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(71) Applicants: **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy St, Cambridge, MA 02138 (US). **MASSACHUSETTS EYE AND EAR INFIRMARY** [US/US]; 243 Charles Street, Boston, MA 02114 (US). **THE SCHEPENS EYE RESEARCH INSTITUTE, INC.** [US/US]; 20 Staniford Street, Boston, MA 02114 (US).

(72) Inventors: **FLOREA, Michael**; 28 Wendell Street, Apt. 8, Cambridge, MA 02138 (US). **WAGERS, Amy, J.**; 30 Banks St, Cambridge, MA 02138 (US). **VANDENBERGHE, Luk**; 14 Indian Hill Road, Weston, MA 02493 (US).

(74) Agent: **WARREN, Lisa, M.**; Morse, Barnes-Brown & Pendleton, P.C., CityPoint, 480 Totten Pond Road, 4th Floor, Waltham, MA 02451 (US).

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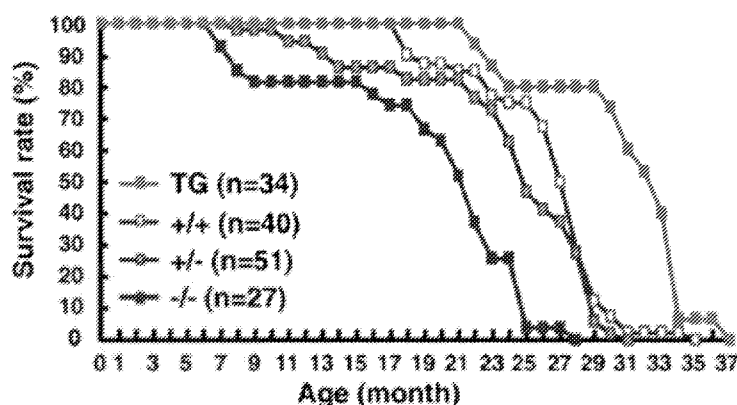


FIG. 1A

(57) Abstract: The disclosure provides viral vector delivery systems for use in treating diseases or disorders in a subject to whom the viral vector delivery systems are administered, as well as to methods of making and using the viral vector delivery systems.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/029757

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/76; A61K 35/761; A61K 38/18; A61K 38/45; A61K 48/00 (2021.01)
CPC - A61K 38/1866; A61K 38/45; A61K 48/0058; C12N 15/86 (2021.08)

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	US 2016/0319303 A1 (UNIVERSITAT AUTÒNOMA DE BARCELONA) 03 November 2016 (03.11.2016) entire document	1-6, 10-13, 29-34, 38-41, 63, 92-97
X	WO 2019/165050 A1 (GENERATION BIO CO) 29 August 2019 (29.08.2019) entire document	124-127
A	US 2016/0362692 A1 (ISIS INNOVATION LIMITED) 15 December 2016 (15.12.2016) entire document	1-6, 10-13, 29-34, 38-41, 63, 92-97, 124-127
A	US 2018/0214416 A1 (URANO et al) 02 August 2018 (02.08.2018) entire document	1-6, 10-13, 29-34, 38-41, 63, 92-97, 124-127
A	US 2013/0122082 A1 (NATIONAL YANG MING UNIVERSITY et al) 16 May 2013 (16.05.2013) entire document	1-6, 10-13, 29-34, 38-41, 63, 92-97, 124-127
P, X	OMICHI et al. "Hair Cell Transduction Efficiency of Single- and Dual-AAV Serotypes in Adult Murine Cochleae," Methods & Clinical Development, 12 May 2020 (12.05.2020), Vol. 17, Pgs. 1167-1177	1-6, 10-13, 29-34, 38-41, 63, 92-97, 124-127

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"D" document cited by the applicant in the international application	"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
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"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	

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/029757

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:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/029757

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.: 14-28, 42-62, 66, 67, 78-91, 100-118, 128-135
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 extra sheet(s)

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.:
1-6, 10-13, 29-34, 38-41, 63, 92-97, 124-127

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/US2021/029757

Claim(s) Nos. 14-28, 42-62, 66, 67, 78-91, 100-118, and 128-135 are unsearchable under Article 17 and have not been included with any invention.

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 need to be paid.

Group I+: claims 1-13, 29-41, 63-65, 68-77, 92-99, and 119-127 are drawn to engineered adenoviral vectors and methods comprising the same.

The first invention of Group I+ is restricted to a viral vector delivery system comprising two AAV serotypes engineered for delivery of a single gene, a non-silencing promoter, and an miRNA target site, wherein the first viral serotype is selected to be AAV1, the second viral serotype is selected to be AAV2, the gene is selected to be A2M (alpha-2-macroglobulin), an miRNA target site selected to be miRNA-1 and methods comprising the same. It is believed that claims 1-6, 10-13, 29-34, 38-41, 63, 92-97, and 124-127 read on this first named invention and thus these claims will be searched without fee to the extent that they read on AAV1, AAV2, ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase), and miRNA-1.

Applicant is invited to elect additional AAV serotypes, single genes, and miRNA target sites to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a viral vector delivery system comprising two AAV serotypes engineered for delivery of a single gene, a non-silencing promoter, and an miRNA target site, wherein the first viral serotype is selected to be AAV3, the second viral serotype is selected to be AAV#B, the gene is selected to be A2M (alpha-2-macroglobulin), an miRNA target site selected to be miRNA-24 and methods comprising the same. Additional AAV serotypes, single genes, and miRNA target sites will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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/examined.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element responsible for treating aging-Srelated disease or conditions requiring the selection of alternative viral serotypes where "the two or more viral serotypes are selected from Table 1," genes where "wherein the gene is selected from Table 2," and miRNA target sites where "the miRNA target site is selected from the group consisting of miRNA-1, miRNA-24, miRNA-29, miRNA-30c, miRNA-33, miRNA-122, miRNA-124, miRNA-128, miRNA-133, miRNA-144, miRNA-148a, miRNA-208a, miRNA-208b, miRNA- 223, and miRNA-499."

Additionally, even if Groups I+ were considered to share the technical features of a viral vector delivery system comprising two or more viral serotypes engineered for delivery of a single gene; a method of treating or preventing a disease or disorder comprising administering to a subject a viral vector delivery system comprising at least two viral serotypes engineered for delivery of a single gene; a viral vector delivery system comprising two or more AAV serotypes engineered for delivery of a single gene, a non-silencing promoter, at least one miRNA target site, the gene, a viral vector delivery system comprising at least one viral serotype engineered for delivery of a single gene; a method of extending the lifespan of a subject comprising administering to a subject a viral vector delivery system comprising at least one viral serotype engineered for delivery of a single gene; a method of treating or preventing a disease or disorder comprising administering to a subject a viral vector delivery system comprising at least one viral serotype engineered for delivery of a single gene; a method of treating Wolfram Syndrome II comprising administering an effective amount of Cisd2 to a subject suffering from Wolfram Syndrome II; a method of identifying a pre-determined level of gene transfer in one or more target tissues of a subject comprising: obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting gene transfer level in the one or more target tissues; obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the resulting gene transfer level in the one or more target tissues; and interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of gene transfer in the one or more target tissues; a method of identifying a pre-determined level of transgene expression in one or more target tissues of a subject comprising: obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting transgene expression level in the one or more target tissues; obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the resulting transgene expression level in the one or more target tissues; and interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of transgene expression in the one or more target tissues. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2016/0319303 A1 to Universitat Autònoma De Barcelona discloses a viral vector delivery system (adeno-associated viral vectors (AAV) that allow the delivery of polynucleotides of interest to specific types of adipose cells, Para. [0006]) comprising two or more viral serotypes (the AAV vector is a pseudotyped AAV vector (i.e. the vector comprises sequences or components originating from at least two distinct AAV serotypes), Para. [0078]) engineered for delivery of a single gene (AAV into the host cell, the single-stranded DNA containing the transgene is converted by the host cell ...the AAV may be engineered, Para. [0172]); a method of treating or preventing a disease or disorder (a method for the treatment or prevention of a disease that requires the expression of a polynucleotide of interest in a subject, Para. [0102]) comprising administering to a subject a viral vector delivery system comprising at least two viral serotypes engineered for delivery of a single gene (the administration to said subject of an adeno-associated viral vector comprising a recombinant viral genome, Para. [0102]; the AAV vector is a pseudotyped AAV vector (i.e. the vector comprises sequences or components originating from at least two distinct AAV serotypes), Para. [0078]; AAV into the host cell, the single-stranded DNA containing the transgene is converted by the host cell ...the AAV may be engineered, Para. [0172]); a viral vector delivery system comprising two or more AAV serotypes (the AAV vector is a pseudotyped AAV vector (i.e. the vector comprises sequences or components originating from at least two distinct AAV serotypes), Para. [0078]) engineered for delivery of a single gene (AAV into the host cell, the single-stranded DNA containing the transgene is converted by the host cell ...the AAV may be engineered, Para. [0172]), a non-silencing promoter (to improve the transgene expression attained by the mini/aP2 and mini/UCP1 promoters, CAG promoter are used in conjunction with tissue-specific miRNA target sequences, Para. [0142]), at least one miRNA target site (tissue-specific miRNA target sequences, Para. [0142]), the gene (AAV genome such as a transgene to be delivered to a mammalian, Para. [0024]), a viral

vector delivery system comprising at least one viral serotype (adeno-associated viral vectors (AAV) that allow the delivery of polynucleotides of interest to specific types of adipose cells, Para. [0006]) engineered for delivery of a single gene (AAV into the host cell, the single-stranded DNA containing the transgene is converted by the host cell ...the AAV may be engineered, Para. [0172]) a method of extending the lifespan of a subject (control of progression of the disease also involves an extension of survival, compared with the expected survival if treatment is not applied, Para. [0062]).

Further, US 2018/0214416 A1 to Urano et al. teaches a method of treating Wolfram Syndrome II (methods of treating or preventing Wolfram Syndrome in a subject in need thereof Para. [0007]; loss of function of WSF2 results in increased apoptosis of beta -cells in Wolfram syndrome subjects, Para. [0081]) a subject suffering from Wolfram Syndrome II (methods of treating or preventing an ER stress disorder in a subject in need thereof, Para. [0006]; methods of treating or preventing Wolfram Syndrome in a subject in need thereof Para. [0007]; loss of function of WSF2 results in increased apoptosis of beta -cells in Wolfram syndrome subjects, Para. [0081]);

Further, US 2013/0122082 A1 to National Yang Ming University et al. teaches comprising administering an effective amount of Cisd2 to a subject (comprising administering to a mammal in need of such treatment a therapeutically effective amount of a Cisd2 gene, Para. [0020]).

Further, WO 2019/165050 A1 to Generation Bio Co. teaches a method of identifying a pre-determined level of gene transfer in one or more target tissues of a subject (a method for controlled expression of a transgene, Para. [0015]; transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]) comprising: obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting gene transfer level in the one or more target tissues (transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]; select a dose low on the dose-response curve of the ceDNA for the initial administration, Para. [00203]); obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the resulting gene transfer level in the one or more target tissues (using the ceDNA vector plasmid titration to plot a standard curve, a regression line equation is then used to calculate the quantity of the ceDNA vector band, which can then be used to determine the percent of total input represented by the ceDNA vector, Para. [00505]; transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]); and interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of gene transfer in the one or more target tissues (effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems, Para. [00204]; transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]); a method of identifying a pre-determined level of transgene expression in one or more target tissues of a subject (a method for controlled expression of a transgene, Para. [0015]; transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]) comprising: obtaining a dose-response curve characterizing the relationship between an amount of a vector administered to the subject and a resulting transgene expression level in the one or more target tissues (transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]; select a dose low on the dose-response curve of the ceDNA for the initial administration, Para. [00203]); obtaining a linear or non-linear equation characterizing the relationship between the amount of vector administered to the subject and the resulting transgene expression level in the one or more target tissues (using the ceDNA vector plasmid titration to plot a standard curve, a regression line equation is then used to calculate the quantity of the ceDNA vector band, which can then be used to determine the percent of total input represented by the ceDNA vector, Para. [00505]; transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]); and interpolating or extrapolating a required dose of a gene delivery system to achieve a defined level of transgene expression in the one or more target tissues (effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems, Para. [00204]; transgene expression at a desired pre-determined level, Para. [0026]; delivery methods ...to penetrate into target tissue cells, Para. [00382]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.