PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	02/11/2018
Applicant's or agent's file reference 9869SG5138	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SG2018/050438	29/08/2018	29/08/2017

International Patent Classification (IPC)
C12N 5/0775 (2010.01)

Applicant
AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH

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 be considered to be a written opinion of the International Preliminary Examination 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/SG	Date of completion of this opinion	Authorized officer
Intellectual Property Office of Singapore 51 Bras Basah Road #01-01 Manulife Centre Singapore 189554 Email: pct@ipos.gov.sg	31/10/2018 *(day/month/year)*	Chen Xiuli (Dr) IPOS Customer Service Tel. No.: (+65) 6339 8616

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. I Basis of this 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 purpose 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 tile.

   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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

   Although a sequence listing has been filed or furnished, it was not used for the purposes of the search.

   Since only one version or copy of sequence listing has been filed or furnished, the statements under item 4 are not required.

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International application No.
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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

1. Statement

Novelty (N)	Claims	1-23	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	1-23	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	1-23	YES
	Claims	NONE	NO

2. Citations and explanations:

The following citations are referred to in this written opinion. Full bibliographic details are provided in the International Search Report:

D1 – ZARIFI F. ET AL, 2017
D2 – WO 2016/006712 A1
(a machine translation was used for the purpose of establishing the written opinion)
D3 – HASEHIRA K. ET AL, 2016
D4 – WO 2008/087260 A1
D5 – SALNER A.L. ET AL, 1982
D6 – ITAKURA Y. ET AL, 2016
D7 – TURINETTO V. ET AL, 2016

D4 discloses an evaluation of the status of a mesenchymal stem cell preparation, by detecting the presence of an elongated glycan structure or a group, at least two, of glycan structures in the preparation. It does not disclose monitoring senescence in the mesenchymal stem cell preparation.

D5 discloses the use of lectin peanut agglutinin (PNA) for sorting bone marrow stem cells (Abstract).

D6 is a study on cellular senescence-dependent glycan changes in human dipoloid fibroblasts using a lectin microarray. It discloses that the cellular senescence process was related to the change in glycan composition of the cell surface and the glycan changes occur before morphological changes. For example, α2-3 sialylated O-glycan structures are decreased with cellular senescence and Gal-β1,3-GalNac structures are increased with cellular senescence. It concludes that knowledge of glycan modifications on aging (stem) cells will be useful in identification of appropriate therapeutic cells.

D7 is a review article disclosing that it is of primary importance to monitor the occurrence of senescent phenotype in clinically expanded hMSCs and presents strategies for *in-vitro* senescence monitoring (Abstract; Table 1).

1. Novelty

Claims 1-23 are novel and therefore comply with PCT Article 33(2).

None of D1-D7 individually discloses all the features in claims 1-23. Therefore, these claims appear to be novel.

2. Inventive step

Claims 1-23 involve an inventive step and therefore comply with PCT Article 33(3).

D1 discloses a method of detecting the lectin profile of MSCs, where a subpopulation of MSCs with small nuclei and moderate staining for lectin probe of PNA is found to have higher proliferation rate. It also directs to the use of

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

lectins for separating this MSC subpopulation with higher proliferation rate from the other subpopulations, e.g. partially differentiated progenitor cells (Discussion on pages 80-81; Conclusion).

D2 discloses that early passage (P2-P6) adipose derived hMSCs with high differentiation potential and proliferative capacity possess higher fluorescence intensity indicating higher lectin binding (to α2-6 sialylated N-glycan structures) as compared to late passage (P25-P29) hMSCs with decreased differentiation potential and proliferative capacity (Example 2). However, D2 focuses on the discrimination of MSCs based on differentiation potential rather than replicative senescence using these lectin probes for α2-6 sialylated N-glycan structures (page 4, last para., page 5, para. 1, pages 6-7, page 10, last para.).

Similar to D2, D3 discloses a higher percentage of α2-6 sialylated N-glycan structures as expressed in early passage adipose derived hMSCs (P3, P5) compared to late passage cells (P26, P28), where α2-6 sialylation indicates differential potential of hMSCs. It additionally discloses the percentage of sialyl-T-antigen (sT-antigen as used in present application) to be higher in late passage hMSCs over early passage hMSCs (page 803, right col. para. 1, page 798, left col. para. 2-3).

Although the late passages of cells have loose or flat cell proliferation curve and impaired differentiation (e.g. D2: page 48 para. 1) or have higher percentage of sT-antigen (e.g. D3), i.e. similar properties to present P21 bone marrow MSCs (senescent group) (para. [0056], [0058] of present application), it is not necessary that late passages of cells with impaired differentiation are senescent cells, which have other defined characteristics. Hence, the late passages of cells disclosed in D2 and D3 are not considered as senescent cells.

Each of D1-D3 mainly differs from claims 1 and 19 in that each document is silent on the use of (lectin) probe binding to a glycan for determining or separating the proliferating/non-senescent MSCs from the senescent MSCs.

Although it is generally known from studies that cellular senescence process is related to the change in glycan composition of the cell surface and the glycan changes occur before morphological changes (e.g. D6), these studies have not been carried out on MSCs. Further, none of the documents whether individually or in combination with other documents clearly teaches or directs to the use of relative difference in glycan expression for determining or separating the proliferating/non-senescent MSCs from the senescent MSCs.

Notwithstanding the lack of support (see Box No. VIII), claims 1 and 19 therefore appear to be not obvious. By virtue of dependency, claims 2-18 and 20-23 are also not obvious.

3. Industrial applicability

Claims 1-23 are industrially applicable and therefore comply with PCT Article 33(4).

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:

Support

Claims 1 and 19 do not comply with PCT Article 6.

Claims 1 and 19 relate to the use of a probe that binds to any glycan on the cell surface of the MSCs. However, it is noted that only PNA, which only binds to glycan T-antigen (Gal-β1,3-GalNAc), is verified to discriminate between senescent and non-senescent MSCs using live cell immunostaining (present para. [0055], [0057]; Fig. 14B). Moreover, the N-glycans between proliferating and senescent MSC were structurally similar to each other, i.e. no statistically significant changes in any observed N-glycan structures (present para. [0061]; Fig. 17B). Accordingly, it is uncertain whether the use of a probe that binds to any glycan is sufficiently able to discriminate between the senescent and non-senescent MSCs. It is also considered that there is undue burden on the skilled person required to identify each and every other probe(s) that can discriminate between the two states in view of the limited disclosure.

As such, claims 1 and 19 lack support across their breadth.