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(54) Title: COMPOSITIONS AND METHODS FOR PREPARING VIRAL VECTORS

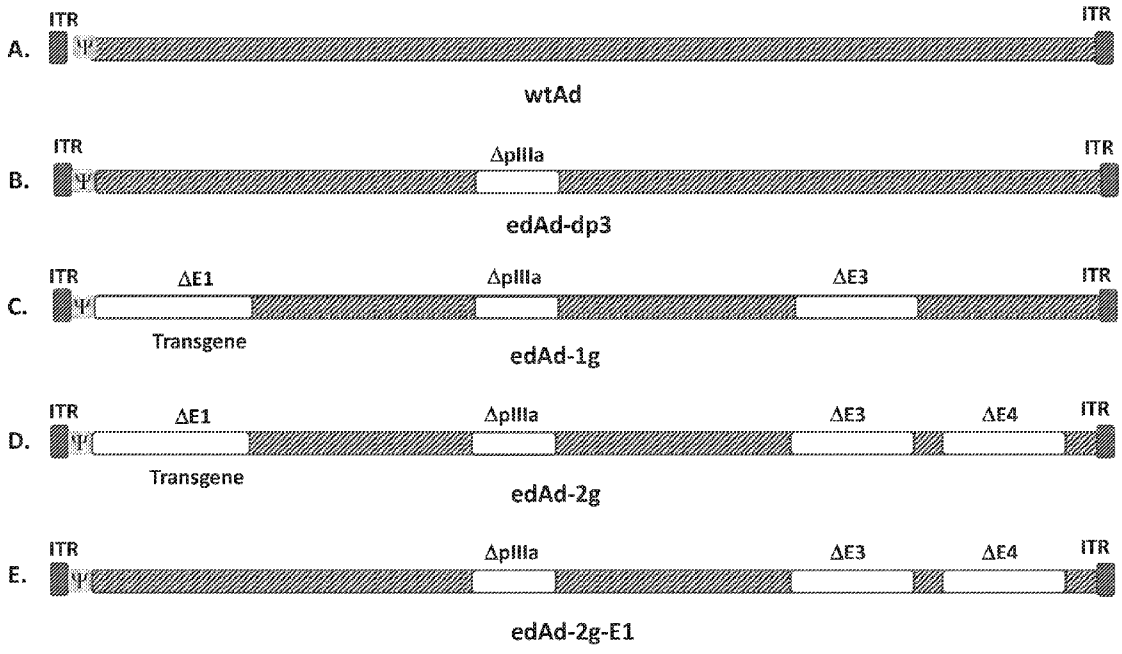

Figure 3

(57) Abstract: A method for preparing an infectious, recombinant virus vector comprises the steps of: (a) infecting host cells with a first virus comprising an encapsidation defective adenovirus (edAd), the edAd comprising a first defective virus genome; (b) incubating the infected host cells in a culture medium for a period of time sufficient for producing infectious virus particles; and (c) recovering infectious virus particles secreted into a culture supernatant, wherein the edAd or the host cells comprise a second defective virus genome engineered to express a target gene of interest, wherein the edAd or the host cells comprise nucleic acid sequences sufficient for expressing adenovirus (Ad) helper genes necessary for replication of the defective virus DNA; and wherein the edAd or the host cells comprise nucleic acid sequences sufficient from expressing helper functions necessary for producing infectious, replication defective virus particles corresponding to the second virus.

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[Continued on next page]

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09 July 2020 (09.07.2020)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/55182

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/86; C12N 15/861; C12N 15/864; C12N 15/63 (2020.01)

CPC - C12N 15/861; C12N 15/8645; C12N 15/63; C12N 2710/10321; C12N 2710/10344; C12N 2750/14143

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	CARAVOKYRI et al. Constitutive episomal expression of polypeptide IX (pIX) in a 293-based cell line complements the deficiency of pIX mutant adenovirus type 5. J Virol November, November 1995, Vol 69, No 11, Pages 6627-6633. Especially abstract, pg 6627 para 2 col 1, pg 6631 col 2 para 1.	1, 2, 7-10, 19 ---------- 11, 12
X	VON SEGGERN et al. Complementation of a fibre mutant adenovirus by packaging cell lines stably expressing the adenovirus type 5 fibre protein. J Gen Virol, Vol 79, Pt 6, Pages 1461-1468. Especially abstract.	1, 2, 19
X	KROUGLIAK et al. Development of cell lines capable of complementing E1, E4, and protein IX defective adenovirus type 5 mutants. Hum Gene Ther, December 1995, Vol 6, No 12, Pages 1575-1586. Especially abstract.	1, 2, 7-10, 19
Y	US 6,251,677 B1 (WILSON et al.) 26 June 2001 (26.06.2001). Especially col 3 ln 2-4; claim 1.	11, 12

☐ 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
"D" document cited by the applicant in the international application
"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
19 March 2020	29 MAY 2020
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 Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/55182

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:

ISA/225 mailed on 09 December 2019. The applicant did not, within the prescribed time limit, pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b). Accordingly, ISA/US cannot consider the sequence listing submitted on 04 February 2020

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/55182

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:
----Go to 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.:
Claims 1, 2, 7-12, 19, limited to enapsidation essential protein capsid protein IX (pIX) and recombinant virus AAV.

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
Information on patent family members

International application No.
PCT/US 19/55182

Continuation of Box III: Observations where Unity of Invention is lacking.

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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-15, 19-20, drawn to an encapsidation defective adenovirus (edAd) for the production of a recombinant virus.

The encapsidation defective virus will be searched to the extent that the encapsidation essential protein is the first named, capsid protein IX (pIX)(claim 2) and the heterologous (recombinant virus) gene(s) are the first named, recombinant AAV (claim 11), It is believed that claims 1, 2, 7-12, 19 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass capsid protein pIX and recombinant AAV. Additional encapsidation essential proteins and heterologous viral genomes will be searched upon payment of additional fees. Applicant must specify the claims that encompass any encapsidation essential proteins and heterologous viral genomes. 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. An exemplary election would be: encapsidation essential protein capsid protein precursor IIIa and recombinant virus gag and pol proteins of lentivirus (claims 1-5, 7-10, 14, 19-20).

Group II: Claims 16-18, drawn to a method of producing a recombinant virus (RV).

The inventions listed as Groups I+ and II 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+ has the special technical feature of a composition of an encapsidation defective adenovirus, not required by Group II.

Group II has the special technical feature of a method of producing a recombinant virus (e.g., AAV), not required by Group I+.

Among the inventions listed as Groups I+ are the specific encapsidation essential proteins recited therein. Each invention requires a specific encapsidation essential protein not required by any other inventions.

Common Technical Feature:

1. Group I+ claims share the common technical feature of claim 1.

2. Group I+ and II share the common technical feature of a producer cell one or more edAds comprising an RV genome.

However, said common technical features do not represent a contribution over the prior art, and are disclosed by the publication titled "Constitutive episomal expression of polypeptide IX (pIX) in a 293-based cell line complements the deficiency of pIX mutant adenovirus type 5" by Caravokyri et al. (hereinafter "Caravokyri") [published J Virol, November 1995, Vol 69, No 11, Pages 6627-6633], in view of US 6,251,677 B1 to Wilson et al. (hereinafter "Wilson").

As to common technical feature #1 (claim 1), Caravokyri discloses an encapsidation defective adenovirus (edAd) for the production of a recombinant virus, comprising: an edAd genome with one or more mutations that result in (1) significantly reduced production or non-production of one or more encapsidation essential proteins (abstract; "This report describes the use of eukaryotic episomal vectors based on the Epstein-Barr virus replicon to generate cells which stably express pIX. These cells provide pIX that is efficiently incorporated into virions that are genetically pIX-"; pg 6631 col 2 para 1; "In this study, we investigated the possibility that constitutive expression of pIX in a cell line would provide a functional pool of molecules for encapsidation of pIX- Ad5 genomes ... This finding is consistent with the fact
that pIX is required stoichiometrically for stabilization of the group-of-nine hexon assemblies that make up the 20 faces of the Ad particle").

As to common technical feature #2, Caravokyri discloses a producer cell one or more edAd (abstract; pg 6631 col 2 para 1). Caravokyri does not disclose that the adenoviral vector comprises an RV genome. However, adenoviral vectors that comprise an RV genome were well-known in the art, as disclosed by Wilson (claim 1; "A recombinant hybrid virus comprising:
(a) adenovirus sequences comprising the adenovirus 5' and 3' cis-elements necessary for replication and virion encapsidation; (b) adeno-associated virus (AAV) sequences comprising the 5' and 3' inverted terminal repeats (ITRs) of an AAV, said AAV sequences flanked by the adenovirus sequences of (a); and (c) a selected transgene operatively linked to sequences which regulate its expression in a target cell, said gene and regulatory sequences flanked by the AAV sequences of (b); wherein the adenovirus sequences comprise a functional deletion in the adenovirus E1 gene, the adenovirus E3 gene, and the adenovirus E4 gene, and wherein the hybrid virus is provided with sufficient adenovirus sequences to permit packaging into a capsid and infection of a target cell"). It would have been obvious that the adenovirus genome disclosed by Wilson could have incorporated a pIX gene deletion, for instance as disclosed by Caravokyri, when used in an appropriate producer cell line.

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+ and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.