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(54) Title: METHODS TO ISOLATE HUMAN MESENCHYMAL STEM CELLS

(57) Abstract: A method of obtaining a population of PDGFR α ⁺ CD51⁺ CD146^{high} stem cells is provided. Compositions comprising PDGFR α ⁺ CD51⁺ CD146^{high} stem cells, and methods of use of a population of PDGFR α ⁺ CD51⁺ CD146^{high} stem cells, are also provided.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/48851

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 5/00, 5/02 (2014.01)

USPC - 435/377, 375, 325

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12 N 5/00, 5/02; C12Q 1/02 (2014.01)

USPC: 435/377, 375, 325, 29, 395, 384

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; ProQuest; 'cd146 OR mcam OR muc18,' 'population OR plural', 'stem cell' or undifferentiated cell', 'cd51 OR integrin alpha V OR itgav OR vnr OR vitronectin receptor,' 'Pdgfr' or platelet derived growth factor receptor'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	QIAN, H et al. Primary Mesenchymal Stem And Progenitor Cells From Bone Marrow Lack Expression Of CD44 Protein. The Journal of Biological Chemistry. 31 May 2012, Vol. 287, pages 25795-25807; column 1, page 25796, paragraph 1; abstract; column 2, page 25795, paragraph 1; column 1, page 25800, paragraph 3; column 1, page 25796, paragraph 4; column 2, page 25797, paragraph 3; figure 1B; column 2, page 25795, paragraph 2; column 2, page 25797, paragraph 4.	1, 2, 3/1, 3/2, 14, 17/14 ----- 15, 16, 17/15, 17/16
Y	US 2010/0172885 A1 (PITTINGER, MF et al.) July 8, 2010; abstract; paragraphs [0012], [0033], [0034]	15, 16, 17/15, 17/16
A	GRONTHOS, S et al. The Growth Factor Requirements Of STRO-1-Positive Human Bone Marrow Stromal Precursors Under Serum-Deprived Conditions In Vitro. Blood. 15 February 1995. Vol. 85; pages 929-940; figure 8B.	15, 16, 17/15, 17/16

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/48851

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-13, 18-33
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Please See Supplemental Page-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No. required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I: Claims 1-3, 14-17

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

-***-Continuation of Box No. III: Observations Where Unity Of invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-3 and 14-17 are directed toward a method of obtaining a population of stem cells comprising identifying PDGFR-alpha+, CD51+ cells, or PDGFR-alpha+ CD51+ CD146+ cells in a heterogeneous population of cells, and recovering the PDGFR-alpha+, CD51+ cells, or PDGFR-alpha+ CD51+ CD146+ cells, so as to obtain the population of stem cells; and an isolated population of PDGFR-alpha+, CD51+ mesenchymal stem cells, wherein the population is 25% or greater PDGFR-alpha+, CD51+ cells or an isolated population of PDGFR-alpha+, CD51+ CD146+ mesenchymal stem cells, wherein the population is 25% or greater PDGFR-alpha+, CD51+ CD146+ cells.

Group II, Claims 34-36 are directed toward a method of expanding a population of HSC or progenitor cells comprising co-culturing the cells with PDGFR-alpha+, CD51+ mesospheres in an amount sufficient to efficiently expand the population of HSC or progenitor cells.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a method of obtaining a population of stem cells comprising identifying PDGFR-alpha+, CD51+ cells, or PDGFR-alpha+, CD51+ CD146+ cells in a heterogeneous population of cells, and recovering the PDGFR-alpha+, CD51+ cells, or PDGFR-alpha+ CD51+ CD146+ cells, so as to obtain the population of stem cells, and an isolated population of PDGFR-alpha+, CD51+ mesenchymal stem cells, wherein the population is 25% or greater PDGFR-alpha+, CD51+ cells or an isolated population of PDGFR-alpha+, CD51+ CD146+ mesenchymal stem cells, wherein the population is 25% or greater PDGFR-alpha+, CD51+ CD146+ cells, which are not present in Group II, the special technical features of Group II including a method of expanding a population of HSC or progenitor cells comprising co-culturing the cells with PDGFR-alpha+, CD51+ mesospheres in an amount sufficient to efficiently expand the population of HSC or progenitor cells.

Groups I-II share the technical features including a population of stem cells comprising PDGFR-alpha+ CD51+ mesenchymal cells.

However, these shared technical features are previously disclosed by the article entitled 'Primary Mesenchymal Stem And Progenitor Cells From Bone Marrow Lack Expression Of CD44 Protein' by Qian, et al. (hereinafter 'Qian'). Qian discloses a population of stem cells comprising PDGFR-alpha+ CD51+ mesenchymal stem cells (a population of stem cells comprising PDGFR-alpha+ CD51+ mesenchymal stem cells; in mice, platelet-derived growth factor receptor alpha (PDGFR-alpha), stem cell antigen-1 (SCA1), CD51, and Nestin are expressed on freshly isolated BM stromal cell populations enriched with MSCs(6-8); page 25795, column 2, paragraph 1).

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Qian reference, unity of invention is lacking.