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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:
4 August 2011

(54) Title: METHOD, SYSTEM AND COMPOSITION FOR OPTICALLY INDUCING CARDIOMYOCYTE CONTRACTION

(57) Abstract: The present invention provides a method, system and composition for screening drug candidates for cardiotoxicity and for novel drugs that effect cardiomyocyte contractility and function. The invention provides an efficient and reliable screening assay to detect the effect of new and potential drug candidates on cardiomyocyte calcium flux, membrane depolarization, and/or the propagation of action potentials.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/58986

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/00; C12N 5/071; (2011.01)
USPC - 435/4; 435/373
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)
IPC(8): C12Q 1/00; C12N 5/071; (2011.01)
USPC: 435/4; 435/373

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/4, 347, 455, 287.1, 288.7, 292.1; 506/10

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google Scholar
Search terms used: cardiomyocyte ion channel culture fibroblast connexin light sensitive protein inducible promoter "gap junction" (HeLa OR CHO OR HEK293 OR NIH/3T3) (Channelrhodopsin OR ChR-1 OR ChR-2 OR Channel rhodopsin)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0054954 A1 (FOLEY et al.) 26 February 2009 (26.02.2009), entire document, especially: para [0003], [0004], [0026], [0054], [0070], [0097], [0099], [0140]-[0151]	1-8
Y	US 2009/0257990 A1 (FELD et al.) 15 October 2009 (15.10.2009), entire document, especially: para [0003], [0027]-[0028], [0169], [0284], [0304], [0391]	1-8
A	US 2004/0137621 A1 (ROSEN et al.) 15 July 2004 (15.07.2004), entire document	1-8

☐ Further documents are listed in the continuation of Box C. ☐

* 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
12 April 2011 (12.04.2011)	0 2 JUN 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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:

------Please see Supplemental 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.:
Group I: Claims 1-8

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/US 10/58986

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This application contains the following inventions or groups of inventions which are not so linked as to from a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-8 , drawn to a culture composition

Group II: Claims 9-27 method of screening a drug candidate for cardiotoxicity, a system for screening a drug candidate, and a method of optically inducing cardiomyocyte contraction.

The groups listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

Groups share the technical feature of claim 1. However, this is not an improvement over the prior art.

Claim 1 lacks an inventive step under PCT Article 33(3) as being obvious over US 2009/0054954 A1 to FOLEY et al., Feb 2009 (hereinafter "Foley"), in view of US 2009/0257990 A1 to FELD et al, Oct 2009. (hereinafter "Feld").

As per claim 1, Foley independently teaches teaches two culture compositions a) and b) (para [0151]--"Donor cells can be expanded in vitro to provide an expanded population of donor cells for administration")
(a) comprising: a cardiomyocyte (para [0140]--"Under specific culture conditions, [embryonic stem] cells differentiate into...cardiomyocytes"; [0141]--"Donor cells within the scope of the invention include...embyronic stem cells"); and
(b) comprising: a non-cardiomyocyte cell (para [0141]--"Donor cells within the scope of the invention include but are not limited to bone marrow-derived cells, e.g., mesenchymal cells and stromal cells, smooth muscle cells, fibroblasts"); wherein at least one non-cardiomyocyte cell comprises a heterologous nucleic acid encoding a light sensitive protein (para [0026]--"A "recombinant viral vector" refers to a viral vector comprising one or more heterologous genes or sequences"; [0099]--"recombinant virus or recombinant cells encoding a light sensitive protein may be administered"; [0004]--"the vector is introduced to mammalian cells ex vivo...the vector is introduced to cardiac cells"); and
optionally at least one noncardiomyocyte cell comprises a heterologous nucleic acid encoding a connexin protein (para [0097]--"promoters and/or enhancers which are not specific for cardiac cells or muscle cells, e.g., RSV promoter, may be employed...Cx40 gene, and Cx43 gene"--Cx40 and Cx43 are connexin protein genes).

However, Foley does not specifically teach a culture composition comprising both a cardiomyocyte and a non-cardiomyocyte, and with the proviso that the cardiomyocyte does not include a light sensitive protein. Feld teaches a culture composition comprising both a cardiomyocyte and a non-cardiomyocyte (para [0284]--"the present inventors utilized a cell culture model system which included fibroblasts which were transfected with ion channel coding sequences and co-cultured with cardiomyocytes").

Although Foley and Feld do not specifically teach with the proviso that the cardiomyocyte does not include a light sensitive protein, Feld does teach that the "co-cultures enabled [us] to test the effects of the ion channel expressing fibroblast on the electrophysiological function of the myocardial cells and to test the effects of various molecules which regulate channel permeability" (para [0284]), and Foley further teaches that the light sensitive protein-comprising non-cardiomyocyte culture composition is utilized for testing and manipulating ion channel regulation (para [0003]). Based on the teachings of Feld and Foley, one of ordinary skill in the art would have found obvious that testing the effect of a heterologous light sensitive protein, introduced via a non-cardiomyocyte, on cardiomyocyte ion channel regulation would be more effective if the protein was not also endogenous to the cardiomyocyte. Further, it would have been obvious to one of ordinary skill in the art to combine the co-culture system of Feld with the light sensitive protein-comprising non-cardiomyocyte of Foley to more effectively control light-induced ion channel manipulation of cardiomyocytes, thus providing a more efficacious treatment of arrhythmia and other cardiac conditions modulated by ion flux (Foley: para [0002]; Feld: para [0027]-[0028]).

Accordingly, unity of invention is lacking.