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(54) Title: MIRNAS IN JOINT DISEASE

(57) Abstract: The present invention concerns microRNAs (miRNA), in particular mir-22, as an indicator of a tissue status or a disease such as osteoarthritis (OA) and as a target molecule for the discovery of a substance for the treatment of a joint disease. Further provided are methods and compounds for investigation, analysis and/or treatment of a joint disease, especially OA.

Description

miRNAs in Joint Disease

Field of the Invention

The present invention concerns microRNAs (miRNA), in particular mir-22, as an indicator of a tissue status or a disease such as osteoarthritis (OA) and as a target molecule for the discovery of a substance for the treatment of a joint disease. Further provided are methods and compounds for investigation, analysis and/or treatment of a joint disease, especially OA.

Background of the Invention

Arthritis is the most common joint disease in humans which is marked by pain, inflammation, redness, and swelling of the joints. Several different forms of arthritis are known. Rheumatoid arthritis (RA), a chronic, systemic inflammatory disorder, primarily affects synovial joints, whereas ankylosing spondylitis (AS), also a chronic inflammatory arthritis, mainly affects the joints of spine and pelvis. Juvenile idiopathic arthritis (JIA) is characterised in an inflammation of the synovium of the joint and effects children of an age below 16 years. Gout is characterised by recurring attacks of acute inflammatory arthritis which are caused by elevated levels of uric acid in the blood. Septic arthritis is caused by an infection of the joint and psoriatic arthritis may develop in patients suffering from psoriasis. Osteoarthritis (OA) is characterised by mechanical abnormalities of the joint such as the degradation of the joints articular cartilage and subchondral bone.

OA is one of the most common diseases of the western world with more than 21 million diagnosed symptomatic cases in the USA, Japan, and Germany in 2000. Due to the aging of the population, an ever-increasing population of OA patients has to be faced, whose quality of life is severely affected. In addition, OA carries a tremendous socio-economic burden with direct and indirect costs. The current treatment modalities concentrate on the management of the pain associated with OA, since pharmacological treatment modalities able to slow, stop or even reverse the course of OA and its effects, are long since sought for but still missing.

OA can be viewed as the clinical and pathological outcome of a range of disorders that result in structural and functional failure of synovial joints. It occurs when the dynamic equilibrium between the breakdown and the repair of joint tissues is overwhelmed. Structural failure of articular cartilage can result from abnormal mechanical strains injuring healthy cartilage, as well as from failure of pathologically impaired cartilage degenerating under the influence of physiological and mechanical strains. OA is characterised by a progressive degeneration of the articular cartilage, ultimately leading to a functional inability of the affected joint. During the chronical progression of OA the articular cartilage is destroyed setting free the underlying bone tissue, ultimately necessitating the surgical replacement of the affected joint.

Besides the destruction of the cartilage, pathological degenerations of the synovial membranes and the ligaments are taking place. Inflammation takes place in OA as a secondary effect.

Morphological changes observed in OA include cartilage erosion as well as a variable degree of synovial inflammation. These changes are attributed to a complex network of biochemical factors, including proteolytic enzymes that lead to a breakdown of the cartilage macromolecules, in particular collagen II, X and aggrecan. Cytokines such as IL-1 and TNF-α which are produced by activated synoviocytes, mononuclear cells or by articular cartilage itself, significantly up regulate matrix-metalloproteinases (MMPs) and cytokine gene expression, and blunt compensatory synthesis pathways.

The exact etiology of OA is still not resolved, but some contributing factors are associated with the onset and progression of the disease. The most important factor is age, but also periodic overload, obesity, joint laxity, and a genetic predisposition play an important role. The degradation of cartilage matrix components is generally agreed to be due to an increased synthesis and activation of extracellular proteinases, mainly MMPs, and cytokines amplifying degenerative processes.

Diagnosis of OA is commonly based on clinical examination of the joints in patients showing a gradual onset of symptoms and signs, with x-rays confirming the

diagnosis. Typical changes observable via x-ray include a narrowing of the joint space, marginal osteophytes, subchondral sclerosis, subchondral cyst formation, bony remodelling, and joint effusions. Laboratory tests for the diagnosis of OA are not available as the markers routinely used remain normal in OA. These tests are only useful in order to differentiate OA from others forms of arthritis, especially inflammatory types of arthritis. The diagnosis of OA is thus, only possible at a stage where the degradation of the joint already progressed far enough to be visible on an x-ray. Early onsets of the disease are not detectable as of yet, preventing a timely treatment that could avoid the destruction of the joint in the first place. Improved tools allowing for an identification of OA at an early stage are thus, still desired.

A causal pharmaceutical therapy leading to a decline of the degenerative symptoms is not possible, at present, because there exists no disease-modifying OA-drug (DMOAD). Accordingly, the contemporary disease management is reduced to the administration of analgesic pharmaceuticals, mainly non-steroidal anti-inflammatory drugs (NSAIDs), to relieve pain and thus, to improve quality of life.

As a consequence of the drawbacks of the state of the art there is a great need of improved diagnostic methods allowing for the identification of early stages of the disease and thus for the prevention and/or early curative treatment of OA.

Summary of the Invention

It is thus, the object of the present invention to provide improved methods for the identification of the disease as well as of substances and compositions suitable for an improved treatment. This is solved by the different aspects of present invention in providing methods and substances on the basis of miRNAs that are differentially expressed during the differentiation process of chondrocytes and cultured bone marrow stem cells (BMCSs).

The present invention is based on the surprising finding of the inventors that specific inhibition of mir-22 in chondrocytes increased chondrogenic markers whilst the overexpression of mir-22 in chondrocytes lead to an decrease of chondrogenic markers.

The invention provides in a first aspect a mir-22 for use as an indicator of a tissue status or a disease and in a second aspect, the use of mir-22 as an indicator of a tissue status or a disease.

In a third aspect, the invention provides a method of identifying an alteration of a tissue status or a risk for developing and/or identifying the presence and/or monitoring progression of a disease, preferably a joint disease, in an individual, comprising detecting the level of mir-22 in a sample.

In a fourth aspect, the present invention provides a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, preferably a joint disease, in an individual, comprising the steps of (a) determining the level of mir-22 in a sample of the individual, and (b) determining the dosage of a pharmaceutical depending on the level of mir-22 in the tested sample.

In a fifth aspect, the present invention provides a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a joint disease, comprising the steps of (a) determining the level of mir-22 in a sample, (b) determining the level of mir-22 in one or more reference samples, (c) examining the tested sample as to whether the level of mir-22 present in said sample is different from the level in the one or more reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-22 in the tested sample is different from the level in the one or more reference samples.

In a sixth aspect, the present invention provides a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a joint disease, comprising the steps of (a) determining the level of mir-22 in a tested sample, (b) determining the level of mir-22 in one or more reference samples, and (c) examining the tested sample as to whether the level of mir-22 present in said sample is different from the level in the one or more reference samples, wherein the tested sample was exposed differently to said substance than the one or more reference samples.

In a seventh aspect, the present invention provides the use of mir-22 in a method according to any of the aspects three to six.

In an eighth aspect, the present invention provides a kit for use in a method according to any of the aspects three to six., comprising one or more means of detecting mir-22.

In a ninth aspect, the present invention provides for the use of the kit of the eighth aspect in a method according to any of the aspects three to six.

In a tenth aspect, the present invention provides one or more nucleic acids for detecting a mir-22 gene, gene product or functionally active variant thereof in a method according to any of the aspects three to six.

In an eleventh aspect, the present invention provides a peptide, polypeptide or protein for detecting a mir-22 gene, gene product or functionally active variant thereof in a method according to any of the aspects three to six.

In a twelfth aspect, the present invention provides for the use of a nucleic acid according to aspect ten, or of a peptide, polypeptide or protein according to aspect eleven in a method according to any of the aspects three to six.

In a thirteenth aspect, the present invention provides a method of screening for a mir-22 antagonist, wherein the method comprises the steps of (a) providing mir-22 or the mir-22 gene, (b) providing a test compound, and (c) measuring or detecting the influence of the test compound on mir-22 or the mir-22 gene.

In a fourteenth aspect, the present invention provides a mir-22 antagonist for the alteration of a tissue status or the prevention or treatment of a joint disease.

In a fifteenth aspect, the present invention provides a pharmaceutical comprising the mir-22 antagonist according to the fourteenth aspect.

In a sixteenth aspect, the present invention provides a method of alteration of a tissue status or prevention or treatment of a joint disease, wherein a therapeutically effective amount of the pharmaceutical according to aspect fifteen is administered to an individual at risk of developing or suffering from a joint disease.

Brief Description of the Figures

Fig.1a: mir-22 expression in chondrocyte pellets during the cultivation period

Fig.1b: expression of mir-22, mir-140, mir-146a and mir-199a in chondrocyte pellets during the cultivation period

Fig.2: mir-22 expression in mesenchymal stem cell pellets during the cultivation period

Fig.3: Effects of mir-22 overexpression on the expression levels of three marker genes and 1 marker protein in three different experiments: collagen II (A); aggrecan (B); Sox9 (C); collagen X (D); CD-RAP (E),

Fig.4: Effects of mir-22 knock down on the expression levels of three marker genes and 1 marker protein in three different experiments: collagen II (A); aggrecan (B); Sox9 (C); collagen X (D); CD-RAP (E)

Fig.5: Effects of mir-22 knock down on the expression levels of three marker genes and 1 marker protein in the presence of IL-1β in three different experiments: collagen II (A); aggrecan (B); Sox9 (C); collagen X (D);

Fig.6: Relative changes in the expression of three marker genes and 1 marker protein in response to mir-22 overexpression or knock-down (A) and effect of IL-1β treatment on mir-22 knock-down (B);

Fig 7: pLenti9 mrp IRES eGFP

Detailed Description of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland). Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by

reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", is understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. "Nucleic acid" molecules are understood as a polymeric or oligomeric macromolecule made from nucleotide monomers. Nucleotide monomers are composed of a nucleobase, a five-carbon sugar (such as but not limited to ribose or 2'-deoxyribose), and one to three phosphate groups. Typically, a polynucleotide is formed through phosphodiester bonds between the individual nucleotide monomers. In the context of the present invention referred to nucleic acid molecules include but are not limited to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and mixtures thereof such as e.g. RNA-DNA hybrids. The terms "polynucleotide" and "nucleic acid" are used interchangeably herein. The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584).

Nucleic acids may be degraded by endonucleases or exonucleases, in particular by DNases and RNases which can be found in the cell. It is, therefore, advantageous to modify the nucleic acids in order to stabilize them against degradation, thereby ensuring that a high concentration of the nucleic acid is maintained in the cell over a long period of time (Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Typically, such stabilization can be obtained by introducing one or more internucleotide phosphorus groups or by introducing one or more non-phosphorus internucleotides.

Suitable modified internucleotides are compiled in Uhlmann and Peyman (1990), supra (see also Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid which can be employed in one of the

uses according to the invention contain, for example, methyl phosphonate, phosphorothioate, phosphoramidate, phosphorodithioate and/or phosphate esters, whereas non-phosphorus internucleotide analogues contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also the intention that this modification should improve the durability of a pharmaceutical composition which can be employed in one of the uses according to the invention.

Nucleic acids may be selected from the group consisting of a polynucleotide probe, a primer(s) (e.g. a primer pair), preferably a primer(s) for polymerase chain reaction (PCR), reverse transcription (RT) reaction, or DNA sequencing, a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a glycol nucleic acid (GNA), a threose nucleic acid (TNA), a microRNA (miRNA), and a small interfering RNA (siRNA).

The term "probe" as used herein refers to a single-strand oligonucleotide which is typically used for the detection of target RNA and/or DNA sequences that is complementary to the sequence of the probe. A probe hybridizes to single-stranded nucleic acid (DNA or RNA) whose nucleotide sequence allows for nucleotide pairing due to complementarity between the probe and the target sequence. The length of a probe depends on the intended use as well as the required specificity of the probe. Typically, a probe is 20-500 (i.e. 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500) nucleotides long, preferably 20-100 nucleotides, more preferably 20-50. For detection of microRNA probes are between 12 and 30 nucleotides. Probes are used in various experimental set ups such as but not limited to Southern and Northern Blots, for real-time PCR and In Situ Hybridization (ISH) as well as for microarray experiments. A probe may be unlabeled, directly labelled, or indirectly labelled, such as with biotin to which a streptavidin complex may later bind. Said label may be a molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, suitable labels include 32P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and other entities which are or can be made detectable. A label may be incorporated into nucleic acids at any position, e.g. at the 3' end, at the 5' end or internally. The term "probe" also encompasses nucleic acids differing in the composition of their backbone

such as but not limited to peptide nucleic acids (PNAs), locked nucleic acids (LNAs), glycol nucleic acids (GNAs) and threose nucleic acids (TNAs).

The term "primer" as used herein refers to a single-strand oligonucleotide which typically serves as a starting point for DNA-replicating enzymes. A primer binds to or hybridises with a DNA template and typically comprises a sequence being complementary to the DNA sequence to which it is supposed to bind. A primer may also comprise additional sequences e.g. sequences serving as nuclease cleavage sites (e.g. Bam H1, Hind III, etc.). The length of a primer is chosen depending on the intended use. For instance, primers used for the amplification of DNA in Polymerase-Chain Reactions (PCR) typically have a length of at least 10 nucleotides, preferably between 10 to 50 (i.e. 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50) nucleotides, more preferably between 15 and 30 nucleotides. Shorter primers of at least 5 nucleotides are used for sequencing of DNA templates. Also encompassed in the term "primer" are "degenerate primers" which are a mixture of similar, but not identical primers. A primer may be tagged or labelled with a marker molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means.

As used herein, the term "microRNA" and variations such as "miRNA" and "miR" include human miRNAs, mature single stranded miRNAs, precursor miRNAs (pre-miR), and variants thereof, which may be naturally occurring. In some instances, the term "miRNA" also includes primary miRNA transcripts and duplex miRNAs. Unless otherwise noted, when used herein, the name of a specific miRNA refers to the mature miRNA. For example, mir-22 refers to a mature miRNA sequence derived from pre-mir-22. Sequences for miRNAs, including human mature and precursor sequences (also called stem-loop sequences), are reported in the following database: miRBase - the microRNA Database, accessible under the following links: http://microrna.sanger.ac.uk or http://www.mirbase.org/

Publications comprising miRNA sequences are: Griffiths-Jones et al., Nucleic Acids Research, 2008, 36, Database Issue, D154-D158; Griffiths-Jones et al., Nucleic Acids Research, 2006, 34, Database Issue, D140-D144; Griffiths-Jones, Nucleic Acids Research, 2004, 32, Database Issue, D109-D111, Griffiths-Jones et al., Nucleic Acids Research, 2011, Vol. 39, Database Issue, D152–D157).

The sequence of mir-22:
Homo sapiens mir 22 stem-loop sequence hsa-mir-22 (accession number: MI0000078):
GGCUGAGCCGCAGUAGUUCUUCAGUGGCAAGCUUUAUGUCCUGACCCAGCUAA AGCUGCCAGUUGAAGAACUGUUGCCCUCUGCC (SEQ ID NO:1)

Mature sequence:
hsa-miR-22 (accession number: MIMAT0000077):
AAGCUGCCAGUUGAAGAACUGU (SEQ ID NO:2)

Minor sequence:
hsa-miR-22* (accession number: MIMAT0004495):
AGUUCUUCAGUGGCAAGCUUUA (SEQ ID NO:3)

This miRNA sequence is predicted based on homology to a verified miRNA from mouse ["Identification of novel genes coding for small expressed RNAs" Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T Science. 294:853-858(2001), "Reduced accumulation of specific microRNAs in colorectal neoplasia" Michael MZ, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ Mol Cancer Res. 1:882-891(2003)]. Its expression was later verified in human ["Kaposi's sarcoma-associated herpes virus expresses an array of viral microRNAs in latently infected cells" Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR Proc Natl Acad Sci U S A. 102:5570-5575(2005)., "A mammalian microRNA expression atlas based on small RNA library sequencing" Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M Cell. 129:1401-1414(2007).

MicroRNAs (miRNAs) are endogenous, small RNA molecules that are implied in the regulation of gene expression at the translation level. Moreover, they are part of the cell's RNA interference (RNAi) mechanism. MiRNAs are encoded by genes

transcribed from DNA but are not translated into protein (non-protein-coding RNA). They have been first discovered in *caenorhabditis elegans* and exist in both plants and animals. The sequences of many miRNAs are highly conserved among different species, suggesting that the miRNA pathway is part of a relatively old and important regulatory mechanism (Grosshans et al. J Cell Biol 156: 17-21 (2002)). MiRNAs have been found to regulate about 30% of mammalian genes (Czech, NEJM 354:1194-1195 (2006); Mack, Nature Biotech. 25:631-638 (2007); Eulalio, et al., Cell 132:9-14 (2008)). Recently, it has been found that miRNAs repress protein production by blocking translation or causing transcript degradation, thereby regulating gene expression. A single miRNA may target 250-500 different mRNAs, proving this class of RNA to be an extremely important mediator of a wide range of cellular functions. Many of the genes regulated by miRNAs are disease-causing genes. Thus, any miRNA-modulating functionality has great therapeutic potential.

In animals, miRNAs are first expressed from the genome as RNA transcripts called primary miRNAs (pri-miRNAs). They are transcribed by RNA Polymerase II, and form hairpin structures. In the nucleus, the dsRNA-specific ribonuclease Drosha processes the pri-miRNAs into shorter, about 70- to 100-nucleotide long stem-loop structures known as pre-miRNAs, which are then exported out into the cytoplasm, likely by Exportin-5 (Exp5). (Yi, et al. Genes Dev. 17: 3011-3016 (2003)). In the cytoplasm, Dicer, a member of the RNase III ribonuclease family, cleaves the pre-miRNA into a double-stranded guide/passenger (miRNA/miRNA*) duplex with 3' overhangs at both ends. The two strands of the miRNA duplex often have mismatches from imperfect complementarity in their sequences and when they separate, a mature miRNA, in many cases, between about 19 and 23 nucleotides long, is bound by the RNA-induced silencing complex (RISC) or a similar protein complex. RISC is also the protein complex that effects target-specific mRNA degradation mediated by small or short interfering RNAs (siRNAs).

At present, it is believed that once it is selected by a catalytic component of RISC, argonaute, as the guide strand, the mature miRNA is integrated into the RISC complex, and binds to a messenger RNA (mRNA) molecule that has a significantly, though often not perfectly, complementary sequence. The passenger strand, miRNA*, may be degraded. Translation of the mRNA bound by the miRNA-RISC complex is then

repressed, resulting in reduced expression of the corresponding gene. In some cases, the bound mRNA is cleaved or deadenylated and degraded. For certain miRNAs, a single precursor contains more than one mature miRNA sequence. In other instances, multiple precursor miRNAs contain the same mature sequence.

The terms "protein" and "polypeptide" are used interchangeably herein and refer to any peptide-linked chain of amino acids, regardless of length or post-translational modification. Proteins usable in the present invention (including protein derivatives, protein variants, protein fragments, protein segments, protein epitopes and protein domains) can be further modified by chemical modification. This means such a chemically modified polypeptide comprises other chemical groups than the 20 naturally occurring amino acids. Examples of such other chemical groups include without limitation glycosylated amino acids and phosphorylated amino acids. Chemical modifications of a polypeptide may provide advantageous properties as compared to the parent polypeptide, e.g. one or more of enhanced stability, increased biological half-life, or increased water solubility. Chemical modifications applicable to the variants usable in the present invention include without limitation: PEGylation, glycosylation of non-glycosylated parent polypeptides, or the modification of the glycosylation pattern present in the parent polypeptide.

As used herein, the term "variant" is to be understood as a polynucleotide or protein which differs in comparison to the polynucleotide or protein from which it is derived by one or more changes in its length or sequence. The polypeptide or polynucleotide from which a protein or nucleic acid variant is derived is also known as the parent polypeptide or polynucleotide. The term "variant" comprises "fragments" or "derivatives" of the parent molecule. Typically, "fragments" are smaller in length or size than the parent molecule, whilst "derivatives" exhibit one or more differences in their sequence in comparison to the parent molecule. Also encompassed modified molecules such as but not limited to post-translationally modified proteins (e.g. glycosylated, biotinylated, phosphorylated, ubiquitinated, palmitoylated, or proteolytically cleaved proteins) and modified nucleic acids such as methylated DNA. Also mixtures of different molecules such as but not limited to RNA-DNA hybrids, are encompassed by the term "variant". Typically, a variant is constructed artificially, preferably by gene-technological means whilst the parent polypeptide or

polynucleotide is a wild-type protein or polynucleotide. However, also naturally occurring variants are to be understood to be encompassed by the term "variant" as used herein. Further, the variants usable in the present invention may also be derived from homologs, orthologs, or paralogs of the parent molecule or from artificially constructed variant, provided that the variant exhibits at least one biological activity of the parent molecule, i.e. is functionally active.

Alternatively or additionally, a "variant" as used herein, can be characterised by a certain degree of sequence identity to the parent polypeptide or parent polynucleotide from which it is derived. More precisely, a protein variant in the context of the present invention exhibits at least 80% sequence identity to its parent polypeptide. A polynucleotide variant in the context of the present invention exhibits at least 80% sequence identity to its parent polynucleotide. The term "at least 80% sequence identity" is used throughout the specification with regard to polypeptide and polynucleotide sequence comparisons. This expression preferably refers to a sequence identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide.

The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5877), with hmmalign (HMMER package, http://hmmer.wustl.edu/) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673-80) available e.g. on http://www.ebi.ac.uk/Tools/clustalw/ or on http://www.ebi.ac.uk/Tools/clustalw2/index.html or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on http://www.ebi.ac.uk/Tools/clustalw/ or http://www.ebi.ac.uk/Tools/clustalw2/index.html. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul

et al. (1990) J. Mol. Biol. 215: 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12, to obtain polynucleotide sequences that are homologous to those nucleic acids which encode mir-22. BLAST protein searches are performed with the BLASTP program, score = 50, word length = 3, to obtain amino acid sequences homologous to mir-22. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1:I54-I62) or Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise.

"Hybridization" can also be used as a measure of sequence identity or homology between two nucleic acid sequences. A nucleic acid sequence encoding miRNAs or a portion thereof, can be used as a "hybridization probe" according to standard hybridization techniques. The hybridization of a mir-22 probe to DNA or RNA from a test source is an indication of the presence of the mir-22 DNA or RNA in the test source. Hybridization conditions are known to those skilled in the art and can be found, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y., 6.3.1-6.3.6, 1991. "Moderate hybridization conditions" are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1X SSC, 0.1% SDS at 50°C. "Highly stringent conditions" are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1 % SDS at 65°C.

The term "tissue" as used herein, refers to an ensemble of cells of the same origin which fulfil a specific function concertedly. Examples of a tissue include but are not limited to bone, cartilage, connective tissue, muscle tissue, nervous tissue, and epithelial tissue. Multiple tissues together form an "organ" to carry out a specific function. Examples of an organ include but are not limited to joint, skeleton, muscle, blood, brain, heart, liver, kidney, stomach, and skin. For example, a joint is formed of

many different tissues such as but not limited to bone, cartilage, synovium, muscle, ligament, and tendon.

The term "joint" as used herein, refers to a location where two or more bones make contact. The term may refer to a movable joint or an unmovable joint. Further, a joint may be a fibrous joint wherein the bones are attached by dense connective tissue rich in collagen or a cartilaginous joint wherein the bones are connected by cartilage. A joint may also be a synovial joint wherein the bones are not directly connected but have a synovial cavity and are attached by a capsule of articular cartilage which is associated with accessory ligaments and tendons. Synovial joints include but are not limited to ball-and-socket-joints, such as the hip or shoulder joints; ellipsoid joints and flat joints, such as the joints of the wrist; hinge joints, such as elbows, knees, ankles or the joints of fingers and toes; saddle joints such as the joint of the thumb; and pivot joint, such as the joint of atlas and axis.

The terms "tissue status", "status of a tissue", "tissue state" and "state of a tissue" are used interchangeably herein referring to the condition of a tissue. The state of a tissue may be characterised by a specific morphology of such tissue or may be characterised by the expression of one or more specific molecules such as but not limited to peptides, proteins, and nucleic acids, or combinations thereof. The status of a tissue may be regarded as "healthy" or "normal" in case it resembles the condition of such tissue when being free from illness or injury and efficiently fulfilling its specific function. The status of a tissue may be regarded as "degenerative", "diseased" or "abnormal" in case such tissue fails to fulfil its function due to an illness or injury. Additionally or alternatively, the status of a tissue may be regarded as "degenerative", "diseased" or "abnormal" in case the morphology of the tissue or its molecule expression pattern is "altered" or "changed" in comparison to normal tissue. Accordingly, the morphology of a tissue or the expression pattern of specific molecules in a tissue may be an indicator for the state of a tissue. Examples of a tissues status include but are not limited to tissue degradation such as cartilage degradation, bone degradation, and degradation of the synovium, tissue inflammation such as cartilage inflammation, or inflammation of the synovium, tissue remodelling such as bone remodelling or cartilage remodelling, sclerosis, liquid accumulation, or proliferative tissue such as proliferation in wound healing processes, cyst formations, or in cancer.

The term "disease" and "disorder" are used interchangeably herein, referring to an abnormal condition, especially an abnormal medical condition such as an illness or injury, wherein a tissue, an organ or an individual is not able to efficiently fulfil its function anymore. Typically, but not necessarily, a disease is associated with specific symptoms or signs indicating the presence of such disease. The presence of such symptoms or signs may thus, be indicative for a tissue, an organ or an individual suffering from a disease. An alteration of these symptoms or signs may be indicative for the progression of such a disease. A progression of a disease is typically characterised by an increase or decrease of such symptoms or signs which may indicate a "worsening" or "bettering" of the disease. The "worsening" of a disease is characterised by a decreasing ability of a tissue, organ or organism to fulfil its function efficiently, whereas the "bettering" of a disease is typically characterised by an increase in the ability of a tissue, an organ or an individual to fulfil its function efficiently. A tissue, an organ or an individual being at "risk of developing" a disease is in a healthy state but shows potential of a disease emerging. Typically, the risk of developing a disease is associated with early or weak signs or symptoms of such disease. In such case, the onset of the disease may still be prevented by treatment. Examples of a disease include but are not limited to traumatic diseases, inflammatory diseases, infectious diseases, cutaneous conditions, endocrine diseases, intestinal diseases, neurological disorders, joint diseases, genetic disorders, autoimmune diseases, and various types of cancer.

The term "joint disease" as used herein, refers to the abnormal condition of the joints, in particular those due to injurious, traumatic, degenerative, inflammatory, infectious or autoimmune causes. Joint diseases include but are not limited to arthritis, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), juvenile idiopathic arthritis (JIA), gout, septic arthritis, psoriatic arthritis, and osteoarthritis (OA), cancer, such as chondrosarcoma, osteosarcoma, fibrosarcoma, and multiple myeloma, tendinitis, bursitis, fractures, and damage to cartilage or bone.

"Symptoms" of a disease are implication of the disease noticeable by the tissue, organ or organism having such disease and include but are not limited to pain, weakness, tenderness, strain, stiffness, and spasm of the tissue, an organ or an individual.

"Signs" or "signals" of a disease include but are not limited to the change or alteration

such as the presence, absence, increase or elevation, decrease or decline, of specific indicators such as biomarkers or molecular markers, or the development, presence, or worsening of symptoms.

The term "indicator" as used herein, refers to a sign or signal for a condition or is used to monitor a condition. Such a "condition" refers to the biological status of a cell, tissue or organ or to the health and/or disease status of an individual. An indicator may be the presence or absence of a molecule, including but not limited to peptide, protein, and nucleic acid, or may be a change in the expression level or pattern of such molecule in a cell, or tissue, organ or individual. An indicator may be a sign for the onset, development or presence of a disease in an individual or for the further progression of such disease. An indicator may also be a sign for the risk of developing a disease in an individual. For instance, indicators relating to arthritic diseases such as OA include but are not limited to transcription factors such as Sox-5, Sox-6, Sox-9, Nfat1, pitx1, FoxO, HIF2A, SAF-1, RUNX-2, cytokines such as IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, TNF-α, NF-κB, and INF-γ, phosphatases such as Alkaline Phosphatase (ALP), proteases such as metalloproteases (e.g. MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, MMP-14), aggrecanases (e.g. ADAM-8, ADAM-12, ADAM-TS4, ADAM-TS5) and cysteinproteasen (e.g. cathepsin B, cathepsin K, cathepsin S, calpain, caspases-3, caspase-9), tissue inhibitors of metalloproteinases (e.g. TIMP-1, TIMP-2, TIMP-3, TIMP-4), extracellular matrix components, such as collagens (e.g. collagen II, VI, IX, X, XI), proteoglycans (e.g. heparan sulfate, chondroitin sulfate, keratan sulfate), aggrecan, elastin, hyaluronic acid, fibronectin, laminin, CD-RAP, CDMPs, chondromodulin and pleiotrophin. Typically, in a tissue, organ, or individual suffering from arthritis, in particular OA, one or more of these indicators are expressed or activated to a higher or lower level than in a tissue, organ, or individual not suffering from arthritis. For instance, the levels of the extracellular matrix components collagen II and X, as well as aggrecan and CD-RAP are lowered in a tissue, organ, or individual suffering or at risk of developing OA in comparison a tissue, organ, or individual not suffering or at risk of developing OA. Further the level of the transcription factor Sox9 as well as the tissue inhibitors of metalloproteinases TIMP-1 and TIMP-4 are decreased in a tissue, organ, or individual suffering or at risk of developing OA in comparison a tissue, organ, or individual not suffering or at risk of developing OA. In contrast, the levels of the

cytokine IL-1, IL-6 and TNF-α as well as of the metalloproteases MMP-1, MMP-2, MMP-3 and MMP-9, and phosphatase ALP are enhanced in a tissue, organ, or individual suffering or at risk of developing OA in comparison a tissue, organ, or individual not suffering or at risk of developing OA.

In the context of the present invention, preferred indicators of a tissue status or a disease are miRNAs, including but not limited to mir-21, mir-22, mir-140, mir-146a, and mir-199a. Mir-22 is particularly preferred as an indicator of diseases such as arthritis, in particular OA, in an individual.

As used herein, an "individual" means any mammal, reptile or bird that may benefit from the present invention. Preferably, an individual is selected from the group consisting of laboratory animals (e.g. mouse, rat or rabbit), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, duck, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas and human beings. It is particularly preferred that the "individual" is a human being.

The term "sample" or "sample of interest" are used interchangeably herein, referring to a part or piece of a tissue, organ or individual, typically being smaller than such tissue, organ or individual, intended to represent the whole of the tissue, organ or individual. Upon analysis a sample provides information about the tissue status or the health or diseased status of an organ or individual. Examples of samples include but are not limited to fluid samples such as blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, or solid samples such as tissue extracts, cartilage, bone, synovium, perichondrium, capsule, and connective tissue. Further examples of samples are cell cultures or tissue cultures such as but not limited to cultures of cartilage, bone, synovium, bone marrow cells, chondrocytes, chondroblasts, chondroclasts, synovial cells, osteocytes, osteoclasts, osteoblasts stem cells and/or mesenchymal stem cells. Analysis of a sample may be accomplished on a visual or chemical basis. Visual analysis includes but is not limited to microscopic imaging or radiographic scanning of a tissue, organ or individual allowing for morphological evaluation of a sample. Chemical analysis includes but is not limited to the detection of the presence or absence of specific indicators or alterations in their amount or level.

The term "reference sample" as used herein, refers to a sample which is analysed in a substantially identical manner as the sample of interest and whose information is compared to that of the sample of interest. A reference sample thereby provides a standard allowing for the evaluation of the information obtained from the sample of interest.

A reference sample may be derived from a healthy or normal tissue, organ or individual, thereby providing a standard of a healthy status of a tissue, organ or individual. Differences between the status of the normal reference sample and the status of the sample of interest may be indicative of the risk of disease development or the presence or further progression of such disease or disorder.

A reference sample may be derived from an abnormal or diseased tissue, organ or individual thereby providing a standard of a diseased status of a tissue, organ or individual. Differences between the status of the abnormal reference sample and the status of the sample of interest may be indicative of a lowered risk of disease development or the absence or bettering of such disease or disorder.

A reference sample may also be derived from the same tissue, organ, or individual as the sample of interest but has been taken at an earlier time point. Differences between the status of the earlier taken reference sample and the status of the sample of interest may be indicative of the progression of the disease, i.e. a bettering or worsening of the disease over time. A reference sample was taken at an earlier or later time point in case a period of time has lapsed between taking of the reference sample and taking of the sample of interest. Such period of time may represent years (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 years), months (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months), weeks (e.g. 1, 2, 3, 4, 5, 6, 7, 8 weeks), days (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 days), hours (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours), minutes (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 minutes), or seconds (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 seconds).

The terms "lowered" or "decreased" level of an indicator, e.g. mir-22, refer to the level of such indicator in the sample being reduced in comparison to the reference or reference sample. The terms "elevated" or "increased" level of an indicator, e.g. mir-22, refer to the level of such indicator in the sample being higher in comparison to the

reference or reference sample. E.g. a miRNA that is detectable in higher amounts in synovial fluid of one individual suffering from OA than in synovial fluid of individuals not suffering from OA, has an elevated level. For mir-22, an elevated level in a sample (such as synovial fluid) indicates the presence of OA or increased susceptibility or increased probability to develop OA.

A reference sample may be "treated differently" or "exposed differently" than a sample of interest in case both samples are treated in a substantially identical way except from a single factor. Such single factors include but are not limited to the time of exposure, the concentration of exposure, or the temperature of exposure to a certain substance. Accordingly, a sample of interest may be exposed to a different dosage of a certain substance than the reference sample or may be exposed for a different time interval than the reference sample or may be exposed at a different temperature than the reference sample. Different dosages to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold increased or decreased dosage of the dosage the reference sample is exposed to. Different exposure times to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold longer or shorter time period than the exposure of the reference. Different temperatures of exposure to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold increased or decreased temperature than the exposure of the reference. In a non-limiting example a sample of interest may be exposed to a 10-fold increased concentration of a substance than the reference sample. The analysis of both samples is then conducted in a substantially identical manner allowing determining the effects, i.e. a beneficial or an adverse effect, of the increased concentration of such substance on the sample of interest. The skilled person will appreciate that this example applies mutatis mutandis to different ranges of concentrations, different exposure times, and/or different temperatures at exposure.

The term "agonist" as used herein refers to a substance that causes an action in a tissue, organ or individual such as receptor-signalling, gene expression, protein synthesis, and protein degradation. Typically, agonists act by binding to the active site or to allosteric sites of a receptor molecule thereby, triggering a specific reaction.

Examples for agonists include but are not limited to nucleic acid molecules, such as mRNA or miRNA, or proteins, such as hormones, cytokines, growth factors, neurotransmitters, and transcription factors.

The term "antagonist" as used herein refers to a substance blocking the action of an agonist. Typically, antagonists act by binding to the active site or to allosteric sites of a receptor molecule, or interact with unique binding sites not normally involved in the regulation of the activity of the receptor. Typically, an antagonist competes with the agonist at structurally-defined binding sites or alters the binding site of the agonist in a manner that the agonist is not able to cause the action it would normally cause due to its binding. The antagonist activity may be reversible or irreversible depending on the longevity of the interaction of the antagonist–receptor complex. Examples for antagonists include but are not limited to nucleic acid molecules, such as siRNAs or miRNAs, or proteins such as hormones, cytokines, growth factors or neurotransmitter, antibodies, or transcription factors.

The term "mimic" as used herein refers to a substance that imitates the action of a target molecule (e.g. a target molecule such as an agonist or an antagonist in a tissue, organ or individual) such as receptor-signalling, gene expression, protein synthesis, and protein degradation. Accordingly, a mimic may be structurally different from the target molecule (e.g. agonist or antagonist) it imitates but may still effect the same specific reaction, e.g. by binding to the active site or to allosteric sites of a receptor molecule (i.e. by exerting at least one of the activities of the agonist or antagonist). Typically, a mimic fulfils the same function as the respective agonist or antagonist, i.e. is functionally equivalent. For example, a mimic of mir-22 is a substance that exerts at least one of the activities of mir-22, has the function of mir-22 and/or imitates the action of mir-22.

The term "receptor" as used herein refers to a molecule such as a protein or polynucleotide, to which one or more specific signalling molecules bind. Signalling molecules may act as agonist or antagonist including without limitation nucleic acid molecules, such as siRNAs or miRNAs, or proteins such as hormones, cytokines, growth factors or neurotransmitter, antibodies or transcription factors. Receptors may be localised at the plasma membrane of a cell, within the cytoplasm and/or in intracellular compartments.

As used herein, "treat", "treating" or "treatment" of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in an individual that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in individuals that were previously symptomatic for the disorder(s).

As used herein, "prevent", "preventing", "prevention", or "prophylaxis" of a disease or disorder means preventing that such disease or disorder occurs in patient.

The terms "pharmaceutical", "medicament" and "drug" are used interchangeably herein referring to a substance and/or a combination of substances being used for the identification, prevention or treatment of a tissue status or disease.

"Pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "active ingredient" refers to the substance in a pharmaceutical composition or formulation that is biologically active, i.e. that provides pharmaceutical value. A pharmaceutical composition may comprise one or more active ingredients which may act in conjunction with or independently of each other.

The active ingredient can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as but not limited to those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The terms "preparation" and "composition" are intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it.

The term "carrier", as used herein, refers to a pharmacologically inactive substance such as but not limited to a diluent, excipient, or vehicle with which the therapeutically active ingredient is administered. Such pharmaceutical carrier can be liquid or solid.

Liquid carrier include but are not limited to sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered intracavitary.

Solid form preparations are suitable for oral administration and include powders, tablets, pills, capsules, lozenges, cachets, suppositories, and dispersible granules. A solid excipient can be one or more substances, which may also act as diluents, flavouring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

In powders, the excipient is preferably a finely divided solid, which is in a mixture with the finely divided active ingredient. In tablets, the active ingredient of the present invention is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% to 80%, more preferably from 20% to 70% of the active compound.

Suitable pharmaceutical "excipients" include starch, glucose, lactose, sucrose, gelatine, pectin, dextrin, malt, rice, flour, chalk, silica gel, magnesium carbonate, sodium stearate, magnesium stearate, glycerol monostearate, talc, sodium chloride, tragacanth, methylcellulose, sodium carboxymethylcellulose, dried skim milk, glycerol, propylene, glycol, water, ethanol a low melting wax, cocoa butter, and the like.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Examples of suitable pharmaceutical carriers are also described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The term "adjuvant" refers to agents that augment, stimulate, activate, potentiate, or modulate the immune response to the active ingredient of the composition at either the cellular or humoral level, e.g. immunologic adjuvants stimulate the response of the immune system to the actual antigen, but have no immunological effect themselves.

Examples of such adjuvants include but are not limited to inorganic adjuvants (e.g. inorganic metal salts such as aluminium phosphate or aluminium hydroxide), organic adjuvants (e.g. saponins or squalene), oil-based adjuvants (e.g. Freund's complete adjuvant and Freund's incomplete adjuvant), cytokines (e.g. IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, and INF-γ) particulate adjuvants (e.g. immuno-stimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (e.g. monophosphoryl lipid A, or muramyl peptides), synthetic adjuvants (e.g. non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), or synthetic polynucleotides adjuvants (e.g polyarginine or polylysine).

As used herein, "administering" includes *in vivo* administration, as well as administration directly to tissue *ex vivo*, such as vein grafts. In the context of the present invention oral, topical, transdermal and/or intracavitary (e.g. intravenous) administration is preferred.

A "therapeutically effective amount" is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

Embodiments of the Invention

In a first aspect the present invention provides mir-22 for use as an indicator of a tissue status or a disease. The surprising finding of the inventors that the presence or absence of mir-22 effects the level of known disease indicators (such as transcription factors, extracellular matrix components, and proteases) evidences that the level of mir-22 correlates to the presence, absence or progression of a tissue status or a disease. Thus, mir-22 is indicative of a tissue status or a disease. The presence, absence or altered amounts of mir-22 are a sign for the presence, absence or progression of a tissue status or disease. In the context of the present invention, an altered tissue status is preferably characterised by the tissue exhibiting different mir-22 level than normal tissue or the same tissue at an earlier time point. A disease status is preferably characterised by an organ or individual exhibiting different mir-22 level in

comparison to a normal organ or individual or in comparison to the same organ or individual at an earlier time point.

In a second aspect the present invention relates to the use of mir-22 as an indicator of a tissue status or a disease. Due to the different expression levels of mir-22 during disease development and/or progression, mir-22 is used as an indicator of a tissue status or a disease.

In further embodiments of the first and/or second aspect of the present invention the level of mir-22 is indicative of (a) a tissue status or a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) the suffering of an individual from an altered tissue status or a disease and/or (d) the progression or a stage of a tissue status or a disease, in an individual. The level of mir-22 is the expression level of mir-22 or the amount of mir-22 present. Accordingly, the expression level of mir-22 and/or the amount of mir-22 present are indicative of (a) a tissue status or a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) the suffering of an individual from an altered tissue status or a disease and/or (d) the progression or a stage of a tissue status or a disease, in an individual. In more preferred embodiments the level of mir-22 is the level of mir-22 in an individual or in a sample of an individual.

In further embodiments an alteration in the mir-22 level indicates a change in tissue status or disease such as the worsening or bettering of a tissue status or disease. Preferably, an elevated level of mir-22 is indicative of a worsening of a tissue status or disease. A lowered level of mir-22 is indicative of a bettering of a tissue status or disease. An alteration in the mir-22 level is also indicative of the risk of developing an altered tissue status or a disease, more preferably an elevated level of mir-22 is indicative of the risk of developing a degenerative tissue status or disease. An altered mir-22 level, preferably an elevated mir-22 level, is also indicative of an individual suffering from an altered tissue status or a disease. Furthermore, an altered mir-22 level, e.g. an elevated or lowered level of mir-22, indicates the progression or a stage of a tissue status or a disease, in an individual. Preferably an elevated mir-22 level is indicative of a worsening of a tissue status or disease.

It is particularly preferred that the level of mir-22 is indicative of a tissue status wherein the tissue is selected from the group consisting of bone, cartilage, connective tissue,

muscle tissue, nervous tissue, and epithelial tissue. Preferably the tissue status is tissue degradation such as but not limited to cartilage degradation, bone degradation, and degradation of the synovium, tissue inflammation such as cartilage inflammation, or inflammation of the synovium, tissue remodelling such as bone remodelling or cartilage remodelling, sclerosis, liquid accumulation, or proliferative tissue such as proliferation in wound healing processes, cyst formations, or in cancer. It is particularly preferred that the tissue status relates to tissue degradation or tissue inflammation, more preferably to cartilage degradation, degradation of the synovium, cartilage inflammation and/or inflammation of the synovium.

It is further preferred that the level of mir-22 is indicative of a disease wherein the disease is a joint disease such as but not limited to arthritis, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), juvenile idiopathic arthritis (JIA), gout, septic arthritis, psoriatic arthritis, and osteoarthritis (OA), cancer, such as chondrosarcoma, osteosarcoma, fibrosarcoma, and multiple myeloma, tendinitis, bursitis, fractures, and/or damage to cartilage or bone. In particularly preferred embodiments the disease is arthritis, more preferably osteoarthritis.

In a third aspect the present invention provides for a method of identifying (a) an alteration of a tissue status or the presence of a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-22. Preferably, mir-22 is detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to mir-22 RNA or cDNA.

Preferably, the level of mir-22 is the level of mir-22 in an individual or in a sample of an individual. Preferably, the level of mir-22 is the expression level of mir-22 or the amount of mir-22 present in an individual or in a sample of an individual.

It is further preferred that the individual is a mammal, reptile or bird. More preferably, the individual is selected from the group consisting of laboratory animals (e.g. mouse, rat or rabbit), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, duck, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including macacae, chimpanzees, bonobos, gorillas and human beings. It is particularly preferred that the individual is a human being.

The sample of an individual is one or more solid samples such as but not limited to tissue extracts, cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue, or one or more fluid samples such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid. In more preferred embodiments the sample is selected from the group consisting of cartilage, synovium, capsule, blood, plasma, serum, urine, and synovial fluid. In particularly preferred embodiments the sample is cartilage, synovium, serum, and/or synovial fluid.

The level of mir-22 is detected via any known method using known means for detection of nucleic acids such as specific nucleic acids or polypeptides able to binding the miRNA in question. Suitable methods include but are not limited to Polaymerase-Chain-Reaction (PCR) techniques such as reverse transcriptase PCR or quantitative real time PCR, hybridisation techniques such as Northern Blot, in situ hybridization, or Chip hybridisation, immunohistological or immunohistochemical or immunocytochemical techniques, such as Western Blots, Immunofluorescence or ELISA, Mass Spectrometry (MS), such as LC/MS, High-Performance Liquid Chromatography (HPLC), in particular Ion Exchange HPLC after labelling with a complementary strand.

The means of detecting the level of mir-22 present in an individual or in a sample of an individual include but are not limited to those typically used in above mentioned methods. These include but are not limited to one or more sets of primers capable of specifically detecting mir-22 RNA or cDNA, (e.g. for use in a quantitative RT PCR), one or more nucleic acid probes able to specifically hybridise to mir-22 RNA or cDNA under standard conditions (e.g. for use in Northern Blot or Chip hybridisation techniques), one or more antibodies able to specifically detect mir-22 (e.g. for use in immunohistological or immunohistochemical or immunochemical techniques, e.g. detection of mir-22 in histological tissue sections or mir-22 immobilized on suitable carriers like membranes, chips, ELISA plates etc.).

In further preferred embodiments of the present invention the level of mir-22 in said individual or sample of an individual is compared to the level of mir-22 in one or more references and/or reference samples. Preferably, the reference is selected from the group consisting of a healthy individual, a diseased individual or the same individual as the tested individual at an earlier or later time point. Additionally or alternatively, the

reference is a value representative for the level of mir-22 in absence of an altered tissue status or disease, in presence of an altered tissue status or disease or for an increased or decreased risk of developing an altered tissue status or disease.

The reference sample is preferably derived from a healthy individual, a diseased individual, or from the same individual as the sample of interest. Where the reference sample was taken from the same individual as the sample of interest, the reference sample was preferably taken at an earlier or later time point then the sample of interest. The time period which has lapsed between taking of the reference sample and taking of the sample of interest preferably represents years (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 years), months (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months), weeks (e.g. 1, 2, 3, 4, 5, 6, 7, 8 weeks), days (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 days), hours (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours), minutes (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 minutes), or seconds (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 seconds). Alternatively or additionally, the reference sample is a reference sample with a level of mir-22 representative for a healthy individual or representative for the presence or absence of an altered tissue status or disease or representative for an increased or decreased risk of developing an altered tissue status or disease.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, an elevated level of mir-22 in the sample of interest in comparison to said reference indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual. In embodiments, wherein the reference is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, a similar level of mir-22 indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, a similar level of mir-22 in the sample of interest in comparison to said reference indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, an lowered level of mir-22 in the sample of interest in comparison to said reference indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, a similar level of mir-22-a indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, an elevated level of mir-22 indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status

or a disease or a value representative of the presence of an altered tissue status or disease, a lowered level of mir-22 indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, an elevated level of mir-22-a in the sample of interest indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a lowered level of mir-22-a in the sample of interest indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a similar level of mir-22-a in the sample of interest indicates (a) a similar risk to develop a deteriorated tissue status or a disease and/or (b) a stagnation in the progression of a deteriorated tissue status or a disease, and/or (c) a persistence of the deteriorated tissue status or the disease in the individual.

In a fourth aspect the present invention provides a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of (a)determining the level of mir-22 in a sample of the individual, and optionally determining the level of mir-22 in a reference or reference sample for comparison with the level of mir-22 in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the level of mir-22 in the sample of interest, optionally depending on the comparison of the levels of mir-22 in the sample of interest and the reference or reference sample.

In the context of the present invention, levels of mir-22 are preferably detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to the mir-22 gene, gene product(s) and/or functionally active variant(s) thereof.

Samples can be fluid or solid, preferably fluid sample are body fluid sample such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, and solid sample are a tissue sample, such as but not limited to cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue. The reference sample is preferably derived from a healthy individual, a diseased individual, or from the same individual as the sample of interest. Where the reference sample was taken from the same individual as the sample of interest, the reference sample was preferably taken at an earlier or later time point then the sample of interest. Alternatively or additionally, the reference sample is a reference sample with a level of mir-22 representative for a healthy individual or representative for the presence or absence of an altered tissue status or disease or representative for an increased or decreased risk of developing an altered tissue status or disease.

In preferred embodiments the necessity for maintenance or alteration, i.e. an increase or decrease, of the dosage of the pharmaceutical is determined upon determination of the mir-22 levels in the sample, preferably in comparison to a reference or reference sample.

It is envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and a lowered level of mir-22 in the

sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or

of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.
In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.
In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.
In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased or of a decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In a fifth aspect the present invention provides a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of (a) determining the level of mir-22 in a sample, (b) determining the level of mir-22 in one or more references or reference samples, (c) examining the tested sample as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-22 in the sample of interest is different from the level in the one or more references or reference samples.
Levels of mir-22 are preferably detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able

to specifically bind and/or hybridise to the mir-22 gene, gene product(s) and/or functionally active variant(s) thereof. Samples can be fluid or solid, preferably fluid sample are body fluid sample such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, and solid sample are a tissue sample, such as but not limited to cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue.

In preferred embodiments the dosage of the pharmaceutical is maintained or altered, i.e. increase or decrease, upon determination of the mir-22 levels in the sample in comparison to the one or more reference or reference sample.

It is envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or decreased.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained, decreased or minimized.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status

or a disease or a value representative of the presence of an altered tissue status or disease, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or decreased.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or decreased.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained, increased or decreased.

In a sixth aspect the present invention provides a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of (a) determining the level of mir-22 in a sample of interest, (b) determining the level of mir-22 in one or more references or reference samples, and (c) examining the sample of interest as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples. Preferably, levels of mir-22 are detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to the mir-22 gene, gene product(s) and/or functionally active variant(s) thereof. Samples can be fluid or solid, preferably fluid sample are body fluid sample such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, and solid sample are a tissue sample, such as but not limited to cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue.

In preferred embodiments the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease is determined upon determination of the mir-22 levels in the sample, preferably in comparison to a reference or reference sample.

It is envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined,

an adverse effect of said substance on a tissue status or the development of a disease is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, a beneficial effect of said substance on a tissue status or the development of a disease is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-22 representative of the absence of an altered tissue status or disease, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, it is determined that said substance has no effect or only very limited effect on a tissue status or the development of a disease.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, it is determined that said substance has no effect or only very limited effect on a tissue status or the development of a disease.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, a beneficial effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and an elevated level of mir-22 in the sample of interest in comparison to said

reference or reference sample is determined, an adverse effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, an elevated level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, an adverse effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a lowered level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, a beneficial effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a similar level of mir-22 in the sample of interest in comparison to said reference or reference sample is determined, it is determined that said substance has no effect or only very limited effect on a tissue status or the development of a disease.

In a seventh aspect the present invention provides the use of mir-22 in a method according to any of the previous aspects of the present invention.

Accordingly, the use of mir-22 is provided in a method of identifying (a) an alteration of a tissue status or the presence of a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-22, as described in detail above. The use of mir-22 is also provided in a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of (a) determining the level of mir-22 in a sample of the individual, and optionally determining the level of mir-22 in a reference or reference sample for comparison with the level of mir-22 in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the level of mir-22 in the sample of interest, optionally depending on the comparison of the levels of mir-22 in the sample of interest and the reference or

reference sample, as described in detail above. Furthermore, the use of mir-22 is envisaged in a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of (a) determining the level of mir-22 in a sample, (b) determining the level of mir-22 in one or more references or reference samples, (c) examining the tested sample as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-22 in the sample of interest is different from the level in the one or more references or reference samples, as described in detail above. Also the use of mir-22 in a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of (a) determining the level of mir-22 in a sample of interest, (b) determining the level of mir-22 in one or more references or reference samples, and (c) examining the sample of interest as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples, as described in detail above, is provided for. The use of mir-22 in either of the above indicated methods comprises the use of the mir-22 gene, mir-22 gene product(s) and/or functionally active variant(s) thereof.

In preferred embodiments of any of the aspects of the present invention, the sample of interest is a solid and/or fluid sample. The solid sample is preferably a tissue sample and the fluid sample a body fluid sample. It is further preferred that the tissue sample is selected from the group consisting of cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue, and the fluid sample is selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid. In more preferred embodiments the sample is selected from the group consisting of cartilage, synovium, capsule, blood, plasma, serum, urine, and synovial fluid. In particularly preferred embodiments the sample cartilage, synovium, serum, and/or synovial fluid. Alternatively or additionally, the sample is a cell culture or tissue culture sample, preferably selected from the group consisting of cartilage, bone, synovium,

bone marrow cells, chondrocytes, chondroblasts, chondroclasts, synovial cells, osteocytes, osteoclasts, osteoblasts, stem cells and/or mesenchymal stem cells.

In an eighth aspect the present invention provides a kit for use in any of the aspects of the present invention, comprising one or more means of detecting mir-22. In preferred embodiments of the kit, the one or more means for detecting mir-22 are means for determining the expression levels of mir-22, more preferably on a gene and/or RNA level. It is particularly preferred that the one or more means for detecting mir-22 are selected from the group consisting of nucleic acid, preferably DNA or RNA, peptide, and protein, preferably monoclonal or polyclonal antibodies. Preferably, means for detecting mir-22 are either naturally occurring nucleic acid such as DNA or RNA or chemically modified nucleic acids with complementarity to the sequence of mir-22 and/or its precursor.

In further preferred embodiments the kit further comprises (a) a container, and/or (b) a data carrier. It is particularly preferred that the data carrier comprises information such as but not limited to (i) instructions concerning methods for identifying the risk for developing and/or identifying the presence and/or monitoring progression of a joint disease, (ii) instructions for use of the means for detecting mir-22, preferably in a sample, more preferably in a sample from an individual and/or of the kit, (iii) quality information such as information about the lot/batch number of the means for detecting mir-22 and/or of the kit, the manufacturing or assembly site or the expiry or sell-by date, information concerning the correct storage or handling of the kit, (iv) information concerning the composition of the buffer(s), diluent(s), reagent(s) for detecting mir-22 and/or of the means for detecting mir-22, (v) information concerning the interpretation of information obtained when performing the above-mentioned methods identifying and/or monitoring progression of a joint disease, (vi) a warning concerning possible misinterpretations or wrong results when applying unsuitable methods and/or unsuitable means, and/or (vii) a warning concerning possible misinterpretations or wrong results when using unsuitable reagent(s) and/or buffer(s).

In a ninth aspect the present invention provides the use of the kit of the eighth aspect in a method of any of the methods of the present invention is provided for.

Accordingly, the use of said kit is provided in a method of identifying (a) an alteration of a tissue status or the presence of a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-22, as described in detail above. The use of said kit is also provided in a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of (a) determining the level of mir-22 in a sample of the individual, and optionally determining the level of mir-22 in a reference or reference sample for comparison with the level of mir-22 in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the level of mir-22 in the sample of interest, optionally depending on the comparison of the levels of mir-22 in the sample of interest and the reference or reference sample as described in detail above. Furthermore, the use of said kit is envisaged in a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of (a) determining the level of mir-22 in a sample, (b) determining the level of mir-22 in one or more references or reference samples, (c) examining the tested sample as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-22 in the sample of interest is different from the level in the one or more references or reference samples as described in detail above. Also the use of said kit in a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of (a) determining the level of mir-22 in a sample of interest, (b) determining the level of mir-22 in one or more references or reference samples, and (c) examining the sample of interest as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples, as described in detail above, is provided for.

In a tenth aspect the present invention provides for one or more nucleic acids for detecting a mir-22 gene, gene product(s) or functionally active variant(s) thereof in a method of any of the aspects of the present invention as described in detail above. In preferred embodiments the nucleic acid is a nucleic acid either occurring naturally such as DNA or RNA, or chemically modified. Preferably, the nucleic acid is selected from the group consisting of a nucleic acid probe, a polyamide or peptide nucleic acid (PNA), microRNA (miRNA), small interfering RNA (siRNA), locked nucleic acid (LNA), primers for polymerase chain reaction (PCR), primers for reverse transcription (RT) reaction, and primers for DNA sequencing. It is particularly preferred that such nucleic acid exhibits a strong binding to mir-22. Thus, exemplary LNAs of about 8 nucleotides are suitable for detecting mir-22, as long as the sequence is unique within the entirety of microRNA sequences.

In an eleventh aspect the present invention provides a peptide, polypeptide or protein for detecting a mir-22 gene, gene product(s), functionally active variant(s) thereof and/or a label on a microRNA in a method of any of the aspects of the present invention as described in detail above. In preferred embodiments the peptide, polypeptide or protein is a protein ligand, preferably an antibody, a fragment or derivate thereof, a darpin or an anticalin, or the polypeptide or peptide is a probe, preferably a mass spectrometry probe.

In preferred embodiments the protein ligand is an antibody that has been prepared recombinantly and, where appropriate, modified, such as chimaeric antibodies, humanized antibodies, multifunctional antibodies, bispecific or oligospecific antibodies, single-stranded antibodies and F(ab) or $F(ab)_2$ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213 or WO 98/24884). Additionally or alternatively to the classical antibodies protein scaffolds against mir-22 are used as protein ligands, e.g. anticalins which are based on lipocalin (Beste et al. (1999) Proc. Natl. Acad. Sci. USA, 96, 1898-1903). The natural ligand-binding sites of the lipocalins, for example the retinol-binding protein or the bilin-binding protein, can be altered, for example by means of a “combinatorial protein design” approach, in such a way that they bind to selected haptens, here to mir-22 (Skerra, 2000, Biochim. Biophys. Acta, 1982, 337-50). Other known protein scaffolds are known as being

alternatives to antibodies for molecular recognition (Skerra (2000) J. Mol. Recognit., 13, 167-187).

In a twelfth aspect the present invention provides for the use of the nucleic acid of the tenth aspect of the present invention and/or of a peptide, polypeptide or protein of the eleventh aspect of the present invention in a method of any of the aspects of the present invention. In preferred embodiments the nucleic acids is used in naked form, in the form of gene transfer vectors or complexed with liposomes or gold particles. Examples of gene transfer vectors are viral vectors, for example adenoviral vectors or retroviral vectors (Lindemann et al. (1997), Mol. Med., 3, 466-76; Springer et al. (1988) Mol. Cell., 2, 549-58). Complexes with liposomes usually achieve a very high efficiency of transfection, in particular of skin cells (Alexander and Akhurst, 1995, Hum. Mol. Genet. 4:146-a79-85). In lipofection, small, unilamellar vesicles composed of cationic lipids are prepared by ultrasonicating the liposome suspension. The DNA is bound ionically on the surface of the liposomes in a ratio which is such that a positive net charge remains and all the plasmid DNA is complexed by the liposomes. In addition to the DOTMA (1,2-dioleyloxypropyl-3-trimethylammonium bromide) and DOPE (dioleoylphosphatidylethanolamine) lipid mixtures employed by Felgner, P. L. et al. (1987), Proc. Natl. Acad. Sci USA, 84, 7413 - 7414, a large number of lipid formulations have by now been synthesized and tested for their efficiency in transfecting a variety of cell lines (Behr et al. (1989) Proc. Natl. Acad. Sci. USA, 86, 6982-6986; Zhao and Huang (1991), Biochim. Biophys. Acta, 1189, 195-203; Felgner et al. (1994) J. Biol. Chem., 269, 2550-2561). Examples of the lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulphate or DOGS (dioctadecylamidoglycylspermine). Auxiliary substances which increase the transfer of nucleic acids into the cell can, for example, be proteins or peptides which are bound to the DNA or synthetic peptide-DNA molecules which enable the nucleic acid to be transported into the nucleus of the cell (Schwartz et al. (1999) Gene Therapy 6:282; Brandén et al. (1999) Nature Biotech., 17, 784). Auxiliary substances also include molecules which enable nucleic acids to be released into the cytoplasm of the cell (Planck et al. (1994) J. Biol. Chem., 269, 12918; Kichler et al. (1997) Bioconj. Chem, 8, 213) or, for example liposomes (Uhlmann and Peyman (1990), supra). Another, particularly suitable form can be obtained by applying the above-described nucleic

acids to gold particles and firing these particles into tissue or cells using what is termed a “gene gun” (Wang et al. (1999) J. Invest. Dermatol. 112:775-81, Tuting et al. (1998) J. Invest. Dermatol. 111:183-8).

In a thirteenth aspect the present invention provides for the use of mir-22 or the mir-22 gene or a functionally active variant thereof as a target molecule for the discovery of a mir-22 antagonist or mimic thereof. In preferred embodiments mir-22 or the mir-22 gene or a functionally active variant thereof are brought into direct or indirect contact with a test compound and the influence of the test compound on mir-22 or the mir-22 gene is measured or detected. Preferably the test compound is a chemical molecule such as but not limited to non-polymeric organic compounds, lipids, carbohydrates, peptides, preferably peptides with about 10 to about 80 amino acids, in particular with 10 to 25 amino acids and oligonucleotides, preferably with about 10 to about 90 nucleotides, in particular with 15 to 25 nucleotides. Especially preferred are small chemical molecules, in particular non-polymeric organic compounds, either synthesized in a laboratory or found in nature, with a preferred molecular weight of about 200 g/mole to about 1500 g/mole, in particular 400 g/mole to 1000 g/mole. Preferably, the test compound alters the level of mir-22 such as increases or decreases the expression level or the amount of mir-22 present. In the context of the present invention test compounds decreasing the level of mir-22 are particularly preferred.

A method of screening for a mir-22 antagonist or mimic thereof is provided for in a fourteenth aspect of the present invention, comprising the steps of (a) providing mir-22 or the mir-22 gene, (b) providing a test compound, and (c) measuring or detecting the influence of the test compound on mir-22 or the mir-22 gene.

A mir-22 antagonist or mimic thereof for the alteration of a tissue status or the prevention or treatment of a joint disease is provided for in a fifteenth aspect.

In the context of present invention, an antagonist for mir-22 or mimic thereof can be any molecule or composition that lowers or inhibits the function or lowers the expression level or amount of mir-22 present. Examples comprise, but are not limited to: 1. A nucleic acid, such as an oligonucleotide, oligoribonucleotides, especially dsRNA, ssRNA, siRNA, miRNA, shRNA etc, as known in the art, 2. An antagonistic peptide or protein, such as a protein destabilising or inhibiting the function of the

miRNA in question, an antagonistic antibody or fragment thereof or a transcriptional antagonist of the miRNA in question, and/or 3. a small molecule compound with antagonistic action. Alternatively or additionally, the antagonist of the present invention can be in the form of a natural product extract, either in crude or in purified form. The extract can be produced according to standard procedures, such as water and/or alcohol and/or organic solvent extraction and/or column chromatography and/or precipitation from an animal, plant or microbial source, like snake poison, leaves or microbial fermentation broths.

The use of such antagonist or mimic thereof for the treatment of a joint disease, in particular a degenerative joint disease such as osteoarthritis, is a further aspect of the present invention. It is preferred that the mir-22 antagonist or mimic thereof is used to treat joint pain, in particular by reducing the joint pain in degenerative joint diseases. In addition, the mir-22 antagonist is used for the production of a medicament for the treatment of a joint disease and/or for the treatment of the joint pain as specified above.

A pharmaceutical comprising a mir-22 antagonist or mimic thereof for use in the prevention or treatment of a joint disease, is provided for in a sixteenth aspect. In preferred embodiments the pharmaceutical further comprises a pharmaceutically acceptable carrier and/or excipient and optionally one or more additional active substances. The additional active substances may be effective against the same or againt a different disease or disorder or condition. Accordingly, additional active substances comprise those having a beneficial effect in the treatment of a joint disease and/or for the treatment of the joint pain as specified above. Furthermore, additional active substances directed at the treatment of different diseases or disorder or conditions suitable to be administered in combination with the active ingredient of the present invention are comprised. Exemplified, additional active substances affecting further miRNAs and/or their gene product(s), such as agonist or antagonists to individual miRNAs, are encompassed. Additional active substances also comprise food supplements such as but not limited to vitamins, minerals, fiber, fatty acids, or amino acids, considered beneficial in a persons diet and/or in the treatment of a joint disease and/or for the treatment of the joint pain as specified above and/or in any other disease, disorder or condition.

For the production of the pharmaceutical the mir-22 antagonists or mimic thereof of the present invention are usually formulated with one or more pharmaceutically acceptable additives or auxiliary substances, such as physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease antagonists, preferably aprotinin, ε-aminocaproic acid or pepstatin A or sequestering agents such as EDTA, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc. depending on the kind of administration. Suitable further additives are, for example, detergents, such as, for example, Triton mir-22-100 or sodium desoxycholate, but also polyols, such as, for example, polyethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic compounds, such as, for example, amino acids such as glycine or in particular taurine or betaine and/or a protein, such as, for example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

The physiological buffer solution preferably has a pH of approx. 6.0-8.0, expecially a pH of approx. 6.8-7.8, in particular a pH of approx. 7.4, and/or an osmolarity of approx. 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pH of the medicament is in general adjusted using a suitable organic or inorganic buffer, such as, for example, preferably using a phosphate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4-(2-hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3-morpholino-1-propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

For the production of the pharmaceutical the mir-22 antagonist or mimic thereof of the present invention may also be formulated with a nanoparticle, a liposome or other lipid complexation, or may be conjugated to a protein, peptide, nucleic acid (e.g. an aptamer) or gel, e.g. based on hyaluronic acid, or similar slow release formulations. The pharmaceutical is preferably in unit dosage form. In such form the preparation may be subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packaged tablets, capsules, and

powders in vials or ampoules. Also, the unit dosage form can be a capsule, an injection vial, a tablet, a cachet, or a lozenge itself, or it can be the appropriate number of any of these in packaged form.

In a seventeenth aspect the present invention provides for a method of alteration of a tissue status or prevention or treatment of a joint disease, wherein a therapeutically effective amount of the pharmaceutical of the sixteenth aspect is administered to an individual at risk of developing or suffering from a joint disease. In preferred embodiments the pharmaceutical is administered orally, topically, transdermal or intracavitary. Intracavitary administration includes but is not limited to intravenous and intra-articular administration.

The pharmaceutical can be administered in a conventional manner, e.g. by means of oral dosage forms, such as, for example, tablets or capsules, by means of the mucous membranes, for example the nose or the oral cavity, in the form of dispositories implanted under the skin, by means of injections, infusions or gels which contain the medicaments according to the invention. It is further possible to administer the medicament topically and locally in order to treat the particular joint disease as described above, if appropriate, in the form of liposome complexes. Furthermore, the treatment can be carried out by means of a transdermal therapeutic system (TTS), which makes possible a temporally controlled release of the medicaments. TTS are known for example, from EP 0 944 398 A1, EP 0 916 336 A1, EP 0 889 723 A1 or EP 0 852 493 A1. Injection solutions are in general used if only relatively small amounts of a solution or suspension, for example about 1 to about 20 ml, are to be administered to the body. Intracavitary administration includes but is not limited to infusions and injections. Infusion solutions are in general used if a larger amount of a solution or suspension, for example one or more litres, are to be administered. Since, in contrast to the infusion solution, only a few millilitres are administered in the case of injection solutions, small differences from the pH and from the osmotic pressure of the blood or the tissue fluid in the injection do not make themselves noticeable or only make themselves noticeable to an insignificant extent with respect to pain sensation. Dilution of the formulation according to the invention before use is therefore in general not necessary. In the case of the administration of relatively large amounts, however, the formulation according to the invention should be diluted briefly before administration to

such an extent that an at least approximately isotonic solution is obtained. An example of an isotonic solution is a 0.9% strength sodium chloride solution. In the case of infusion, the dilution can be carried out, for example, using sterile water while the administration can be carried out, for example, via a so-called bypass

In preferred embodiments of any aspect of the present invention is the tissue status is tissue damage, preferably selected from the group consisting of damage to bone, cartilage, synovium, perichondrium, capsule, and connective tissue and/or is characterised by altered level of regulatory molecules, signalling molecules, and/or degenerative molecules present in the tissue.

In further preferred embodiments of any aspect of the present invention is the disease is a joint disease, preferably selected from the group consisting of osteoarthritis, rheumatoid arthritis, psoriatic arthritis, septic arthritis, Ankylosing spondylitis, Bursitis (inflammation), Dermatomyositis, Fibromyalgia, Gouty arthritis, Juvenile chronic arthritis (Still's disease), Mixed Connective Tissue Disease, Polymyalgia rheumatica, Polymyositis, Reactive arthritis (Reiter's Syndrome), Scleroderma, Shoulder tendinitis, Sjorgen's Sydrome, Systemic Lupus Erythemetosis and/or is characterised by a physical or metabolic injury of the joint indicated by an inflammation of the joint or a degradation of the joint, more preferably wherein the disease is osteoarthritis.

Further, non-limiting aspects and embodiments of the present invention are described below.

The diagnostic methods involve measuring the levels of one or more miRNAs in patient samples and using the test results to diagnose and/or predict an optimal treatment regimen for the patient. Compositions described in the invention include antagonists of mir-22., that function as miRNA antagonists that can be introduced to a patient to treat and reduce one or more of the conditions associated with joint diseases, especially with osteoarthritis.

The diagnostic methods involve measuring the levels of one or more miRNAs in patient samples and using the test results to diagnose and/or predict an optimal treatment regimen for the patient. Compositions described in the invention include agonists of mir-22 / antagonists of mir-22, that function as miRNAs / miRNA

agonist/antagonists that can be introduced to a patient to treat and reduce one or more of the conditions associated with inflammatory diseases, especially with osteoarthritis.

In the context of one or more of the present aspects of the invention (e.g. such as using the miRNA as biomarker), the detection of the miRNA can be performed by any known method using known means for detection of nucleic acids such as specific nucleic acids able to binding the miRNA in question. One aspect of present invention concerns the use of a means for detecting the miRNA in question amount in a biological sample for the manufacture of a diagnostic means for diagnosing a malfunction of the carbohydrate or lipid metabolism.

The means to detect the amount (i.e. the steady state level) of the miRNA in question present in a biological sample can be any means to detect the miRNA in question. This can be for example a set of primers capable of specifically detecting the miRNA in question or the cDNA, e.g. for use in a quantitative RT PCR. Another means can e.g. be a nucleic acid probe able to specifically hybridise to miRNA in question mRNA or cDNA under standard conditions, e.g. for use in Northern Blot or Chip hybridisation techniques. Another means can e.g. be an antibody able to specifically detect the miRNA in question e.g. for use in immunohistological or immunohistochemical or immunochemical techniques (e.g. detection of miRNA in question in histological tissue sections or miRNA in question immobilized on suitable carriers like membranes, chips, ELISA plates etc.).

According to one embodiment, the invention concerns a method for identifying a joint disease such as OA or an increased risk for developing a joint disease, such as OA in an individual, which comprises examining a sample taken from the individual, as to whether the level of mir-22 and/or the activity of mir-22 present in said sample is different from that of one or more reference samples. The presence of an elevated level indicating an increased risk of said individual to suffer from or to develop a joint disease such as OA.

The terms "lowered level" or "elevated level" refer to the amount of the miRNA in a sample of tissue or tissue fluid known to have, under certain circumstances, detectable levels If the miRNA in question (such as cellular or tissue extract, synovial fluid, serum or plasma), especially from an individual to be tested, compared to the amount of the miRNA in comparable sample from a cohort or cohorts that do not have the joint

disease, especially OA. E.g. a miRNA that is detectable in higher amounts in synovial fluid of one individual suffering from OA than in synovial fluid of individuals not suffering from OA, has an elevated level. For mir-22, an elevated level in a patient sample (such as synovial fluid) indicates the presence of OA or increased susceptibility or increased probability to develop OA.

In some cases, the level of the miRNA marker will be compared to a control to determine whether the level is reduced or elevated. The control may be an external control, such as the miRNA in a sample from a patient known to be free of the joint disease especially OA. In other circumstances, the external control may be the miRNA in a sample from a tissue/tissue fluid known not to have detectable amounts (or always the same amount, irrespective of the disease-state of the individual) of the miRNA in question or a known amount of a synthetic RNA. An internal control may be a miRNA from the tissue/tissue fluid sample being tested. The identity of a miRNA control may be the same as or different from the patient serum or plasma miRNA being measured.

In the context of present invention, an antagonist for a miRNA can be any molecule or composition that lowers or inhibits the function or amount of the miRNA in question. Examples comprise, but are not limited to:

1. A nucleic acid, such as an oligonucleotide, oligoribonucleotides, especially dsRNA, ssRNA, siRNA, miRNA, shRNA, ssDNA, ssDNA/RNA hybrids or chemically modified nucleic acids etc., as known in the art.

2. An antagonistic peptide or protein, such as a protein destabilising or inhibiting the function of the miRNA in question, an antagonistic antibody or fragment thereof or a transcriptional antagonist of the miRNA in question,

3. A small molecule compound with antagonistic action,

4. A composition comprising one or more of 1-4 and possibly an excipient.

One subject matter of the present invention is, therefore, the use of a antagonist for the treatment of a joint disease, in particular a degenerative joint disease such as osteoarthritis. The miRNA antagonist can also be used to treat the joint pain, in particular by reducing the joint pain in degenerative joint diseases. In addition, the miRNA antagonist can be used for the production of a medicament for the treatment of a joint disease and/or for the treatment of the joint pain as specified above.

According to the present invention the term “chemical molecule” encompasses non-polymeric organic compounds, lipids, carbohydrates, peptides, preferably peptides with about 10 to about 80 amino acids, in particular with 10 to 25 amino acids and oligonucleotides, preferably with about 10 to about 90 nucleotides, in particular with 15 to 25 nucleotides. Especially preferred are small chemical molecules, in particular non-polymeric organic compounds, either synthesized in a laboratory or found in nature, with a preferred molecular weight of about 200 g/mole to about 1500 g/mole, in particular 400 g/mole to 1000 g/mole.

Alternatively the antagonist of the present invention can be in the form of a natural product extract, either in crude or in purified form. The extract can be produced according to standard procedures, such as water and/or alcohol and/or organic solvent extraction and/or column chromatography and/or precipitation from an animal, plant or microbial source, like snake poison, leaves or microbial fermentation broths.

The term “binding protein” or “binding peptide” refers to a class of proteins or peptides which bind and inhibit mir-22 including, without limitation, polyclonal or monoclonal antibodies, antibody fragments and protein scaffolds directed against mir-22, e.g. anticalins which are directed against mir-22.

The procedure for preparing an antibody or antibody fragment is effected in accordance with methods which are well known to the skilled person, e.g. by immunizing a mammal, for example a rabbit, with mir-22, where appropriate in the presence of, for example, Freund’s adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al. (1981) The New England Journal of Medicine: 1344-1349). The polyclonal antibodies which are formed in the animal as a result of an immunological reaction can subsequently be isolated from the blood using well known methods and, for example, purified by means of column chromatography. Monoclonal antibodies can, for example, be prepared in accordance with the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293-299).

According to the present invention the term antibody or antibody fragment is also understood as meaning antibodies or antigen-binding parts thereof, which have been prepared recombinantly and, where appropriate, modified, such as chimaeric antibodies, humanized antibodies, multifunctional antibodies, bispecific

or oligospecific antibodies, single-stranded antibodies and F(ab) or $F(ab)_2$ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213 or WO 98/24884).

As an alternative to the classical antibodies it is also possible, for example, to use protein scaffolds against mir-22, e.g. anticalins which are based on lipocalin (Beste et al. (1999) Proc. Natl. Acad. Sci. USA, 96, 1898-1903). The natural ligand-binding sites of the lipocalins, for example the retinol-binding protein or the bilin-binding protein, can be altered, for example by means of a "combinatorial protein design" approach, in such a way that they bind to selected haptens, here to mir-22 (Skerra, 2000, Biochim. Biophys. Acta, 1982, 337-50). Other known protein scaffolds are known as being alternatives to antibodies for molecular recognition (Skerra (2000) J. Mol. Recognit., 13, 167-187).

The term "nucleic acids against the mir-22 gene or mir-22 itself" refers to double-stranded or single stranded DNA or RNA which, for example, inhibit or activate the expression of the mir-22 gene or the activity of mir-22 and includes, without limitation, antisense nucleic acids, aptamers, siRNAs (small interfering RNAs), miRNAs, shRNAs (short hairpin RNAs) and ribozymes.

The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584). Aptamers are nucleic acids which bind with high affinity to a polypeptide, here mir-22. Aptamers can be isolated by selection methods such as SELEmir-22 (see e.g. Jayasena (1999) Clin. Chem., 45, 1628-50; Klug and Famulok (1994) M. Mol. Biol. Rep., 20, 97-107; US 5,582,981) from a large pool of different single-stranded RNA molecules. Aptamers can also be synthesized and selected in their mirror-image form, for example as the L-ribonucleotide (Nolte et al. (1996) Nat. Biotechnol., 14, 1116-9; Klussmann et al. (1996) Nat. Biotechnol., 14, 1112-5). Forms which have been isolated in this way enjoy the advantage that they are not degraded by naturally occurring ribonucleases and, therefore, possess greater stability.

Nucleic acids may be degraded by endonucleases or exonucleases, in particular by DNases and RNases which can be found in the cell. It is, therefore, advantageous to modify the nucleic acids in order to stabilize them against

degradation, thereby ensuring that a high concentration of the nucleic acid is maintained in the cell over a long period of time (Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Typically, such a stabilization can be obtained by introducing one or more internucleotide phosphorus groups or by introducing one or more non-phosphorus internucleotides.

Suitable modified internucleotides are compiled in Uhlmann and Peyman (1990), supra (see also Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid which can be employed in one of the uses according to the invention contain, for example, methyl phosphonate, phosphorothioate, phosphoramidate, phosphorodithioate and/or phosphate esters, whereas non-phosphorus internucleotide analogues contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also the intention that this modification should improve the durability of a pharmaceutical composition which can be employed in one of the uses according to the invention.

The use of suitable antisense nucleic acids is further described e.g. in Zheng and Kemeny (1995) Clin. Exp. Immunol., 100, 380-2; Nellen and Lichtenstein (1993) Trends Biochem. Sci., 18, 419-23, Stein (1992) Leukemia, 6, 697-74 or Yacyshyn, B. R. et al. (1998) Gastroenterology, 114, 1142).

The production and use of siRNAs as tools for RNA interference in the process to down regulate or to switch off gene expression, here mir-22 gene expression, is e.g. described in Elbashir, S. M. et al. (2001) Genes Dev., 15, 188 or Elbashir, S. M. et al. (2001) Nature, 411, 494.

Ribozymes are also suitable tools to inhibit the translation of nucleic acids, here the RAK gene, because they are able to specifically bind and cut the mRNAs. They are e.g. described in Amarzguioui et al. (1998) Cell. Mol. Life Sci., 54, 1175-202; Vaish et al. (1998) Nucleic Acids Res., 26, 5237-42; Persidis (1467) Nat. Biotechnol., 15, 921-2 or Couture and Stinchcomb (1996) Trends Genet., 12, 510-5.

The nucleic acids described are preferably meant to be used or act as antagonists of mir-22.

For the production of the medicament the mir-22 antagonists of the present invention are usually formulated with one or more pharmaceutically acceptable additives or auxiliary substances, such as physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease antagonists, preferably aprotinin, ε-aminocaproic acid or pepstatin A or sequestering agents such as EDTA, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc. depending on the kind of administration.

Suitable further additives are, for example, detergents, such as, for example, Triton mir-22-100 or sodium desoxycholate, but also polyols, such as, for example, polyethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic compounds, such as, for example, amino acids such as glycine or in particular taurine or betaine and/or a protein, such as, for example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

The physiological buffer solution preferably has a pH of approx. 6.0-8.0, expecially a pH of approx. 6.8-7.8, in particular a pH of approx. 7.4, and/or an osmolarity of approx. 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pH of the medicament is in general adjusted using a suitable organic or inorganic buffer, such as, for example, preferably using a phosphate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4-(2-hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3-morpholino-1-propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

The medicament can be administered in a conventional manner, e.g. by means of oral dosage forms, such as, for example, tablets or capsules, by means of the mucous membranes, for example the nose or the oral cavity, in the form of dispositories implanted under the skin, by means of injections, infusions or gels which contain the medicaments according to the invention. It is further possible to administer the medicament topically and locally in order to treat the particular joint disease as described above, if appropriate, in the form of liposome complexes. Furthermore, the treatment can be carried out by means of a transdermal

therapeutic system (TTS), which makes possible a temporally controlled release of the medicaments. TTS are known for example, from EP 0 944 398 A1, EP 0 916 336 A1, EP 0 889 723 A1 or EP 0 852 493 A1.

Injection solutions are in general used if only relatively small amounts of a solution or suspension, for example about 1 to about 20 ml, are to be administered to the body. Infusion solutions are in general used if a larger amount of a solution or suspension, for example one or more litres, are to be administered. Since, in contrast to the infusion solution, only a few millilitres are administered in the case of injection solutions, small differences from the pH and from the osmotic pressure of the blood or the tissue fluid in the injection do not make themselves noticeable or only make themselves noticeable to an insignificant extent with respect to pain sensation. Dilution of the formulation according to the invention before use is therefore in general not necessary. In the case of the administration of relatively large amounts, however, the formulation according to the invention should be diluted briefly before administration to such an extent that an at least approximately isotonic solution is obtained. An example of an isotonic solution is a 0.9% strength sodium chloride solution. In the case of infusion, the dilution can be carried out, for example, using sterile water while the administration can be carried out, for example, via a so-called bypass.

The above-described nucleic acids can be used in naked form, in the form of gene transfer vectors or complexed with lipids (especially cationic lipids) or gold particles, they may be formulated as liposomes (wherein the nucleic acid is e.g. carried as cargo inside a membraneous lipidlayer such as a lipid bilayer), or may be based on nanoparticles e.g. on dextrane, peptides, or other polymers.

Examples of gene transfer vectors are viral vectors, for example adenoviral vectors or retroviral vectors (Lindemann et al. (1997), Mol. Med., 3, 466-76; Springer et al. (1988) Mol. Cell., 2, 549-58). Complexes with liposomes usually achieve a very high efficiency of transfection, in particular of skin cells (Alexander and Akhurst, 1465, Hum. Mol. Genet. 4:146-a79-85). In lipofection, small, unilamellar vesicles composed of cationic lipids are prepared by ultrasonicating the liposome suspension. The DNA is bound ionically on the surface of the liposomes in a ratio which is such that a positive net charge remains and all the plasmid DNA is complexed by the liposomes. In addition to the DOTMA (1,2-dioleyloxypropyl-3-

trimethylammonium bromide) and DOPE (dioleoylphosphatidylethanolamine) lipid mixtures employed by Felgner, P. L. et al. (1987), Proc. Natl. Acad. Sci USA, 84, 7413 - 7414, a large number of lipid formulations have by now been synthesized and tested for their efficiency in transfecting a variety of cell lines (Behr et al. (1989) Proc. Natl. Acad. Sci. USA, 86, 6982-6986; Zhao and Huang (1991), Biochim. Biophys. Acta, 1189, 195-203; Felgner et al. (1994) J. Biol. Chem., 269, 2550-2561). Examples of the lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulphate or DOGS (dioctadecylamidoglycylspermine).

Auxiliary substances which increase the transfer of nucleic acids into the cell can, for example, be proteins or peptides which are bound to the DNA or synthetic peptide-DNA molecules which enable the nucleic acid to be transported into the nucleus of the cell (Schwartz et al. (1999) Gene Therapy 6:282; Brandén et al. (1999) Nature Biotech., 17, 784). Auxiliary substances also include molecules which enable nucleic acids to be released into the cytoplasm of the cell (Planck et al. (1994) J. Biol. Chem., 269, 12918; Kichler et al. (1997) Bioconj. Chem, 8, 213) or, for example liposomes (Uhlmann and Peyman (1990), supra).

Another, particularly suitable form can be obtained by applying the above-described nucleic acids to gold particles and firing these particles into tissue or cells using what is termed a “gene gun” (Wang et al. (1999) J. Invest. Dermatol. 112:775-81, Tuting et al. (1998) J. Invest. Dermatol. 111:183-8).

Another subject matter of the present invention is the use of mir-22 or the mir-22 gene as a target for the discovery of a mir-22 antagonist for the treatment of a joint disease, in particular a degenerative joint disease such as osteoarthritis or and joint disease such as rheumatoid arthritis, and/or for the treatment of joint pain, in particular by reducing the joint pain in degenerative joint diseases. Preferably the mir-22 antagonist can be used in form of a medicament as described above.

Accordingly, the present invention refers also to a method of screening a mir-22 antagonist, wherein the method comprises the steps of:

(a) providing mir-22 or the mir-22 gene,

(b) providing a test compound, and

(c) measuring or detecting the influence of the test compound on mir-22 or the mir-22 gene.

In general, mir-22 or the mir-22 gene is provided e.g. in an assay system and brought directly or indirectly into contact with a test compound, in particular a biochemical or chemical test compound, e.g. in the form of a chemical compound library. Then, the influence of the test compound on mir-22 or the mir-22 gene is measured or detected. Thereafter, suitable antagonists can be analyzed and/or isolated. For the screening of chemical compound libraries, the use of high-throughput assays are preferred which are known to the skilled person or which are commercially available.

According to the present invention the term “chemical compound library” refers to a plurality of chemical compounds that have been assembled from any of multiple sources, including chemically synthesized molecules and natural products, or that have been generated by combinatorial chemistry techniques.

In general, the influence of the test compound on mir-22 or the mir-22 gene is measured or detected in a heterogeneous or homogeneous assay. As used herein, a heterogeneous assay is an assay which includes one or more washing steps, whereas in a homogeneous assay such washing steps are not necessary. The reagents and compounds are only mixed and measured.

Suitable functional assays may be based on the gene expression of mir-22.

In particular, the present invention relates to the following aspects:

1. Mir-22 for use as an indicator of a tissue status or a disease.

2. Use of Mir-22 as an indicator of a tissue status or a disease.

3. Mir-22 according to aspect 1 or use of mir-22 according to aspect 2 , wherein the level of mir-22 is indicative of

(a) a tissue status or a disease and/or

(b) the risk of developing an altered tissue status or a disease and/or

(c) the suffering of an individual from an altered tissue status or a disease and/or

(d) the progression or a stage of a tissue status or a disease, in an individual.

4. A method of identifying
(a) an alteration of a tissue status or the presence of a disease and/or
(b) the risk of developing an altered tissue status or a disease and/or
(c) monitoring the progression or the stage of a tissue status or a disease
in an individual, comprising detecting the level of mir-22.

5. Mir-22 or use of mir-22 according to aspect 3 or method of aspect 4, wherein the level of mir-22 is the level of mir-22 in an individual or in a sample of an individual.

6. Mir-22 or use of mir-22 or method according to aspect 5 further comprising comparing the level of mir-22 in said individual or sample to the level of mir-22 in one or more references or reference samples.

7. Mir-22 or use of mir-22 or method according to aspect 6, wherein the reference is selected from the group consisting of a healthy individual, a diseased individual or the same individual as the tested individual at an earlier or later time point or a value representative for the level of mir-22 in absence of the altered tissue status or disease, in presence of an altered tissue status or disease or for an increased or decreased risk of developing an altered tissue status or disease.

8. Mir-22 or use of mir-22 or method according to aspect 7, wherein the reference sample is selected from the group consisting of a reference sample derived from a healthy individual, a reference sample derived from a diseased individual, a reference sample derived from the same individual as the sample of interest taken at an earlier or later time point, and a reference sample with a level of mir-22 representative for a healthy individual or representative for the presence or absence of an altered tissue status or disease or representative for

an increased or decreased risk of developing an altered tissue status or disease.

9. Mir-22 or use of mir-22 or method according to aspect 7, wherein the reference is a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or a level of mir-22 representative of the absence of an altered tissue status or disease, or mir-22 or use of mir-22 or method according to aspect 8, wherein the reference sample is derived from a healthy individual or from an individual with a decreased risk of developing an altered tissue status or disease or comprises a level of mir-22 representative of a healthy individual or of a status of disease-absence or for a decreased risk of developing an altered tissue status or disease, wherein an elevated level of mir-22 indicates

(a)the presence of a deteriorated tissue status or a disease and/or

(b)an increased risk to develop a deteriorated tissue status or a disease and/or

(c) the progression of a deteriorated tissue status or a disease

in the individual.

10. Mir-22 or use of mir-22 or method according to aspect 7, wherein the reference is a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease or mir-22 or use of mir-22 or method according to aspect 8, wherein the reference sample is derived from a diseased individual or from an individual with an increased risk of developing an altered tissue status or disease or comprises a level or amount of mir-22 representative for a diseased individual or for a status of disease-presence or for an increased risk of developing an altered tissue status or disease, wherein a similar level of mir-22 indicates

(a)the presence of a deteriorated tissue status or a disease and/or

(b)an increased risk to develop a deteriorated tissue status or a disease and/or

(c)the progression of a deteriorated tissue status or a disease in the individual.

11. Mir-22 or use of mir-22 or method according to aspect 7, wherein the reference is the same individual at an earlier time point, or mir-22 or use of mir-22 or method according to aspect 8 wherein the reference sample is derived from the same individual as sample of interest and was taken at an earlier time point, wherein

(i)an elevated level of mir-22 in the sample of interest indicates

(a) the presence of a deteriorated tissue status or a disease and/or

(b) an increased risk to develop a deteriorated tissue status or a disease and/or

(c) the progression of a deteriorated tissue status or a disease in the individual.

,

(ii)a lowered level of mir-22 in the sample of interest indicates

(a) an alteration of the tissue status or an improvement or absence of a disease and/or

(b) a decreased risk to develop a deteriorated tissue status or a disease and/or

(c) a declined progression of the deteriorated tissue status or the disease,

(iii) a similar level of mir-22 in the sample of interest indicates

(a) a similar risk to develop a deteriorated tissue status or a disease and/or

(b) a stagnation in the progression of a deteriorated tissue status or a disease, and/or

(c) a persistence of the deteriorated tissue status or the disease in the individual.

12. A method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of

(a)determining the level of mir-22 in a sample of the individual, and optionally determining the level of mir-22 in a reference or reference sample for comparison with the level of mir-22 in the sample of interest, and

(b)determining the dosage of a pharmaceutical depending on the level of mir-22 in the sample of interest, optionally depending on the comparison of the levels of mir-22 in the sample of interest and the reference or reference sample.

13. A method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of

(a) determining the level of mir-22 in a sample,

(b)determining the level of mir-22 in one or more references or reference samples,

(c)examining the tested sample as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, and

(d)adapting the dosage of a pharmaceutical depending on whether the level of mir-22 in the sample of interest is different from the level in the one or more references or reference samples.

14. A method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of

(a) determining the level of mir-22 in a sample of interest,

(b)determining the level of mir-22 in one or more references or reference samples, and

(c)examining the sample of interest as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples,

wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples.

15. Mir-22 or use of mir-22 according to any of aspects 5 to 11 or method according to any of aspects 5 to 14, wherein the sample of interest is tissue and/or fluid.

16. Mir-22 or use of mir-22 or method of aspect 15, wherein the tissue sample is selected from the group consisting of tissue extract, synovial tissue and cartilage, and the body fluid sample is selected from the group consisting of synovial fluid, serum plasma and urine.

17. Use of mir-22 in a method according to any of aspects 4 to 16.

18. A kit for use in a method according to any of aspects 4 to 16, comprising one or more means of detecting mir-22.

19. The kit according to aspect 18 wherein the one or more means for detecting mir-22 are means for determining the expression levels of mir-22, preferably on a gene and/or RNA level.

20. The kit according to aspect 18 or 19, wherein the one or more means for detecting mir-22 are selected from the group consisting of nucleic acid, preferably DNA or RNA, a mixture of both or chemically modified nucleic acids, peptide, and protein, preferably monoclonal or polyclonal antibodies.

21. The kit according to any of aspects 18 to 20, wherein the kit further comprises

(a) a container, and/or

(b) a data carrier, wherein the data carrier comprises information such as

(i) instructions concerning methods for identifying the risk for developing and/or identifying the presence and/or monitoring progression of a joint disease

(ii)instructions for use of the means for detecting mir-22, preferably in a sample, more preferably in a sample from an individual and/or of the kit,
(iii)quality information such as information about the lot/batch number of the means for detecting mir-22 and/or of the kit, the manufacturing or assembly site or the expiry or sell-by date, information concerning the correct storage or handling of the kit,
(iv)information concerning the composition of the buffer(s), diluent(s), reagent(s) for detecting mir-22 and/or of the means for detecting mir-22,
(v)information concerning the interpretation of information obtained when performing the above-mentioned methods identifying and/or monitoring progression of a joint disease,
(vi)a warning concerning possible misinterpretations or wrong results when applying unsuitable methods and/or unsuitable means, and/or
(vii) a warning concerning possible misinterpretations or wrong results when using unsuitable reagent(s) and/or buffer(s).

22. Use of the kit of any of aspects 18 to 21 in a method according to any of aspects 4 to 16.

23. One or more nucleic acids for detecting a mir-22 gene, gene product or functionally active variant thereof in a method according to any of aspects 4 to 16.

24. The nucleic acid according to aspect 23, wherein the nucleic acid is selected from the group consisting of a locked nucleic acid (LNA), nucleic acid probe, a polyamide or peptide nucleic acid (PNA), microRNA (miRNA), small interfering RNA (siRNA), primers for polymerase chain reaction (PCR), primers for reverse transcription (RT) reaction, and primers for DNA sequencing.

25. The nucleic acid according to aspect 24 comprising the nucleotide sequence of at least 8 nucleotides.

26. A peptide, polypeptide or protein for detecting a mir-22 gene, gene product or functionally active variant thereof in a method according to any of aspects 4 to 16.

27. The peptide, polypeptide or protein according to aspect 25, wherein the protein or polypeptide is a protein ligand, preferably an antibody, a fragment or derivate thereof, a darpin or an anticalin, or wherein the polypeptide or peptide is a probe, preferably a mass spectrometry probe.

28. Use of a nucleic acid according to any of aspects 23 to 25, or of a peptide, polypeptide or protein according to aspect 26 or 27 in a method according to any of aspects 4 to 16.

29. Use of mir-22 or the mir-22 gene or a functionally active variant thereof as a target molecule for the discovery of a mir-22 antagonist.

30. The use according to aspect 30, wherein mir-22 or the mir-22 gene or a functionally active variant thereof is brought into direct or indirect contact with a test compound and the influence of the test compound on mir-22 or the mir-22 gene is measured or detected.

31. A method of screening for a mir-22 antagonist, wherein the method comprises the steps of:

(a) providing mir-22 or the mir-22 gene,

(b) providing a test compound, and

(c) measuring or detecting the influence of the test compound on mir-22 or the mir-22 gene.

32. A mir-22 antagonist for the alteration of a tissue status or the prevention or treatment of a joint disease.

33. A pharmaceutical comprising a mir-22 antagonist for use in the prevention or treatment of a joint disease, preferably an inflammatory joint disease.

34. The pharmaceutical of aspect 33, further comprising a pharmaceutically acceptable carrier and/or excipient and optionally one or more additional active substances.

35. A method of alteration of a tissue status or prevention or treatment of a joint disease, wherein a therapeutically effective amount of the pharmaceutical according to aspect 33 or 34 is administered to an individual at risk of developing or suffering from a joint disease.

36. The method of aspect 35, wherein the pharmaceutical is administered orally, topically, transdermal or intracavitary, preferably intravenous or intraarticular.

37. Mir-22 or use of mir-22 according to any of aspects 5 to 11 or method according to any of aspects 5 to 14, and 35 to 36, use of the kit of aspect 22, the nucleic acid of any of aspects 23 or 25, the peptide polypeptide or protein of aspect 26 or 27, or the use of the nucleic acid, peptide, polypeptide or protein of aspect 28, or the mir-22 antagonist of aspect 32, wherein the tissue status is tissue damage, preferably selected from the group consisting of damage to bone, cartilage, synovium, perichondrium, capsule, and connective tissue and/or is characterised by altered level of regulatory molecules, signalling molecules, and/or degenerative molecules present in the tissue.

38. Mir-22 or use of mir-22 according to any of aspects 5 to 11 or method according to any of aspects 5 to 14, and 35 to 36, use of the kit of aspect 22, the nucleic acid of any of aspects 23 to 25, the peptide polypeptide or protein of aspect 26 or 27, or the use of the nucleic acid, peptide, polypeptide or protein of aspect 28, or the mir-22 antagonist of aspect 32, wherein the disease is a joint

disease, preferably selected from the group consisting of osteoarthritis, rheumatoid arthritis, psoriatic arthritis, septic arthritis, Ankylosing spondylitis, Bursitis (inflammation), Dermatomyositis, Fibromyalgia, Gouty arthritis, Juvenile chronic arthritis (Still's disease), Mixed Connective Tissue Disease, Polymyalgia rheumatica, Polymyositis, Reactive arthritis (Reiter's Syndrome), Scleroderma, Shoulder tendinitis, Sjorgen's Sydrome, Systemic Lupus Erythemetosis and/or is characterised by a physical or metabolic injury of the joint indicated by an inflammation of the joint or a degradation of the joint, more preferably wherein the disease is osteoarthritis.

Further aspects of the present invention are the following:

1. [Prognostic Method] A method of identifying an increase in risk for joint disease in a subject comprising:

a) measuring the level of mir-22 in a biological sample from the subject; and

b) comparing the level of mir-22 in the sample to the level of mir-22 in a suitable control

wherein an increase in the level of mir-22 in the sample is determined as compared to the control, thereby identifying the subject as having an increased risk for joint disease.

2. [Diagnostic Method] A method for diagnosing a joint disease in a subject, the method comprising:

a)measuring the level of mir-22 in a biological sample from the subject; and

b)comparing the level of mir-22 in the sample to the level of mir-22 in a suitable control

wherein an increase in the level of mir-22 in the sample is determined as compared to the control, thereby identifying the subject as having the joint disease.

3. [Patient Stratification] A method for treating a subject having a joint disease, the method comprising:

a)measuring the level of mir-22 in a biological sample from the subject;

b)comparing the level of mir-22 in the sample to the level of mir-22 in a suitable control; and

c)administering to the patient a pharmaceutical composition for treatment of joint disease when an increase in the level of mir-22 in the sample is determined as compared to the control, thereby treating the subject.

4. [Dosage Optimization] A method for determining the dosage of a pharmaceutical for treating a subject having a joint disease, the method comprising:

a)measuring the level of mir-22 in a biological sample from the subject;

b)comparing the level of mir-22 in the sample to the level of mir-22 in a suitable control; and

c)increasing the dosage of a pharmaceutical composition when an increase in the level of mir-22 in the sample is determined as compared to the control or decreasing the dosage of the pharmaceutical composition when a decrease in the level of mir-22 in the sample is determined as compared to the control,

thereby determining the dosage of the pharmaceutical in the subject.

5. [Screening Assays] A method for determining the effect of a substance or test compound on a joint disease, the method comprising:

a)measuring the level of mir-22 in a sample treated with the substance or test compound;

b)comparing the level of mir-22 in the treat sample to the level of mir-22 in a suitable control; and

c)identifying the substance as having an adverse effect if an increase in the level of mir-22 in the sample is determined as compared to the control

or identifying the substance as a having a beneficial effect if a decrease in the level of mir-22 in the sample is determined as compared to the control,

thereby determining the effect of the substance or test compound on joint disease.

6. The method of any one of aspects 1-4, wherein the level of mir-22 is detected using one or more nucleic acid probes.

7. The method of any one of aspects 1-6, wherein the suitable control is a biological sample from a healthy subject.

8. The method of aspect 6, wherein the suitable control is a sample that is not treated with substance.

9. The method of any one of the previous aspects, wherein the joint disease is osteoarthritis.

10. [Method of treatment with mir-22 antagonist] A method of treating a joint disease in a subject comprising administering a mir-22 antagonist to the subject, thereby treating joint disease in the subject.

11. The method of aspect 10, wherein the mir-22 antagonist is selected from the group consisting of an siRNA, a shRNA, an antisense RNA, and a ribozyme.

12. The method of aspect 10, wherein the mir-22 antagonist, is substantially complementary to at least a portion of the precursor form of mir-22 (SEQ ID NO:1).

13. The method of aspect 10, wherein the mir-22 antagonist is substantially complementary to at least a portion of the mature form of mir-22 (SEQ ID NO:2) or mir-22* (SEQ ID NO:3).

14. The method of aspect 10, wherein the miRNA-146a antagonist is a nucleic acid molecule of about 6 to about 20 nucleotides in length.

15. The method of aspect 10, wherein miRNA-146a antagonist is a LNA-modified or PNA-modified nucleic acid molecule.

16. The method of aspect 10, wherein the joint disease is osteoarthritis.

17. The method of aspect 10, wherein administration of the miR-146 antagonist results in the increase in one or more chondrogenic markers in chondrocytes of the subject.

18. The method of aspect 17, wherein the one or more chondrogenic markers are selected from the group consisting of collagen II, aggrecan, Sox9, collagen X, ALP, CD-Rap, MMP-1 and MMP-3.

19. The method of aspect 11, wherein the mammal is a human.

20. A kit comprising one or more means of detecting mir-22 and a suitable control.

The invention is described in more detail in the examples and figures that are not to be understood as limiting the scope of present invention.

Examples

Example 1: mir-22 expression profile in chondrocyte and stem cells during their cultivation

The expression of mir-22 was determined in primary chondrocytes and bone marrow derived mesenchymal stem cells (BMSC) during their cultivation in order to assess the normal fluctuation in the expression level of mir-22 in these cells over the indicated time periods.

The chondrocytes were cultivated in pellet culture for 0, 1, 4, 7 or 14 days in DMEM medium supplemented with 2mM L-glutamine (Sigma-Aldrich), 1× non essential amino acids (Sigma-Aldrich), 10nM dexamethasone (Sigma-Aldrich), 10µg/ml insulin, 5.5µg/ml transferrin, 5ng/ml sodium selenite (Sigma-Aldrich), 44µg/ml ascorbic acid

(Sigma-Aldrich) and 10ng/ml TGF-β1 (R&D Systems). BMSC were cultivated in pellet culture for 0, 1, 4, 7, 14 or 21 days in the same medium. Chondrocytes and BMSC were harvested and each pellet was processed individually for RNA isolation. For the analysis of secreted proteins supernatants were collected and pooled over the entire culture period. For extraction of the RNA, Pellets were harvested after seven days of culture and separately homogenized with the Mixermill (Retsch). A proteinase K digest was performed onto the homogenized cells. Total RNA including miRNA was eluted from the Qiagen miRNeasy Plus Micro kit. The RNA was reverse transcribed into cDNA using the Reverse Transcriptase Kit and RNase Inhibitor (Life Technologies) according to the manufacturer's instructions. The mir-22 expression profile in chondrocyte and BMSC was determined via miRNA specific quantitative real time PCR using Taqman probes according to protocols well-known to the skilled person. The ABI Taqman® microRNA Low density arrays (TLDA, Applied Biosystems, Foster City, CA) were used to profile 668 miRNAs and additional reference genes. The assay includes three steps: a multiplex RT, a preamplification and a singleplex TaqMan PCR. All steps were performed according to the manufacturer's instructions. The Real-time PCR was done on an AB 7900HT Sequence Detection System. For expression analysis the comparative Ct method was applied.

Fig. 1a illustrates the mir-22 expression during the cultivation period in chondrocyte pellets and Fig. 2 illustrates the mir-22 expression during the cultivation period in BMSC. An Expression of mir-22 could be detected in chondrocyte pellets at a cycle threshold of about 23 cycles (ct = 23) whilst the ct for BMSCs was 23 throughout the entire period of the experiment. The cultivation conditions were chosen such as to reflect conditions of healthy differentiated cartilage. As can be gained from Fig. 1a and Fig.2, the expression level of mir-22 remained stable in both cell types during the culture period and only exhibited very little fluctuation. This stable expression stands in contrast to the increase of expression of mir-199a and mir-140 over time in parallel experiments conducted under the same conditions (Fig. 1b), two micro RNAs that have both been identified by present inventors to be markers of the physiological cartilage state; on the other hand the expression of mir-146a, that has been shown by present inventors to be associated with osteoarthritic cartilage, shows the same stability of expression throughout the cultivation period as mir-22. Thus, the expression level of

mir-22 is lower in physiological cartilage than that of markers of differentiated, physiological (i.e. non-osteoarthritic) cartilage. Accordingly, a lowered level of mir-22 expression in contrast to that of markers of differentiated, physiological (i.e. non-osteoarthritic) cartilage is indicative for the absence of OA.

Example 2: Overexpression and Knock down of mir-22 in BMSC

The expression of the proteins collagen II, aggrecan, Sox9, collagen X, ALP, and CD-Rap is know to be altered in arthritic joints. Their respective expression levels are thus, indicative of the presence or absence of OA. To investigate the role of mir-22 in OA, the effects of its overexpression or knockdown on these molecules, measured either on mRNA (collagen II, aggrecan, Sox9, and collagen X) or on protein level (CD-Rap) are analysed in BMSC after chondrogenic differentiation.

In three independent experiments (V15, V18, V20) BMSC were infected with pseudoviral particles comprising either the DNA encoding for mir-22 (herein further referred to as: mir-22) or MRP ((herein further referred to as: empty vector) Mir-22 had been cloned into pLenti9, a lentiviral vector containing partial sequences of HIV1 LTRs (see map below). Pseudoviral particles were produced as a custom service by Vectalys (Toulouse, France). Infection of BMSCs was carried out with a multiplicity of infection (MOI) of five. For enhancement of infection 8µg/ml Polybrene was added and the plates were centrifuged for 90 min at 500×g at 32°C. On the following day high-density pellets consisting of 2.5×10^5 cells were formed by centrifugation (300×g, 5 min) in 96 well conical bottom plates (Eppendorf).

Cells were harvested after 7 days of pellet culture and their RNA isolated following the same protocol as given in Example 1. cDNA synthesis was performed using the Reverse Transcriptase Kit and RNase Inhibitor (Life Technologies) according to the manufacturer's instructions. The expression levels of the four chondrogenic markers collagen II, aggrecan, Sox9, and collagen X were determined via quantitative real time PCR. TaqMan reactions were performed using TaqMan® Universal PCR Master Mix and the FAMTM-labelled gene specific assays for the target genes ACAN, COL2A1, COL10A1, and SOX9 (see table). All gene specific assays consist of FAM™ dye-labeled TaqMan® MGB probes. PCR was performed using the ABI Prism 7900 (Life Technologies). Ribosomal protein L37a (RPL37a) was used as reference gene. For RPL37a the following sequences were selected: reference forward primer: GGCACTGTGGTTCCTGCAT, reference reverse primer: ACAGCGGAAGTGGTATTGTACGT. The MGB reference probe was labeled with VIC and had the sequence: CCGCCAGCCACTGTCT. For expression analysis the comparative Ct method was applied.

Target gene	Gene symbol	Accession No.	Gene specific assay ID
Aggrecan	ACAN	NM_013227	Hs00153936_m1
Collagen, type II, alpha 1	COL2A1	NM_033150	Hs00264051_m1

Collagen, type X, alpha 1	COL10A1	NM_000493	Hs00166657_m1
SRY (sex determining region Y)-box 9	SOX9	NM_000346	Hs00165814_m1

The effects of mir-22 overexpression on the expression levels of four different marker proteins (i.e. collagen II, aggrecan, Sox9, and collagen X) in three different, independent experiments (i.e. V15, V18, V20) are illustrated in Fig. 3 (see Fig. 3 A-D). Each experiment comprised four samples (n = 4).

The expression levels of the marker protein CD-RAP was tested in experiments V15, V18 and V20 (see Fig. 3 E) using ELISA according to protocols well-known to the skilled person. Protein levels were quantified in pellet culture supernatants using ELISA kits according to the manufacturer's instructions: CD-RAP (MIA) (Roche). For each single pellet culture supernatants obtained at every medium change within the whole culture period of three weeks were collected and pooled for determination of protein levels by ELISA for each single pellet. The amount of culture medium applied to the pellets during culture was identical for each pellet.

The expression levels of all five marker proteins tested were significantly reduced upon overexpression of mir-22 in comparison to the expression levels determined in the control cells. The overexpression of mir-22 appears to down-regulated the expression of these proteins. High mir-22 levels are thus indicative of reduced expression levels of these markers. Accordingly, elevated level of mir-22 are indicative of the presence of OA in comparison to normal controls.

In three different experiments (V21, V22, V23) mir-22 was knocked-down in BMSC (herein further referred to as: mir-22 inh) using miRIDIAN Hairpin Inhibitor hsa-mir-22 (Dharmacon) and the effects on the expression of the four marker proteins collagen II, aggrecan, Sox9, and collagen X were observed in comparison to control cells. BMSCs were treated with miRIDIAN Hairpin Inhibitor hsa-mir-22 at a concentration of 30 nM in monolayer culture. miRIDIAN microRNA Hairpin Inhibitor Negative Control #1 (Dharmacon) served as negative control. On the following day high-density pellets consisting of 2.5×10^5 cells were formed by centrifugation (300×g, 5 min) in 96 well conical bottom plates (Eppendorf). Pellets were cultured for a maximum of 3 week. All

following steps of analysis were performed as already described for over-expression of mir-22.

The effects of the mir-22 knock-down on the expression levels of fife marker proteins (i.e. collagen II, aggrecan, Sox9, collagen X, and CD-RAP) in three different, independent experiments (i.e. V21, V22, V23) are illustrated in Fig. 4 A-E. Per experiment four samples were examined (n = 4). The expression levels of all four marker proteins tested were increased upon knock-down of mir-22 in comparison to the expression levels determined in the control cells. Thus, lowering of mir-22 levels led to increased levels of marker proteins of physiological cartilage. Accordingly, lowered levels of mir-22 are indicative of the absence of OA in comparison to controls. The expression levels of mir-22 are thus indicative of the physiological or pathological state of cartilage, especially with respect to the presence or absence of osteoarthritis. It can further be concluded that antagonists or inhibitors of mir-22 (expression or function) can be used for the treatment or prevention of OA by increasing the expression of proteins that are indicative and beneficial for the physiological state of cartilage.

Example 3: Knock down of mir-22 in BMSC and additional treatment with IL-1

IL-1 is a pro-inflammatory cytokine which is capable of inducing chondrocytes and synovial cells to synthesize MMPs. Furthermore, IL-1 suppresses the expression of the type II and IX collagens in the cartilage matrix (Pujol JP 1998) and diminishes the synthesis of proteoglycans. Its presence has been confirmed in OA joints. The effects of an IL-1 treatment on the expression levels of the marker proteins collagen II, aggrecan, Sox9, and collagen X in differentiated BMSC is thus, determined.

To investigate the effect of IL-1, in experiments V22 and V23 additional samples were prepared wherein the cells were additionally treated with the cytokine IL-1. BMSC pellet cultures were prepared as described earlier. After 7 days of differentiation (as described before) the pellets were further cultivated in the absence of TGFβ and in the presence of 0.05 ng/ml IL-1β (R&D Systems). RNA was extracted after 7 days of IL-1β

stimulation, 2 weeks after the start of the pellet culture. Supernatants were collected for 2 weeks during IL-1β stimulation (total culture period of 3 weeks).

The effects of the IL-1 treatment on the mir-22 knock-down are illustrated in Fig. 5 A-D. Upon IL-1 treatment expression levels of collagen II, aggrecan, Sox9, and collagen X, were slightly increased in comparison to the expression levels determined in the control cells. This increase was however, lower than the increase in expression levels without IL-1 (see Example 2 above and Fig. 4) indicating a partial reversal of the mir-22 knock-down effect.

Fig. 6 further illustrates the percentage changes of the expression levels of the four marker proteins collagen II, aggrecan, Sox9, and collagen X, due to overexpression or knock-down of mir-22 in comparison to the non-treated controls (A) as well as the effects of the IL-1 treatment on the mir-22 knockdown (B).

Sequence Listing – Free Text Information

SEQ ID NO: 1 Homo sapiens mir22 stem-loop sequence hsa-mir-22 (accession number: MI0000078)

SEQ ID NO: 2 hsa-miR-22 (accession number: MIMAT0000077)

SEQ ID NO: 3 hsa-miR-22* (accession number: MIMAT0004495)

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Claims

1. Mir-22 for use as an indicator of a tissue status or a disease.

2. Use of mir-22 as an indicator of a tissue status or a disease.

3. A method of identifying
(a) an alteration of a tissue status or the presence of a disease and/or
(b) the risk of developing an altered tissue status or a disease and/or
(c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-22.

4. A method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of
(a) determining the level of mir-22 in a sample of the individual, and optionally determining the level of mir-22 in a reference or reference sample for comparison with the level of mir-22 in the sample of interest, and
(b)determining the dosage of a pharmaceutical depending on the level of mir-22 in the sample of interest, optionally depending on the comparison of the levels of mir-22 in the sample of interest and the reference or reference sample.

5. A method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of
(a) determining the level of mir-22 in a sample,
(b) determining the level of mir-22 in one or more references or reference samples,
(c) examining the tested sample as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples, and

(d)adapting the dosage of a pharmaceutical depending on whether the level of mir-22 in the sample of interest is different from the level in the one or more references or reference samples.

6. A method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of
(a) determining the level of mir-22 in a sample of interest,
(b)determining the level of mir-22 in one or more references or reference samples, and
(c)examining the sample of interest as to whether the level of mir-22 present in said sample of interest is different from the level in the one or more references or reference samples,
wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples.

7. Use of mir-22 in a method according to any of claims 3 to 6.

8. A kit for use in a method according to any of claims 3 to 6, comprising one or more means of detecting mir-22.

9. Use of the kit of claim 8 in a method according to any of claims 3 to 6.

10. One or more nucleic acids for detecting a mir-22 gene, gene product or functionally active variant thereof in a method according to any of claims 3 to 6.

11. A peptide, polypeptide or protein for detecting a mir-22 gene, gene product or functionally active variant thereof in a method according to any of claims 3 to 6.

12. Use of a nucleic acid according to claim 10, or of a peptide, polypeptide or protein according to claim 11 in a method according to any of claims 3 to 6.

13. Use of mir-22 or the mir-22 gene or a functionally active variant thereof as a target molecule for the discovery of a mir-22 antagonist.

14. A method of screening for a mir-22 antagonist, wherein the method comprises the steps of:

(a) providing mir-22 or the mir-22 gene,

(b) providing a test compound, and

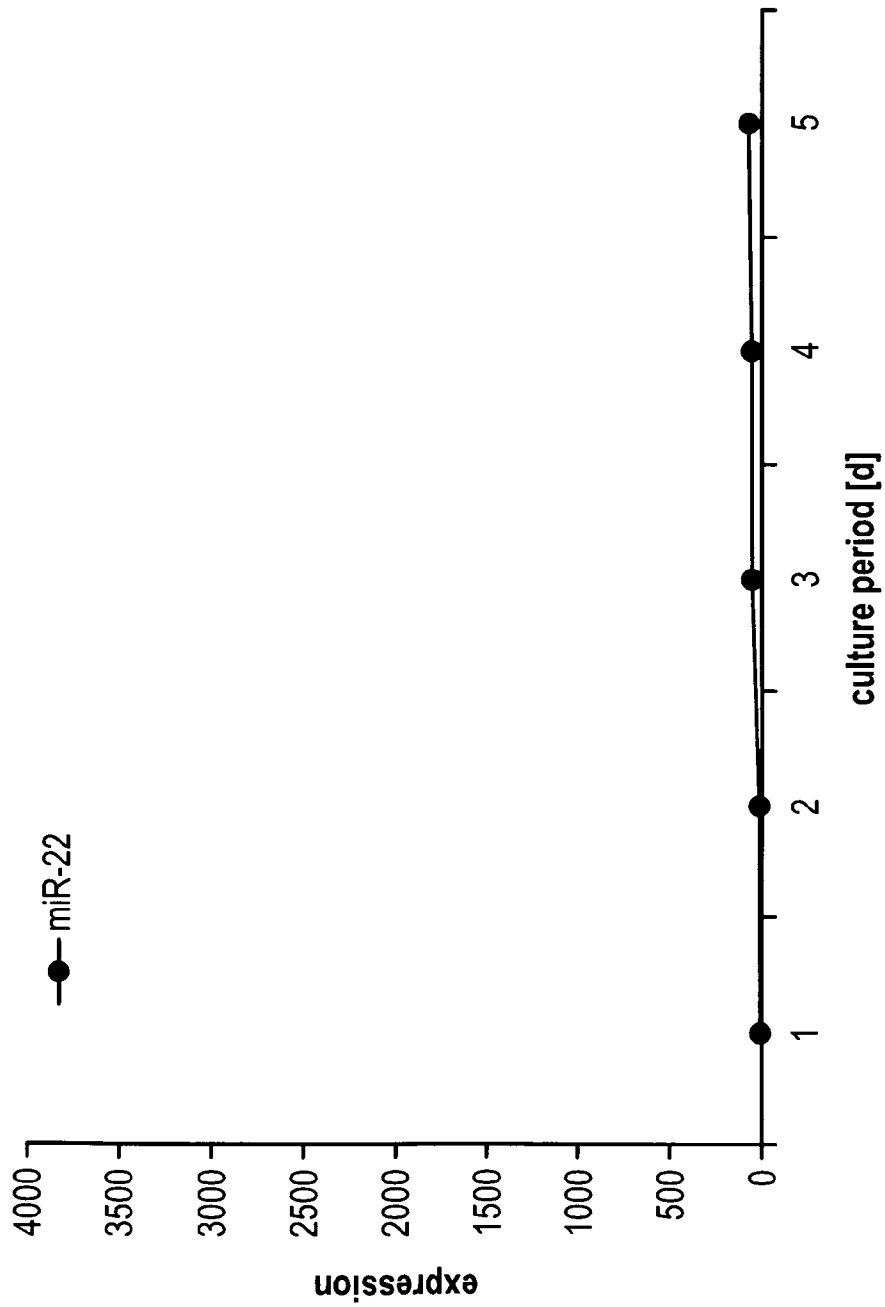
(c) measuring or detecting the influence of the test compound on mir-22 or the mir-22 gene.

15. A mir-22 antagonist for the alteration of a tissue status or the prevention or treatment of a joint disease.

16. A pharmaceutical comprising a mir-22 antagonist for use in the prevention or treatment of a joint disease, preferably an inflammatory joint disease.

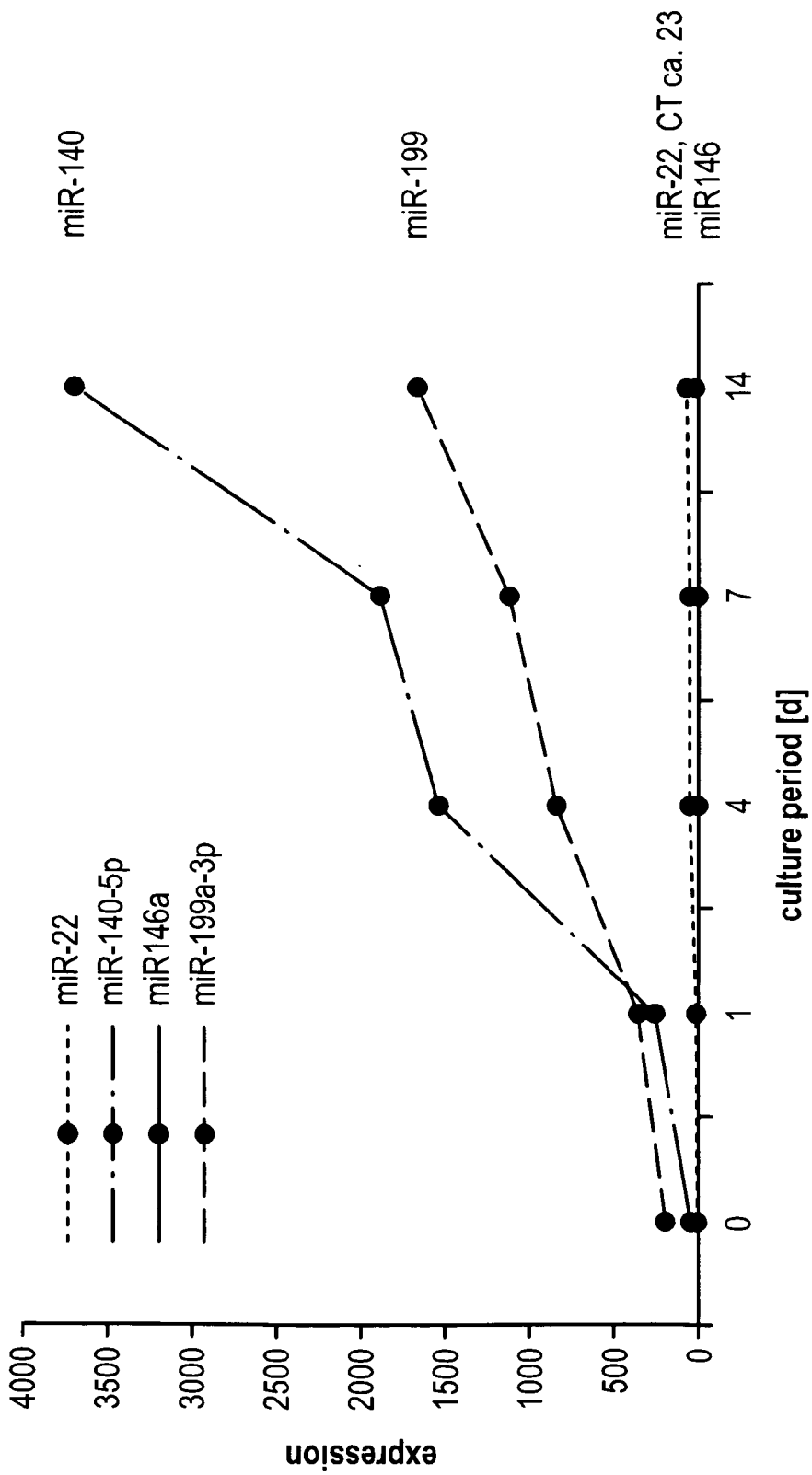
17. A method of alteration of a tissue status or prevention or treatment of a joint disease, wherein a therapeutically effective amount of the antagonist according to claim 15 or the pharmaceutical according to claim 16 is administered to an individual at risk of developing or to an individual suffering from a joint disease.

Fig. 1a



WO2010/287

Fig. 1b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miR-199
miR-22, CT ca. 23
miR146
miR-22
miR-140-5p
miR146a
miR-199a-3p
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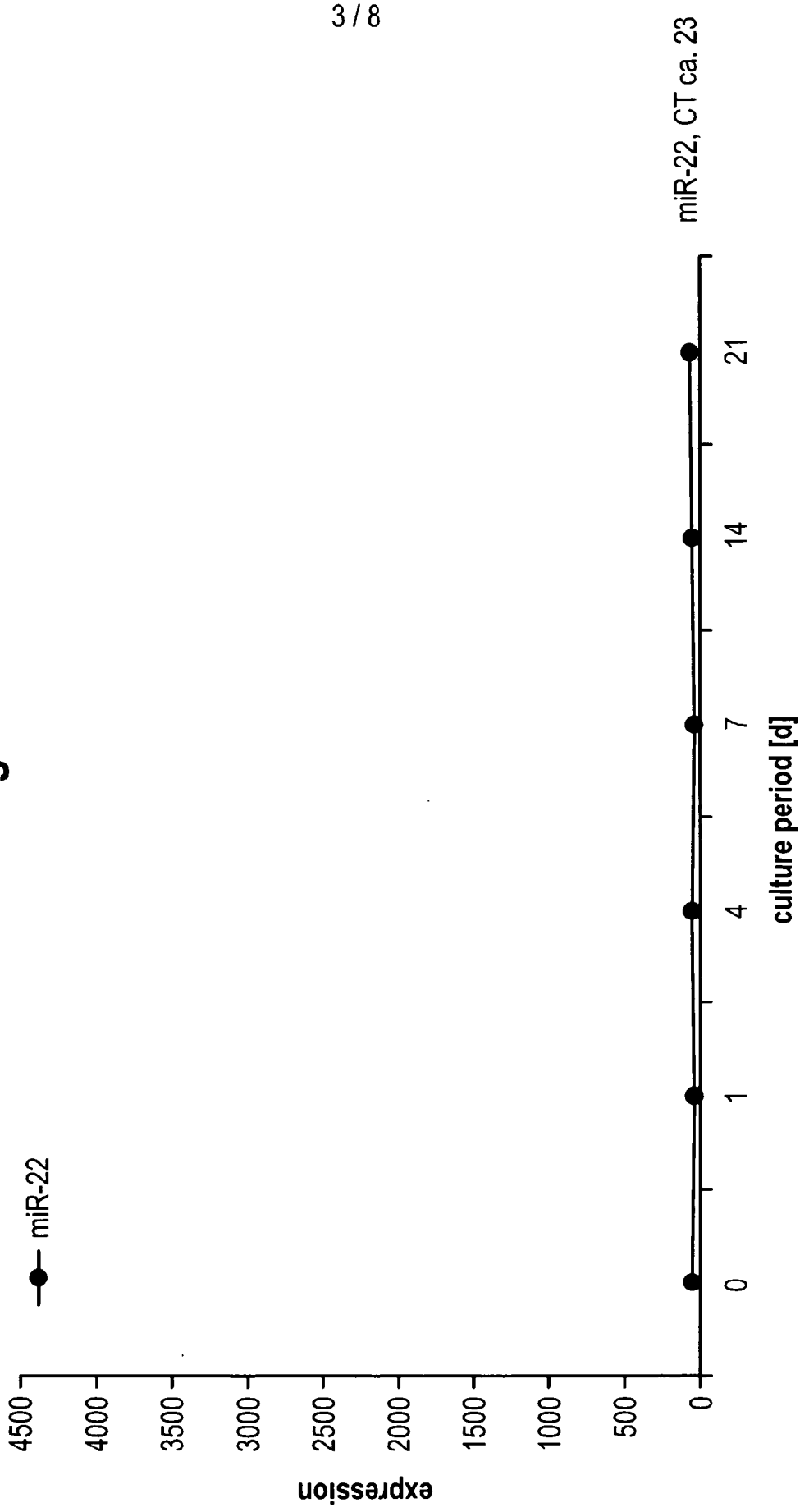
1000
1500
2000
2500
3000
3500
4000
0
1
4
7
14

Fig. 2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miR-22
expression
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3000
2500
2000
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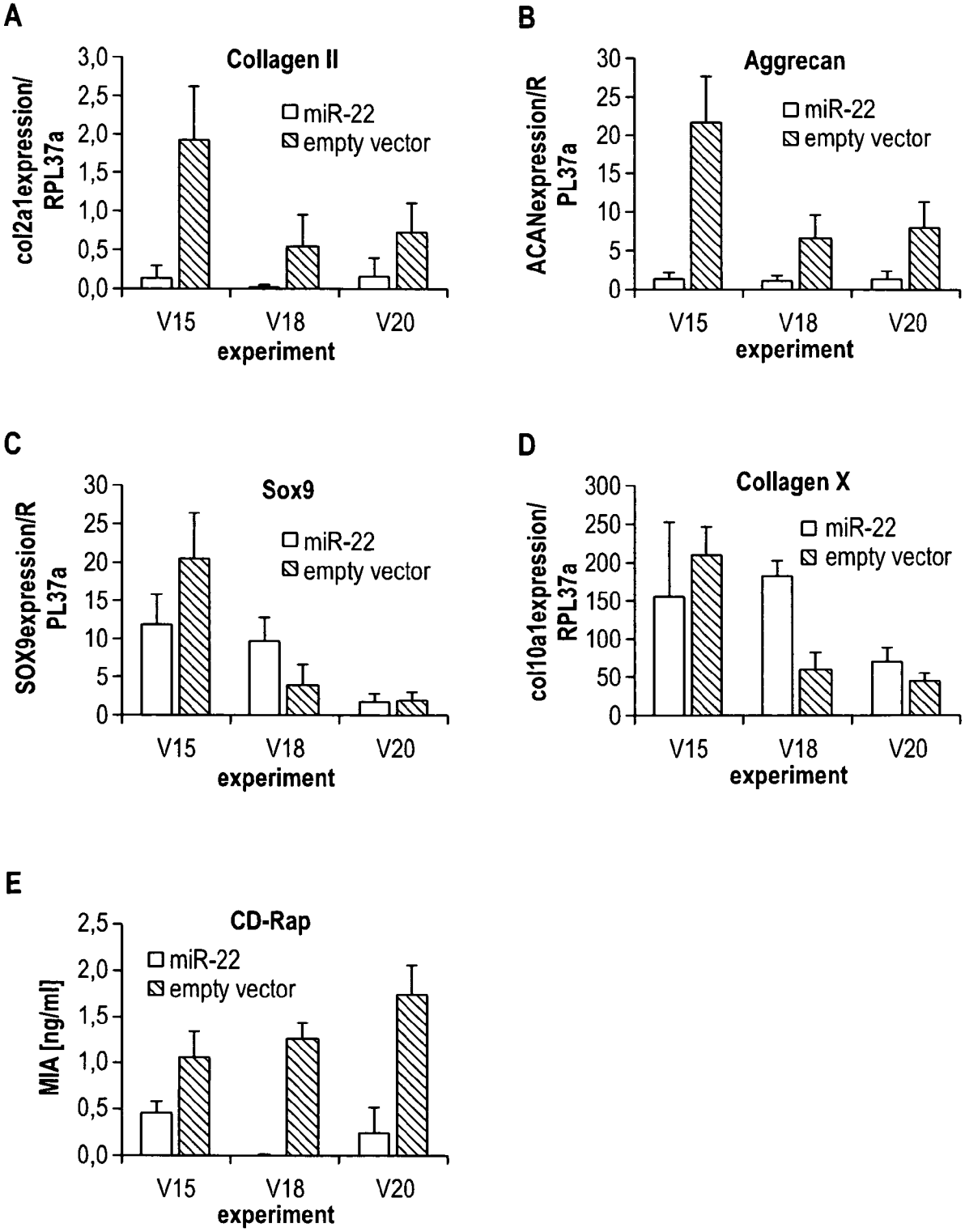
500
0
0
1
4
7
14
21

Fig. 3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2,0
1,5
1,0
0,5
0,0
miR-22
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V15
V18
V20
experiment
B
Aggrecan
ACANexpression/R PL37a
30
25
20
15
10
5
0
miR-22
empty vector
V15
V18
V20
experiment
C
Sox9
SOX9expression/R PL37a
30
25
20
15
10
5
0
miR-22
empty vector
V15
V18
V20
experiment
D
Collagen X
col10a1expression/ RPL37a
300
250
200
150
100
50
0
miR-22
empty vector
V15
V18
V20
experiment
E
CD-Rap
MIA [ng/ml]
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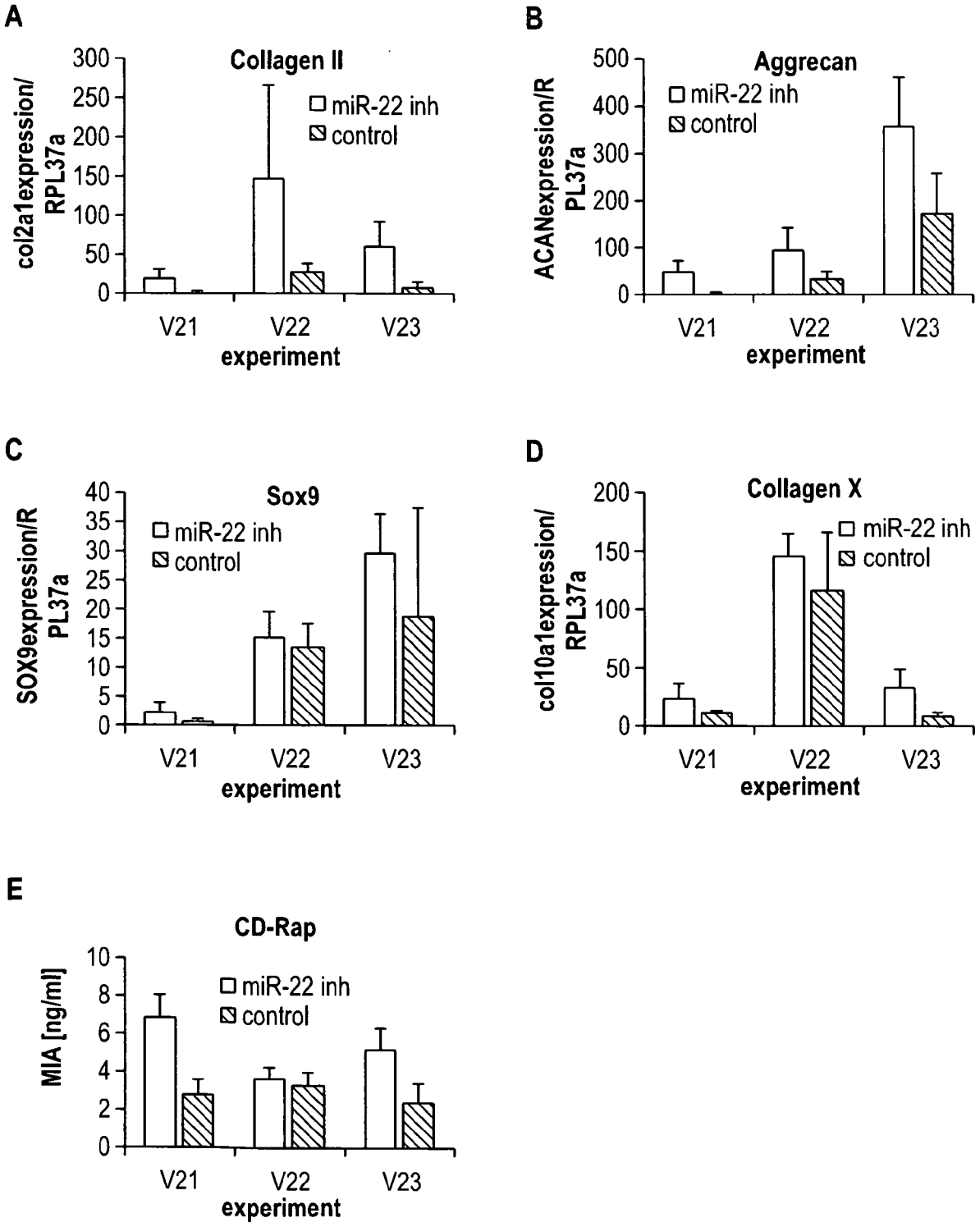
V15
V18
V20
experiment

Fig. 4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miR-22 inh
control
V21
V22
V23
experiment
B
Aggrecan
ACANexpression/R PL37a
500
400
300
200
100
0
miR-22 inh
control
V21
V22
V23
experiment
C
Sox9
SOX9expression/R PL37a
40
35
30
25
20
15
10
5
0
miR-22 inh
control
V21
V22
V23
experiment
D
Collagen X
col10a1expression/ RPL37a
200
150
100
50
0
miR-22 inh
control
V21
V22
V23
experiment
E
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MIA [ng/ml]
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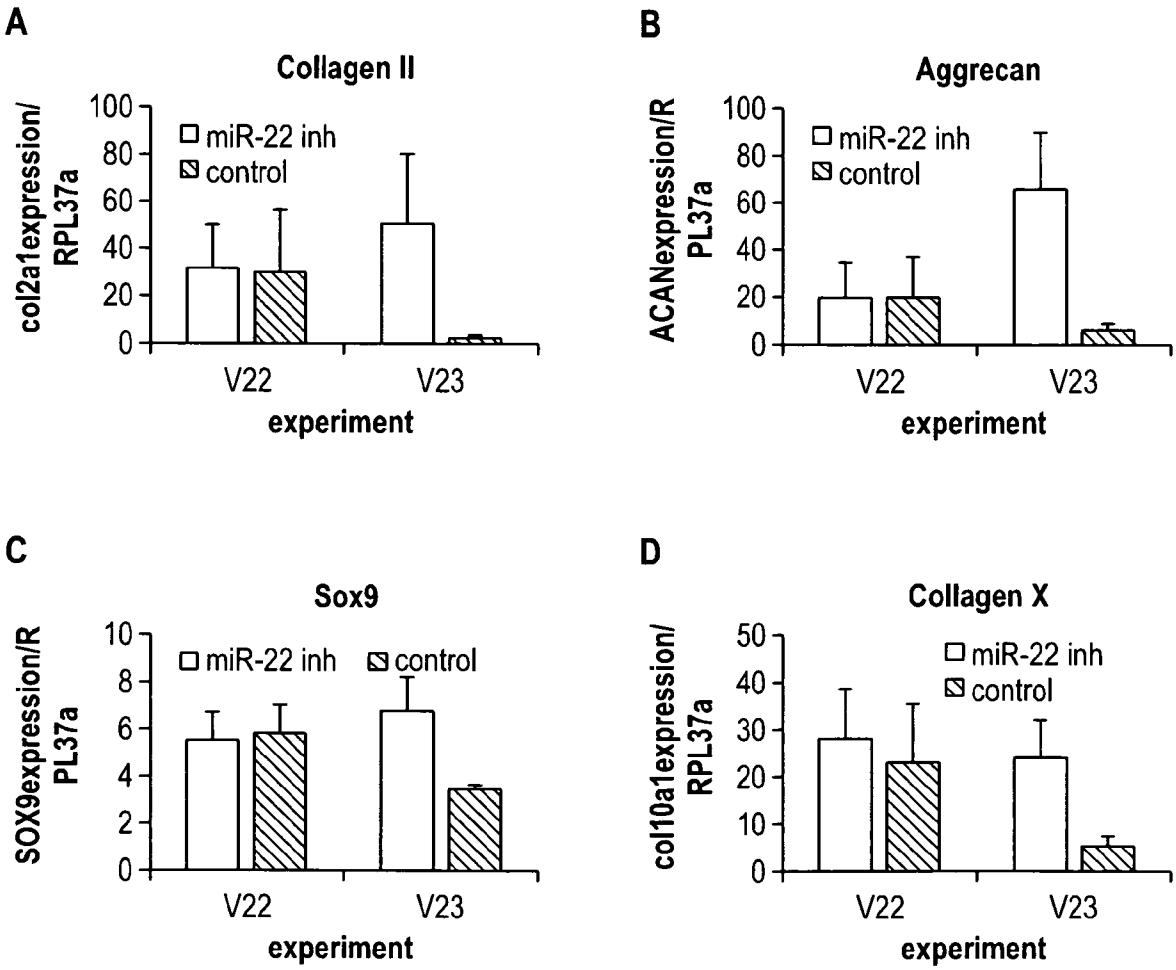
4
2
0
miR-22 inh
control
V21
V22
V23
experiment

Fig. 5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col2a1expression/ RPL37a
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60
40
20
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miR-22 inh
control
V22
V23
experiment
B
Aggrecan
ACANexpression/R PL37a
100
80
60
40
20
0
miR-22 inh
control
V22
V23
experiment
C
Sox9
SOX9expression/R PL37a
10
8
6
4
2
0
miR-22 inh
control
V22
V23
experiment
D
Collagen X
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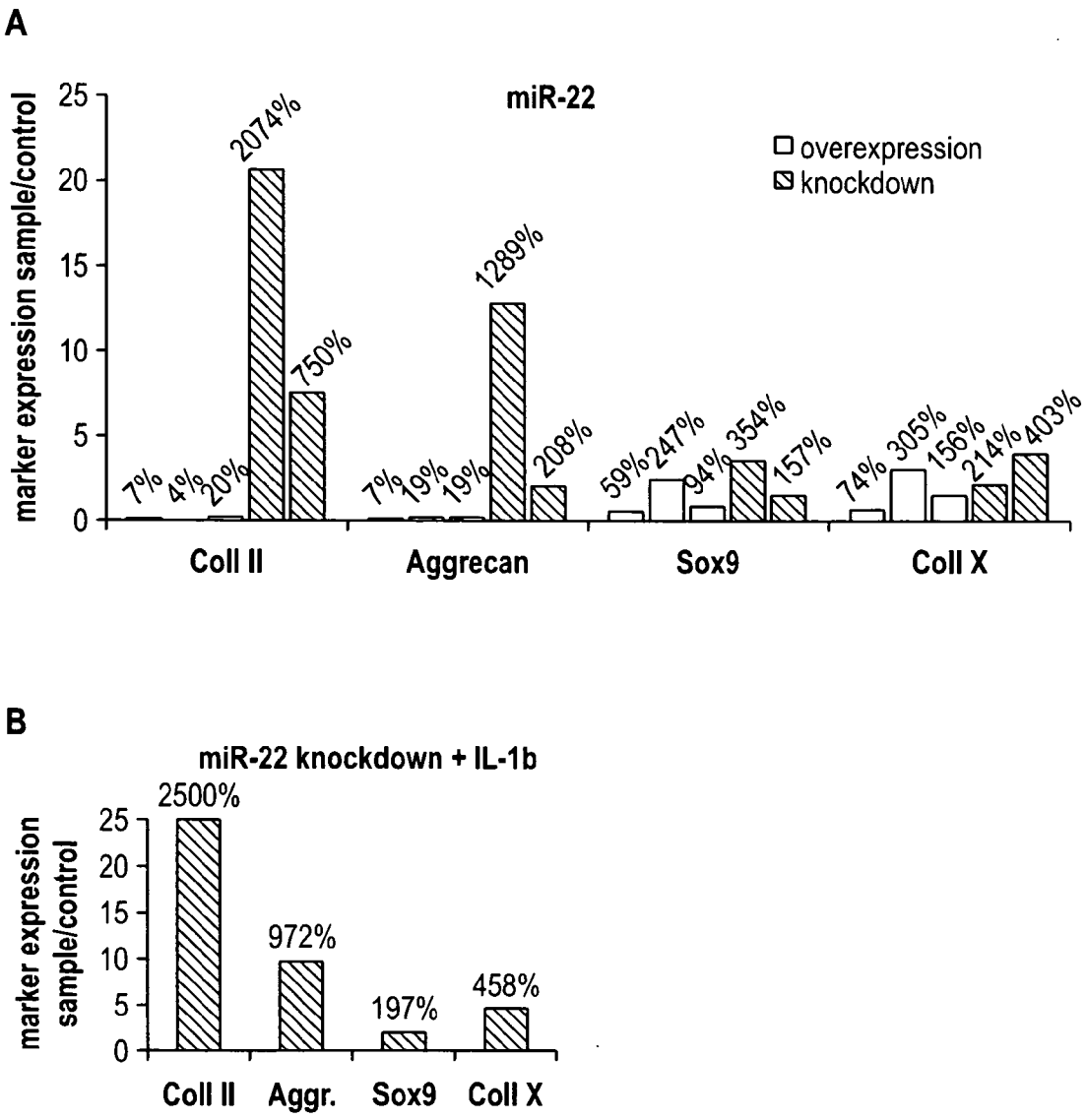
miR-22 inh
control
V22
V23
experiment

Fig. 6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knockdown
marker expression sample/control
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15
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4%
20%
2074%
750%
7%
19%
19%
1289%
208%
59%
247%
94%
354%
157%
74%
305%
156%
214%
403%
Coll II
Aggrecan
Sox9
Coll X
B
miR-22 knockdown + IL-1b
marker expression sample/control
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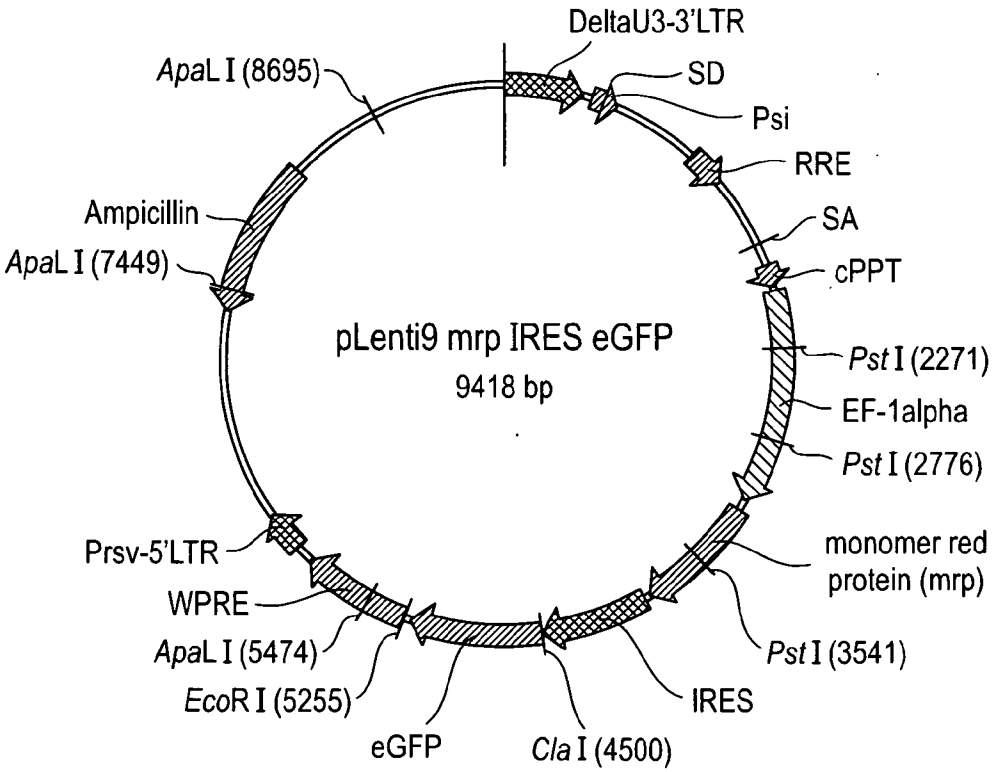
972%
197%
458%
Coll II
Aggr.
Sox9
Coll X

Fig. 7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Psi
RRE
SA
cPPT
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EF-1alpha
Pst I (2776)
monomer red protein (mrp)
Pst I (3541)
IRES
Cla I (4500)
eGFP
EcoR I (5255)
ApaL I (5474)
WPRE
Prsv-5'LTR
ApaL I (7449)
Ampicillin
ApaL I (8695)
pLenti9 mrp IRES eGFP
9418 bp

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/073033

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/7088
ADD.

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)
C12N A61K

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)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, EMBL, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ILIOPOULOS DIMITRIOS ET AL: "Integrative MicroRNA amd Proteomic Approaches Identify Novel Osteoarthritis and Their Collaborative Metabolic and Inflammatory Networks", PLOS ONE, PUBLIC LIBRARY OF SCIENCE, vol. 3, no. 11, 17 November 2008 (2008-11-17), pages E3740-E3749, XP002589646, ISSN: 1932-6203, DOI: 10.1371/JOURNAL.PONE.0003740 figures 1,3,6 -----	1-17
X	WO 2008/116267 A1 (CRC FOR ASTHMA AND AIRWAYS LTD [AU]; FOSTER PAUL STEPHEN [AU]; MATTES) 2 October 2008 (2008-10-02) claims 1,10,11,13,22,24 -----	1-17

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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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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"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
9 May 2012	16/05/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALCARAZ M J ET AL: "New molecular targets for the treatment of osteoarthritis", BIOCHEMICAL PHARMACOLOGY, PERGAMON, OXFORD, GB, vol. 80, no. 1, 1 July 2010 (2010-07-01), pages 13-21, XP027027185, ISSN: 0006-2952 [retrieved on 2010-03-03] page 19, left-hand column	1-17
X	WO 2010/056737 A2 (MIRNA THERAPEUTICS INC [US]; PATRAWALA LUBNA [US]; TANG DEAN G; KELNAR) 20 May 2010 (2010-05-20) claims 1,31,50,54	1-10, 12-15,17
X	WO 2008/142567 A2 (KAROLINSKA INST INNOVATIONS AB [SE]; PIVARCSI ANDOR [SE]; SONKOLY ENIK) 27 November 2008 (2008-11-27) claims 1,14,38,55	1-15,17
X	J ZHANG ET AL: "microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumourigenicity", BRITISH JOURNAL OF CANCER, vol. 103, no. 8, 12 October 2010 (2010-10-12), pages 1215-1220, XP55026413, ISSN: 0007-0920, DOI: 10.1038/sj.bjc.6605895 page 1216, left-hand column; figure 4	1-3, 6-10,12
X	J XIONG ET AL: "Tumor-suppressive microRNA-22 inhibits the transcription of E-box-containing c-Myc target genes by silencing c-Myc binding protein", ONCOGENE, vol. 29, no. 35, 2 September 2010 (2010-09-02), pages 4980-4988, XP55026414, ISSN: 0950-9232, DOI: 10.1038/onc.2010.241 figures 1-3	1-3, 6-10, 12-15
X	M. SUN ET AL: "Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells", MOLECULAR CANCER THERAPEUTICS, vol. 7, no. 3, 1 March 2008 (2008-03-01), pages 464-473, XP55026412, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-07-2272 page 469 - page 470; figures 3-5	1-3, 6-10, 12-15

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ANNALISA PALMIERI ET AL: "Peptide-15 Changes miRNA Expression in Osteoblast-Like Cells", IMPLANT DENTISTRY, vol. 17, no. 1, 1 January 2008 (2008-01-01), pages 100-108, XP55022180, ISSN: 1056-6163, DOI: 10.1097/ID.0b013e318166d182 page 101, right-hand column	1-17
A	D. P. PANDEY ET AL: "miR-22 Inhibits Estrogen Signaling by Directly Targeting the Estrogen Receptor mRNA", MOLECULAR AND CELLULAR BIOLOGY, vol. 29, no. 13, 1 July 2009 (2009-07-01), pages 3783-3790, XP55026416, ISSN: 0270-7306, DOI: 10.1128/MCB.01875-08 the whole document	1-17
A	K. TSUZAKA ET AL: "Micro RNA Expression Profiling in Peripheral Blood Mononuclear Cells From Rheumatoid Arthritis or Healthy Controls", ARTHRITIS & RHEUMATISM, vol. 58, no. 9, Suppl. S, September 2008 (2008-09), page S513, XP55026620, Retrieved from the Internet: URL:https://acr.confex.com/acr/2008/webprogram/Paper2755.html> [retrieved on 2012-05-09] abstract	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/073033

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008116267 A1	02-10-2008	AU 2008232316 A1	02-10-2008
		EP 2136846 A1	30-12-2009
		US 2010113562 A1	06-05-2010
		WO 2008116267 A1	02-10-2008
WO 2010056737 A2	20-05-2010	US 2010179213 A1	15-07-2010
		WO 2010056737 A2	20-05-2010
WO 2008142567 A2	27-11-2008	EP 2152898 A2	17-02-2010
		US 2010202973 A1	12-08-2010
		WO 2008142567 A2	27-11-2008