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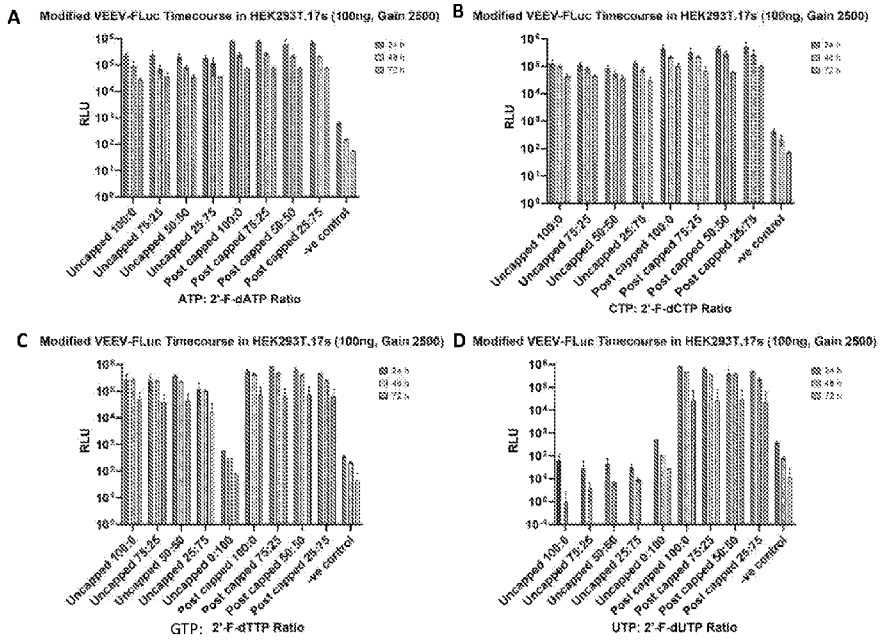
(54) Title: RNA MOLECULE

Figure 18



(57) **Abstract:** The invention relates to RNA molecules, to methods for preparing RNA molecules, and to methods for translating RNA molecules into protein. The invention extends to improved methods for forming RNA by in vitro transcription, and to the resultant RNA molecules. Furthermore, the invention relates to novel methods for enhancing the expression and/or translation of RNA, i.e. protein expression, and to methods for improving the stability of an RNA molecule. The invention also involves reducing the activation of innate sensing, interferon generation and/or degradation of an RNA molecule in a host. The invention also incorporates the use of the RNA molecules in vaccines and other therapeutic pharmaceutical compositions, and their use in immunisation and therapy, such as RNAi, gene therapy, gene editing and protein replacement.

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[Continued on next page]

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A. CLASSIFICATION OF SUBJECT MATTER
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ADD.

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)
C12P A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAYZER, J.M. ET ALL: "In vivo activity of nuclease-resistant siRNAs", RNA, vol. 10, no. 5, May 2004 (2004-05), pages 766-771, XP002598150, DOI: 10.1261/RNA.5239604 the whole document see especially: abstract page 768, column 1, lines 9-32; figure 2 ----- -/--	1-9, 17-19, 21,23-28

[x] Further documents are listed in the continuation of Box C.

[x] 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

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Date of the actual completion of the international search	Date of mailing of the international search report
16 August 2023	25/08/2023

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/093574 A1 (MODERNA THERAPEUTICS, INC.) 19 June 2014 (2014-06-19) the whole document see especially: page 80, paragraph 246 - page 81, paragraph 249 pages 368-374; example 13; table 18 pages 389-394; example 20; table 32	1-9, 17-19, 21,23-28
X	WO 2020/051507 A1 (BROAD INSTITUTE, INC.; MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 12 March 2020 (2020-03-12) the whole document see especially: pages 222-224; example 7	1-9, 17-19, 21,23-28
A	AURUP, H. ET AL.: "Translation of 2'-modified mRNA in vitro and in vivo", NUCLEIC ACIDS RESEARCH, vol. 22, no. 23, 1994, pages 4963-4968, XP002096818, the whole document	1-9, 17-19, 21,23-28
A	ZHU, B. ET AL.: "Synthesis of 2'-Fluoro RNA by Syn5 RNA polymerase", NUCLEIC ACIDS RESEARCH, vol. 43, no. 14, E94, 18 August 2015 (2015-08-18), pages 1-11, XP093058497, DOI: 10.1093/nar/gkv367 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4538805/pdf/gkv367.pdf> the whole document see especially: page 3, column 1, lines 13-33	1-9, 17-19, 21,23-28
X	WO 2008/078180 A2 (ARCHEMIX CORP.) 3 July 2008 (2008-07-03) the whole document see especially: page 29, paragraph 88 - page 32, paragraph 91 page 41, paragraph 107 - page 43, paragraph 111 pages 62-67; examples 2, 3 pages 73-84; examples 5-9 figures 5-10	1-9, 11-22, 24-28 47

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHELLISERRYKATTIL, J. & ELLINGTON, A.D.: "Evolution of a T7 RNA polymerase variant that transcribes 2'-O-methyl RNA", NATURE BIOTECHNOLOGY, vol. 22, no. 9, 8 August 2004 (2004-08-08), pages 1155-1160, XP037159668, DOI: 10.1038/NBT1001	1-9, 11-22, 24-28
A	the whole document see especially: abstract page 1157, column 2, line 51 - page 1158, column 21, line 2 page 1159; figure 3	47

X	ZÜST, R. ET AL.: "Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5", NATURE IMMULOGY, vol. 12, no. 2, 9 January 2011 (2011-01-09), pages 137-143, XP037065924, DOI: 10.1038/NI.1979 cited in the application the whole document	1-9, 11-22, 24-28

X	SIOUD, M.: "Single-stranded small interfering RNA are more immunostimulatory than their double-stranded counterparts: A central role for 2'-hydroxyl uridines in immune responses", EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 36, no. 5, 11 April 2006 (2006-04-11), pages 1222-1230, XP071222725, DOI: 10.1002/EJI.200535708 the whole document	1-9, 11-22, 24-28

X	KUGE, H. ET AL.: "Cap ribose methylation of c-mos mRNA stimulates translation and oocyte maturation in Xenopus laevis", NUCLEIC ACIDS RESEARCH, vol. 26, no. 13, July 1998 (1998-07), pages 3208-3214, XP055605998, DOI: 10.1093/nar/26.13.3208 the whole document	47

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHOI, J. ET AL.: "2'-O-methylation in mRNA disrupts tRNA decoding during translation elongation", NATURE STRUCTURAL & MOLECULAR BIOLOGY, vol. 25, no. 3, 19 February 2018 (2018-02-19), pages 208-216, XP036447846, DOI: 10.1038/S41594-018-0030-Z cited in the application the whole document	1-9, 11-22, 24-28
X	SCHWANS, J.P. ET AL.: "A Packing-Density Metric for Exploring the Interior of Folded RNA Molecules", ANGEWANDTE CHEMIE, vol. 116, no. 23, June 2004 (2004-06), pages 3095-3099, XP071333926, DOI: 10.1002/ANGE.200353575 the whole document see especially: page 3095; figure 1 page 3096, column 1, lines 19-28	1-10, 17-19, 21,24-28
X	WO 2010/048590 A1 (ALNYLAM PHARMACEUTICALS, INC.) 29 April 2010 (2010-04-29) the whole document see especially: page 4, paragraph 17 page 7, paragraph 27 pages 124-125; claims 1, 21	1-10, 17-19, 21,24-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2023/050508

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:

see additional 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 an 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.:

10-16, 20, 22, 47(completely); 1-9, 17-19, 21, 23-28(partially)

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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-9, 17-19, 21, 23-28(all partially)

one or more modified nucleotide triphosphate (NTP) for use in:
(i) enhancing the expression and/or translation of an RNA molecule comprising the one or more modified NTP,
(ii) enhancing the stability of an RNA molecule comprising the one or more modified NTP, and/or
(iii) reducing the activation of innate sensing, interferon generation and/or degradation of an RNA molecule comprising the one or more modified NTP,
wherein the one or more modified NTP comprises a 2'-substituted group in which the OH group normally at the 2' position is replaced with a halogen

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2. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with an optionally substituted aromatic group, wherein the aromatic group is optionally substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl

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3. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with a NH2

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4. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with a N3

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5. claims: 1-9, 17-19, 21, 23-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with a H

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6. claims: 11-16, 20, 22, 47(completely); 1-9, 17-19, 21, 24-28(partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

normally at the 2' position is replaced with an optionally substituted O-alkyl, wherein the alkyl is optionally substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl;
one or more 2'-O-methyl modified nucleotide triphosphate (NTP) in an RNA molecule for use in enhancing the translation of the RNA molecule

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7. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with an optionally substituted O-alkenyl, wherein the alkenyl is optionally substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl

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8. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with an optionally substituted O-alkynyl, wherein the alkynyl is optionally substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl

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9. claims: 10(completely); 1-9, 17-19, 21, 24-28(partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with an optionally substituted alkyl, wherein the alkyl is optionally substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl

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10. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with an optionally substituted alkenyl, wherein the alkenyl is optionally substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl

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11. claims: 1-9, 17-19, 21, 24-28(all partially)

idem as subject 1 but limited to one or more modified NTP comprising a 2'-substituted group in which the OH group normally at the 2' position is replaced with an optionally substituted alkynyl, wherein the alkynyl is optionally

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

substituted with halogen, oxo, OR, CN, NR2 or SR, wherein R is H or C1-6 alkyl, C2-6 alkenyl or C2-6 alkynyl

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12. claims: 29-39

method of preparing a modified RNA molecule, wherein the method comprises contacting, in the presence of at least 20 mM magnesium ions:
(i) a template nucleic acid sequence,
(ii) an RNA polymerase, and
(iii) a plurality of nucleotide triphosphates (NTPs), one or more of which is a modified nucleotide triphosphate (NTP),
wherein the RNA polymerase transcribes the template nucleic acid sequence to form an RNA molecule comprising at least 20 nucleotides, and
wherein at least 25% of the constituent nucleotides in the RNA molecule are modified;
use of 20 mM magnesium ions in a transcription reaction to prepare a modified RNA molecule comprising at least 20 nucleotides,
wherein at least 25% of the constituent nucleotides in the RNA molecule are modified

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13. claims: 40-46

RNA molecule obtained or obtainable by the method according to any one of claims 29-39;
pharmaceutical composition comprising the RNA molecule according to claim 40 and a pharmaceutically acceptable vehicle;
method of preparing the pharmaceutical composition according to claim 41 comprising contacting the RNA molecule according to claim 40 and a pharmaceutically acceptable vehicle;
RNA molecule according to claim 40 or the pharmaceutical composition according to claim 41 for use as a medicament;
RNA molecule according to claim 40 or the pharmaceutical composition according to claim 41 for use in treating, preventing or ameliorating a disease in a subject;
vaccine composition comprising the RNA molecule according to claim 40 or the pharmaceutical composition according to claim 41;
RNA molecule according to claim 40, the pharmaceutical composition according to claim 41, or the vaccine according to claim 45 for use in stimulating an immune response in a subject

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2023/050508

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014093574 A1	19-06-2014	EP 2931914 A1	21-10-2015
		US 2015315541 A1	05-11-2015
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