PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2022/059774	12.10.2022	15.10.2021

International Patent Classification (IPC) or both national classification and IPC
INV. A61K39/12 A61P31/20

Applicant
PFIZER INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Marinoni J-C Telephone No. +31 70 340-0

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2022/059774

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

4. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established to the extent that a meaningful opinion could be formed without a WIPO Standard ST.26 compliant sequence listing.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7, 9, 11, 13-15, 21, 22, 30
	No:	Claims	1-6, 8, 10, 12, 16-20, 23-29, 31, 32
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-32
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 **Introduction**

The present application relates to mRNA vaccines formulated in lipid nanoparticles, wherein the mRNA expresses the varicella zoster virus (VZV) glycoprotein gE, and the use of the same as vaccine.

2 **Cited documents**

Reference is made to the following documents:

D1 WO 2018/170270 A1 (MODERNATX INC [US]) 20 September 2018

D2 WO 2017/070601 A1 (MODERNATX INC [US]) 27 April 2017

D3 MONSLOW MORGAN A ET AL: "Immunogenicity generated by mRNA vaccine encoding VZV gE antigen is comparable to adjuvanted subunit vaccine and better than live attenuated vaccine in nonhuman primates", VACCINE, ELSEVIER, vol. 38, no. 36, 20 July 2020, pages 5793-5802

D4 WO 2020/246750 A2 (CATHOLIC UNIV KOREA IND ACADEMIC COOPERATION FOUNDATION [KR] ET AL.) 10 December 2020

D5 CHEN SAM ET AL: "Dexamethasone prodrugs as potent suppressors of the immunostimulatory effects of lipid nanoparticle formulations of nucleic acids", JOURNAL OF CONTROLLED RELEASE, vol. 286, 17 July 2018, pages 46-54

D6 EYGERIS YULIA ET AL: "Deconvoluting Lipid Nanoparticle Structure for Messenger RNA Delivery", NANO LETTERS, vol. 20, no. 6, 6 May 2020, pages 4543-4549

D7 JUSTIN M. RICHNER ET AL: "Modified mRNA Vaccines Protect against Zika Virus Infection", CELL, vol. 168, no. 6, 1 March 2017, pages 1114-1125.e10

3 **Novelty (Art. 33(2) PCT)**

3.1 **D1** discloses mRNA-LNP vaccines expressing a VZV gE protein having 100% identity with the protein of SEQ ID No. 1 of the present application (see Example 11). The DNA and mRNA molecules (**D1**, SEQ ID No. 1 and SEQ ID

No. 123, respectively) share less than 90% identity with the nucleic acid sequences of SEQ ID 12-145 of the present application. However, **D1** discloses nucleic acid sequences which share more than 90% identity with the sequences of SEQ ID No. 13 of the present application (**D1**, SEQ ID No. 7, 25, 27, 28, 96, 97, 129, 130, *inter alia* which all share 99.46% identity to present SEQ ID No. 13) with SEQ ID No. 14 (**D1**, SEQ ID No. 60, 62, 63, 101 inter alia which share 92.96% identity with SEQ ID No. 14), with SEQ ID No. 15 (SEQ ID No. 137 and 150 share 96.89% identity with present SEQ ID No. 15 while **D1**, SEQ ID No. 63 and 101 share 93.59% identity with present SEQ ID No. 15), etc... The mRNA molecules of **D1** have a 5'UTR, a 3'UTR, and a poly-A tail, wherein 5'-UTR: GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC (SEQ ID NO: 138, which differs from the nucleotide sequences of the 5'UTRs recited at present **claim 9**), the 3 '-UTR has the sequence UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCC UCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 139, which differ from the nucleotide sequences of the 3'UTRs recited at present **claim 11**) and the polyA tail has the sequence AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG (SEQ ID NO: 140 which differs from the polyA tails recited at present **claim 13** having SEQ ID No. 287 or 315), see Example 13, page 257, lines 2-10 and wherein some of the mRNAs have a cap sequence m7G(5')ppp(5')G-2'-0-methyl, represented by G* in the sequences listed in Table 2 (pages 240ff), indicated as VZV mRNA sequences (SEQ ID No. 92 to SEQ ID No. 137). The mRNA is formulated in a DLin-MC3 -DMA based LNP (see Example 15; Fig. 3), wherein the mRNA contain methylpseudouridine modification or not. From the Examples, the exact composition of the LNP is not readily apparent, but LNPs comprising DLin-MC3-DMA; cholesterol; 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC); and polyethylene glycol (PEG)2000-DMG are explicitly mentioned (see page 165, par. 52 and 70). Codon optimization is also suggested (see from page 47, line 29 to page 48, line 23 and claim 50). **D1** also discloses variants of the gE protein that localize to the cell membrane, the Golgi apparatus or are secreted (see Example 14). **D1** does not mention the cationic lipid (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315). **D1** is therefore prejudicial to the novelty of the subject-matter of present **claims 1-4, 8, 10, 12, 16-19, 25-29, 31, 32.**

3.2 The disclosure of **D2** is essentially the same as the disclosure of **D1**. **D3** is a scientific article which corresponds to the patent applications of **D1** and **D2**. With respect to the VZV gE mRNA cationic LNP formulation, **D3** refers to **D7** which indicates that the LNP comprises a 50:10:38.5:1.5 ration of an unidentified ionizable lipid (but which has to be cationic since **D3** explicitly mentions cationic LNPs at par. 2.1), DSPC, cholesterol and an unidentified PEG-lipid. **D3** is therefore prejudicial to the subject-matter of **claims 1-4, 8 , 10, 12, 16-20, 23-29, 31, 32**.

3.3 **D4** discloses VZV gE RNA vaccines wherein the mRNA comprises the coding sequence of VZV (Varicella-Zoster-virus) gE as a coding region (SEQ ID NO: 12), and a SV40 late polyadenylation signal, see Preparation Example 14. SEQ ID No. 12 of **D4** shares 99.89% identity with the DNA of SEQ ID No. 12 of the present application. The RNA is used to immunize C57BL/6 mice previously primed with 2000 PFU of live-attenuated VZV vaccine including recombinant VZV gE protein (260-927 nucleotides of Oka/SK strain, GenBank: 702 AF272392.1 for mimicking the immune system of VZV seropositive individuals. The mice received 2 times at 4 week interval, 20 µg of RNA nucleic acid molecule (pCrPV-VZV) by intramuscular injection, see Example 8, Table 6, Group G4 and group G5, wherein the RNA in G5 is combined with the CA-1 stabilizer of Formula 2. **D4** suggests codon optimization (par. [0084]), the use of modified nucleotides (par. [0049]) and the use of 5'UTRs and 3'UTRs (par. [0088]-[0091] and par. [0097] and [0098]. **D4** is therefore prejudicial to the novelty of the subject-matter of **claims 1-6, 12, 25-29, 31, 32**.

3.4 **D5** and **D6** are cited with respect to the essential presence of cholesterol and certain cholesterol derivatives in LNPs in the context of mRNA delivery in vaccine applications (see **Item VIII, par. 2** below).

4 **Inventive step (Art. 33(3) PCT)**

4.1 Some specific combinations of VZV gE mRNA sequence and LNPs characterized by specific lipid components in specific relative amounts, may be found inventive based on comparative experimental data provided in the Examples.

4.2 However, none of the present claims are directed to any specific VZV gE mRNA-LNP combination for which an inventive step could be acknowledged.

4.3 The reason is that the remaining claims either suggest lists of possible 5'UTR, 3'UTR or polyA sequences, specific GC contents, presence of certain (broadly

defined) lipids, or lists of specific lipids, which individually or in unspecific combinations cannot account for an inventive step.

4.4 **Claims 1-32** therefore do not meet the requirements of Art. 33(3) PCT.

Re Item VIII

Certain observations on the international application

1 **Claim 16** defines that "the RNA comprises at least one modified nucleotide". The language "*modified nucleotide*" is not fully clear because the nature and extent of the modification of the (unspecified) nucleotide(s) is not mentioned in the claim. Furthermore, it appears that this language results in the scope of the claim encompassing subject-matter which is incommensurate with the contribution of the Applicant to the art, wherein said contribution is limited to specific modified nucleotides (*i.e.* methylpseudouridine, see **claim 17**). The claim is therefore not clear, contrary to the requirements of Art. 6 PCT.

2 **Claim 20** specifies that the LNP comprises a "*steroid or steroid analog*". The only compound identified in the description or used in the examples as "*a steroid or steroid analog*" is cholesterol. The Applicant have not shown any data rendering plausible the use of any steroid analog other than cholesterol (including estradiol, testosterone and anabolic steroids such as oxandrolone, triterpenes, phytosterols, and phytoestrogens such as flavones and isoflavones, some lignans, and stilbenes, steroid analogs lacking ring B such as vitamin D_3), steroid analogs lacking ring C or steroid analogs lacking ring D, C19 substituted steroid analogs, prednisolone, betamethasone, dexamethasone, hydrocortisone). **D5** discloses mRNA-LNP particles comprising LD003 (a dexamethasone prodrug), but the LNPs also comprise cholesterol (see paragraph 2.3 "Preparation of lipid nanoparticles"). Furthermore, **D6** discloses mRNA vaccines comprising LNPs wherein the cholesterol has been replaced with C24 alkyl cholesterol derivatives such as S-sitosterol, fucosterol, stigamstanol and campestrol. **D6** insists that "*cholesterol, a major constituent within LNPs, contributes to their morphology that influences gene delivery*", see the abstract. Cholesterol (or cholesterol derivatives, provided that a basis exists in the application as filed for such compounds) are therefore an essential feature of the LNP in the context of mRNA vaccination.

3 The term "*about*" in **claim 30** is vague and renders the claim unclear, contrary to the requirements of Art. 6 PCT.