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— *with international search report (Art. 21(3))*
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08 February 2018 (08.02.2018)

(54) Title: GENE THERAPY METHODS FOR AGE-RELATED DISEASES AND CONDITIONS

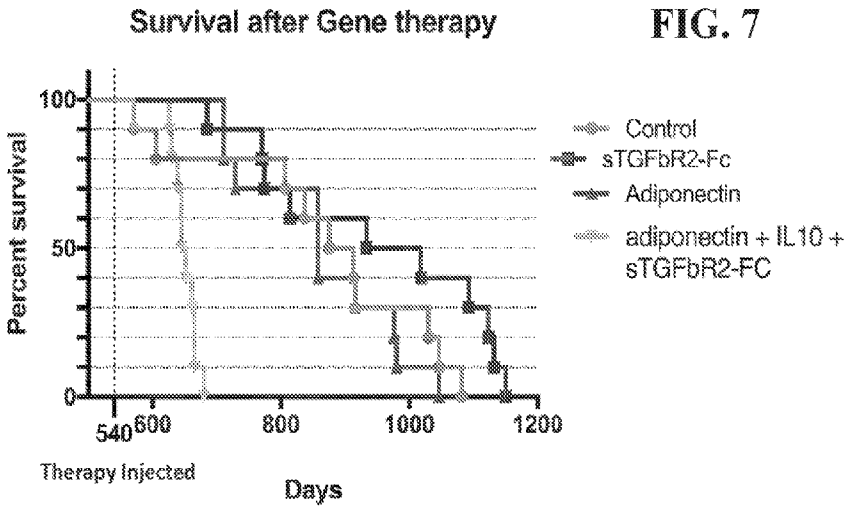

FIG. 7

(57) Abstract: Methods of gene therapy are provided for treating or preventing age-related diseases or conditions by regulating one or more functional proteins associated with age-related diseases or conditions.

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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:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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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.: 3-12, 14-15, 24-47, 49-50, 55-57, 61-62
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:
This application contains the following inventions or groups of inventions which are not so linked as to form 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.

----------Please see continuation in first extra 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.:
1-2, 13, 51, 52, 100-104, 109-110, 120, 121, 124 limitd to adiponectin and ADcy5.

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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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/00, C12Q 1/68 (2017.01)
CPC - C12Q 2600/136, C12Q 1/6883, C12N 15/113, C12N 2320/30

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0213512 A1 (ELLISON et al.,) 31 July 2014 (31.07.2014) para [0078-0079]; para [0083]	1, 2, 100, 101, 104, 109 --- 102, 103, 110, 121, 124
X --- Y	YUAN ET AL., A Versatile Adeno-Associated Virus Vector Producer Cell Line Method for Scalable Vector Production of Different Serotypes. HUMAN GENE THERAPY, May, 2011, Vol. 22, pp 613?624. Abstract; p614, col 2, para 2; p615, col 1, last para-col 2, first para; p615, col 2, last para-p616, col 1, first para	13 --- 102, 103, 110, 121
X --- Y	US 2006/0239972 A1 (ZOLOTUKHIN et al.,) 26 October 2006 (26.10.2006) para [0008-0010]; para [0052], [0057]	51, 120 --- 52
Y	WO 2008/033518 A2 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 20 March 2008 (20.03.2008) para [00482]	52
Y	US 2010/0003218 A1 (DUAN et al.,) 7 January 2010 (07.01.2010) Abstract; para [0051]; para [0089]	124

☐ 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 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
27 September 2017 (27.09.2017)	11 DEC 2017
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. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

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Continuation of:
Box No. III Observations where unity of invention is lacking

Group I+, Claims 1-2, 13, 51-54, 58-60, 96, 97, 100-104, 109-116, 120-124, 135-136, directed to a viral vector comprising: a gene capable of expressing a mammalian protein or an inhibitor mRNA product. The viral vector will be searched to the extent that the mammalian protein encompasses adiponectin; and the inhibitor mRNA product encompasses, ADcy5 (the first named over express protein and inhibitory mRNA product listed in Table 1). It is believed that claims 1-2, 13, 51, 52, 100-104, 109-110, 120, 121, 124, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass adiponectin and ADcy5. Additional mammalian protein(s) and inhibitor mRNA product(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected mammalian protein(s) and inhibitor mRNA product(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a mammalian protein GSTK1 and an inhibitor mRNA product, Agtr1a (claims 1-2, 13, 51-54, 58-60, 96, 97, 100-104, 109-115, 120-124, 135-136,).

[Note, claims 53-54, 58-60, 96, 97, 111-115, 122-123, 135-136 are excluded from the first invention because they comprise more than one nucleic acid sequences encoding mammalian proteins]

Group II+, Claims 16-23, 48, 98, 99, 105-108, 117-119, 144-185, directed to a first viral vector comprising a fusion protein comprising a soluble Transforming Growth Factor Beta Receptor II (sTGFbeta-R2) and an Ig Fc domain; and a second viral vector comprising a mammalian protein; and method of use. The composition may be searched, for example, to the extent that the second viral encompasses a mammalian protein Nrf2 (see claim 48). It is believed that claims 16-23, 48, 98, 99, 105-108, 117-119, 144, read on this exemplary invention. Additional mammalian heterologous protein(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected heterologous protein(s). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a heterologous protein Klotho (Claims 145-163, 184)

Group III+, Claims 63-76, 95, 125-134, directed to a method of treating age-related diseases, comprising administering viral expression vectors to a subject comprising heterologous functional proteins and/or heterologous inhibitor RNA sequences. Group IV+ will be searched upon payment of additional fees. The method may be searched, for example, to the extent that the treatment encompasses the heterologous protein, adiponectin; and an inhibitor RNA targeting ADcy5. It is believed that claims 63-65, 125, 126, read on this exemplary invention. Additional heterologous protein(s) and inhibitor RNA(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected heterologous protein(s) and inhibitor RNA(s). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a heterologous protein AMPK and inhibitor RNA targeting Cebpalpha (Claims 63-66, 127)

Group IV+, Claims 77-94, 137-138, directed to a method of treating obesity and type II diabetes, comprising administering viral expression vectors to a subject comprising heterologous functional proteins. Group V+ will be searched upon payment of additional fees. The method may be searched, for example, to the extent that the heterologous functional protein encompasses FGF21. It is believed that claims 77, 81, 82, 84, 87, 88, 90, 91, 94 read on this exemplary invention. Additional heterologous protein(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected heterologous protein(s). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a heterologous protein Atg5. (Claims 78, 80, 83, 84. 85, 93).

Group V, Claims 139-143, directed to a method of integrating foreign DNA into a genomic nucleic acid sequence of a eukaryotic cell comprising providing to the eukaryotic cell one or more guide RNA sequences and Cas9 enzyme.

The inventions listed as Groups I+, II+ III+, IV+ and V 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:

Special Technical Features

Group I+ and II+ include the special technical feature of a composition of matter, not required by the methods of Groups III+, IV+ and V. Group I+ includes the special technical feature of a gene capable of expressing a mammalian protein or an inhibitor mRNA product, recited therein. Each invention of Group I+ requires a specific mammalian protein and/or a specific inhibitor mRNA product, not required by any of the other inventions in Group I+; or the inventions of Groups II+, III+, IV+ and V.

Group II+ includes the special technical feature of viral vectors comprising a nucleic acid for sTGFbeta-R2 fusion protein and a nucleic acid for a second mammalian protein, recited therein. Each invention of Group II+ requires a specific second mammalian protein, not required by any of the other inventions in Group II+; or the inventions of Groups I+, III+, IV+ and V.

Group III+ includes the special technical feature of a method of treating age-related diseases, comprising administering viral expression vectors to a subject comprising heterologous functional proteins and/or heterologous inhibitor RNA sequences, recited therein. Each invention of Group III+ requires a specific heterologous functional protein and a specific heterologous inhibitor RNA sequence, not required by any of the other inventions in Group III+; or the inventions of Groups I+, II+, IV+ and V.

Group IV+ includes the special technical feature of a method of treating obesity and type II diabetes, comprising administering viral expression vectors to a subject comprising heterologous functional proteins, recited therein. Each invention of Group IV+ requires a specific heterologous functional protein, not required by any of the other inventions in Group IV+; or the inventions of Groups I+, II+, III+ and V.

Group V includes the special technical feature of a method of using guide RNA and Cas9, not required by any of the other inventions in I+, II+, III+ and IV+.

----continued on next sheet----

INTERNATIONAL SEARCH REPORT

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Continuation of:
Box No. III Observations where unity of invention is lacking

Common Technical Features

The inventions of Groups I+, II+, III+, IV+ and V share the technical feature of a viral vector comprising one or more nucleic acid sequence encoding a functional protein.

The inventions of Groups I+ and III+ share the technical feature of a viral vector comprising one or more nucleic acid sequence encoding an inhibitor mRNA.

The inventions of Groups II+, III+ and IV+ share the technical feature of a method of treating a disorder.

However, these shared technical features do not represent a contribution over prior art, in view of US 2009/0214637 A1 (Musatov).

Musatov teaches (see claims 1, 13) a viral vector comprising: a first nucleic acid sequence (abstract, use of vectors such as a recombinant adeno-associated virus (AAV) to deliver a at least a portion of a gene that can increase or decrease expression of a therapeutic protein of interest, e.g., in cells in a specific region of the brain associated with metabolic disorder.) comprising a gene capable of expressing a mammalian protein (Claim 11, providing at least a portion of a Hip2 gene...incorporating the Hip2 gene into a vector; transfecting the vector into at least one neuron of the hypothalamus) or an inhibitor mRNA product (Claim 3, providing a polynucleotide sequence that functions as at least one of a shRNA, a siRNA, and a RNA.; Claim 5, glucose-responsive neurons.), wherein the first nucleic acid sequence is operably linked to a first regulatory sequence for expression of the product in mammalian cells (para [0081], The promoter can be any desired promoter, selected based on the level of expression required of the gene operably linked to the promoter and the cell type in which the vector is used.).

Musatov teaches (see claim 76) a method of treating a mammal for obesity and type II diabetes (abstract, novel methods and compositions for treating metabolic disorders. Some aspects pertain to the use of gene therapy to treat diseases related to metabolic disorders, such as diabetes, obesity, high blood pressure, wasting syndrome, cachexia and atherogenic dyslipidemia.).

US 2015/0018405 A1 to BAR-ILAN UNIVERSITY (hereinafter 'Bar-Ilan') teaches (see claim 63) a method of treating age-related diseases or conditions (abstract, providing treatment of disease, in particular age-related disease, through increasing or decreasing the activity of SIRT6 protein.).
The inventions of Groups II+ share the technical feature of a first viral vector comprising a fusion protein comprising a soluble Transforming Growth Factor Beta Receptor II (sTGFbeta-R2) and an Ig Fc domain. US 2010/0098668 A1 (Seth) teaches (see claim 16) "selective expression of a protein that specifically binds to TGF-.beta., such as a soluble form of the TGF-.beta. receptor-II, alone or fused to a stabilizing peptide such as Fc (sTGF.beta.RIIFc) is disclosed" (para [0009]).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the inventions.

Groups I+, II+, III+, IV+ and V therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.