

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.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/EP2023/064899

International filing date (day/month/year)
03.06.2023

Priority date (day/month/year)
03.06.2022

International Patent Classification (IPC) or both national classification and IPC
INV. C07K14/435 A61K38/18

Applicant
BOEHRINGER INGELHEIM INTERNATIONAL GMBH

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 43bis.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 66.1bis(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:



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Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

Sonnerat, Isabelle

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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.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.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 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>1-50</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-23</u>
	No: Claims	<u>24-50</u>
Industrial applicability (IA)	Yes: Claims	<u>1-50</u>
	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

1 References

Reference is made to the following documents:

- D1 WO 2021/148411 A1 (BOEHRINGER INGELHEIM INT [DE];
 MEDIZINISCHE HOCHSCHULE HANNOVER [DE]), 29 July 2021
- D2 ZHAO LONGWEI ET AL: "Production of bioactive recombinant human
 myeloid-derived growth factor in Escherichia coli and its mechanism on
 vascular endothelial cell proliferation", JOURNAL OF CELLULAR AND
 MOLECULAR MEDICINE, vol. 24, no. 2, 22 November 2019, pages
 1189-1199
- ; -& ZHAO LONGWEI ET AL: "Supporting Information- Production of
 bioactive recombinant human myeloid-derived growth factor in Escherichia
 coli and its mechanism on vascular endothelial cell proliferation",
 JOURNAL OF CELLULAR AND MOLECULAR MEDICINE,
 vol. 24, no. 2, 1 January 2020, pages 1189-1199

Re Item V

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

2 Novelty and Inventive Step (Articles 33(2) and 33(3) PCT)

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 24-50 does not involve an inventive step in the sense of Article 33(3) PCT.

- 2.1 Claim 24 is drafted as a product by process claim and hence, is to be construed as a claim to the product as such. It is noted that a product is not rendered novel merely by the fact that it is produced by means of a new process, but must be novel and inventive per se.

The product of claim 24 is a composition comprising a protein of 143 amino acids having the sequence of SEQ ID No. 1 or 2.

A protein of 143 amino acids having the sequence of SEQ ID No. 1 or 2 is not disclosed in the prior art. Claim 24 is thus new (Article 33(2) PCT).

However, the claim can not be considered inventive in the sense of Article 33(3) PCT for the following reasons:

- 2.2 D1 is regarded as being the prior art closest to the subject-matter of claim 24, and discloses human myeloid-derived growth factor (MYDGF) lacking the N-terminal signal peptide (SEQ ID No. 1, 142 amino acids), which is identical with SEQ ID No. 1 and 2 of the application except that it does not contain a N-terminal alanine and/or serine residue. The document further discloses compositions comprising the MYDGF, such as a pharmaceutical composition and the use of MYDGF or the pharmaceutical composition for treating fibrosis, hypertrophy or heart failure (cf. claims 1, 2, 4, 14 and 15).
- 2.3 The subject-matter of claim 24 therefore differs from this known compositions in that the MYDGF comprises 143 amino acids and has the amino acid sequence of SEQ ID No. 1 or 2.
- 2.4 No unexpected effect is linked to said difference.
- 2.5 It is not shown in the application that MYDGF, which lacks the signal peptide and comprises an alanine or serine residue at the N-terminus has any effect compared to the MYDGF of D2, which is identical to SEQ ID No. 1 and 2 of the application, but does not comprise an alanine or serine residue at the N-terminus.
- 2.6 It is merely shown, that MYDGF comprising an alanine or serine residue at the N-terminus can be produced in high yield by E. coli and recovered from the inclusion bodies with high purity.
- This effect however is not linked to the MYDGF per se, but is only achieved upon expression of the protein in E. coli.
- 2.7 Therefore, the only problem that is solved by the subject-matter of claim 24 is the provision of a further composition comprising mature MYDGF. The mere provision of such a composition to which no expected technical effect or useful property is linked does however not require inventive skills. Claim 24 is therefore not inventive (Article 33(3) PCT).
- 2.8 Dependent claims 25-36, as well as claims 37-50 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 novelty and/or inventive step, because they are either known from the prior art such as the composition being a pharmaceutical composition, as claimed in claim 38, or the use of the composition for treating heart failure, hypertrophy or fibrosis, as claimed in claim 44 (cf. D2) or are within the scope of the normal experience and competence of a skilled person. Claims 25-50 are thus also not inventive (Article 33(3) PCT).

- 2.9 Insofar as claims 1-23 are clear (cf. below), they can be considered novel and inventive (Articles 33(2) and 33(3) PCT), because the production of mature MYDGF with an amino acid sequence of SEQ ID No. 1 or 2 by expression thereof in a bacterial host cell, cultivation of the cell, isolation of inclusion bodies containing the protein from the host cell, solubilisation and refolding of the protein is not disclosed or suggested therein.
- 2.9.1 D2 is regarded as being the prior art closest to the subject-matter of claim 1, and discloses the expression of human MYDGF with a C-terminal His-tag from vector pET31b in *E. coli* cells, lysis of the cells and purification of soluble MYDGF from the supernatant by nickel chelate chromatography (cf. paragraphs 2.1; 2.2 and 3.1). The MYDGF consists of 151 amino acids and is identical to SEQ ID No. 1 except that it contains a methionine residue at the N-terminus and a C-terminal his tag (cf. figure S2 c).
- 2.9.2 The subject-matter of claim 1 therefore differs from this known method in that the MYDGF contains an alanine or serine residue at the N-terminus, does not contain a his-tag and is produced in the form of inclusion bodies.
- 2.9.3 The effect that is linked to said difference is a higher productivity of MYDGF (cf. table 5b of the application).
- 2.9.4 The problem to be solved by the present invention may therefore be regarded as the provision of an alternative method for producing MYDGF.
- 2.9.5 The solution proposed in claim 1 of the present application can be considered to involve an inventive step (Article 33(3) PCT), because the skilled person would have had no incentive from the prior art to express a variant of mature human MYDGF in which the N-terminal V residue in position +1 is preceded by an A or an S residue (SEQ ID No's. 1 and 2) in *E. coli* and because it could not have been expected that expression of such a variant in *E. coli* would result in a higher productivity compared to cell mass compared to the method of D2.
- 2.9.6 Claims 2-23 all refer back to claim 1 and therefore are also novel and inventive.

Re Item VIII

Certain observations on the international application

3 Clarity (Article 6 PCT)

- 3.1 It is clear from the description on page 26, paragraph 2 or page 26 paragraph 3 that the following feature is essential to the definition of the invention: the host cell being a bacterial cell.

Since independent claim 1 does not contain these features it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

Claim 1 further lacks clarity (Article 6 PCT), because proteins are usually produced in form of inclusion bodies by bacteria and not, for example, by yeasts which are encompassed by claim 1.

- 3.2 Claim 1 does not meet the requirements of Article 6 PCT because the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter in terms of the result to be achieved ("a protein which after maturation consists of") which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result (cf. claim 4).
- 3.3 The same also applies for the same reason to claim 2.
- 3.4 Claim 45 refers back to the medical use of the pharmaceutical composition of claim 44 and again defines the medical use of the composition. Claim 45 thus lacks clarity (Article 6 PCT).