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(54) Title: DIAGNOSIS AND TREATMENT OF LUPUS NEPHRITIS

(57) Abstract: The present invention relates to markers useful in connection with diagnosis, prognosis, and treatment of subjects suffering from an inflammatory disease, in particular SLE and/or lupus nephritis.



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DIAGNOSIS AND TREATMENT OF LUPUS NEPHRITIS

TECHNICAL FIELD

The present invention relates to the field of diagnosis, prognosis and treatment
5 optimization of inflammatory diseases and disorders – in particular lupus nephritis in humans.

BACKGROUND

Lupus nephritis is the most prevalent and severe manifestation of systemic lupus
erythematosus (SLE) and is characterized by deposition of immune complexes and kidney
inflammation. About half of the SLE subjects experience signs of lupus nephritis. The medical
10 therapy for lupus nephritis depends on the severity of the disease. For mild disease,
corticosteroids are, in general, prescribed. More severe disease requires treatment with
immunosuppressant agents, such as e.g. biological drugs.

Lupus nephritis is ultimately diagnosed by means of kidney biopsies. Efficient and
reliable non-invasive diagnostic methods have not yet been provided. There is thus a need in
15 the art for non-invasive, or essentially non-invasive, diagnostic methods that are preferably
simple and/or fast, and/or reliable and/or cheap. There is furthermore a need in the art for
non-invasive, or essentially non-invasive, preferably simple and cheap methods of monitoring
disease activity and/or means for optimizing and adjusting treatment regimen for subjects
suffering from lupus nephritis.

20 Thus, there is a continuing need to identify new molecular diagnostic or prognostic
markers associated with lupus nephritis. There is also a need to treat subjects that have SLE
(including lupus nephritis). Such subjects may be identified by their expression of biomarkers
associated with lupus nephritis.

SUMMARY

25 As described herein the inventors provide methods useful for improving diagnosis
and therapy of auto-immune diseases, in particular SLE and lupus nephritis.

In a first aspect, the present invention provides a method for monitoring/detecting
the response of a subject to an anti-inflammatory agent, wherein said method comprises
30 obtaining information on the level of expression of two or more M2 macrophage genes in a
sample from said subject, wherein expression of said two or more genes is indicative of a
response of the subject to the anti-inflammatory agent.

In a further aspect, the present invention provides a method for identifying a subject with an increased probability of glomerular inflammation, wherein said method comprises obtaining information on the level of expression of two or more M2 macrophage genes in a sample from said subject, wherein expression of said two or more genes indicates that a subject with a glomerular inflammation has been identified.

In a further aspect, the present invention provides a kit comprising (i) at least one probe/detecting agent for detecting expression of two or more M2 macrophage genes in a urine sample, and (ii) instructions for use of the kit.

In a further aspect, the present invention provides a method of treating lupus nephritis in a subject, comprising administering a pharmaceutically effective amount of an anti-inflammatory drug, preferably a biological drug, to a subject having increased expression of two or more M2 macrophage biomarkers relative to a control subject.

In a further aspect, the present invention provides a method of treating lupus nephritis in a subject, wherein said method comprises the step of detecting two or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA, or RT-PCR.

In a further aspect, the present invention provides a method of treating lupus nephritis in a subject, wherein said method comprises: a) identifying a subject with SLE; b) determining that the subject expresses two or more M2 macrophage biomarkers; c) selecting a biological anti-inflammatory drug as a treatment for the subject; and d) administering the drug to the subject.

In a final aspect, the present invention provides a method of treating lupus nephritis in a subject, wherein said method comprises determining that the subject expresses two or more M2 macrophage biomarkers by detecting two or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA or RT-PCR.

DESCRIPTION

The present invention is associated with methods for identifying subjects, preferably
5 SLE subjects, with glomerular inflammation (lupus nephritis) as well as methods for
monitoring therapeutic effect of an anti-inflammatory compound in a lupus nephritis subject.

In another embodiment, methods of treatment are provided to subjects identified as
having SLE and/or lupus nephritis by virtue of their expression of the biomarker(s) disclosed
herein.

10 In another embodiment, a diagnostic kit for testing the presence or absence of M2
macrophage biomarker(s) is provided.

In another embodiment, a method of determining the therapeutic effectiveness of a
drug administered for treatment of SLE (including lupus nephritis) is provided.

Definitions

15 “Systemic lupus erythematosus” (SLE) is an immune-complex related disorder
characterized by chronic IgG antibody production directed at ubiquitous self antigens such as
anti-dsDNA. The central mediator of disease in SLE is the production of auto-antibodies
20 against self-proteins/tissues and the subsequent generation of immune-mediated
inflammation. Auto-antibodies either directly or indirectly mediate inflammation. The effects of
SLE are systemic (e.g., kidney, lung, musculoskeletal system, muco-cutaneous, eye, central
nervous system cardiovascular system, gastrointestinal tract, bone marrow, blood), rather
than localized to a specific organ, although glomerulonephritis may result in some cases (i.e.
25 lupus nephritis). Multiple chromosomal loci have been associated with the disease and may
contribute towards different aspects of the disease, such as anti-dsDNA antibodies and
glomerulonephritis. Efficacy in SLE in human disease and in appropriate mouse models is
measured by the ability of the therapeutic entity to decrease auto-antibodies (Eg: anti-
dsDNA) and/or by decrease in renal pathology (enhanced kidney function), leading to
30 amelioration of disease symptoms.

The term “macrophage” refers to cells produced by the differentiation of monocytes.
Monocytes and macrophages are phagocytes. Macrophages function in both non-specific
defence (innate immunity) as well as help initiate specific defence mechanisms (adaptive

immunity) of vertebrate animals. Their role is to phagocytize cellular debris and pathogens, either as stationary or as mobile cells. They also stimulate lymphocytes and other immune cells to respond to pathogens. Macrophages can be identified by specific expression of a number of proteins including CD14, CD40, CD11b, F4/80 (mice)/EMR1 (human), lysozyme M, MAC-1/MAC-3 and CD68. Macrophages polarize into an "M1" or an "M2" effector cell subtype depending on various signals in their environment. M1 macrophages undergo a classical activation program induced by pathogen recognition and characterized by antimicrobial and pro-inflammatory effector functions. M2 macrophages undergo an alternative activation program that differentiates them into an effector cell with tissue remodelling and immuno regulatory properties.

M2 macrophages has a role in the pathogenesis of experimental lupus nephritis studied in mice (J Immunol. 2012 May 1;188(9):4568-80. M2 specific markers are described in more detail in Annu. Rev. Immunol. 2009, vol 27, p. 451-483. The inventors of the present invention have shown that M2 macrophage mRNA/protein is present in urine samples from lupus nephritis subjects. The inventors have herein identified a number of preferred mRNA/protein M2 macrophage specific markers (Examples of M2 macrophage specific biomarkers according to the invention are listed in table 1, preferred M2 macrophage biomarkers include. CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, CD84, and MSR1) collectively referred to as the "M2 macrophage markers", identified in urine from lupus nephritis patients with both progressed and moderate disease activity. Such markers, alone or preferably in any combination, may be used as a clinical disease activity marker for SLE/lupus nephritis. Generally, more accurate results are provided in connection with monitoring/detection of more than one (two, three, four, five, six, seven, eight, nine, ten, or more) M2 macrophage markers in a sample according to the present invention. One explanation for this finding is that the markers, in particular on the mRNA level, can have a very low quality and it may therefore be safer and yield a more consistent and accurate result if more than one M2 macrophage markers (e.g. one, two, three, four, five, six, seven, eight, nine, ten, or more) are detected/measured.

The term "SLEDAI" (SLE disease activity index) is used by practitioners to assess the disease state in SLE subjects. SLEDAI score is increased by an average of 3 points in a flare. It follows that the ultimate goal of any treatment of SLE/lupus nephritis is to decrease SLEDAI score.

Quantification of the M2 macrophage biomarkers disclosed herein can be used for following active renal disease in lupus nephritis patients. In general, any measurable

decrease in expression of one, two, or three M2 macrophage biomarkers identified herein can be used as an indication that the drugs used for treating lupus nephritis are efficient. Other urine markers can also potentially be used for following effect of drugs administered to lupus nephritis patients – such as e.g. MCP1 in urine. Lupus nephritis markers present in blood or serum include anti dsDNA, complement factors, such as e.g. C3, C4 as well as anti C1q. Alternatively, evaluation by microscopy of kidney biopsies can also be informative about disease progression as well as assessment of treatment efficacy.

A “subject” is a person that is affected by a particular disease (SLE/lupus nephritis) and/or an SLE patient that is suspected to have glomerular inflammation. A “control subject” is a subject that does not have a disease of interest. In some embodiments, the disease of interest is SLE, preferably lupus nephritis.

The term “array” or “micro-array” refers to an arrangement of hybridisable elements, preferably polynucleotide probes (e.g., oligonucleotides), on a substrate. The substrate can be, for example and without limitation, a solid substrate, such as a glass slide, or a semi-solid substrate, such as a nitrocellulose membrane.

The terms “level of expression” or “expression level” are generally used interchangeably and refer to the amount of a polynucleotide or an amino acid product or protein in a biological sample. “Expressed genes” include those that are transcribed into a polynucleotide as mRNA and then translated into a protein, and also those that are transcribed into RNA but not translated into a protein (for example, transfer and ribosomal RNAs). Levels of expression indicative of glomerular inflammation as determined e.g. on protein level in urine include e.g. 25 pg/ml, or 50 pg/ml, or 75 pg/ml, or 100 pg/ml, or 150 pg/ml or 200 pg/ml, 250 pg/ml, or 300 pg/ml. Determination of expression on mRNA level can be made e.g. by quantitative RT-PCR. Generally, the level of expression of the one or more M2 macrophage biomarkers according to the invention will decrease in connection with efficient treatment, e.g. treatment with anti-inflammatory drugs. Based on this information, optionally in combination with his or her experience, the clinicians can decide on treatment regimen and/or modification of a treatment regimen for the individual subject/patient.

The term “biomarker” as used herein refers to an indicator of a state of a subject; as such biomarkers can be useful for evaluating the disease state of a subject including diagnosis and evaluating response of treatment in an individual. A biomarker is a molecular entity, which can be detected in a biological sample from the subject. Biomarkers include, but

are not limited to, DNA, RNA/mRNA, protein, carbohydrate, and other biochemical entities or moieties, including combinations thereof, for example a glycolipid or a glycoprotein-based molecular marker. "Diagnostic markers" and "prognostic markers" are types of "biomarkers" that indicates that the presence or absence or level of a molecular entity, which can provide information on diagnosis and/or prognosis, including such as the response to one or more types of therapy. Biomarkers may be suited for diagnostics and/or following disease development and treatment response and/or predicting a clinical response to a therapy. In connection with the present invention, expression of biomarkers/markers can be detected on mRNA or protein level. mRNA can be detected using standard methods for assessing mRNA (e.g. multiplex PCR, qRT-PCR, RT-PCR, or cDNA micro-array chip – (e.g. "GeneChip" from Affymetrix or equivalent)). Protein can be detected using conventional protein quantification techniques (e.g. ELISA, Luminex/bioplex, MSD, Western-blot, Biacore, mass spectrometry, antibody arrays and oligonucleotide based aptamer analysis – Somalogic or equivalent) or analysis of protein present on cells or fragment of cells (e.g. flow cytometry, fluorescent activated cell sorting and various forms of microscopy; light microscopy, laser scanning cytometry, fluorescence microscopy, confocal microscopy). Likewise, various probes/detecting agents can be used for detecting said markers, including oligonucleotide probes/primers, micro-array chip probes, antibodies specific for the markers, etc. Examples of suitable probes for various M2 macrophage biomarkers are listed in table 1.

The term "sample" or "biological sample" as used herein refers to a composition that is obtained from or derived from a subject. Preferably, the sample is expected or known to contain a biomarker of interest. Generally, in some embodiments, a sample is obtained or derived from a fluid sample. Types of fluid samples include, without limitation: a urine sample, a blood sample, a serum sample, a plasma sample, a saliva sample, a mucus sample, a sweat sample, a semen sample, a tear sample, a skin sample (e.g. a mouth swab), etc. Fluid samples typically have the advantage of being obtainable in a non-invasive or essentially non-invasive manner or using a non-invasive or an essentially non-invasive method. As used herein, a sample may contain compounds which are not naturally intermixed in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

More specifically, the M2 macrophage markers may be obtained from various samples. In one embodiment, the M2 macrophage markers may be obtained from a kidney tissue sample. In a preferred embodiment, the M2 macrophage markers may be obtained

from a blood or serum sample or from a urine sample, which advantageously is a non-invasive procedure.

A "control sample" refers to a biological sample from a control subject (e.g., an individual or donor) that does not have the disease or condition for which a method or composition of the invention is being used to identify (SLE/lupus nephritis). For example, in some embodiments, a control sample may be a sample, preferably a urine sample, from an individual that does not have SLE or lupus nephritis.

The term "diagnosis" is used herein to refer to the identification or classification of a molecular or pathological state, disease or condition. For example, "diagnosis" may refer to identification of a particular type of inflammatory disease or disorder or a specific autoimmune disease, such as e.g. SLE or lupus nephritis.

The term "subject response" or "response" can be assessed using any endpoint indicating a benefit to the subject, including, without limitation hereto, a) reduction of disease progression, and/or b) reduction in the number of disease episodes and/or symptoms; and/or c) reduction in SLEDAI score; and/or d) reduction in kidney inflammation; and/or e) reduction of expression of M2 macrophage markers in a sample; f) microscopy of a kidney sample; and/or g) decreased mortality at a given point of time following treatment, etc.

"Personalized medicine" is a medical model that proposes the customization of healthcare, with all decisions and practices being tailored to the individual subject by use of subject specific information, such as e.g. genetic information, expression profile information, etc..

Drugs suitable for treating anti-inflammatory diseases such as SLE and lupus nephritis include methotrexate, dexamethasone, and prednisone as well as various biological drugs. Drugs already in use in autoimmunity include immune modulators such as IFN-beta, Orencia (CTLA4-Ig), Humira (anti-TNF), Cimzia (anti-TNF, PEG Fab), Tysabri (α4-integrin mAb), Simponi, Rituxan/MabThera, Actemra/RoActemra, Kineret, Non-steroidal anti-inflammatory drugs (NSAIDs) like Aspirin, Ibuprofen etc, Corticosteroids, disease-modifying antirheumatic drugs (DMARDs) like Plaquenil, Azulfidine, Methotrexate etc, Copaxone (glatirimer acetate), Gilneya (fingolimod), Antibiotics like Flagyl, Cipro, Topical (skin applied) medications including topical corticosteroids, vitamin D analogue creams (Dovonex), topical retinoids (Tazorac), moisturizers, topical immunomodulators (tacrolimus and pimecrolimus),

coal tar, anthralin, and others, Raptiva, Ustekimumab, light therapy like PUVA, UVB, CellCept (mycophenolate mofetil).

It is also envisaged that other biological drugs can be used to reduce inflammation in SLE/lupus nephritis patients, incl. e.g. neutralizing anti IL-21 antibodies, neutralizing anti
5 IL-20 antibodies, neutralizing C5aR antibodies, neutralizing NKG2a antibodies, neutralizing DR3 antibodies (and other neutralizing TNFR antibodies), neutralizing NKG2D antibodies, neutralizing NKG2A antibodies, neutralizing TREM-1 antibodies, neutralizing TNF-alpha antibodies, neutralizing CD20 antibodies, neutralizing GM-CSF antibodies, neutralizing IL-6 antibodies, neutralizing CCR1 antibodies, neutralizing Syk antibodies, neutralizing IL17A
10 antibodies, neutralizing JAK1/2/3 antibodies, neutralizing INF-alpha antibodies, etc. Biologics are created by biologic processes, rather than being chemically synthesized.

“Biological drugs”/“biologics” according to the present invention can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances. Biological drugs are isolated from a variety of natural sources (rather than being chemically
15 synthesized) - human, animal, or microorganism - and may be produced by biotechnology methods and other technologies. Biological drugs according to the present invention are preferably derived from recombinant proteins that may optionally be subject to post-translational modification, such as e.g. conjugation with polymeric compounds and or processing to yield fragments of said recombinant proteins (e.g. processing of an antibody to
20 Fab fragments).

The term “antibody” includes antigen-binding fragments of antibodies, such as e.g. Fab fragments. Antigen binding fragments may be covalently linked to a polymeric moiety in order to e.g. increase the circulatory half life of said fragment. Antibodies are preferably human antibodies or antibodies comprising a human framework (such as e.g. a “humanized”
25 antibody).

The term “treatment”, as used herein, refers to the medical therapy of any human or other animal subject in need thereof. Said subject is expected to have undergone physical examination by a medical or veterinary medical practitioner, who has given a tentative or definitive diagnosis which would indicate that the use of said specific treatment may be
30 beneficial to the health of said human or other animal subject. The timing and purpose of said treatment may vary from one individual to another, according to the status quo of the

subject's health. Thus, said treatment may be prophylactic, palliative, symptomatic and/or curative.

Embodiment 1: A method of treating lupus nephritis in a subject, comprising administering a pharmaceutically effective amount of an anti-inflammatory drug, preferably a biological drug, to a subject having increased expression of one, two, three or more M2 macrophage biomarkers relative to a control subject.

Embodiment 2: A method of treating lupus nephritis in a subject, wherein said method comprises the step of detecting one, two, three, or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA, or RT-PCR.

Embodiment 3: A method of treating lupus nephritis in a subject, wherein said method comprises: a) identifying a subject with SLE; b) determining that the subject expresses one, two, three or more M2 macrophage biomarkers; c) selecting a biological anti-inflammatory drug as a treatment for the subject; and d) administering the drug to the subject.

Embodiment 4: A method of treating lupus nephritis in a subject, wherein said method comprises determining that the subject expresses one, two, three, or more M2 macrophage biomarkers by detecting one, two, three or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA or RT-PCR.

Embodiment 5: A method of treating lupus nephritis in a subject, wherein said method comprises determining that the subject expresses one, two, three or more M2 macrophage biomarkers and: a) detecting the biomarkers in a sample, preferably a urine sample obtained from said subject, by contacting said sample with a primer and extending said primer using individually-labeled dideoxynucleotides.

Embodiment 6: A method of treating lupus nephritis in a subject, wherein said method comprises determining that the subject expresses one, two, three or more M2 macrophage biomarkers and: a) detecting the biomarkers in a sample, preferably a urine sample obtained from said subject, by contacting said sample with one or more M2 macrophage marker antibodies.

Embodiment 7: A method according to any one of embodiments 1-6, wherein the anti-inflammatory drug is a biological drug, such as a neutralizing antibody selected from the group consisting of: an TNF-alpha antibody, an IL-21 antibody, an IL-20 antibody, a C5aR antibody, a TNFR antibody, a DR3 antibody, an NKG2D antibody, a NKG2A antibody, a
5 TREM-1 antibody, a CD20 antibody, a GM-CSF antibody, a IL-6 antibody, a CCR1 antibody, a Syk antibody, a IL17A antibody, a JAK1/2/3 antibody, and an INF-alpha antibody.

Embodiment 8: A method according to any one of embodiments 1-7, wherein the M2 macrophage biomarker(s) is(are) selected from one, two, three or more biomarkers selected from table 1, preferably selected from the list consisting of: CD36, CD86, CPVL,
10 MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, CD84, and MSR1.

Embodiment 9: A method for determining the therapeutic effectiveness of an anti-inflammatory drug, preferably a biological drug, for treatment of lupus nephritis in a subject, comprising: detecting one, two, three, or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA, or RT-PCR,
15 wherein the presence of one, two, three, or more M2 macrophage biomarkers is indicative of the anti-inflammatory drug being therapeutically effective to treat lupus nephritis in said subject.

Embodiment 10: A method for determining the therapeutic effectiveness of an anti-inflammatory drug, preferably a biological drug, for treatment of lupus nephritis in a subject, comprising:
20

a) detecting one, two, three or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by contacting said sample with a M2 macrophage specific primer; and

25 b) extending said primer using individually-labeled dideoxynucleotides;
wherein the presence of an extension product bearing the label associated with the complementary sequence corresponding to the biomarker is indicative of anti-inflammatory drugs being therapeutically effective to treat lupus nephritis in said subject.

30 **Embodiment 11:** A method for determining the therapeutic effectiveness of an anti-inflammatory drug, preferably a biological drug, for treatment of lupus nephritis in a subject, comprising: determining the presence of one, two, three or more M2 macrophage biomarkers in a sample obtained from said subject by contacting said sample with an anti M2

macrophage biomarker antibody, wherein the presence of said biomarker(s) is indicative of compound A being therapeutically effective to treat disease X in said subject.

Embodiment 12: A method for monitoring/detecting the response of a subject to an anti-inflammatory agent, preferably a biological drug, wherein said method comprises obtaining information on the level of expression of one, two, three, four, five, or more M2 macrophage genes in a sample, preferably a urine sample from said subject, wherein expression of said genes is indicative of a response of the subject to the anti-inflammatory agent.

Embodiment 13: A method for identifying a subject with an increased probability of glomerular inflammation (e.g. lupus nephritis), wherein said method comprises obtaining information on the level of expression of one, two, three, four, five or more M2 macrophage genes in a sample, preferably a urine sample from said subject, wherein expression of said genes indicates that a subject with a glomerular inflammation has been identified.

Embodiment 14: A method according to the invention, wherein said M2 macrophage genes are selected from table 1, preferably from the list consisting of: CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, and CD84.

Embodiment 15: A method according to the invention, wherein said method is a non-invasive method or an essentially non-invasive method.

Embodiment 16: A method according to the invention, wherein the subject is an SLE subject, preferably an SLE patient.

Embodiment 17: A method according to the invention, wherein said subject is a lupus nephritis subject.

Embodiment 18: A method according to the invention, wherein expression of M2 macrophage markers is detected on mRNA level, preferably using RT-PCT.

Embodiment 19: The method according to the invention, wherein expression of M2 macrophage markers is detected on protein level, preferably using ELISA or Western blot.

Embodiment 20: A kit comprising (i) at least one probe/detecting agent for detecting expression of one, two, three, four, five, or more M2 macrophage genes in a biological sample, and (ii) instructions for use of the kit.

5 **Embodiment 21:** A kit according to the invention, wherein said probes are specific for one, two, three, four, five, or more M2 macrophage genes selected from table 1, preferably selected from the list consisting of: CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, and CD84.

10 **Embodiment 22:** A kit according to the invention, wherein the sample is a urine sample.

Embodiment 23: A kit according to any one of claims 10-12, wherein said kit further comprises a urine preservation agent.

15

EXAMPLES

Example 1

20 Urine samples were collected from n=20 lupus nephritis patients at three separate time-points along with samples from healthy controls at a single time-point. Total RNA was collected from the urine samples using the urine RNA isolation kit from Zymo Research using the standard protocol. The isolated total RNA was further processed according to the Affymetrix standard protocol for hybridisation to the human HGU133plus2 micro array and
25 scanned on an Affymetrix array scanner. The data was analysed in the QluCore Omics Explorer. In order to define macrophage M2 markers the data was compared to array data from a public dataset available at the NCBI GEO database (GSE5099). The results from this comparative analysis are presented in table 1.

30 **Table 1:** M2 Macrophage Affymetrix GeneChip probes ("Affymetrix probe ID") were used to identify M2 macrophage biomarkers.

Affymetrix probe ID	M2 macrophage biomarker
215049_x_at	CD163
206488_s_at	CD36
205686_s_at	CD86
208146_s_at	CPVL

201487_at	CTSC
211776_s_at	EPB41L3
230559_x_at	FGD4
227265_at	FGL2
204834_at	FGL2
205022_s_at	FOXN3
1554018_at	GPNMB
205419_at	GPR183
203821_at	HBEGF
38037_at	HBEGF
217478_s_at	HLA-DMA
208306_x_at	HLA-DRB1
212454_x_at	HNRPD
200598_s_at	HSP90B1
209185_s_at	IRS2
212830_at	MEGF9
1555728_a_at	MS4A4A
219607_s_at	MS4A4A
224358_s_at	MS4A7
223343_at	MS4A7
212251_at	MTDH
218231_at	NAGK
223405_at	NPL
221210_s_at	NPL
210004_at	OLR1
213142_x_at	PION
212640_at	PTPLB
212636_at	QKI
225188_at	RAPH1
225189_s_at	RAPH1
218909_at	RPS6KC1
212154_at	SDC2
220924_s_at	SLC38A2
207332_s_at	TFRC
201506_at	TGFB1
203888_at	THBD
237252_at	THBD
212352_s_at	TMED10
221218_s_at	TPK1
210645_s_at	TTC3
202664_at	WIPF1
219228_at	ZNF331
227613_at	ZNF331

The integrity of mRNA in urine is very low. Initially, the inventors explored conventional mRNA isolation protocols (e.g. Trizol®) but were not successful in isolating any mRNA. The outcome of any mRNA based analysis is therefore dependent on how the mRNA is isolated. The inventors utilized a urine RNA purification system from Zymo Research Corporation (ZR Urine RNA Isolation Kit™). This kit comprises a filter that captures soluble RNA and cells and cell debris material and thereby enable isolation of a consistent mRNA quality from urine samples. The procedure is carried out in two steps 1. urine filtration followed by lysis and elution of the filtered content; 2 collection and isolation of the total RNA in column based gel matrix.

Urine can furthermore be preserved by stabilisation systems from e.g. Sierra Molecular Corporation (GeneLock®) or Norgen Biotek Corporation (Urine Collection and preservation tubes) prior to RNA isolation. Use of stabilization systems is an advantage is the urine sample is taken in a non-clinical setting, e.g. at home.

CLAIMS

1. A method for monitoring/detecting the response of a subject to an anti-inflammatory agent, wherein said method comprises obtaining information on the level of expression of two or more M2 macrophage genes in a sample from said subject, wherein expression of said two or more genes is indicative of a response of the subject to the anti-inflammatory agent.
2. A method for identifying a subject with an increased probability of glomerular inflammation, wherein said method comprises obtaining information on the level of expression of two or more M2 macrophage genes in a sample from said subject, wherein expression of said two or more genes indicates that a subject with a glomerular inflammation has been identified.
3. A method according to any one of the preceding claims, wherein said M2 macrophage genes are selected from the list consisting of: CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, and CD84.
4. A method according to any one of the preceding claims, wherein the sample is a urine sample.
5. A method according to any one of the preceding claims, wherein the subject is an SLE patient.
6. A method according to any one of the preceding claims, wherein said subject is a lupus nephritis patient.
7. A method according to any one of the preceding claims, wherein expression of M2 macrophage markers is detected on mRNA level.
8. The method according to any one of the preceding claims, wherein expression of M2 macrophage markers is detected on protein level.
9. A kit comprising (i) at least one probe/detecting agent for detecting expression of two or more M2 macrophage genes in a urine sample, and (ii) instructions for use of the kit.

10. A kit according to claim 9, wherein said probes are specific for two or more M2 macrophage genes selected from the list consisting of: CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, and CD84.

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11. A kit according to any one of claims 9-10, wherein said kit further comprises a urine preservation agent.

12. A method of treating lupus nephritis in a subject, comprising administering a pharmaceutically effective amount of an anti-inflammatory drug, preferably a biological drug, to a subject having increased expression of two or more M2 macrophage biomarkers relative to a control subject.

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13. A method of treating lupus nephritis in a subject, wherein said method comprises the step of detecting two or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA, or RT-PCR.

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14. A method of treating lupus nephritis in a subject, wherein said method comprises: a) identifying a subject with SLE; b) determining that the subject expresses two or more M2 macrophage biomarkers; c) selecting a biological anti-inflammatory drug as a treatment for the subject; and d) administering the drug to the subject.

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15. A method of treating lupus nephritis in a subject, wherein said method comprises determining that the subject expresses two or more M2 macrophage biomarkers by detecting two or more M2 macrophage biomarkers in a sample, preferably a urine sample obtained from said subject, by micro-array, ELISA or RT-PCR.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/066164

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

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

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

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

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

see additional sheet

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

3, 10(completely); 1, 2, 4-9, 11-15(partially)

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/066164

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
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)
C12Q

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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/238094 A1 (CHAUSSABEL DAMIEN [US] ET AL) 11 October 2007 (2007-10-11) abstract; claims 1-28 paragraph [0016] - paragraph [0018] paragraph [0036] paragraph [0046] - paragraph [0047]; table 2 paragraph [0103] -----	1-15
X	US 2010/267033 A1 (ABBAS ALEXANDER [US] ET AL) 21 October 2010 (2010-10-21) paragraph [0008] - paragraph [0018] paragraph [0041] - paragraph [0042] paragraph [0095] paragraph [0081] ----- -/-	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

1 October 2013

Date of mailing of the international search report

13/12/2013

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/066164

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/285458 A1 (BANKAITIS-DAVIS DANUTE [US]) 11 November 2010 (2010-11-11) paragraph [0012] - paragraph [0014]; table 2 paragraph [0026] - paragraph [0029] paragraph [0043] paragraph [0061] paragraph [0102] - paragraph [0105] paragraph [0135] - paragraph [0136] -----	1-5,7-15
X	US 2008/292622 A1 (BURKLY LINDA C [US] ET AL) 27 November 2008 (2008-11-27) paragraph [0003] - paragraph [0006] paragraph [0011] - paragraph [0022] paragraph [0026] - paragraph [0028] -----	1-5,7,8, 10-15
X	"Affymetrix Human Genome U133 Plus 2.0 Array [CDF: HGU133Plus2_Hs_ENSG v12.1.0]", GEO 19 April 2010 (2010-04-19), XP002668741, Retrieved from the Internet: URL: http://www.ncbi.nlm.gov/geo/queria/acc.cgi?acc=GPL9987 [retrieved on 2010-04-19] the whole document -----	9,10
A	US 2003/228617 A1 (AUNE THOMAS M [US] ET AL) 11 December 2003 (2003-12-11) paragraph [0009] - paragraph [0013] paragraph [0026] - paragraph [0060] paragraph [0228]; table 2 -----	1-15
A	CROW M K ET AL: "MICROARRAY ANALYSIS OF GENE EXPRESSION IN LUPUS", ARTHRITIS RESEARCH AND THERAPY, BIOMED CENTRAL, LONDON, GB, vol. 5, no. 6, 1 January 2003 (2003-01-01), pages 279-287, XP001189382, ISSN: 1478-6354, DOI: 10.1186/AR1015 abstract; tables 1-2 -----	1-15
A	FERNANDO O. MARTINEZ ET AL: "Alternative Activation of Macrophages: An Immunologic Functional Perspective", ANNUAL REVIEW OF IMMUNOLOGY, vol. 27, no. 1, 1 April 2009 (2009-04-01), pages 451-483, XP055063397, ISSN: 0732-0582, DOI: 10.1146/annurev.immunol.021908.132532 cited in the application the whole document ----- -/--	1-15

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/066164

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Y. IWATA ET AL: "Aberrant Macrophages Mediate Defective Kidney Repair That Triggers Nephritis in Lupus-Susceptible Mice", THE JOURNAL OF IMMUNOLOGY, vol. 188, no. 9, 1 May 2012 (2012-05-01), pages 4568-4580, XP055052787, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1102154 cited in the application abstract -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/066164

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			WO 2004046098 A2 03-06-2004

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 3, 10(completely); 1, 2, 4-9, 11-15(partially)

Method for monitoring/detecting response to an anti-inflammatory agent;
Method for identifying a subject with an increased probability of glomerular inflammation;
Kit comprising at least one probe therefor;
Methods of treating lupus nephritis in a subject; comprising detecting the expression of two or more M2 macrophage genes, preferably selected from CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52 and CD84.

2. claims: 1, 2, 4-9, 11-15(all partially)

Method for monitoring/detecting response to an anti-inflammatory agent;
Method for identifying a subject with an increased probability of glomerular inflammation;
Kit comprising at least one probe therefor;
Methods of treating lupus nephritis in a subject; comprising detecting the expression of two or more M2 macrophage genes, preferably selected from Table 1.

3. claims: 1, 2, 4-9, 11-15(all partially)

Method for monitoring/detecting response to an anti-inflammatory agent;
Method for identifying a subject with an increased probability of glomerular inflammation;
Kit comprising at least one probe therefor;
Methods of treating lupus nephritis in a subject; comprising detecting the expression of two or more M2 macrophage genes, preferably selected from CD36, CD86, CPVL, MS4A4A, SDC2, THBD, ZNF331, CD163, CD52, CD84 and MSR1.
