

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference T08322WO /MH	FOR FURTHER ACTION		See item 4 below
International application No. PCT/EP2020/055017	International filing date (<i>day/month/year</i>) 26 February 2020 (26.02.2020)	Priority date (<i>day/month/year</i>) 27 February 2019 (27.02.2019)	
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237			
Applicant TIGENIX, S.A.U.			

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*. I(a).
2. This REPORT consists of a total of 8 sheets, including this cover sheet.

In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	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 Article 35(2) with regard to novelty, inventive step or 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
4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 25 August 2021 (25.08.2021)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Nora Lindner e-mail pct.team5@wipo.int

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: see form PCT/ISA/220

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/EP2020/055017	International filing date (day/month/year) 26.02.2020	Priority date (day/month/year) 27.02.2019
International Patent Classification (IPC) or both national classification and IPC INV. C12N5/0775 A61K35/28 G01N33/569		
Applicant TIGENIX, S.A.U.		

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:  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 Paresce, Donata 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. 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>1-9, 11, 13</u>
	No: Claims	<u>10, 12, 14, 15</u>
Inventive step (IS)	Yes: Claims	<u>1-9, 11, 13</u>
	No: Claims	<u>10, 12, 14, 15</u>
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 EP 3 219 321 A1 (TIGENIX S A U [ES]) 20 September 2017 (2017-09-20)
- D2 WO 2014/093948 A1 (UNIV RUTGERS [US]) 19 June 2014 (2014-06-19)
- D3 M KRAMPERA: "Mesenchymal stromal cell 'licensing': a multistep process",
LEUKEMIA,
vol. 25, no. 9, 1 September 2011 (2011-09-01), pages 1408-1414,
XP055137780,
ISSN: 0887-6924, DOI: 10.1038/leu.2011.108
- D4 DELAROSA OLGA ET AL: "Requirement of IFN-gamma-mediated
indoleamine 2,3-dioxygenase expression in the modulation of lymphocyte
proliferation by human adipose-derived stem cells",
TISSUE ENGINEERING, LARCHMONT, NY, US,
vol. 15, no. 10, 7 October 2009 (2009-10-07), pages 2795-2806,
XP002563369,
ISSN: 1076-3279, DOI: 10.1089/TEN.TEA.2008.0630
[retrieved on 2009-04-03]
- D5 HOCINE R. HOCINE ET AL: "Minimizing the risk of allo-sensitization to
optimize the benefit of allogeneic cardiac-derived stem/progenitor cells",
SCIENTIFIC REPORTS,
vol. 7, no. 1, 24 January 2017 (2017-01-24), XP055607403,
DOI: 10.1038/srep41125
- D6 OLGA DELAROSA ET AL: "Mesenchymal stem cells as therapeutic agents
of inflammatory and autoimmune diseases",
CURRENT OPINION IN BIOTECHNOLOGY,
vol. 23, no. 6, 1 December 2012 (2012-12-01), pages 978-983,
XP055096416,
ISSN: 0958-1669, DOI: 10.1016/j.copbio.2012.05.005

1 Novelty

1.1 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of claim(s) 10, 12, 14-15 is not new.

1.2 D1 (an earlier application from the same applicants) discloses the following;

"Expanded **allogeneic adipose tissue-derived stromal stem cells** for use in a method of treating a refractory complex perianal fistula in a patient having Crohn's disease" (see claim 1).

1.3 The following distinguishing features of the claim are not known from D1:

1.4 D1 does not disclose a method method for selecting a stem cell (SC) population suitable for allogeneic therapy, wherein a sample of an SC population is cultured in the presence of an IFN- γ concentration capable of inducing maximal HLA-Class I expression in said SC population (test sample) and separately culturing a sample of the SC population in the absence of IFN- γ (control sample), inducing complement-dependent cytotoxicity (CDC) and determining the concentration of HLA-Class I antibody that induces 50% of the maximal CDC (EC_{50} value) in the test sample and the control sample; and selecting the SC population for allogeneic therapy if the ratio of the EC_{50} value of the control sample to the EC_{50} value of the test sample is less than 1.25, or is at least 3.5 ng/ml.

1.5 The subject matter of **claims 1-9, 11, 13 is therefore new** with respect to the document D1 and the claims satisfy the requirements of Article 33(2) PCT with respect to novelty.

1.6 However, it is considered that the SC population of claim 10 (and dependent claims thereof) are not distinguishable from SC populations that have not been selected. These claims at present are not considered novel over D1.

2 Inventive Step

2.1 Claim 1 differs in subject matter from D1 in respect of the distinguishing features given above.

2.2 The technical effect of this difference leads to the following objective problem:

2.3 The problem is the provision of a stem cell population suitable for use in allogeneic stem cell therapy.

2.4 The solution is a method of selecting stem cells which are optimally activated for a specific treatment (the cells are activated then selected based on a certain EC value).

- 2.5 Methods for "priming" or activating mesenchymal stem cells or adipose derived stem cells with cytokines before transplantation to enhance the immunosuppressive capacity of the cells was known in the prior art, see D2, see p. 3, claim 1 and 25, D3 (p. 1410) or D4, p. 2796.
- 2.6 However, these documents do not disclose methods of selecting specific stem cells after activation.
- 2.7 D5 discloses a study wherein,
"In this study, we used human cardiac-derived stem/progenitor cells (hCPC) to examine the proneness of cardiac stem/progenitor cells to DSA-HLA induced rejection. hCPC are stem cells with mixed phenotype expressing pluripotency as well as early cardiac lineage transcription factors. We developed a tailored in vitro flow cytometry-based assay that allowed us to determine the antigen specificity and the binding strength of circulating DSA-HLA and the antibody-mediated injuries to hCPC. We show that the presence of DSA-HLA-I with high/intermediate binding strength is detrimental for allogeneic hCPC promoting their death. In contrast, DSA-HLA-I with low binding strength or DSA-HLA-II are not. Furthermore, we found a significant correlation between the occurrence of CDC and ADCC by the developed flow cytometry-based assay and the binding strength of DSA-HLA determined by the standard Luminex-based assay. Thus, DSA-HLA-I-sensitization could contribute to the loss of hCPC upon their infusion. A systematic immuno-monitoring of DSA-HLA by Luminex-based assay would provide an immune educated choice of these off-the-shelf allogeneic hCPC, which might permit a prolonged persistence to activate endogenous regeneration and optimize repair impaired heart function" (see p. 2).
- 2.8 D5, however, does not disclose the method of claim 1.
- 2.9 D6 (XP055096416) does mention that,
"Therefore, the immunological status and the inflammatory environment at the time of MSCs administration may be critical in order to obtain the expected clinical benefits. It is reasonable, therefore, to believe that an 'active inflammatory profile' at the time of treatment would be most appropriate to trigger full therapeutic capabilities of MSCs. Unfortunately, our knowledge regarding what an ideal 'active inflammatory profile' should be (i.e. activity or ratio of certain cell subsets or cytokines at local or systemic level) remains uncertain and may vary from disease to disease" (see p. 979).
- 2.10 D6, however, does not suggest a method of identifying the "ideal active inflammatory profile".

- 2.11 Therefore, the subject matter of **claims 1-9, 11, 13** appears to be new and to involve an inventive step, Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

3 Clarity

- 3.1 The application does not fulfil the requirements of Article 6 PCT because the subject matter of at least one of the claims lacks clarity to such an extent that an interpretation of the application must be made in order to arrive at an opinion with regard to novelty and inventive step. In particular the following clarity issue is noted:
- 3.2 Claim 10 is not clear. The claimed product is defined in terms of parameters which depend on a certain process (as defined in claim 1). Claim 10 does specify that the EC50 value is determined as set forth in claim 1, however this is not sufficient to clearly define the actual cell population. Any population of stem cells suitable for allogeneic therapy is considered to fall under the scope of this claim (see Novelty above).
- 3.3 Furthermore, the wording of claim 10 does not clearly define the scope of the claim. It is not clear if the SC population is "suitable for allogeneic therapy" (product claim) or for use in the treatment of patients (purpose limited medical use claim).
- 3.4 The SC population as defined in claim 11 could be considered clear (the SC population is selected by the method of any of claims 1 to 9), however, the cells should be defined and not only as preferable options.
- 3.5 Although claim 15 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.