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(88) Date of publication of the international search report:

28 November 2019 (28.11.2019)

(54) Title: MITOCHONDRIAL RNA IMPORT FOR TREATING MITOCHONDRIAL DISEASE

(57) Abstract: Disclosed is an RNA allotopic strategy to complement and genetically rescue mitochondrial gene defects. This approach can permit rescue of a mitochondrial DNA mutant by allotopic expression of a full-length recoded mitochondrial RNA that is transcribed in the nucleus, successfully imported into the mitochondria, and expressed there.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/85, A61K 48/00, C12N 15/85 (2019.01)
CPC - C12N 15/85, A61K 48/005, C12N 15/8509, C12N 2840/00

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.
Y	Val et al., Organelle trafficking of chimirc ribozymes and genetic manipulation of mitochondria, Nucleic Acids Research, 15 July 2011, Vol. 39, No.21; pgs 9262-9274; pg 9266, col 2, para 2	1-3, 8-9 and 11-13
Y	WO 2017/178633 A1 (RIJK ZWAAN ZAADTEELT EN ZAADHANDEL B.V.) 19 October 2017 (19.10.2017); pg 18, ln 21-27; pg 18, ln 31-32	1-3, 8-9 and 11-13
Y	US 2016/0289673 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 6 OCTOBER 2016 (06.10.2016); para [0060]	3, 8-9, 13

☐ 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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search
5 August 2019

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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.: 4-7, 10, 14-27, 31-40, 44-50
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 searched, the appropriate additional search fees must be paid.

Group I: claims 1-3, 8-9, 11-13, drawn to an expression cassette.

Group II: claims 28-30, 41-43, 51-55, drawn to a method of complementing a defect in a mutated mitochondrial protein in a cell.

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:

*****Continued in Supplemental Box*****

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.:
1-3, 8-9, 11-13

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

Special Technical Features

Group I includes the special technical feature of a composition comprising an expression cassette, not required by Group II.

Group II includes the special technical feature of a method comprising contacting a cell with an expression cassette, not required by Group I.

Common Technical Features

The inventions of Groups I and II share the technical feature of an expression cassette comprising, in a 5' to 3' order, an RNA polymerase II promoter, a mitochondrial tRNA RNE element, a first open reading frame, and a translation termination signal.

However, these shared technical features do not represent a contribution over prior art in view of the journal article entitled "Organelle trafficking of chimeric ribozymes and genetic manipulation of mitochondria by Val et al. (hereinafter "Val") (Nucleic Acids Research, 2011, Vol. 39, No. 21, pg 9262-9274) and WO 2017/178633 A1 to RIJK ZWAAN ZAADTEELT EN ZAADHANDEL B.V. (hereinafter "RZZZ").

Val teaches (instant claim 11) an expression cassette comprising, in a 5' to 3' order, an RNA polymerase II promoter, a first open reading frame, and a translation termination signal (pg 9266, col 2, para 2 "The strategy we developed for nuclear expression and mitochondrial import of RNAs of interest in plant cells is summarized in Figure 2. Plant cells are nuclearly transformed with constructs encoding a passenger sequence attached to the 5'-end of the TYMV PKTLS. These sequences are under the control of an RNA polymerase II promoter (constitutive or inducible) and terminator, yielding polyadenylated transcripts. To generate RNAs with the aminoacylatable TYMV TLS at the 3'-end, the sequence encoding the cis-cleaving ribozyme of the Hepatitis delta virus (cHDV) (27) is inserted into the constructs between the TYMV PKTLS cDNA and the terminator. Expression of the nuclear transgene thus generates a 5'-capped transcript comprising, successively, the passenger RNA, the PKTLS, the cHDV and the poly(A) tail. "; Figure 2 shows polymerase II and polymerase termination signal with a reading sequence in between.), however fails to teach a mitochondrial tRNA RNE element after the promoter and before the first open reading frame.

RZZZ teaches a mitochondrial tRNA RNE element that can be located at various locations in the vector (pg 18, ln 21-27 "The localisation signal is suitably selected from the group comprising a nuclear localisation signal, a chloroplast targeting signal, a mitochondrial targeting signal. The nucleic acid sequence encoding a modified, non-naturally occurring RNA- guided endonuclease protein may optionally further comprises a FokI endonuclease domain or another protein domain. The tRNA-like structure can be... tRNAGlu"; pg 18, ln 31-32 "The tRNA-like structure is located in the 3'UTR of the transcript, in the 5'UTR of the transcript, or in the coding sequence of the transcript."). Given that RZZZ teaches wherein the mitochondrial tRNA RNE element can be placed at various points between the coding transcripts, it would have been obvious to an artisan of ordinary skill in the art to experiment with including the tRNA RNE element at different positions in the vector taught by Val, including after the promoter and before the first open reading frame in the vector construct.

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

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

Item 4 (continued):

Claims 4-7, 10, 14-27, 31-40, 44-50 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).