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WO 2012/010905 A3

(54) Title: ASSAYS FOR SCREENING DRUG SAFETY

(57) Abstract: The present invention relates to methods of screening test substances in order to evaluate their toxic or protective effects on mammalian cells. In particular, the invention relates to the methods of obtaining an indication of the cardiotoxic or cardioprotective effect of a test substance by detecting or measuring the production of mi RNA by mammalian cells which have been contacted with the test substance.

INTERNATIONAL SEARCH REPORT

International application No PCT/GB2011/051391

A. CLASSIFICATION OF SUBJECT MATTER INV. C12Q1/68 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				
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, 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	WO 2008/042231 A2 (CHILDRENS MEDICAL CENTER [US]; IKEDA SADAKATSU [US]; PU WILLIAM [US];) 10 April 2008 (2008-04-10) page 2 page 58, line 16 - page 59, line 8 claims 1-14	24-32,37		
X	US 2009/005336 A1 (WANG ZHIGUO [CA]) 1 January 2009 (2009-01-01) paragraphs [0002], [0152] - [0155] claims 11,14,18 ----- -/--	1,2,4-8, 10-20,36		
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;"><input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.</td> <td style="width: 50%; border: none;"><input checked="" type="checkbox"/> See patent family annex.</td> </tr> </table>			<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.			
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"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			
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Schmitt-Humbert, C			

INTERNATIONAL SEARCH REPORT

International application No.
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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, 2, 4-8, 10-20, 24-32, 36, 37(all 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/GB2011/051391

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HORIE TAKAHIRO ET AL: "Acute doxorubicin cardiotoxicity is associated with miR-146a-induced inhibition of the neuregulin-ErbB pathway.", CARDIOVASCULAR RESEARCH 1 SEP 2010 LNKD-PUBMED:20495188, vol. 87, no. 4, 21 May 2010 (2010-05-21), pages 656-664, XP002659069, ISSN: 1755-3245 abstract</p>	<p>1,2,4-8, 10-20, 24-32, 36,37</p>
X	<p>----- WO 2009/012468 A2 (UNIV COLORADO [US]; PORT JONATHAN DAVID [US]; SUCHAROV CARMEN [US]; BR) 22 January 2009 (2009-01-22) claim 1</p>	<p>30-32</p>
X	<p>----- IKEDA SADAKATSU ET AL: "Altered microRNA expression in human heart disease", PHYSIOLOGICAL GENOMICS, AMERICAN PHYSIOLOGICAL SOCIETY, US, vol. 31, no. 3, 1 November 2007 (2007-11-01), pages 367-373, XP009098819, ISSN: 1094-8341 abstract table 2 page 368, column 2, last paragraph - page 369, column 1, paragraph 1</p>	<p>1,2,4</p>
X	<p>----- MATKOVICH SCOT J ET AL: "Reciprocal regulation of myocardial microRNAs and messenger RNA in human cardiomyopathy and reversal of the microRNA signature by biomechanical support", CIRCULATION, LIPPINCOT WILLIAMS AND WILKINS, BALTIMORE, US, vol. 119, no. 9, 23 February 2006 (2006-02-23), pages 1263-1271, XP007914419, ISSN: 1524-4539, DOI: 10.1161/CIRCULATIONAHA.108.813576</p>	<p>1,2,4</p>
Y	<p>abstract table 2 tables S3a, S3b</p>	<p>6-8, 10-20</p>
Y	<p>----- WO 2008/058394 A1 (UNIV BRITISH COLUMBIA [CA]; HAYDEN MICHAEL [CA]; CARLETON BRUCE [CA];) 22 May 2008 (2008-05-22) claims 1,2,5,6,11,14,15</p>	<p>6-8, 10-20</p>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2011/051391

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008042231 A2	10-04-2008	US 2009306181 A1 WO 2008042231 A2	10-12-2009 10-04-2008

US 2009005336 A1	01-01-2009	NONE	

WO 2009012468 A2	22-01-2009	AU 2008275877 A1 CN 101802227 A EP 2179060 A2 JP 2010533503 A KR 20100049079 A US 2010267804 A1 WO 2009012468 A2	22-01-2009 11-08-2010 28-04-2010 28-10-2010 11-05-2010 21-10-2010 22-01-2009

WO 2008058394 A1	22-05-2008	AU 2007321678 A1 CA 2669131 A1 EP 2102392 A1 EP 2312024 A1 JP 2010508858 A US 2011312907 A1 WO 2008058394 A1	22-05-2008 22-05-2008 23-09-2009 20-04-2011 25-03-2010 22-12-2011 22-05-2008

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, 2, 4-8, 10-20, 24-32, 36, 37(all partially)

Methods of obtaining an indication of the presence of subclinical myocardial injury in a subject comprising detecting/measuring the levels or amount of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-27a; a method for screening for test compounds which are capable or reducing a cardiotoxic effect of a medicament based on the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p wherein one of said miRNAs is miR-27a; methods of obtaining an indication of the cardiotoxic or cardioprotective effect of a test substance based on detecting/measuring the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-27a; methods of obtaining an indication of the susceptibility of a subject to a cardiac disease based on detecting/measuring the levels or amounts of one or more miRNAs, particularly the level or amount of miR-27a; methods for obtaining an indication of the optimal treatment for a subject having cardiac disease or disorder based on detecting/measuring the levels or amounts of one or more miRNAs, particularly the level or amount of miR-27a; methods for obtaining an indication of the prognosis of a subject having a cardiac disease or disorder based on detecting/measuring the levels or amounts of one or more miRNAs, particularly the level or amount of miR-27a and a method of screening for genes involved in one or more cardiac diseases or disorders based on the identification of one or more miRNAs which are overexpressed in subjects suffering from one or more cardiac diseases or disorders, wherein the miRNA is preferably miR-27a.

2. claims: 1, 2, 4-8, 10-20, 24-32, 36, 37(all partially)

Methods of obtaining an indication of the presence of subclinical myocardial injury in a subject comprising detecting/measuring the levels or amount of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-1; a method for screening for test compounds which are capable or reducing a cardiotoxic effect of a medicament based on the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-1; methods of obtaining an indication of the cardiotoxic or cardioprotective effect of a test substance based on detecting/measuring the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-27a; methods of obtaining an indication of the susceptibility of a subject to a cardiac disease based on detecting/measuring the levels or amounts of miR-1; methods for obtaining an indication of the optimal treatment for a subject having cardiac disease or disorder based on detecting/measuring the levels or amounts of miR-1; methods for obtaining an indication of the prognosis of a subject having a cardiac disease or disorder based on detecting/measuring the levels or amounts of miR-1 and a method of screening for genes involved in one or more cardiac diseases or disorders based on the identification of one or more miRNAs which are overexpressed in subjects suffering from one or more cardiac diseases or disorders, wherein the miRNA is miR-1.

3-4. claims: 1, 2, 4-8, 10-20, 24-32, 36, 37(all partially)

Methods of obtaining an indication of the presence of subclinical myocardial injury in a subject comprising detecting/measuring the levels or amount of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-X; a method for screening for test compounds which are capable or reducing a cardiotoxic effect of a medicament based on the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-X; methods of obtaining an indication of the cardiotoxic or cardioprotective effect of a test substance based on detecting/measuring the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-X; methods of obtaining an indication of the susceptibility of a subject to a cardiac disease based on detecting/measuring the levels or amounts of miR-X; methods for obtaining an indication of the optimal treatment for a subject having cardiac disease or disorder based on detecting/measuring the levels or amounts of miR-X; methods for obtaining an indication of the prognosis of a subject having a cardiac disease or disorder based on detecting/measuring the levels or amounts of miR-X and a method of screening for genes involved in one or more cardiac diseases or disorders based on the identification of one or more miRNAs which are overexpressed in subjects suffering from one or more cardiac diseases or disorders, wherein the miRNA is miR-X; wherein in invention 3, miR-X is miR-146a and in invention 4, miR-X is miR-542-3p

5-8. claims: 1-3, 5-9, 11-20, 24-32, 36, 37(all partially)

Methods of obtaining an indication of the presence of

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

subclinical myocardial injury in a subject comprising detecting/measuring the levels or amount of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-X; a method for screening for test compounds which are capable or reducing a cardiotoxic effect of a medicament based on the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-X; methods of obtaining an indication of the cardiotoxic or cardioprotective effect of a test substance based on detecting/measuring the level of one or more miRNAs selected from miR-27a, miR-1, miR-21, miR-133a, miR-133b, miR-146a, miR-181 and miR-542-3p, wherein one of said miRNAs is miR-X; methods of obtaining an indication of the susceptibility of a subject to a cardiac disease based on detecting/measuring the levels or amounts of miR-X; methods for obtaining an indication of the optimal treatment for a subject having cardiac disease or disorder based on detecting/measuring the levels or amounts of miR-X; methods for obtaining an indication of the prognosis of a subject having a cardiac disease or disorder based on detecting/measuring the levels or amounts of miR-X and a method of screening for genes involved in one or more cardiac diseases or disorders based on the identification of one or more miRNAs which are overexpressed in subjects suffering from one or more cardiac diseases or disorders, wherein the miRNA is miR-X; wherein in invention 5, miR-X is miR-133a; in invention 6, miR-X is miR-133b; in invention 7, miR-X is miR-21 and in invention 8, miR-X is miR-181

9. claims: 21-23, 33(completely); 37(partially)

A method of obtaining an indication of the therapeutic or adverse effect of a test substance which has been administered to a subject comprising detecting the levels or amounts of one or more miRNAs selected from miR-1, miR-21, miR-27a, miR-133a, miR-133b, miR-146a, miR-181 and/or miR-542-3p; a method for obtaining an indication of the exposure of a subject to a toxic substance comprising detecting and/or measuring the levels or amounts of one or more miRNAs, wherein the miRNAs are preferably selected from miR-1, miR-21, miR-27a, miR-133a, miR-133b, miR-146a, miR-181 and/or miR-542-3p.
