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[Continued on next page]

(54) Title: ANGIOGENIN AS A DIAGNOSTIC OR PROGNOSTIC BIOMARKER AND DRUG TARGET IN AGE-RELATED MACULAR DEGENERATION

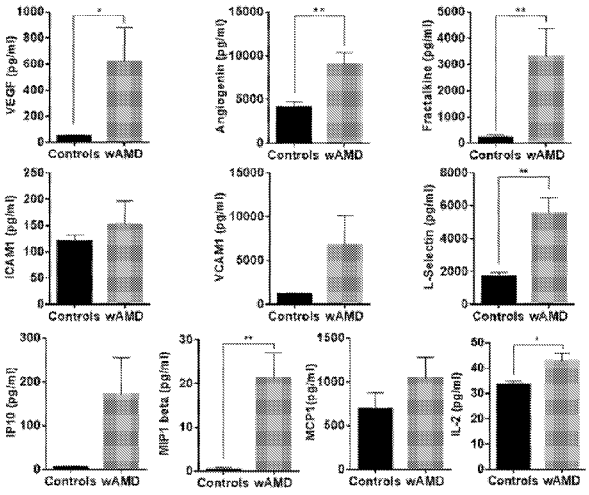

FIG 1

(57) Abstract: The present invention relates to the quantification of various soluble proteins, including angiogenin in aqueous humor and plasma of patients with AMD. The invention further describes the role of angiogenin in the pathogenesis, progression of AMD. Hence, the level of angiogenin serves as a diagnostic and/or prognostic marker in AMD. Angiogenin modulators administered in the form of either intra-vitreal injection or topical solution to patients suffering from AMD can cause the required changes in the levels of angiogenin along with improved prognosis. The invention discloses the quantification of soluble proteins, which is useful for simultaneous detection and quantification of angiogenin along with other molecular factors associated with AMD. The invention also highlights that plausibility of modulating angiogenin function in the management of AMD.

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TITLE OF THE INVENTION

Angiogenin as a diagnostic or prognostic biomarker and drug target in age-related macular degeneration

[0001] DESCRIPTION OF THE INVENTION

[0002] Technical field of the invention

[0003] The present invention relates to the association observed between soluble proteins and ocular condition. The invention, in particular, relates to the association between angiogenin and ocular conditions such as Age-Related Macular Degeneration (AMD), Diabetic Retinopathy (DR), Central Retinal Vein Occlusion (CRVO), Branch Retinal Vein Occlusion (BRVO) and pterygium. The invention further relates to the use of angiogenin as a diagnostic and prognostic biomarker and also the use of angiogenin and modulation of its function as a therapeutic target in the management of AMD.

[0004] Background of the invention

[0005] Angiogenesis is a process, which helps in the formation of new blood vessels in the body. Angiogenin is a protein with ribonuclease activity and is made up of single chain amino acids consisting of 123 amino acids and induces cell migration, proliferation, invasion and angiogenesis.

[0006] AMD is the leading cause of vision loss and blindness in people, which results due to degeneration of the macula, a part of the retina which is responsible for the sharp vision. Degeneration of macula in addition to the formation of new blood vessels is one of the causes for loss of central vision in case of patients affected with AMD.

[0007] There are two types of AMD namely dry AMD and wet AMD. Dry AMD is an early stage of the disease and results from aging and thinning of macular tissues, depositing yellow colored pigmented spots in the macula. This aging and thinning of macular tissues causes inflammation and results in vision loss. Dry AMD further advances into a stage where growth of newer blood vessels below the retina is observed, with leakage of blood and other fluids. This condition is called as wet AMD. The leakage also leads to permanent damage of the retinal cells, which creates blind spots in the central vision in later stage.

[0008] Various proteins including cytokines (Interleukin-6; Interleukin-8), chemokines (MIP-1β, Macrophage Inflammatory Protein 1 beta; MCP-1, Monocyte Chemotactic Protein-1; RANTES, Regulated on Activation, Normal T cell Expressed and Secreted; Fractalkine) IFNs (Interferons), TGF (Transforming Growth Factors), E-selectin, L-selectin, VEGF (Vascular Endothelial Growth Factor) and angiogenin are molecular factors which are present in the eye. The increase in the levels of these proteins are associated with the formation of AMD.

[0009] Various clinical studies have been carried out for detecting angiogenin levels in patients with dry and wet AMD. A German study suggests that there is no significant difference in the angiogenin levels of aqueous humor between control and various stages of AMD. Whereas, a Japanese study states that aqueous humor angiogenin levels are significantly higher in patients with AMD as compared to control group.

[0010] There are various therapies, which are used in the treatment of AMD. Intra-vitreal bevacizumab injection decreases the levels of aqueous VEGF and increases the levels of inflammatory mediators namely IL-6, IL-8, MIP-1beta and MCP-1 levels but without changes in Angiogenin levels.

[0011] Serum angiogenin levels are also monitored in various diseased conditions such as Behcet's disease wherein serum angiogenin levels are significantly higher in the diseased group than the control group. Angiogenin levels are also highly

expressed in cultured fibroblasts of pterygia with severe phenotype, compared to those with normal conjunctiva. Angiogenin levels in diabetic retinopathy (DR) are found to be lower in the vitreous fluid as compared to the diabetic individuals without complications. Patients without diabetic complications have high local and systemic concentration of angiogenin in the serum. Also, a decrease in tyrosine phosphorylation of many vitreous proteins including angiogenin is observed in DR, which may indicate an alternate disease specific function. Furthermore, a significant elevation of IL-8, VEGF and angiogenin in the vitreous but not in serum samples is observed in diabetic individuals compared to control subjects. A significant increase in the vitreous angiogenin levels in eyes with proliferative diabetic retinopathy (PDR) and proliferative vitreo retinopathy (PVR) is observed.

[0012] The US Patent Application **US20140370087** titled *"Novel use of angiogenin"* discloses a pharmaceutical composition that includes angiogenin or a fragment as an active ingredient, which is useful for the prevention and treatment of glaucoma. Angiogenin or its fragments suppresses the inflammatory response by activating the aqueous humor outflow due to which there is an increase in the nitrous oxide (NO) generation which in turn reduces the intraocular pressure. However, the invention is silent with respect to the means by which angiogenin levels are decreased in AMD.

[0013] The Patent Application **WO1998040487** titled *"Angiogenin receptor, compositions and methods related thereto"* discloses the use of nucleic acids, which are used as probes to detect an angiogenin receptor-encoding nucleic acid, and to inhibit expression of the receptor. The invention also provides antibodies for drug screening assays such as CAM assay, blotting techniques, which help in detecting the angiogenin levels and therapeutic and diagnostic methods and compositions related thereto. However, the invention does not describe angiogenin use as diagnostic and/or prognostic marker for AMD.

[0014] The Patent Application **AU2015252112A1** titled "*Use of Complement Pathway Inhibitors to Treat Ocular Diseases*" discloses the treatment of ocular diseases including age-related macular degeneration (ARMD), diabetic retinopathy (DR) and ocular angiogenesis (OA) with the administration of Complement Pathway inhibitors such as anti-Factor D antibody, Factor H or inhibitors that block the action of properdin, factor B, factor Ba, factor Bb, C2, C2a, C3a, C5, C5a, C5b, C6, C7, C8, C9, or C5b-9. However, the invention does not disclose the mechanism of targeting angiogenin for the management of AMD.

[0015] There are different methods available for the quantification of soluble proteins. The quantification of soluble proteins is crucial in understanding the relationship between the biomolecules in the biological fluids and disease pathogenesis. Hence, quantification of soluble proteins determines the relationship between the levels of soluble proteins and stage/progression of disease. It can also serve as diagnostic and prognostic markers for various conditions including ocular diseases.

[0016] Summary of the invention

[0017] The present invention relates to quantification of various soluble proteins such as inflammatory cytokines, chemokines, secreted cell adhesion molecules and pro-angiogenic factors in aqueous humor and plasma, which helps in diagnosis and also in determining the prognosis of AMD. The invention relates to identification of soluble proteins as diagnostic or prognostic markers in analysis of progression of the ocular diseases. The soluble proteins are analyzed in fluids such as aqueous humor, vitreous humor, serum and plasma of patients with AMD.

[0018] The invention utilizes a standard method for measuring or analyzing the levels of various soluble proteins such as inflammatory cytokines, chemokines, growth factors, secreted cell adhesion molecules and pro-angiogenic factors in aqueous humor and plasma using cytometric bead array, which is useful for simultaneous detection and quantification of various soluble factors. The assay is performed and the capture beads and analyte signals are acquired by a flow

cytometer. A comparative study is performed using analyte signal intensities with reference standards to determine absolute concentrations of individual analytes using BD FCAP Array Software. The statistical analysis and graphical representation of the results are performed using GraphPad.

[0019] The comparative levels of VEGF, angiogenin, fractalkine, ICAM-1 (Intercellular Adhesion Molecule-1), VCAM-1 (Vascular Cell Adhesion Molecule), L-selectin, IL-2, IP10, MIP-1 beta and MCP-1 are quantified by cytometric bead array in the aqueous humor of control and AMD patients. The control group comprises aqueous humor from patients with diabetes undergoing cataract surgery with no other associated ocular pathologies. The results indicate that the levels of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules are higher in the aqueous humor of patients with wet and dry AMD as compared to that of the control group.

[0020] The invention indicates the higher level of angiogenin in aqueous humor in patients with AMD compared to controls. The levels of angiogenin in related to the progression of the disease. Angiogenin can act as a diagnostic and prognostic biomarker for diagnosis or prognosis of the ocular diseases such as AMD.

[0021] The invention thus describes that higher levels of angiogenin are found in aqueous humor and plasma in patients with AMD. Angiogenin levels are higher in the plasma of patients with dry AMD. Thus, suggesting angiogenin as a potential drug target in the management of AMD.

[0022] Brief description of the drawings

[0023] The foregoing and other features of embodiments will become more apparent from the following detailed description of embodiments when read in conjunction with the accompanying drawings.

[0024] FIG 1 illustrates the levels of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules in the aqueous humor of patients with wet AMD.

[0025] FIG 2 illustrates the levels of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules in the plasma of patients with dry AMD.

[0026] Detailed description of the invention

[0027] In order to make the matter of the invention clear and concise, the following definitions are provided for specific terms used in the following description.

[0028] The term "*Diagnostic Biomarker*" refers to a chemical/biological/physical entity which by means of specific reactions assists in qualitative and quantitative analysis of the disease.

[0029] The term "*Prognostic Biomarker*" refers to a chemical/biological/physical entity that provides information about the status of a specific disease in either untreated or treated individuals.

[0030] The present invention relates to quantification of various soluble proteins such as inflammatory cytokines, chemokines, secreted cell adhesion molecules and pro-angiogenic factors in aqueous humor and plasma, which helps in diagnosis and also in determining the prognosis of AMD.

[0031] The invention relates to identification of soluble proteins as diagnostic or prognostic markers in analysis of progression of the ocular diseases. The soluble proteins are analyzed in fluids such as aqueous humor, vitreous humor, serum and plasma of patients with AMD.

[0032] The invention utilizes a method for measuring or analyzing the levels of various soluble proteins such as inflammatory cytokines, chemokines, growth factors, secreted cell adhesion molecules and pro-angiogenic factors in aqueous humor and plasma using cytometric bead array, which is useful for simultaneous detection and quantification of various soluble factors. The assay for the aforementioned soluble proteins is performed as per manufacturer's instruction. The capture beads and analyte signals are acquired by a flow cytometer (BD FACSCalibur™) using BD Cell Quest Pro Software. A comparative study is performed using analyte signal intensities with reference standards to determine absolute concentrations of individual analytes using BD FCAP Array Software. The statistical analysis and graphical representation of the results are performed using GraphPad.

[0033] FIG 1 illustrates the levels of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules in aqueous humor of patients with wet AMD. The comparative levels of VEGF, angiogenin, fractalkine, ICAM-1 (Intercellular Adhesion Molecule-1), VCAM-1 (Vascular Cell Adhesion Molecule), L-selectin, IL-2, IP10, MIP-1 beta and MCP-1 are quantified by cytometric bead array using a flow cytometer in the aqueous humor of control and wet AMD patients. Aqueous humor is obtained for analysis immediately following intra-vitreal injection of anti-VEGF. The control group comprises aqueous humor from patients with diabetes undergoing cataract surgery with no other associated ocular pathologies. The results indicate that the levels of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules are higher in the aqueous humor of patients with wet AMD as compared to that of the control group.

[0034] FIG 2 illustrates the levels of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules in plasma of patients with dry AMD. The comparative levels of VEGF, angiogenin, fractalkine, ICAM-1, VCAM-1, L-Selectin, IL-2, IP10, MIP-1beta and MCP-1 are quantified by

cytometric bead array in the plasma of healthy controls and dry AMD patients. The control group comprises plasma from patients with diabetes undergoing cataract surgery with no other associated ocular pathologies. The results indicate that the level of pro-angiogenic factors, cytokines, chemokines and secreted cell adhesion molecules are similar in the plasma of patients with dry AMD as compared to control group. However, there is a significant increase in angiogenin level in plasma of patients with dry AMD compared to other factors that were quantified.

[0035] The increase in the levels of angiogenin in the plasma and aqueous humor of dry and wet AMD, respectively indicates the use of angiogenin as a diagnostic and/or prognostic biomarker, which can be detected in the aqueous humor, vitreous humor, serum or plasma of the patients with AMD. Thus, suggesting angiogenin as a potential drug target in the management of AMD.

[0036] The invention thus describes that higher levels of angiogenin are found in aqueous humor and plasma of patients with AMD. Angiogenin levels are higher in the plasma of patients with dry AMD. Hence, indicating a vital role for angiogenin in the pathogenesis and progression of AMD.

[0037] Claims:

We Claim:

1. A biomarker to identify the risk of ocular conditions, wherein:
    a. the biomarker is a soluble protein;
    b. the biomarker quantified to be used to diagnose and prognosticate disease including eye disease; and
    c. the biomarker is quantified in a fluid of a human.

2. The biomarker as claimed in claim 1, wherein the biomarker is selected from a group comprising VEGF (Vascular Endothelial Growth Factor) VEGF, angiogenin, fractalkine, ICAM-1 (Intercellular Adhesion Molecule-1), VCAM-1 (Vascular Cell Adhesion Molecule), L-selectin, IL-2 (Interleukin-2), IP10 (Inflammatory Protein-10), MIP-1 beta (Macrophage Inflammatory Protein-1beta) and MCP-1 (Monocyte Chemotactic Protein-1).

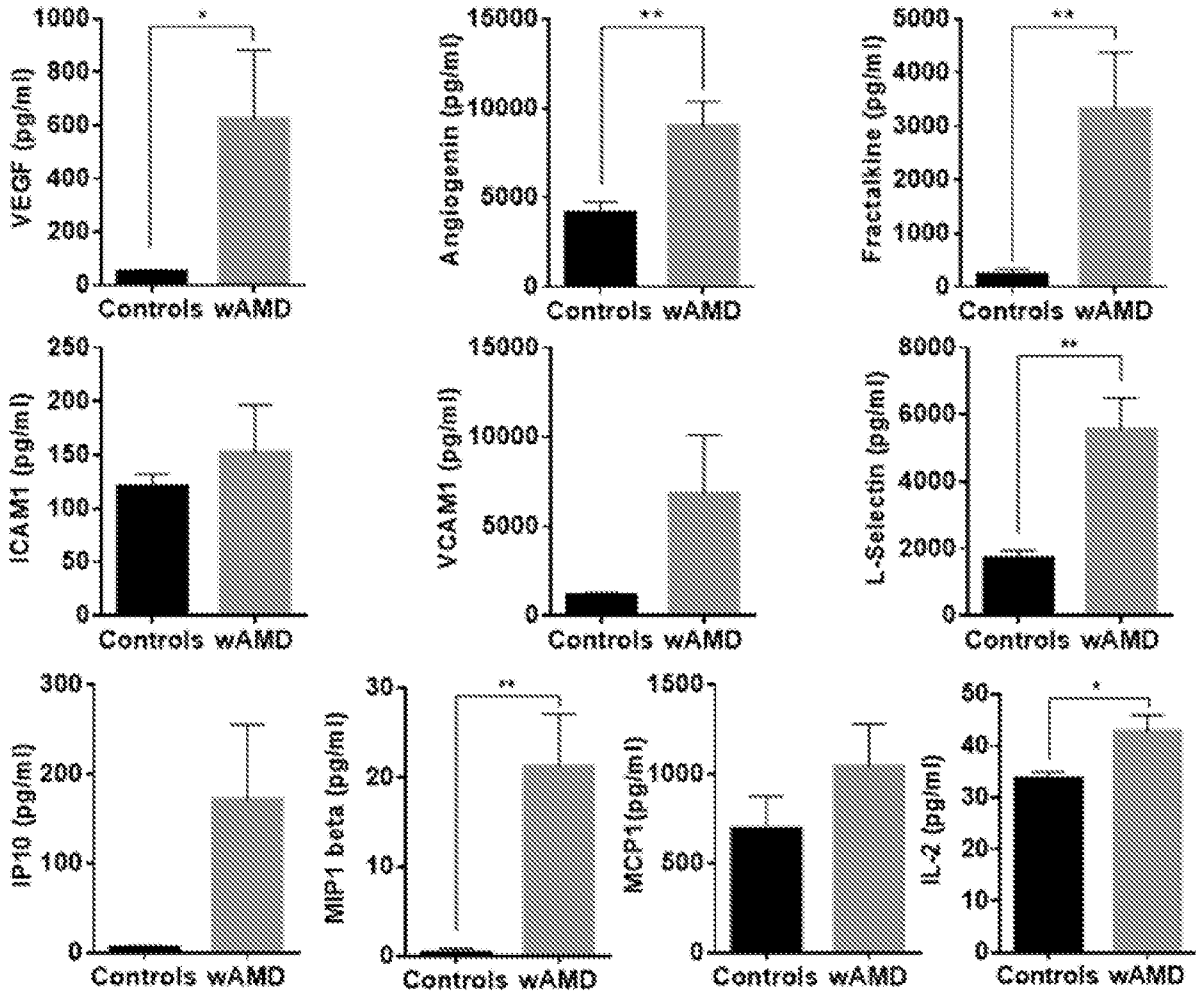
3. The biomarker as claimed in claim 1, wherein the fluid is selected from a group comprising aqueous humor, vitreous humor, serum and plasma.

4. The biomarker as claimed in claim 1, wherein the ocular conditions are selected from a group comprising of dry and wet Age-Related Macular Degeneration (AMD), Diabetic Retinopathy (DR), Central Retinal Vein Occlusion (CRVO), Branch Retinal Vein Occlusion (BRVO) and pterygium.

5. The biomarker as claimed in claim 1, wherein the levels of angiogenin is high in aqueous humor of patient with age-related macular degeneration.

6. The biomarker as claimed in claim 1, wherein the levels of angiogenin in plasma patient with dry age-related macular degeneration is high

compared to control such that angiogenin is suitable as a drug target for treatment of AMD.


VEGF (pg/ml)
1000
800
600
400
200
0
*
Controls wAMD
Angiogenin (pg/ml)
15000
10000
5000
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**
Controls wAMD
Fractalkine (pg/ml)
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2000
1000
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Controls wAMD
ICAM1 (pg/ml)
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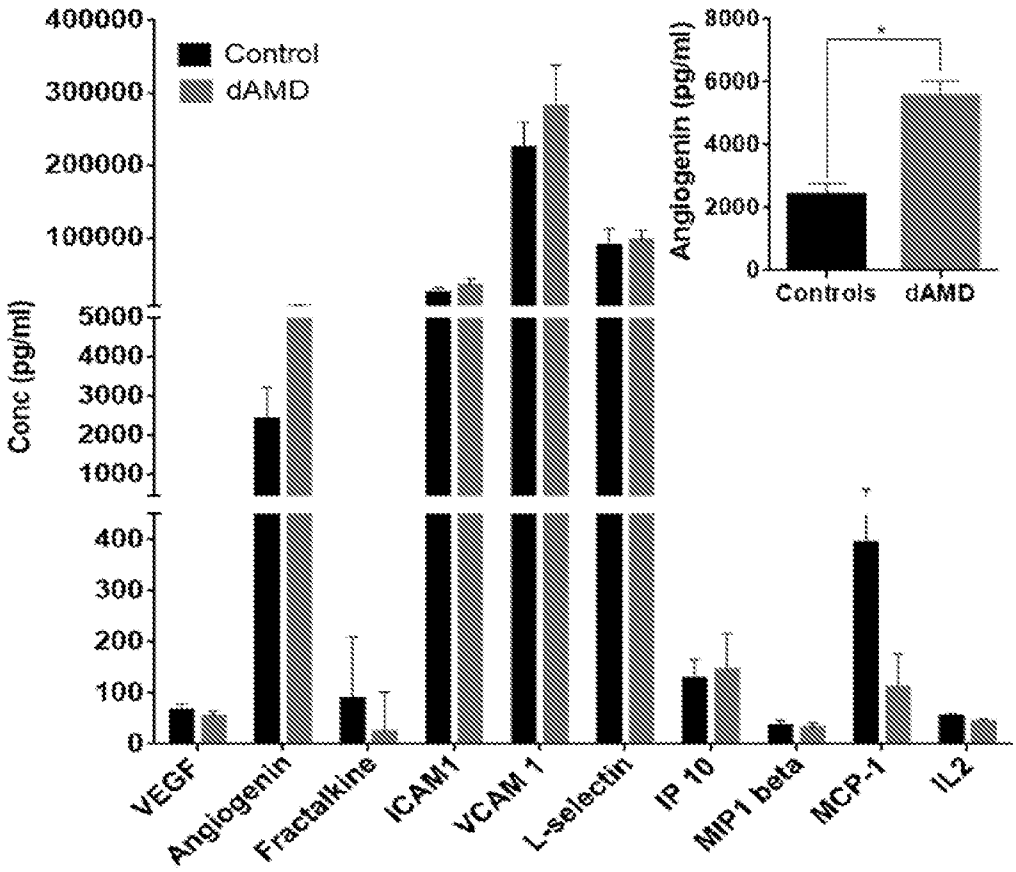
Controls wAMD

FIG 1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300000
200000
100000
5000
4000
3000
2000
1000
400
300
200
100
0
VEGF
Angiogenin
Fractalkine
ICAM1
VCAM 1
L-selectin
IP 10
MIP1 beta
MCP-1
IL2
Angiogenin (pg/ml)
8000
6000
4000
2000
0
*
Controls
dAMD

FIG 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/051998

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/00,C07K14/515,C07K14/54,A61P21/02 Version=2017.01

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)

C07K, A61P

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)

Patseer, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Nagineni, Chandrasekharam N., Vijay K. Kommineni, Abitha William, Barbara Detrick, and John J. Hooks. "Regulation of VEGF expression in human retinal cells by cytokines: Implications for the role of inflammation in agerelated macular degeneration." Journal of cellular physiology 227, no. 1 (2012): 116-126. Pages 116, 119-121	1-6
X	Skeie, Jessica M., Shemin Zeng, Elizabeth A. Faidley, and Robert F. Mullins. "Angiogenin in age-related macular degeneration." Molecular vision 17 (2011): 576. Page 580-581	1-6
X	WO2006055739 A2 (ROBERT SACK) 26 May 2006 Example 2, Claims, Detailed Description	1-6
X	KR101337046 B1 (UNIV CHUNG ANG IND) 6 Dec 2013 Claim 1	1-6
X	US20140370087A1 (CHUNG-ANG UNIVERSITY THE INDUSTRY ACADEMIC-COOPERATION FOUNDATION) 18 Dec 2012 Abstract, Claims	1-6

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"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
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"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	Date of mailing of the international search report
04-08-2017	04-08-2017
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Anushri Kamble Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/051998

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1743031 A4 (CHILDREN S MEDICAL CENTER CORP) May 28, 2008 Para [065] and claims	1-6

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2017/051998

Citation	Pub.Date	Family	Pub.Date
WO 2006055739 A2	26-05-2006	US 2009068162 A1	12-03-2009
US 20140370087 A1	18-12-2012	WO 2013051767 A1	11-04-2013
EP 1743031 A4	28-05-2008	US 2013178386 A1	11-07-2013
		JP 2007535324 A	06-12-2007
		WO 2005103281 A3	06-04-2005