



(51) International Patent Classification:

C07H 21/04 (2006.01) C12P 21/04 (2006.01)

(21) International Application Number:

PCT/US2012/039897

(22) International Filing Date:

29 May 2012 (29.05.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/490,450 26 May 2011 (26.05.2011) US

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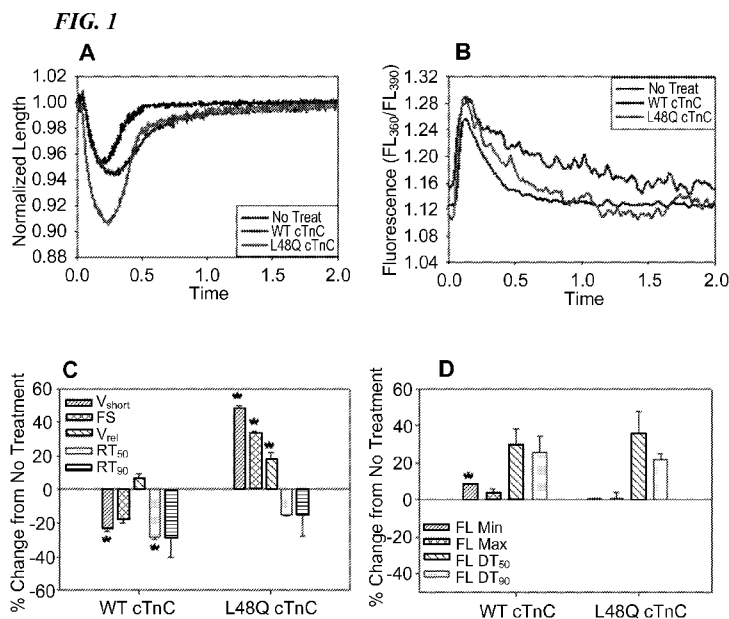
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: CELL AND GENE BASED METHODS TO IMPROVE CARDIAC FUNCTION



(57) Abstract: Compositions and methods for improving cardiac function, myocardial contractility and relaxation in a mammal are provided. Cardiomyocytes transfected with one or more expression vectors comprising a ribonucleotide reductase subunit R1-encoding nucleic acid sequence and a ribonucleotide reductase subunit R2-encoding nucleic acid sequence operably linked to a promoter are grafted to a mammalian myocardium. Also provided are compositions and methods for delivering dATP to a myocardium through grafting of donor cells overexpressing R1 and R2. dATP is thereby produced in situ and delivered through gap junctions established between donor cells and host cardiomyocytes. Alternatively, viral vector(s) having the R1 and R2-encoding construct(s) are administered to the mammal directly. Improvement of cardiac function can also be effected by administration of vectors comprising a nucleic acid sequence encoding a L48Q, 61 Q, or L57Q cTnC variant.

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *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:
28 February 2013

Published:

- *with international search report (Art. 21(3))*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/39897

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07H 21/04; C12P 21/04 (2012.01)

USPC - 536/23.2

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) - C07H 21/04; C12P 21/04 (2012.01) (2012.01)

USPC - 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8) - C12P 21/04, C12N 5/00 (2012.01)

USPC - 435/70.1, 435/325

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, SureChem, GoogleScholar, Dialog

cardiac, cardiomyocytes, myocardium, graft, Ribonucleotide reductase, R1, R2 (M1/M2), dATP, expression vector, adeno-associated viral vector, transduction reporter, human embryonic stem cells, induced pluripotent stem cells (iPSC), fibroblast, infarcted myocardium...

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Regnier et al. 2-Deoxy-ATP Enhances Contractility of Rat Cardiac Muscle. Circ Res. 2000; vol 86, pg 1211-1217. Abstract and pg 1216, col 1, para 2	1-28 and 69-70
Y	Zhou et al. Overexpression of Ribonucleotide Reductase in Transfected Human KB Cells Increases Their Resistance to Hydroxyurea: M2 but not M1 Is Sufficient to Increase Resistance to Hydroxyurea in Transfected Cells. Cancer Research 1995; vol 55, pg 1328-1333. Abstract, pg 1329, col 1, para 5	1-28 and 69-70
Y	US 2008/0152635 A1 (LEE et al.) 26 June 2008 (26.06.2008) Title, Abstract, para[0011], para[0012], para[0013], para[0017], para[0035], para[0078], para[0086], para[0091], para[0159], para[0171]-para[0172]	1-28 and 69-70
Y	US 2007/0117766 A1 (PHILLIPS et al.) 24 May 2007 (24.05.2007) para[0029], para[0031], para[0065], para[0086], para[0103]	5-8 and 23-26 1-28 and 69-70
A	WO 2000/38518 A1 (LEIDEN et al.) 6 July 2000 (06.07.2000) Abstract	1-28 and 69-70
A	US 6,451,594 B1 (CHIEN et al.) 17 September 2002 (17.09.2002) col 2, ln 65- col 3, ln 8	1-28 and 69-70

☐ 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

19 November 2012 (19.11.2010)

Date of mailing of the international search report

18 DEC 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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Facsimile No. 571-273-3201

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)



on paper



in electronic form

b. (time)



in the international application as filed



together with the international application in electronic form



subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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.: 83-88
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:
Claims 83-88 are unsearchable because no electronic sequence listing was submitted in response to the ISA/225 that was mailed on 25 July 2012. The electronic sequence listing filed on 24 August 2012 contained errors and could not be entered into ISA/US's search system/tool.

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 extra sheet for continuation -

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.:
1-28 and 69-70

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.

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International application No.

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Continuation of:

Box NO III. Observations where unity of invention is lacking

Group I: claims 1-28 and 69-70, drawn to a method of improving cardiac function in a mammal comprising:

grafting cardiomyocytes to the myocardium of said mammal, wherein said cardiomyocytes contain a first expression vector comprising a first nucleic acid sequence encoding ribonucleotide reductase subunit R1 and a second expression vector comprising a second nucleic acid sequence encoding ribonucleotide reductase subunit R2, said first nucleic acid sequence and second nucleic acid sequence being operably linked to a promoter that induces overexpression of R1 and R2, thereby generating dATP to improve cardiac function in the mammal.

Group II: claims 29-36 and 63-68, drawn to a method of improving cardiac function in a mammal comprising administering a first viral vector comprising a first nucleic acid sequence encoding ribonucleotide reductase subunit R1 and a second viral vector comprising a second nucleic acid sequence encoding ribonucleotide reductase subunit R2, said first nucleic acid sequence and second nucleic acid sequence being operably linked to a cardiac specific promoter.

Group III: claims 37-62, drawn to a method of improving myocardial contractility and myocardial relaxation in a mammal comprising: grafting cardiomyocytes to the myocardium of said mammal, wherein said cardiomyocytes contain a first expression vector comprising a first nucleic acid sequence encoding ribonucleotide reductase subunit R1 and a second expression vector comprising a second nucleic acid sequence encoding ribonucleotide reductase subunit R2, said first nucleic acid sequence and second nucleic acid sequence being operably linked to a promoter that induces overexpression of R1 and R2, thereby generating dATP to improve myocardial contractility and myocardial relaxation in the mammal.

Group IV: claims 71-82, drawn to a method of improving cardiac function in an individual in need thereof comprising: administering a viral vector comprising a nucleic acid sequence encoding a cTnC variant having a L48Q amino acid substitution, wherein said cTnC variant has an increased binding affinity for Ca²⁺, said nucleic acid sequence being operably linked to a cardiac-specific promoter.

The inventions listed as Groups I through IV 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:

The inventions of Groups I-III do not include the inventive concept of a viral vector comprising a nucleic acid sequence encoding a cTnC variant having a L48Q amino acid substitution, as required by Group IV.

The inventions of Groups I-II do not include the inventive concept of a method for improving myocardial contractility and myocardial relaxation in a mammal, as required by Group III.

The inventions of Groups I do not include the inventive concept of a viral vector and cardiac-specific promoter, as required by Group II.

The inventions of Groups I-IV share the technical feature of a method of improving cardiac function in an individual in need thereof, comprising: administering a vector comprising a nucleic acid sequence. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2008/0152635 A1 to Lee et al. (hereinafter 'Lee'). Lee discloses a method of improving cardiac function in an individual in need thereof (para [0035], providing improved and coordinated electrical coupling with increased efficacy of myocardial contractility), comprising: administering a vector comprising a nucleic acid sequence (para [0012] and [0077]-[0078]). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Groups I-III share the technical feature of a method of improving cardiac function in a mammal comprising: grafting cardiomyocytes to the myocardium of said mammal, wherein said cardiomyocytes contain a first expression vector comprising a first nucleic acid sequence encoding ribonucleotide reductase subunit R1 and a second expression vector comprising a second nucleic acid sequence encoding ribonucleotide reductase subunit R2, said first nucleic acid sequence and second nucleic acid sequence being operably linked to a promoter that induces overexpression of R1 and R2, thereby generating dATP to improve cardiac function in the mammal. This shared technical feature does not represent a contribution over prior art as being obvious over the article entitled '2'-Deoxy-ATP Enhances Contractility of Rat Cardiac Muscle' by Regnier et al. (hereinafter 'Regnier') (Circ Res. 2000; vol 86, pg 1211-1217) in view of the article entitled 'Overexpression of Ribonucleotide Reductase in Transfected Human KB Cells Increases Their Resistance to Hydroxyurea: M2 but not M1 Is Sufficient to Increase Resistance to Hydroxyurea in Transfected Cells' to Zhou et al. (hereinafter 'Zhou') (Cancer Research 1995; vol 55, pg 1328-1333) and Lee, as applied above. Regnier discloses a method of improving cardiac function in a mammal comprising: upregulation of ribonucleotide reductase thereby generating dATP to improve cardiac function in the mammal (abstract and pg 1216, col 1, para 2, Our data thus far suggest that the enhancement of cardiac Fmax makes dATP an attractive candidate for specific improvement of cardiac function. Thus, ribonucleotide reductase, the enzyme responsible for conversion of ATP to dATP, could be considered as a target for therapeutic treatment in heart failure). Zhou teaches a first expression vector comprising a first nucleic acid sequence encoding ribonucleotide reductase subunit R1 and a second expression vector comprising a second nucleic acid sequence encoding ribonucleotide reductase subunit R2, said first nucleic acid sequence and second nucleic acid sequence being operably linked to a promoter that induces overexpression of R1 and R2 (abstract, pg 1329, col 1, para 5, The two vectors pHbeta APr-1-neo/M1 and pHbeta APr-I-neo/M2 were each transfected into the KB wild-type cell to establish a single subunit of RR enzyme in one subclone line, SV40 promoter). Lee discloses a method of improving cardiac function in a mammal (para [0035]) comprising: grafting cardiomyocytes to the myocardium of said mammal (para [0012] and [0086]). One of ordinary skill in the art, in view of the teachings of Regnier, would have been motivated to have introduced the expression vectors comprising ribonucleotide reductase subunit R1 and R2 of Zhou into the cardiomyocytes of Lee for transplanting into myocardium of a subject in need thereof, because Regnier teaches that upregulation of ribonucleotide reductase in the cardiomyocytes improves cardiac function (pg 1216, col 1, para 2) and Lee teaches that transplanted cardiomyocytes could improve and coordinate electrical coupling with increased efficacy of myocardial contractility (para [0035]). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

-----continued in next Supplemental Box-----

INTERNATIONAL SEARCH REPORT

International application No.

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Continuation of:

Box NO III. Observations where unity of invention is lacking

The inventions of Groups II and IV share the technical feature of a viral vector and a cardiac-specific promoter. Lee teaches that viral vector (para [0078]) and a cardiac-specific promoter (para [0079]) is suitable to establish transgenic cardiomyocytes (para [0012], [0078]-[0079]). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I through IV therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.