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/NL2018/050137	06.03.2018	06.03.2017

International Patent Classification (IPC) or both national classification and IPC
INV. A61K38/55 A61P31/14

Applicant
ACADEMISCH ZIEKENHUIS LEIDEN H O D N LUMC

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
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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/NL2018/050137

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:

      ☐ 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).

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. 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.

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	
	No:	Claims	1-15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item IV

Lack of unity of invention

This Authority considers that the application does not meet the requirements of unity of invention and that there are 2 inventions covered by the claims indicated as follows:

Invention (1): claims: 1, 2, 7, 13(completely); 5, 6, 9-12, 15(partially)

An inhibitor comprising a beta-grasp fold, wherein said fold comprises region 1 (amino acids 2-14), region 2 (amino acids 42-49), and region 3 (amino acids 62-76) of the amino acid sequence set forth in SEQ ID NO:1, wherein the inhibitor comprises one or more amino acid substitutions in said regions as compared to the amino acid sequence set forth in SEQ ID NO:1, and wherein said substitutions include an amino acid substitution of A to F at the amino acid position corresponding to 46 of SEQ ID NO: 1 and/or an amino acid substitution of E to Y at the amino acid position corresponding to 64 of SEQ ID NO: 1; the nucleic acid encoding said inhibitor, a vector expressing said nucleic acid, an in vitro cell line/non-human cell/non-human organism expressing said vector, a pharmaceutical composition comprising the inhibitor; its the use for inhibiting the biological activity of a viral protein, and its use in therapy; a method for identifying the inhibitor

Invention (2): claims: 3, 4, 8, 14(completely); 5, 6, 9-12, 15(partially)

An inhibitor comprising a beta-grasp fold, wherein said fold comprises region 1 (amino acids 2-14), region 2 (amino acids 42-49), and region 3 (amino acids 62-76) of the amino acid sequence set forth in SEQ ID NO:1, wherein the inhibitor comprises one or more amino acid substitutions in said regions as compared to the amino acid sequence set forth in SEQ ID NO:1, and wherein said substitution include an amino acid substitution of R to G at the amino acid position corresponding to 74 of SEQ ID NO: 1; the nucleic acid encoding said inhibitor, a vector expressing said nucleic acid, an in vitro cell line/non-human cell/non-human organism expressing said vector, a pharmaceutical composition comprising the inhibitor; its the use for inhibiting the biological activity of a viral protein, and its use in therapy; a method for identifying the inhibitor

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

The problem to be solved by the present application is the provision of ubiquitin variants as inhibitors.

The solutions present in the application to this problem are different for the two groups of inventions:

Invention 1: An inhibitor comprising a beta-grasp fold, wherein said fold comprises region 1 (amino acids 2-14), region 2 (amino acids 42-49), and region 3 (amino acids 62-76) of the amino acid sequence set forth in SEQ ID NO:1, wherein the inhibitor comprises one or more amino acid substitutions in said regions as compared to the amino acid sequence set forth in SEQ ID NO:1, and wherein said substitutions include an amino acid substitution of A to F at the amino acid position corresponding to 46 of SEQ ID NO: 1 and/or an amino acid substitution of E to Y at the amino acid position corresponding to 64 of SEQ ID NO: 1.

Invention 2: An inhibitor comprising a beta-grasp fold, wherein said fold comprises region 1 (amino acids 2-14), region 2 (amino acids 42-49), and region 3 (amino acids 62-76) of the amino acid sequence set forth in SEQ ID NO:1, wherein the inhibitor comprises one or more amino acid substitutions in said regions as compared to the amino acid sequence set forth in SEQ ID NO:1, and wherein said substitution include an amino acid substitution of R to G at the amino acid position corresponding to 74 of SEQ ID NO: 1.

The common concept, namely ubiquitin variants as inhibitors, has been disclosed in the following prior art document:

WO 2012/020289 (UNIV TORONTO [CA]; SIDHU SACHDEV [CA]; BEATTY LINDA [CA]; ERNST ANDREA, 16 February 2012) discloses ubiquitin variants comprising region 1 (amino acids 2-14), region 2 (amino acids 35-49), or region 3 (amino acids 62-72) of the amino acid sequence of ubiquitin; the document discloses (cf. Figs. 5A, 9A, 12A) several ubiquitin variants falling within the scope of present claim 7.

SEQ ID No: 27 of D1 comprises a human ubiquitin variant with a substitution at position 64 (E to Y);

SEQ ID No: 28 of D1 comprises a human ubiquitin variant with a substitution at position 64 (E to Y);

SEQ ID No: 95 of D1 comprised a human ubiquitin variant with a substitution at position 64 (E to Y);

SEQ ID No: 96 of D1 comprises a human ubiquitin variant with a substitution at position 46 (A to F);

SEQ ID No: 139 of D1 comprises a human ubiquitin variant with a substitution at position 64 (E to Y).

The document further discloses that the human ubiquitin variants are inhibiting an ubiquitin enzyme (cf. claim 2); SEQ ID Nos: 27 and 28 are competitive inhibitors of the ubiquitin specific protease USP2 by interfering with the binding of ubiquitin substrates to USP2a (cf. Fig. 5B); SEQ ID Nos: 95 and 96 are competitive inhibitors of the ubiquitin ligase Nedd4 (neural precursor cell expressed developmentally down-regulated protein 4) (cf. Fig. 9B); SEQ ID No: 139 is a competitive inhibitor of the ubiquitin specific protease USP8 (cf. Fig.12B).

Consequently, the subjects (1) to (2) as defined above are no longer linked by a common concept involving a special technical feature, resulting in a lack of unity a posteriori.

Re Item V

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

Reference is made to the following documents; the numbering will be adhered to in the rest of the procedure.

D1 WO 2012/020289 A2 (UNIV TORONTO [CA]; SIDHU SACHDEV [CA]; BEATTY LINDA [CA]; ERNST ANDREA) 16 February 2012 (2012-02-16)

D2 Mark Kemp ET AL: "Recent Advances in the Discovery of Deubiquitinating Enzyme Inhibitors"
In: "PROGRESS IN MEDICINAL CHEMISTRY.", 1 January 2016 (2016-01-01), ELSEVIER, AMSTERDAM., NL, XP055333853, ISSN: 0079-6468
vol. 55, pages 149-192, DOI: 10.1016/bs.pmch.2015.10.002,

- D1 discloses (cf. claim 1) ubiquitin variants comprising region 1 (amino acids 2-14), region 2 (amino acids 35-49), or region 3 (amino acids 62-72) of the amino acid sequence of ubiquitin or a variant thereof.
- D2 gives an overview of deubiquitinating enzyme inhibitors and their use in therapy.

Invention 1: claims: 1, 2, 7, 13(completely); 5, 6, 9-12, 15(partially)

1 **Claims 7, 9-12**

1.1 The subject-matter of claim 7 relates to a human ubiquitin variant with a substitution at position 46 (A to F) and/or a substitution at position 64 (E to Y), which has inhibiting activity.

1.2 The subject-matter of claim 7 is not novel in the sense of Article 33(2) PCT over the teaching of D1.

Document D1 discloses (cf. claim 1) ubiquitin variants comprising region 1 (amino acids 2-14), region 2 (amino acids 35-49), or region 3 (amino acids 62-72) of the amino acid sequence of ubiquitin or a variant thereof; the document discloses (cf. Figs. 6, 13, 21) several ubiquitin variants falling within the scope of present claim 7, having inhibitory activity (cf. claim 2):

- SEQ ID No: 27 of D1 comprises a human ubiquitin variant with a substitution at position 64 (E to Y);
- SEQ ID No: 28 of D1 comprises a human ubiquitin variant with a substitution at position 64 (E to Y);
- SEQ ID No: 95 of D1 comprised a human ubiquitin variant with a substitution at position 64 (E to Y);
- SEQ ID No: 96 of D1 comprises a human ubiquitin variant with a substitution at position 46 (A to F);
- SEQ ID No: 139 of D1 comprises a human ubiquitin variant with a substitution at position 64 (E to Y).

1.3 Dependent claims 9-12 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to novelty and/or inventive step.

2 **Claims 1-2,5-6**

2.1 The subject-matter of claim 1 relates to the use of human ubiquitin variants with a substitution at position 46 (A to F) and/or a substitution at position 64 (E to Y) for inhibiting the biological activity of a viral protein. [The Applicant's attention it drawn to the fact that the wording "preferably" is not limiting the scope of the claim.]

2.2 The subject-matter of claim 1 is novel in accordance with Article 33(2) PCT over the teaching of D1. Document D1 discloses (cf. claim 1; Figs. 6, 13, 21; SEQ ID Nos: 27, 28, 95, 96,139) that the human ubiquitin variants are inhibiting an ubiquitin enzyme (cf. claim 2); SEQ ID Nos: 27 and 28 are competitive inhibitors of the ubiquitin specific protease USP2 by interfering with the binding of ubiquitin substrates to USP2a (cf. Fig. 5B); SEQ ID Nos: 95 and 96 are competitive inhibitors of the ubiquitin ligase Nedd4 (neural precursor cell expressed developmentally down-regulated protein 4) (cf. Fig. 9B); SEQ ID No: 139 is a competitive inhibitor of the ubiquitin specific

protease USP8 (cf. Fig.12B). However, D1 does not disclose that the human ubiquitin variants are inhibiting a viral protein (the proteins inhibited in D1 are of human origin).

2.3 However, the subject-matter of claim 1 cannot be considered inventive in the sense of Article 33(3) PCT in view of closest prior art document D1, since it is obvious to the skilled person that compounds which are inhibiting a specified protein of human origin, will also be effective in inhibiting their viral counterpart.

2.4 Dependent claims 2, 5-6 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to inventive step.

3 **Claim 13**

3.1 The subject-matter of claim 13 is directed to the first medical use claim, i.e. ubiquitin variants for use in therapy. [The Applicant's attention it drawn to the fact that the wording "preferably" is not limiting the scope of claim 13.]

3.2 The subject-matter of claim 13 is not novel in accordance with Article 33(2) PCT over the teaching of D1. Document D1 discloses ubiquitin variants for use in therapy, in particular cancer (cf. [0016]: SEQ ID No:27 and 28 for use in the treatment of cancer, in particular prostate cancer).

3.3 Importantly, it seems that a claim directed to a further medical use, i.e. the ubiquitin variants for use in the treatment and/or prevention of Middle East respiratory syndrome coronoviral (MERS-CoV infection), could be considered as novel and inventive.

4 **Claim 15**

4.1 The subject-matter of claim 15 relates to an screening assay for identifying agents inhibiting the biological activity of a viral ubiquitin binding protein partner using a peptide library.

4.2 The subject-matter of claim 15 is novel in accordance with Article 33(2) PCT over the teaching of D1. D1 discloses the same method, but not for inhibiting a viral ubiquitin binding protein partner (human is the source of origin used in D1).

4.3 However, the subject-matter of claim 15 cannot be considered as involving an inventive step in the sense of Article 33(3) PCT in view of closest prior art document D1, since it is obvious to the skilled person to screen for inhibitors against the biological activity of ubiquitin binding protein partners of viral origin.

Invention 2 - Claims: 3, 4, 8, 14(completely); 5, 6, 9-12, 15(partially)

5 **Claims 8, 9-12**

5.1 The subject-matter of claim 8 relates to a human ubiquitin variant with a substitution at position 74 (R to G) and/or a substitution at position 64 (E to Y), which has inhibiting activity.

5.2 The subject-matter of claim 8 is not novel in the sense of Article 33(2) PCT over the teaching of D1.

Document D1 discloses (cf. claim 1) ubiquitin variants comprising region 1 (amino acids 2-14), region 2 (amino acids 35-49), or region 3 (amino acids 62-72) of the amino acid sequence of ubiquitin or a variant thereof; the document discloses (cf. Figs. 10, 12, 13) several ubiquitin variants falling within the scope of present claim 8, having inhibitory activity (cf. claim 2):

- SEQ ID No: 103 of D1 comprises a human ubiquitin variant with a substitution at position 74 (R to G);
- SEQ ID No: 137 of D1 comprises a human ubiquitin variant with a substitution at position 74 (R to G);
- SEQ ID No: 148 of D1 comprises a human ubiquitin variant with a substitution at position 74 (R to G);

5.3 Dependent claims 9-12 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to novelty and/or inventive step.

6 **Claims 3-4, 5-6**

6.1 The subject-matter of claim 3 relates to the use of human ubiquitin variants with a substitution at position 74 (R to G) for inhibiting the biological activity of a viral protein. [The Applicant's attention it drawn to the fact that the wording "preferably" is not limiting the scope of the claim.]

6.2 The subject-matter of claim 3 is novel in accordance with Article 33(2) PCT over the teaching of D1. Document D1 discloses (cf. claim 1; Figs. 10, 12, 13; SEQ ID Nos: 103, 137, 148) that the human ubiquitin variants are inhibiting

an ubiquitin enzyme (cf. claim 2); SEQ ID No:103 is a inhibitor of the ubiquitin ligase ITCH (cf. Fig. 10B); SEQ ID No:137 is a competitive inhibitor of the ubiquitin specific protease USP8 (cf. Fig. 12B); SEQ ID No:148 is a competitive inhibitor of the ubiquitin specific protease USP21 (cf. Fig.13B). However, D1 does not disclose that the human ubiquitin variants are inhibiting a viral protein (the proteins inhibited in D1 are of human origin).

6.3 However, the subject-matter of claim 3 cannot be considered inventive in the sense of Article 33(3) PCT in view of closest prior art document D1, since it is obvious to the skilled person that compounds which are inhibiting a specified protein of human origin, will also be effective in inhibiting their viral counterpart.

6.4 Dependent claims 4, 5-6 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to inventive step.

7 **Claim 14**

7.1 The subject-matter of claim 14 is directed to the first medical use claim, i.e. ubiquitin variants for use in therapy. [The Applicant's attention it drawn to the fact that the wording "preferably" is not limiting the scope of claim 14.]

7.2 The subject-matter of claim 14 is not novel in accordance with Article 33(2) PCT over the teaching of D1. Document D1 discloses ubiquitin variants for use in therapy, in particular cancer (cf. [0006], [0029], [0115]).

7.3 Importantly, it seems that a claim directed to a further medical use, i.e. the ubiquitin variants for use in the treatment and/or prevention of Crimean-Congo hemorrhagic fever viral infection could be considered as novel and inventive.

8 **Claim 15**

8.1 The subject-matter of claim 15 relates to an screening assay for identifying agents inhibiting the biological activity of a viral ubiquitin binding protein partner using a peptide library.

8.2 The subject-matter of claim 15 is novel in accordance with Article 33(2) PCT over the teaching of D1. D1 discloses the same method, but not for inhibiting a viral ubiquitin binding protein partner (human is the source of origin used in D1).

8.3 However, the subject-matter of claim 15 cannot be considered as involving an inventive step in the sense of Article 33(3) PCT in view of closest prior art document D1, since it is obvious to the skilled person to screen for inhibitors against the biological activity of ubiquitin binding protein partners of viral origin.

Concluding remark

It seems that patentable subject-matter is present in the application, namely the further medical use, i.e. the ubiquitin variants for use in the treatment and/or prevention of Middle East respiratory syndrome coronoviral (MERS-CoV) infection (invention 1) or Crimean-Congo hemorrhagic fever viral infection (invention 2), respectively.