

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 <i>bis</i> .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. PCT/IB2016/057502	International filing date (day/month/year) 09.12.2016	Priority date (day/month/year) 23.12.2015	
International Patent Classification (IPC) or both national classification and IPC INV. A61K39/155 A61K39/12 C07K14/005			
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 notified 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:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	Date of completion of this opinion see form PCT/ISA/210	Authorized Officer Sommer, Birgit Telephone No. +49 89 2399-0	
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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 43bis.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:
 - ☒ 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 13ter.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 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
4. ☐ 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.
5. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

- ☐ the entire international application
- ☒ claims Nos. 1-10, 16-23(all partially)

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):
- ☒ no international search report has been established for the whole application or for said claims Nos. 1-10, 16-23(all partially)
- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:
 - ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
 - ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
 - ☐ 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).
- ☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:
- ☒ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☐ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☐ all parts.
 - ☒ the parts relating to claims Nos. 11-15(completely); 1-10, 16-23(partially)

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

1. Statement
- | | | |
|-------------------------------|-------------|--|
| Novelty (N) | Yes: Claims | <u>11-15(completely); 9, 10, 16, 17(partially)</u> |
| | No: Claims | <u>1-8, 18-23(all partially)</u> |
| Inventive step (IS) | Yes: Claims | <u>11-15(completely); 9, 10, 16, 17(partially)</u> |
| | No: Claims | <u>1-8, 18-23(all partially)</u> |
| Industrial applicability (IA) | Yes: Claims | <u>11-15(completely); 1-10, 16-23(partially)</u> |
| | No: Claims | |
2. Citations and explanations
- see separate sheet**

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)
and / or
2. Non-written disclosures (Rules 43*bis*.1 and 70.9)
see form 210

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

Reference is made to the following documents:

- D1 WO 2014/160463 A1
- D2 WO 2012/158613 A1
- D3 WIDJAJA I et al., PLOS ONE, 24 June 2015, pages 1/19-19/19
- D4 CIMICA V et al., CLIN. VACC. IMMUNOL., vol. 23, 2016, pages 451-459 -& "Supplemental Material"
- D5 WO 2017/040387 A2
- D6 MCLELLAN J S et al., SCIENCE, vol. 342, 2013, pages 592-598 -& "Supplementary Materials"

The present application discloses RSV fusion (F) protein mutants which are stabilized in prefusion-like conformation by engineered disulfide bonds, cavity filling mutations and/or electrostatic mutations.

Re Item II:

The current assessment is based on the assumption that all claims enjoy the priority rights from the filing date of the priority document (23.12.2015), i.e. D4 and D5 mentioned in the International Search Report (ISR) as P- or E-documents are not taken into account.

Re Item IV:

The application does not comply with the requirements of unity as set forth in the PCT regulations (**Article 34(3), Rule 13 PCT**) since the six groups of inventions mentioned in the International Search Report (ISR) are not so linked as to form a single general inventive concept.

The only concept linking all groups of claims is the provision of RSV F protein mutants. The prior art already discloses RSV F protein mutants comprising at least an engineered disulfide bond mutation, a cavity filling mutation and/or an electrostatic mutation (see the documents listed in the International Search Report (ISR), e.g. D1, claims; D2, claims). The technical problem underlying all groups of inventions seems

to be the provision of further RSV F protein mutants. The application solves this problem by providing mutants comprising at least one of an engineered disulfide bond mutation, a cavity filling mutation and/or an electrostatic mutation.

Even if a further common concept could be formulated for groups 1-4 as the provision of further RSV F protein mutants comprising at least one engineered disulfide bond mutation, said concept is also neither novel nor inventive. The technical problem underlying groups 1-4 is formulated as the provision of further RSV F protein mutants comprising at least one disulfide bond mutation. The application solves this problem by providing RSV F protein mutants comprising at least one engineered disulfide bond mutation selected from S55C and L188C, S155C and S290C, T103C and I148C as well as L142C and N371C. However, stabilized RSV F protein mutants comprising an engineered disulfide bond at positions S155C and S290C are already known from the prior art (see the documents cited in the ISR, e.g. D1, claims).

Since the International Searching Authority (ISA) fails to see any common technical feature among the groups of RSV F protein mutants, said technical feature defining an inventive contribution over the prior art, any of groups 1-6 represents a separate invention.

Since only three search fees have been paid, the following comments only refer to the first, third and forth group of inventions, i.e.

(1) to a mutant of a wild-type RSV F protein, wherein the mutant comprises at least one engineered disulfide bond mutation consisting of S55C and L188C, for example mutants pXCS738, pXCS780, pXCS830, pXCS852, pXCS853, pXCS855 and pXCS881, as well as subject-matter related thereto (claims 1-10 and 13-23),

(3) to a mutant of a wild-type RSV F protein, wherein the mutant comprises at least one engineered disulfide bond mutation consisting of T103C and I148C, for example mutants pXCS847 and pXCS851, as well as subject-matter related thereto (claims 1-12 and 15-23) and

(4) a mutant of a wild-type RSV F protein, wherein the mutant comprises at least one engineered disulfide bond mutation consisting of L142C and N371C, for example mutant pXCS 881, as well as subject-matter related thereto (claims 1-10 and 16-23).

Re Item V:

- 1 D1 discloses numerous RSV F protein mutants stabilized by engineered disulfide bonds, cavity-filling mutations and/or electrostatic mutations. In particular, RSV F protein mutants comprising an engineered intra-protomer

disulfide bond are disclosed comprising the mutations S155C and S290C (D1, e.g. claims; examples). Said disulfide bond mutation may be combined with a F, L, W, Y, H or M substitution at position 190 and/or 207 (D1, e.g. claims 6, 33, 34). Moreover, a variant "DS-Cav1-TriC" is disclosed comprising in addition to the S155C and S290C mutation the electrostatic mutations D486H-E487Q-F488W-D489H and the cavity-filling mutations S190F-V207L (D1, e.g. example 9). The DS-Cav1 mutations are further combined with cysteine substitutions in the HRB region (D1, e.g. page 171, lines 1-24; table 23). Further cavity filling and/or electrostatic mutations falling under the specific definitions in claims 7 and 8 of the present application are also disclosed (D1, e.g. page 141 *et seq*, tables 14 and 15). D1 is novelty-destroying for claims 1-8 and 18-23.

D2 discloses RSV F protein mutants stabilized in pre-fusion conformation by engineered disulfide bonds, e.g. mutations 56C and 164C, 57C and 190C or 58C and 164C (D2, e.g. page 14, paragraph 56 - page 18, paragraph 84; claims; example 3, SEQ ID NOS: 4-9). D2 is novelty-destroying for claims 1, 3-5 and 18-23.

D3 discloses the stabilization of F proteins of RSV subtype A in prefusion-like conformation by mutation of heptad repeat B (HRB). A construct Flys.HRBcys-GCN is disclosed comprising the disulfide bond mutations 478C, 486C, 506C and 507C (D3, e.g. abstract; results; discussion). D3 is novelty-destroying for claims 1, 3-5, 18, 20 and 22.

Consequently, none of claims 1-8 and 18-23 meets the requirements of **Article 33(2) PCT**.

2 invention (1): claims 1-10 and 13-23

D1, which is considered as most relevant prior art, discloses RSV F protein mutants stabilized by engineered disulfide bonds, cavity-filling mutations and/or electrostatic mutations. In particular, RSV F protein mutants are disclosed comprising an engineered disulfide bond due to the mutations S155C and S290C (D1, e.g. claims; examples).

The problem to be solved is formulated as the provision of further RSV F protein mutants stabilized in the pre-fusion conformation. The application solves this problem by disclosing RSV F mutants carrying at least an engineered disulfide bond by introducing the mutations S55C and L188C.

Claims 1-8 and 18-23 are not novel and therefore also not inventive in view of D1-D3. Moreover, the introduction of engineered disulfide bonds in order to stabilize RSV F is not inventive *per se*. The prior art already discloses various RSV F mutants carrying disulfide bond mutations (D1, e.g. claims, examples; D2, e.g. example 3; D3, e.g. abstract).

However, the available prior art neither teaches nor suggests RSV F protein mutants comprising the specific combination of mutations S55C and L188C. At least some of said mutants exhibit increased neutralizing antibody responses and/or appear more stable than e.g. the DS-Cav1 mutant of D1 (description, e.g. figures 8 and 9). Therefore, an inventive step can be in principle acknowledged for claims 9, 10, 13-17 insofar as said claims relate to RSV F protein mutants comprising the mutations S55C and L188C (**Article 33(3) PCT**).

3 invention (3): claims 1-12 and 15-23

D1, which is considered as most relevant prior art, discloses RSV F protein mutants stabilized by engineered disulfide bonds, cavity-filling mutations and/or electrostatic mutations. In particular, RSV F protein mutants are disclosed comprising an engineered disulfide bond due to the mutations S155C and S290C (D1, e.g. claims; examples).

The problem to be solved is formulated as the provision of further RSV F protein mutants stabilized in the pre-fusion conformation. The application solves this problem by disclosing RSV F mutants carrying at least an engineered disulfide bond by introducing the mutations T103C and I148C.

Claims 1-8 and 18-23 are not novel and therefore also not inventive in view of D1-D3. Moreover, the introduction of engineered disulfide bonds in order to stabilize RSV F is not inventive *per se*. The prior art already discloses various RSV F mutants carrying disulfide bond mutations (D1, e.g. claims, examples; D2, e.g. example 3; D3, e.g. abstract).

However, the available prior art neither teaches nor suggests RSV F protein mutants comprising the specific combination of mutations T103C and I148C. At least some of said mutants exhibit increased neutralizing antibody responses and/or appear more stable than e.g. the DS-Cav1 mutant of D1 (description, e.g. figures 8 and 9). Therefore, an inventive step can be in principle acknowledged for claims 9-12 and 15-17 insofar as said claims relate to RSV F protein mutants comprising the mutations T103C and I148C (**Article 33(3) PCT**).

4 invention (4): claims 1-10 and 16-23

D6, which is considered as most relevant prior art, discloses RSV F protein mutants stabilized by engineered disulfide bonds, cavity-filling mutations and/or electrostatic mutations. In particular, RSV F protein mutants are disclosed comprising an engineered disulfide bond due to the mutations S155C-S290C or T357C-N371C (D6, e.g. abstract, table 1).

The problem to be solved is formulated as the provision of further RSV F protein mutants stabilized in the pre-fusion conformation. The application solves this problem by disclosing RSV F mutants carrying at least an engineered disulfide bond by introducing the mutations L142C and N371C.

Claims 1-8 and 18-23 are not novel and therefore also not inventive in view of D1-D3.

Moreover, the introduction of engineered disulfide bonds in order to stabilize RSV F is not inventive *per se*. The prior art already discloses various RSV F mutants carrying disulfide bond mutations in general (D1, e.g. claims, examples; D2, e.g. example 3; D3, e.g. abstract) and the mutation N371C in particular (D6, e.g. table 1). Therefore, the provision of a further RSV F protein stabilized by a particular disulfide bond involves an inventive step only if it shows unexpected properties and/or achieves an unexpected technical effect which would make a contribution over the prior art.

It is acknowledged that the available prior art neither teaches nor suggests RSV F protein mutants comprising the specific combination of mutations L142C and N371C.

However, the introduction of said disulfide bond has apparently no surprising technical effect compared to e.g. the disulfide bonds of D1-D3 or D6. The variant pXCS881 carrying the mutations L142C and N371C cannot elicit neutralizing antibody responses and/or is less stable than e.g. the DS-Cav1 mutant of D1 (description, e.g. figures 8 and 9). Therefore, the subject-matter as claimed is considered merely as an arbitrary alternative to the known RSV F protein variants from D1-D3 or D6. The provision of such an arbitrary alternative does not involve an inventive step.

Consequently, claims 9, 10, 16 and 17, insofar as said claims relate to RSV F protein mutants comprising the mutations L142C and N371C, do not involve an inventive step (**Article 33(3) PCT**).

- 5 Claim 23 relates to subject-matter considered by this Authority to be covered by the provisions of **Rule 39.1(iv) / 67.1(iv) PCT**. The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Re Item VIII:

- 6 Present claims 1-9 and 16-23 relate to RSV F protein mutants only defined by reference to a desirable characteristic or property, namely the presence of specific point mutations. The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of **Article 6 PCT** and/or disclosure within the meaning of **Article 5 PCT** for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, claims 1-4 also lack clarity (**Article 6 PCT**). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out only for those parts of the claims which appear to be supported and disclosed. Claims 1-10 and 13-23 have been interpreted according to the examples as relating to the specific RSV F protein mutants pXCS738, pXCS780, pXCS830, pXCS847, pXCS851, pXCS852, pXCS853, pXCS855 and pXCS881. The preliminary examination was also restricted to said interpretation.
- 7 The present application only provides adequate guidance that the specific RSV F protein mutants pXCS780, pXCS830, pXCS847, pXCS851, pXCS852, pXCS853 and pXCS855 exhibit increased stability and/or neutralizing antibody responses compared to RSV F protein mutants of the prior art.
- It is not reasonable to predict that all of the numerous RSV F protein mutants covered by the claims have the properties and uses the applicant ascribes to them in the description. For example, the application itself discloses that at least mutants pXCS738 and pXCS881, which comprise the S55C and S188C mutation and optionally the L142C and N371C mutation, are not able to elicit increased neutralizing antibodies compared to the DS-Cav1 mutant of D1 (description, e.g. figure 8). Therefore, claims 1-10 and 15-23 are not enabled

over the whole scope of the claims and also not technically supported by the description as their scope is broader than justified by the description and drawings (**Article 5/6 PCT**).