

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

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/EP2020/067995

International filing date (day/month/year)
26.06.2020

Priority date (day/month/year)
27.06.2019

International Patent Classification (IPC) or both national classification and IPC
INV. C12N5/071 C07K14/035 C07K14/74 C12N9/12

Applicant
NOVO NORDISK A/S

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

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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. 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>1-15</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-15</u>
	No: Claims	
Industrial applicability (IA)	Yes: Claims	<u>1-15</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

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:

- D1 WO 2019/076149 A1 (CHONGQING PREC BIOTECH COMPANY LIMITED [CN]) 25 April 2019 (2019-04-25)
- D2 WO 2012/145384 A1 (UNIV WASHINGTON CT COMMERCIALI [US]; RUSSELL DAVID W [US]) 26 October 2012 (2012-10-26)
- D3 GERMÁN G GORNALUSSE ET AL: "HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells", NATURE BIOTECHNOLOGY, vol. 35, no. 8, 15 May 2017 (2017-05-15), pages 765-772, XP055640664, New York
ISSN: 1087-0156, DOI: 10.1038/nbt.3860
- D4 WO 2017/079673 A1 (FATE THERAPEUTICS INC [US]) 11 May 2017 (2017-05-11)
- D5 YOUNG GYU KIM ET AL: "Ganciclovir-mediated cell killing and bystander effect is enhanced in cells with two copies of the herpes simplex virus thymidine kinase gene", CANCER GENE THERAPY, vol. 7, no. 2, 1 February 2000 (2000-02-01), pages 240-246, XP055644894, GB
ISSN: 0929-1903, DOI: 10.1038/sj.cgt.7700113
- D6 RAMOS C A ET AL: "An inducible Caspase 9 suicide gene to improve the safety of mesenchymal stromal cel therapies", STEM CELLS (MIAMISBURG),, vol. 28, no. 6, 1 June 2010 (2010-06-01), pages 1107-1115, XP002711238, ISSN: 1066-5099, DOI: 10.1002/STEM.433

D1 discloses CAR T-cells in which for HLA-A, b2m and CIITA or TRAC have been knocked out by TALEN or CRISPR/Cas9 systems. Cells are further transfected with vectors expressing HLA-E, F.

D2 discloses beta2μ ko human cells in which HLA-E expression constructs have been introduced by knock-in. HSV-TK is present on the vectors but the TK genes are then deleted. No CIITA ko is performed.

D3 describes beta2μ ko human ES cells in which HLA-E expression constructs have been introduced by knock-in. The antibiotic resistance hybrid genes HyTK and TKN are present on the beta2μ knock-out vectors.

D4 discloses iPSCs in which several genes that can be knocked out to reduce immunogenicity (such HLA-A,B,C via b2μ ko, TAP1, TAP2, PD1) and several that can be introduced (such as CD16, 41BBL, CD3, CD4, HLA-E, HLA-G) are disclosed. iPSCs in which b2μ and CIITA have been knocked out and HLA-E introduced are exemplified (see Example 15). The genetic manipulation is made with several kind of vectors.

D5 reports that the insertion of two copies of the HSV-TK gene into cells enhances Gangcyclovir-mediated cell killing in away proportional to the copy number of TK.

D6 describes the use of TK as a suicide gene for controlling unwanted proliferation of undifferentiated stem cells in therapy.

Novelty (Art. 33(2) PCT)

Claims 1-15 can be considered novel.

None of the cited documents, thus, discloses the knock-in of two or four HSV-TK genes at distinct and known locations concomitant to the expression of HLA-E and the knock out of endogenous beta2μ.

Inventive step (Art. 33(3) PCT)

Claims 1-15 can also be considered inventive.

D4 is considered the closest prior art.

Although D4 discloses the knock-out of HLA-I expression via deletion of the endogenous b2 μ allele and the introduction of HLA-E, as well as the deletion of CIITA and the introduction of HSV-TK as a suicide gene, this is just a selection among several possible genes that can be introduced or deleted in stem cells.

The problem to be solved can be considered the provision of pluripotent stem cells with decreased immunogenicity and increased safety for therapy.

D5 reports that the insertion of two copies of the HSV-TK gene into cells enhances Gangcyclovir-mediated cell killing in away proportional to the copy number of TK.

However, no indication is given that this system might be used to increase the safety of stem cells modified to decrease their immunogenicity.

The skilled person would not have found any suggestion in the prior art to select the features of the claims to arrive at the claimed subject-matter.

Re Item VIII

Certain observations on the international application

Industrial Applicability (Art 33 (4) PCT)

Claim 8 contains the step of "providing a mammalian cell" which may imply a surgical step. Such a step is considered to fall under the provisions of Rule 39.1(iv)/67.1(iv) PCT. Claims 14 and 15 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv)/67.1(iv) PCT.

For the assessment of present claims 8, 14 and 15, with regard to surgical methods applied to the human body and to methods of treatment of the human body, on the question whether they are industrially applicable, no unified criteria exist in the PCT contracting states.

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.

Patentability, in particular novelty and inventive step, 8, 14 and 15 has been assessed for claim 8 ignoring the step of providing cells and for claims 14 and 15 on the basis of a purpose-limited product claim taking into account the alleged effects of the compound/composition.

The patentability can also be dependent upon the formulation of the claim. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.