application No

PCT/US2007/009975

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006023491 A	02-03-2006	AU 2005277547 A1	02-03-2006
		CA 2576925 A1	02-03-2006
		EP 1789447 A2	30-05-2007
WO 2005079515 A	01-09-2005	AU 2005215024 A1	01-09-2005
		CA 2554965 A1	01-09-2005
		EP 1761562 A2	14-03-2007