ENDOTHELIAL CELLS ACTIVATION BIOMARKERS CHARACTERIZING ANTIBODY MEDIATED REJECTION AND USES THEREOF

FIGURE 5A

Abstract: Described herein are methods and kits for the detection of endothelial cell injury and/or activation and to the diagnostic of transplant antibody mediated rejection (ABMR). The invention further relates to methods and kits for diagnosing endothelial to mesenchymal transition (EndMT). In various embodiments, the methods comprise assessing expression of one, two or three biomarkers selected from Fascin1, Vimentin and Hsp47.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/48 G01N33/68
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)
C12Q 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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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[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) on which is cited to establish the publication date of another citation or other special reason (as specified)
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- "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
- "Z" document member of the same patent family

Date of the actual completion of the international search:
6 August 2013

Date of mailing of the international search report:
25/10/2013

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

Authorized officer:
Schmidt-Yodlee, H
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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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</table>
| X         | M. HAZZAN ET AL: "Epi thel i al-to-Mesenchymal Transi tion
Predi cts Cyclosporin Ne phrotoxi city in
Renal Transplant Recipients", JOURNAL OF THE AMERICAN SOCIETY OF
NEPHROLOGY, vol. 22, no. 7, 1 July 2011 (2011-07-01), pages 1375-1381, XP055074161, ISSN: 1046-6673, DOI: 10.1681/ASN.2010060673
page 1378 - page 1379; figure 5 | 1-13, 25-31, 43 |
| X         | RIE DER F ET AL: "M1186
Endothelial-to-Mesenchymal Transition of
abstract | 1-13, 25-31, 43 |
| X         | M. NING ET AL: "Proteomic Temporal Profile of Human Brain Endothelium After
Oxidative Stress", STROKE, vol. 42, no. 1, 1 January 2011 (2011-01-01), pages 37-43, XP055072963, ISSN: 0039-2499, DOI: 10.1161/STROKEAHA.110.585703
abstract; table 1 | 1-13, 25-31, 43 |
| X         | GHIMI RE GOPAL ET AL: "Endothelial Cell Injury Induced by Intracoronary Sera in
Patients With ST Elevation Myocardial Infarction", JOURNAL OF THE AMERICAN COLLEGE OF
CARDIOLOGY: 58TH ANNUAL SCIENTIFIC SESSION
[retrieved on 2009-03-03] abstract | 1-13, 25-31, 43 |

Form PCT/ISA/210 (continuation of second sheet) (April 2008)
### Documents Considered to Be Relevant

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<tr>
<td>X</td>
<td>ZEISBERG ELISABETH M ET AL: &quot;Endothelial-to-mesenchymal transition contributes to cardiac fibrosis&quot;, NATURE MEDICINE, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 13, no. 8, 1 August 2007 (2007-08-01), pages 952-961, XP002553821, ISSN: 1078-8956, DOI: 10.1038/NM1613 (retrieved on 2007-07-29) cited in the application abstract; figures 1-6</td>
<td>2-13, 25-31, 43</td>
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<tr>
<td>X</td>
<td>BELGE GAZANFER ET AL: &quot;Upregulation of the high mobility group AT-hook 2 gene in acute aorto-iliac dissection is potentially associated with endothelial-to-mesenchymal transition&quot;, HISTOLOGY AND HISTOPATHOLOGY: CELLULAR AND MOLECULAR BIOLOGY, GUTENBERG, ES, vol. 26, no. 8, 1 August 2011 (2011-08-01), pages 1029-1037, XP008163831, ISSN: 0213-3911 Retrieved from the Internet: URL: <a href="http://www.hh.um.es/Abstracts/Vol26/26_8_1029.htm">http://www.hh.um.es/Abstracts/Vol26/26_8_1029.htm</a> abstract; figures 1-4; table 1</td>
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<td>A</td>
<td>EP 2 000 802 Al (Mochida Pharm Co Ltd [JP]) 10 December 2008 (2008-12-10) paragraph [0009]; claims 15-25</td>
<td>1-13, 25-31,43</td>
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<td>A</td>
<td>Liu Y: &quot;Epithelial to Mesenchymal Transition in Renal Fibrogenesis: Pathologic Significance, Molecular Mechanism, and Therapeutic Intervention&quot;, Journal of the American Society of Nephrology, vol. 15, 2004, pages 1-12, XP002709962, DOI: 10.1097/01.ASN.0000106015.29070.E7 abstract; figures 2,3; table 1 page 8</td>
<td>1-13, 25-31,43</td>
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<td>US 2009017019 A1</td>
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<td>WO 2008151078 A1</td>
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<td>WO 2007116779 A1</td>
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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).

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 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.:

   1-13, 25-31 (completely); 43 (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.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 25-31 (completely); 43 (partially)
   method for detecting endothelial cell injury and/or activation in a mammalian subject; method for identifying a mammalian subject showing endothelial injury

1.1. claims: 1-13, 25-31, 43 (partially)
   method according to independent claims 1 and 25, wherein the at least biomarker is Fascin

1.2. claims: 1-13, 25-31, 43 (partially)
   method according to independent claims 1 and 25, wherein the at least biomarker is Vimentin

1.3. claims: 1-13, 25-31, 43 (partially)
   method according to independent claims 1 and 25, wherein the at least biomarker is Hsp47

2. claims: 14-24, 32-38 (completely); 43 (partially)
   method for diagnosing antibody mediated rejection in an allograft; method for preventing progression of antibody mediated tissue injury in a patient with allograft

3. claims: 39-42
   diagnostic kit