(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 October 2015 (22.10.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/160786 A1

(51) **International Patent Classification**:
G01N 33/564 (2006.01) *C07K 16/28* (2006.01)

(21) **International Application Number**:
PCT/US2015/025719

(22) **International Filing Date**:
14 April 2015 (14.04.2015)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/979,322 14 April 2014 (14.04.2014) US

(71) **Applicants**: **BRIGHAM AND WOMEN'S HOSPITAL, INC.** [US/US]; 75 Francis Street, Boston, Massachusetts 02115 (US). **UNIVERSITÄTSMEDIZIN DER JOHANNES GUTENBERG-UNIVERSITÄT MAINZ** [DE/DE]; Langenbeckstrasse 2, 55131 Mainz (DE).

(72) **Inventors**: **KELLEY, Vicki Rubin**; 75 Francis Street, Boston, Massachusetts 02115 (US). **SCHWARTING, Andreas**; Langenbeckstrasse 2, 55131 Mainz (DE). **WEINMANN-MENKE, Julia**; Langenbeckstrasse 2, 55131 Mainz (DE).

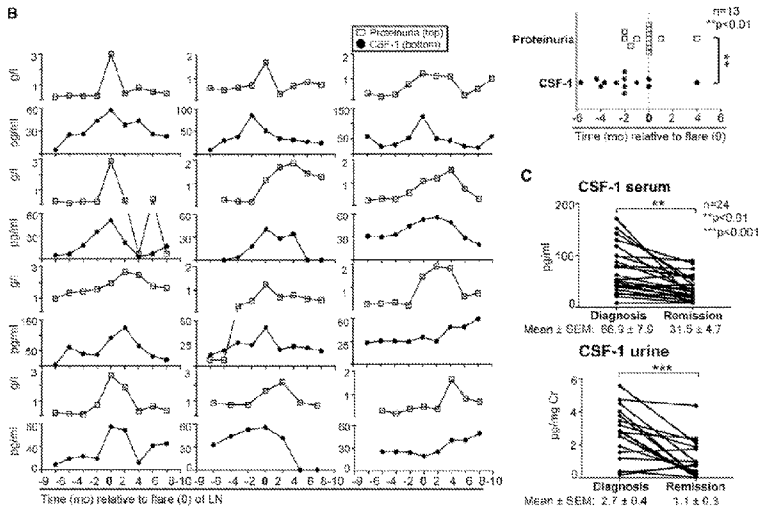
(74) **Agents**: **RESNICK, David S.** et al.; Nixon Peabody LLP, 100 Summer Street, Boston, Massachusetts 02110 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) **Title**: METHOD OF DIAGNOSING, PROGNOSING, AND TREATING LUPUS NEPHRITIS



FIGs. 4A-4C

(57) **Abstract**: The present invention relates to methods of identifying the risk of a subject developing lupus nephritis (LN) by measuring the CSF-1 level in a sample obtained from the subject. For a subject having systemic lupus erythematosus (SLE) or in remission of LN, tracking the CSF-1 levels of the subject over time can permit one to predict the likelihood of the subject developing LN or LN flares. For a subject having LN and receiving treatment for LN, the treatment progress can be monitored by tracking the CSF-1 levels of the subject over time. Furthermore, CSF-1 can be a therapeutic target for treating LN.

WO 2015/160786 A1

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

METHOD OF DIAGNOSING, PROGNOSING, AND TREATING LUPUS NEPHRITIS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/979,322 filed April 14, 2014, the contents of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

[0002] The present invention relates generally to methods of diagnosing, prognosing, and treating lupus nephritis.

BACKGROUND

[0003] Lupus nephritis (LN) is the main cause of morbidity and mortality for patients with systemic lupus erythematosus (SLE). Despite current treatment end-stage renal disease is frequent in LN patients (up to 26%). Moreover, relapses or flares of LN are common (27-66%) and contribute to mortality. Current methods for diagnosing LN in patients with SLE include proteinuria, active sediment and a decrease in glomerular filtration rate with confirmation by a renal biopsy. Clearly, identification of a biomarker that heralds LN prior to structural renal damage and the loss of renal function would provide a window to treat early, and obviate renal tissue injury.

SUMMARY

[0004] As described herein, serum or urine CSF-1 levels are found to be elevated in patients with lupus nephritis (LN). CSF-1 can thus be used as a biomarker for the diagnosis of lupus nephritis prior to renal damage. Moreover, for a subject having SLE or in remission of LN, tracking the CSF-1 levels of the subject over time can permit one to predict the likelihood of the subject developing LN or LN flares. For a subject having LN and receiving treatment for LN, the treatment progress can be monitored by tracking the CSF-1 levels of the subject over time. Furthermore, CSF-1 can be a therapeutic target for treating LN.

[0005] In one aspect, the technology described herein relates to a method of monitoring disease activity in a subject with systemic lupus erythematosus, the method comprising: (i) measuring, at each of two or more time points, a level of CSF-1 in a sample obtained from the subject; (ii) determining a temporal trend of the level of CSF-1 as a function of the

measurements of step (i); and (iii) identifying the subject as (a) having an increased risk of developing lupus nephritis if the temporal trend is generally upward, or (b) having no or minimal risk of developing lupus nephritis if the temporal trend is generally flat or downward. In some embodiments, the level of CSF-1 is measured at three or more time points.

[0006] In one aspect, the technology described herein relates to a method of monitoring disease activity in a subject in remission of lupus nephritis (LN), the method comprising: (i) measuring, at each of two or more time points, a level of CSF-1 in a sample obtained from the subject; (ii) determining a temporal trend of the level of CSF-1 as a function of the measurements of step (i); and (iii) identifying the subject as (a) having an increased risk of developing LN flares if the temporal trend is generally upward, or (b) having no or minimal risk of developing LN flares if the temporal trend is generally flat or downward. In some embodiments, the level of CSF-1 is measured at three or more time points.

[0007] In one aspect, the technology described herein relates to a method of monitoring treatment progress in a subject having lupus nephritis, the method comprising: (i) measuring, at a first time point, a first level of CSF-1 in a first sample obtained from the subject; (ii) administering to the subject a therapeutic agent for treating lupus nephritis; and (iii) measuring, at a second time point, a second level of CSF-1 in a second sample obtained from the subject, wherein the second time point is later than the first time point and after said administering, and wherein if the second level is significantly lower than the first level, then the treatment is considered to be effective. In some embodiments of monitoring treatment progress, the first sample and the second sample are of the same type, each selected from the group consisting of blood, plasma, serum, and urine.

[0008] In some embodiments of any one of the foregoing aspects, the level of CSF-1 is a protein level.

[0009] In some embodiments of any one of the foregoing aspects, the level of CSF-1 is measured by an immunoassay.

[0010] In some embodiments of any one of the foregoing aspects, the sample is contacted with an anti-CSF-1 antibody.

[0011] In some embodiments of any one of the foregoing aspects, the anti-CSF-1 antibody is detectably labeled or capable of generating a detectable signal.

[0012] In some embodiments of any one of the foregoing aspects, the antibody is fluorescently labeled.

[0013] In some embodiments of any one of the foregoing aspects, the level of CSF-1 is measured by measuring a nucleic acid encoding CSF-1.

[0014] In some embodiments of any one of the foregoing aspects, the sample is selected from the group consisting of a blood, plasma, serum, and urine sample.

[0015] In yet another aspect, the technology described herein relates to a method of treating lupus nephritis in a subject, the method comprising administering a therapeutically-effective amount of a CSF-1 inhibitor to the subject. In some embodiments, the CSF-1 inhibitor decreases the expression level of CSF-1 protein. In some embodiments, the CSF-1 inhibitor decreases the activity of CSF-1 protein. In some embodiments, the CSF-1 inhibitor is selected from the group consisting of a small molecule, a nucleic acid, a nucleic acid analog or derivative, a peptide, a peptidomimetic, a protein, an antibody or an antigen-binding fragment thereof, a saccharide, a lipid, a glycosaminoglycan, an extract made from a biological material, and combinations thereof.

[0016] In some embodiments of any one of the foregoing aspects, the subject is a mammal.

[0017] In some embodiments of any one of the foregoing aspects, the mammal is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **FIGs. 1A-1D** demonstrate that serum and urine CSF-1 are elevated in patients with SLE with LN, CLE, serositis, and musculoskeletal manifestations compared with healthy controls (two cohorts) and noninflammatory kidney disease (only Mainz cohort) but are highest in patients with LN. CSF-1 levels in SLE (FIGs. 1A and 1C) compared with healthy controls and SLE with LN, CLE, serositis, and musculoskeletal manifestations (FIGs. 1B and 1D) compared with healthy controls and noninflammatory kidney disease. CSF-1 was quantified using ELISA assay. Values are means±SEM. Analysis was done using Mann–Whitney U test.

[0019] **FIGs. 2A-2D** demonstrate that serum and urine CSF-1 levels reflect intrarenal CSF-1 expression and histopathology disease activity. (FIG. 2A) Left panel: Representative photomicrograph (original magnification, 320) illustrating CSF-1 expression in renal TEC in type IV and type II LN. Graphs of CSF-1 levels in TEC and glomeruli in type IV and type II LN. Right panel: Correlation of serum or urine CSF-1 levels with CSF-1 expression in TEC before therapy. (FIG. 2B) Correlation of intrarenal Mø (CD68+) with CSF-1 in LN and

noninflammatory kidney diseases with serum or urine CSF-1 in LN. (FIG. 2C) Correlation of CSF-1 in kidney, serum, and urine with histopathology activity and chronicity indices in LN. (FIG. 2D) Serum and urine CSF-1 levels stratified according to ISN/RPS classification of LN. CSF-1 and CD68 were detected on renal biopsy specimens by immunostaining and serum or urine CSF-1 quantified using ELISA. Values are means±SEM. Analysis was done using Spearman correlation calculation.

[0020] **FIG. 3** is a set of graphs demonstrating that longitudinally monitoring CSF-1 in serum positively correlates with disease activity in two cohorts. Serum or urine CSF-1 at diagnosis of biopsy-proven LN and during therapy in comparison to conventional clinical disease activity measures. CSF-1 was quantified using ELISA. Values are means±SEM. Analysis was done using the Kruskal–Wallis test for multiple comparisons. CRP, C-reactive protein.

[0021] **FIGs. 4A-4C** are graphs demonstrating that serum and urine CSF-1 levels increase before proteinuria and flares in LN and declined during remission. (FIG. 4A) Composite patient values. Serum and urine CSF-1 levels in LN compared with glomerular dysfunction and serologic measures before (visualized as red), during, and after LN flares. Flare is indicated as 0 time point. CSF-1 was quantified using ELISA. (FIG. 4B) Individual patient values. Left panel: Individual serum CSF-1 levels and proteinuria in patients with LN monitored before flares, at diagnosis, and after flares. Right panel: Comparison of longitudinally tracked elevated CSF-1 (50% greater than normal levels) versus proteinuria (.500 mg/24 hours) for each individual patient at time points before the patient's flare. Prior to the flare, the rise in CSF-1 levels (58±20 days) preceded an increase in proteinuria (4±13 days). (FIG. 4C) Serum and urine CSF1 levels in LN at diagnosis (biopsy proven) compared with remission. Values are means±SEM.

[0022] **FIGs. 5A-5B** are graphs demonstrating that elevated serum and urine CSF-1 are a prognostic indicator of LN. (FIG. 5A) Composite patient values. Longitudinal tracked serum and urine CSF-1 levels in patients with SLE before (visualized as red) and at diagnosis of LN (time of renal biopsy, indicated as 0 time point) and at follow-up visits compared with conventional clinical indices of LN. Most patients had active LN without other lupus manifestation symptoms. Arthralgia, malar rash, and mild cutaneous manifestations were seen in <25% of the evaluated patients with LN. CSF-1 was quantified using ELISA. (FIG. 5B) Individual patient values. Left panel: Display of CSF-1 and proteinuria in each individual longitudinally tracked patient before, at, and after renal biopsy. Right panel: Comparison of elevated CSF-1 (defined as 50% higher than baseline) versus proteinuria (defined as urinary

protein levels of $500 mg/24 hours) at each point before the patient's renal biopsy. Before new-onset LN, the rise in CSF-1 levels (93±15 days) preceded an increase in proteinuria (27±9 days). Values are means±SEM. CRP, C-reactive protein.

[0023] **FIGs. 6A-6C** are graphs demonstrating that serum and urine CSF-1 analysis is highly accurate and specific and values are reproducibly stable. (FIG. 6A) Nonparametric receiver operating characteristic (ROC) curve for serum and urine CSF-1 in patients with SLE (n=217) and patients with LN (n=84) versus healthy controls (n=162). (FIG. 6B) Serum and urine CSF-1 levels urine tracked through 1-4 cycles of freezing and thawing specimens. (FIG. 6C). Left panel: Serum CSF-1 levels from healthy volunteers compared initially and after 3-5 and 6-8 mo. Right panel: Serum CSF-1 levels from SLE patients in remission compared initially and after 3-5, 6-8 and 9-12 mo. Serum and urine CSF-1 levels were quantified using an ELISA.

[0024] **FIG. 7** is a diagram of an exemplary embodiment of a system for performing an assay for determining the level of CSF-1 in a sample obtained from a subject.

[0025] **FIG. 8** is a diagram of an exemplary embodiment of an analysis module as described herein.

[0026] **FIG. 9** is a diagram of an exemplary embodiment of an operating system and applications for a computing system as described herein.

[0027] **FIGs. 10A-10B** are graphs demonstrating that elevated serum and urine CSF-1 are a prognostic indicator of LN. The data are obtained from 3 additional patients for predicting LN in patients without a prior history of this illness.

DETAILED DESCRIPTION

[0028] The invention is based, in part, on the discovery that serum or urine CSF-1 levels are elevated in patients with lupus nephritis (LN). In addition, it was discovered that, in longitudinally tracked patients, elevated serum CSF-1 heralded the initial onset of disease, and a rise in serum or urine CSF-1 predicted recurrences of LN before clinical evidence of glomerular dysfunction and conventional serologic measures, even in patients with other manifestations of systemic lupus erythematosus (SLE).

[0029] In one aspect, the technology described herein relates to a method of monitoring disease activity in a subject with SLE or a subject in remission of LN. In some embodiments, the technology described herein provides a noninvasive means to predict the onset and recurrence of lupus nephritis (LN) before overt renal injury to optimize and individualize treatment. The method comprises (i) measuring, at a first time point, a first level of CSF-1 in

a first sample obtained from the subject; and (ii) measuring, at a second time point, a second level of CSF-1 in a second sample obtained from the subject, wherein the second time point is later than the first time point, and wherein if the second level is significantly higher than the first level, then the subject with SLE is identified as having an increased risk of developing LN, or the subject in remission of LN is identified as having an increased risk of developing LN flares.

[0030] In some embodiments of the methods of monitoring disease activity, the second level is at least 10% higher, at least 20% higher, at least 25% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 60% higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 100% higher, at least 2x higher than the first level. The second level can be measured several hours, days, or months after the measurement of the first level.

[0031] In some embodiments of the methods of monitoring disease activity, longitudinally tracking the level of CSF-1 can be utilized to predict whether a subject with SLE will develop LN. The CSF-1 level can be measured at two or more time points (e.g., 3, 4, 5, 6, 7, 8, 9, or more), permitting a temporal trend of the CSF-1 level to be established. In some embodiments, the temporal trend can be determined by visual inspection. In some embodiments, the temporal trend can be established by performing a mathematical fit to the data to obtain a slope. The temporal trend can show whether the CSF-1 level is upward, flat, or downward over time. An upward temporal trend can indicate that the subject with SLE has an increased risk of developing LN. A flat or downward temporal trend can indicate that the subject with SLE has no or minimal risk of developing LN.

[0032] In some embodiments, an upward temporal trend with a slope of at least 0.1 can indicate that the subject with SLE has an increased risk of developing LN. Preferably, in some embodiments, an upward temporal trend with a slope of at least 0.2 can indicate that the subject with SLE has an increased risk of developing LN. In some embodiments, an upward temporal trend with a slope of at least 0.3 can indicate that the subject with SLE has an increased risk of developing LN. In some embodiments, an upward temporal trend with a slope of at least 0.4 can indicate that the subject with SLE has an increased risk of developing LN. In some embodiments, an upward temporal trend with a slope of at least 0.5 can indicate that the subject with SLE has an increased risk of developing LN.

[0033] Similarly, longitudinally tracking the level of CSF-1 can be utilized to predict whether a subject in remission of LN will develop LN flares. An upward temporal trend can indicate that the subject in remission of LN has an increased risk of developing LN flares. A

flat or downward temporal trend can indicate that the subject in remission of LN has no or minimal risk of developing LN flares.

[0034] In some embodiments, an upward temporal trend with a slope of at least 0.1 can indicate that the subject in remission of LN has an increased risk of developing LN flares. In some embodiments, an upward temporal trend with a slope of at least 0.2 can indicate that the subject in remission of LN has an increased risk of developing LN flares. In some embodiments, an upward temporal trend with a slope of at least 0.3 can indicate that the subject in remission of LN has an increased risk of developing LN flares. In some embodiments, an upward temporal trend with a slope of at least 0.4 can indicate that the subject in remission of LN has an increased risk of developing LN flares. In some embodiments, an upward temporal trend with a slope of at least 0.5 can indicate that the subject in remission of LN has an increased risk of developing LN flares.

[0035] In some embodiments, the subject is identified as having an increased risk of developing LN or LN flares at least 10 days, at least 20 days, at least 30 days, or at least 40 days before the symptom onset. Symptoms of LN include, but are not limited to, swelling of the legs, ankles, and/or feet, weight gain, high blood pressure, dark urine, foamy urine, frothy urine, and the need to urinate during the night.

[0036] It should be noted that when levels of CSF-1 are compared or used to establish a trend, the samples used to measure these CSF-1 levels should be of the same type (e.g., blood, serum, plasma, or urine).

[0037] In yet another aspect, the technology described herein relates to a method of monitoring treatment progress in a subject having lupus nephritis, the method comprising: (i) measuring, at a first time point, a first level of CSF-1 in a first sample obtained from the subject; (ii) administering to the subject a therapeutic agent for treating lupus nephritis; and (iii) measuring, at a second time point, a second level of CSF-1 in a second sample obtained from the subject, wherein the second time point is later than the first time point and after said administering, and wherein if the second level is significantly lower than the first level, then the treatment is considered to be effective.

[0038] In some embodiments of the methods of monitoring treatment progress, longitudinally tracking the level of CSF-1 can also be utilized to assess the treatment efficacy. A downward temporal trend of CSF-1 level can indicate that the treatment is effective, while a flat or upward temporal trend can indicate the treatment is not effective or that the subject is not responsive to the treatment. The magnitude of the slope, as described previously, can be used to determine the treatment efficacy.

[0039] In some embodiments, the methods and assays described herein include (a) transforming the CSF-1 into a detectable target; (b) measuring the amount of the target; and (c) determining the slope of the temporal change in CSF-1 level in the subject.

[0040] As used herein, the term "transforming" or "transformation" refers to changing an object or a substance, e.g., biological sample, nucleic acid or protein, into another substance. The transformation can be physical, biological or chemical. Exemplary physical transformation includes, but not limited to, pre-treatment of a biological sample, e.g., from whole blood to blood serum by differential centrifugation. A biological/chemical transformation can involve at least one enzyme and/or a chemical reagent in a reaction. For example, a DNA sample can be digested into fragments by one or more restriction enzymes, or an exogenous molecule can be attached to a fragmented DNA sample with a ligase. In some embodiments, a DNA sample can undergo enzymatic replication, e.g., by polymerase chain reaction (PCR). In some embodiments, mRNA can be transformed to cDNA for PCR.

[0041] Transformation, measurement, and/or detection of a target molecule, e.g. a CSF-1 mRNA or polypeptide can comprise contacting a sample obtained from a subject with a reagent (e.g. a detection reagent) which is specific for the target, e.g., a CSF-1-specific reagent. In some embodiments, the target-specific reagent is detectably labeled. In some embodiments, the target-specific reagent is capable of generating a detectable signal. In some embodiments, the target-specific reagent generates a detectable signal when the target molecule is present.

[0042] Methods to measure CSF-1 gene expression products are well known to a skilled artisan. Such methods to measure gene expression products, e.g., protein level, include ELISA (enzyme linked immunosorbent assay), western blot, immunoprecipitation, and immunofluorescence using detection reagents such as an antibody or protein binding agents. Alternatively, a peptide can be detected in a subject by introducing into a subject a labeled anti-peptide antibody and other types of detection agent. For example, the antibody can be labeled with a detectable marker whose presence and location in the subject is detected by standard imaging techniques.

[0043] For example, antibodies for CSF-1 are commercially available from vendors such as Fisher Scientific, and can be used for the purposes of the invention to measure protein expression levels, e.g. anti-CSF-1. Alternatively, since the amino acid sequences for CSF-1 are known and publicly available at NCBI website, one of skill in the art can raise their own antibodies against these polypeptides of interest for the purpose of the invention.

[0044] The amino acid sequences of the polypeptides described herein, e.g. CSF-1 have been assigned NCBI accession numbers for different species such as human, mouse and rat. In particular, the NCBI accession number for the amino acid sequence of human CSF-1 is included herein, see SEQ ID NO: 1.

[0045] SEQ ID NO: 1 human CSF-1 polypeptide NCBI Ref Seq: NP_000748

1 mtapgaagrc ppttwlgsll llvcllasrs iteevseycs hmigsghlqs lqrlidsqme

61 tscqitfefv dqeqlkdpvc ylkkafllvq dimedtmrfr dntpnaiaiv qlqelslrlk

121 scftkdyeeh dkacvrtfye tplqllekvk nvfnetknll dkdwnifskn cnnsfaecss

181 qdvvtkpdcn clypkaipss dpasvsphqp lapsmapvag ltwedsegte gssllpgeqp

241 lhtvdpgsak qrpprstcqs feppetpvvk dstiggspqp rpsvgafnpg medildsamg

301 tnwvpeeasg easeipvpqg telspsrpgg gsmqteparp snflsasspl pasakgqqpa

361 dvtgtalprv gpvrptgqdw nhtpqktdhp sallrdppep gsprisslrp qglsnpstls

421 aqpqlsrshs sgsvlplgel egrrstrdrr spaepeggpa segaarplpr fnsvpltdtg

481 herqsegsss pqlqesvfhl lvpsvilvll avggllfyrw rrrshqepqr adspleqpeg

541 spltqddrqv elpv

[0046] In some embodiments, immunohistochemistry ("IHC") and immunocytochemistry ("ICC") techniques can be used. IHC is the application of immunochemistry to tissue sections, whereas ICC is the application of immunochemistry to cells or tissue imprints after they have undergone specific cytological preparations such as, for example, liquid-based preparations. Immunochemistry is a family of techniques based on the use of an antibody, wherein the antibodies are used to specifically target molecules inside or on the surface of cells. The antibody typically contains a marker that will undergo a biochemical reaction, and thereby experience a change of color or other readily detectable property, upon encountering the targeted molecules. In some instances, signal amplification can be integrated into the particular protocol, wherein a secondary antibody, that includes the marker stain or marker signal, follows the application of a primary specific antibody.

[0047] In some embodiments, the assay can be a Western blot analysis. Alternatively, proteins can be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is well known in the art and typically involves iso-electric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. These methods also require a considerable amount of cellular material. The analysis of 2D SDS-PAGE gels can be performed by determining the intensity of protein spots on the gel, or can

be performed using immune detection. In other embodiments, protein samples are analyzed by mass spectroscopy.

[0048] Immunological tests can be used with the methods and assays described herein and include, for example, competitive and non-competitive assay systems using techniques such as Western blots, radioimmunoassay (RIA), ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, immunodiffusion assays, agglutination assays, e.g. latex agglutination, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, e.g. FIA (fluorescence-linked immunoassay), chemiluminescence immunoassays (CLIA), electrochemiluminescence immunoassay (ECLIA, counting immunoassay (CIA), lateral flow tests or immunoassay (LFIA), magnetic immunoassay (MIA), and protein A immunoassays. Methods for performing such assays are known in the art, provided an appropriate antibody reagent is available. In some embodiments, the immunoassay can be a quantitative or a semi-quantitative immunoassay.

[0049] An immunoassay is a biochemical test that measures the concentration of a substance in a biological sample, typically a fluid sample such as urine, using the interaction of an antibody or antibodies to its antigen. The assay takes advantage of the highly specific binding of an antibody with its antigen. For the methods and assays described herein, specific binding of the target polypeptides with respective proteins or protein fragments, or an isolated peptide, or a fusion protein described herein occurs in the immunoassay to form a target protein/peptide complex. The complex is then detected by a variety of methods known in the art. An immunoassay also often involves the use of a detection antibody.

[0050] Enzyme-linked immunosorbent assay, also called ELISA, enzyme immunoassay or EIA, is a biochemical technique to detect the presence of an antibody or an antigen in a sample via enzymatic conversion of an indicator substrate generally to a visible or optically detectable form. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries.

[0051] In one embodiment, an ELISA involving at least one antibody with specificity for the particular desired antigen (e.g., CSF-1 as described herein) can also be performed. A known amount of sample and/or antigen is immobilized on a solid support (usually a polystyrene micro titer plate). Immobilization can be either non-specific (e.g., by adsorption to the surface) or specific (e.g. where another antibody immobilized on the surface is used to capture antigen or a primary antibody). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be

covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bio-conjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. Older ELISAs utilize chromogenic substrates, though newer assays employ fluorogenic substrates with much higher sensitivity.

[0052] In another embodiment, a competitive ELISA is used. Purified antibodies that are directed against a target polypeptide or fragment thereof are coated on the solid phase of multi-well plate, i.e., conjugated to a solid surface. A second batch of purified antibodies that are not conjugated on any solid support is also needed. These non-conjugated purified antibodies are labeled for detection purposes, for example, labeled with horseradish peroxidase to produce a detectable signal. A sample (e.g., a blood sample) from a subject is mixed with a known amount of desired antigen (e.g., a known volume or concentration of a sample comprising a target polypeptide) together with the horseradish peroxidase labeled antibodies and the mixture is then are added to coated wells to form competitive combination. After incubation, if the polypeptide level is high in the sample, a complex of labeled antibody reagent-antigen will form. This complex is free in solution and can be washed away. Washing the wells will remove the complex. Then the wells are incubated with TMB (3, 3′, 5, 5′-tetramethylbenzidene) color development substrate for localization of horseradish peroxidase-conjugated antibodies in the wells. There will be no color change or little color change if the target polypeptide level is high in the sample. If there is little or no target polypeptide present in the sample, a different complex in formed, the complex of solid support bound antibody reagents-target polypeptide. This complex is immobilized on the plate and is not washed away in the wash step. Subsequent incubation with TMB will produce much color change. Such a competitive ELSA test is specific, sensitive, reproducible and easy to operate.

[0053] There are other different forms of ELISA, which are well known to those skilled in the art. The standard techniques known in the art for ELISA are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; and Oellerich, M. 1984, J. Clin. Chem. Clin. Biochem. 22:895-904. These references are hereby incorporated by reference in their entirety.

[0054] Commercial ELISA kits are available through vendors such as R&D Systems (McKinley Place, MN).

[0055] In one embodiment, the levels of a polypeptide in a sample can be detected by a lateral flow immunoassay test (LFIA), also known as the immunochromatographic assay, or strip test. LFIAs are a simple device intended to detect the presence (or absence) of antigen, e.g. a polypeptide, in a fluid sample. There are currently many LFIA tests used for medical diagnostics either for home testing, point of care testing, or laboratory use. LFIA tests are a form of immunoassay in which the test sample flows along a solid substrate via capillary action. After the sample is applied to the test strip it encounters a colored reagent (generally comprising antibody specific for the test target antigen) bound to microparticles which mixes with the sample and transits the substrate encountering lines or zones which have been pretreated with another antibody or antigen. Depending upon the level of target polypeptides present in the sample the colored reagent can be captured and become bound at the test line or zone. LFIAs are essentially immunoassays adapted to operate along a single axis to suit the test strip format or a dipstick format. Strip tests are extremely versatile and can be easily modified by one skilled in the art for detecting an enormous range of antigens from fluid samples such as urine, blood, water, and/or homogenized tissue samples etc. Strip tests are also known as dip stick test, the name bearing from the literal action of "dipping" the test strip into a fluid sample to be tested. LFIA strip tests are easy to use, require minimum training and can easily be included as components of point-of-care test (POCT) diagnostics to be use on site in the field. LFIA tests can be operated as either competitive or sandwich assays. Sandwich LFIAs are similar to sandwich ELISA. The sample first encounters colored particles which are labeled with antibodies raised to the target antigen. The test line will also contain antibodies to the same target, although it may bind to a different epitope on the antigen. The test line will show as a colored band in positive samples. In some embodiments, the lateral flow immunoassay can be a double antibody sandwich assay, a competitive assay, a quantitative assay or variations thereof. Competitive LFIAs are similar to competitive ELISA. The sample first encounters colored particles which are labeled with the target antigen or an analogue. The test line contains antibodies to the target/its analogue. Unlabelled antigen in the sample will block the binding sites on the antibodies preventing uptake of the colored particles. The test line will show as a colored band in negative samples. There are a number of variations on lateral flow technology. It is also possible to apply multiple capture zones to create a multiplex test.

[0056] The use of "dip sticks" or LFIA test strips and other solid supports have been described in the art in the context of an immunoassay for a number of antigen biomarkers. U.S. Pat. Nos. 4,943,522; 6,485,982; 6,187,598; 5,770,460; 5,622,871; 6,565,808, U. S.

patent applications Ser. No. 10/278,676; U.S. Ser. No. 09/579,673 and U.S. Ser. No. 10/717,082, which are incorporated herein by reference in their entirety, are non-limiting examples of such lateral flow test devices. Examples of patents that describe the use of "dip stick" technology to detect soluble antigens via immunochemical assays include, but are not limited to US Patent Nos. 4,444,880; 4,305,924; and 4,135,884; which are incorporated by reference herein in their entireties. The apparatuses and methods of these three patents broadly describe a first component fixed to a solid surface on a "dip stick" which is exposed to a solution containing a soluble antigen that binds to the component fixed upon the "dip stick," prior to detection of the component-antigen complex upon the stick. It is within the skill of one in the art to modify the teachings of this "dip stick" technology for the detection of polypeptides using antibody reagents as described herein.

[0057] Other techniques can be used to detect the level of a polypeptide in a sample. One such technique is the dot blot, and adaptation of Western blotting (Towbin et at., Proc. Nat. Acad. Sci. 76:4350 (1979)). In a Western blot, the polypeptide or fragment thereof can be dissociated with detergents and heat, and separated on an SDS-PAGE gel before being transferred to a solid support, such as a nitrocellulose or PVDF membrane. The membrane is incubated with an antibody reagent specific for the target polypeptide or a fragment thereof. The membrane is then washed to remove unbound proteins and proteins with non-specific binding. Detectably labeled enzyme-linked secondary or detection antibodies can then be used to detect and assess the amount of polypeptide in the sample tested. The intensity of the signal from the detectable label corresponds to the amount of enzyme present, and therefore the amount of polypeptide. Levels can be quantified, for example by densitometry.

[0058] In some embodiments, the level of, e.g., CSF-1, can be measured, by way of non-limiting example, by Western blot; immunoprecipitation; enzyme-linked immunosorbent assay (ELISA); radioimmunological assay (RIA); sandwich assay; fluorescence in situ hybridization (FISH); immunohistological staining; radioimmunometric assay; immunofluoresence assay; mass spectroscopy and/or immunoelectrophoresis assay.

[0059] In certain embodiments, the gene expression products as described herein can be instead determined by determining the level of messenger RNA (mRNA) expression of the genes described herein, e.g. CSF-1. Such molecules can be isolated, derived, or amplified from a biological sample, such as a blood sample. Techniques for the detection of mRNA expression are known by persons skilled in the art, and can include, but are not limited to, PCR procedures, RT-PCR, quantitative RT-PCR Northern blot analysis, differential gene

expression, RNA protection assay, microarray based analysis, next-generation sequencing; hybridization methods, etc.

[0060] In general, the PCR procedure describes a method of gene amplification which is comprised of (i) sequence-specific hybridization of primers to specific genes or sequences within a nucleic acid sample or library, (ii) subsequent amplification involving multiple rounds of annealing, elongation, and denaturation using a thermostable DNA polymerase, and (iii) screening the PCR products for a band of the correct size. The primers used are oligonucleotides of sufficient length and appropriate sequence to provide initiation of polymerization, i.e. each primer is specifically designed to be complementary to a strand of the genomic locus to be amplified. In an alternative embodiment, mRNA level of gene expression products described herein can be determined by reverse-transcription (RT) PCR and by quantitative RT-PCR (QRT-PCR) or real-time PCR methods. Methods of RT-PCR and QRT-PCR are well known in the art.

[0061] In some embodiments, the level of an mRNA can be measured by a quantitative sequencing technology, e.g. a quantitative next-generation sequence technology. Methods of sequencing a nucleic acid sequence are well known in the art. Briefly, a sample obtained from a subject can be contacted with one or more primers which specifically hybridize to a single-strand nucleic acid sequence flanking the target gene sequence and a complementary strand is synthesized. In some next-generation technologies, an adaptor (double or single-stranded) is ligated to nucleic acid molecules in the sample and synthesis proceeds from the adaptor or adaptor compatible primers. In some related technologies, the sequence can be determined, e.g. by determining the location and pattern of the hybridization of probes, or measuring one or more characteristics of a single molecule as it passes through a sensor (e.g. the modulation of an electrical field as a nucleic acid molecule passes through a nanopore). Exemplary methods of sequencing include, but are not limited to, Sanger sequencing, dideoxy chain termination, 454 sequencing, SOLiD sequencing, polony sequencing, Illumina sequencing, Ion Torrent sequencing, sequencing by hybridization, nanopore sequencing, Helioscope sequencing, single molecule real time sequencing, RNAP sequencing, and the like. Methods and protocols for performing these sequencing methods are known in the art, see, e.g. "Next Generation Genome Sequencing" Ed. Michal Janitz, Wiley-VCH; "High-Throughput Next Generation Sequencing" Eds. Kwon and Ricke, Humanna Press, 2011; and Sambrook et al., Molecular Cloning: A Laboratory Manual (4 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012); which are incorporated by reference herein in their entireties.

[0062] The nucleic acid sequences of the CSF-1 gene have been assigned NCBI accession numbers for different species such as human, mouse and rat. For example, the human CSF-1 mRNA (e.g. SEQ ID NO: 2) is known. Accordingly, a skilled artisan can design an appropriate primer based on the known sequence for determining the mRNA level of the respective gene.

[0063] SEQ ID NO: 2 human CSF-1 mRNA NCBI Ref Seq: NM_000757

```
   1 agtgcagcgc agaagacaga gggtgactag gaagacgcgc gagcggggct ggccggccgg
  61 cgggtggggg aggggaggcg ggggaaggcg gctgagtggg cctctggagt gtgtgtgtct
 121 gtgtcagtgt gtgtgtgtgt gtgtgtatgt gtgtgtctgg cgcctggcca gggtgatttc
 181 ccataaacca catgcccccc agtcctctct taaaaggctg tgccgagggc tggccagtga
 241 ggctcggccc ggggaaagtg aaagtttgcc tgggtcctct cggcgccaga gccgctctcc
 301 gcatcccagg acagcggtgc ggccctcggc cggggcgccc actccgcagc agccagcgag
 361 cgagcgagcg agcgagggcg gccgacgcgc ccggccggga cccagctgcc cgtatgaccg
 421 cgccgggcgc cgccgggcgc tgccctccca cgacatggct gggctccctg ctgttgttgg
 481 tctgtctcct ggcgagcagg agtatcaccg aggaggtgtc ggagtactgt agccacatga
 541 ttgggagtgg acacctgcag tctctgcagc ggctgattga cagtcagatg gagacctcgt
 601 gccaaattac atttgagttt gtagaccagg aacagttgaa agatccagtg tgctacctta
 661 agaaggcatt tctcctggta caagacataa tggaggacac catgcgcttc agagataaca
 721 cccccaatgc catcgccatt gtgcagctgc aggaactctc tttgaggctg aagagctgct
 781 tcaccaagga ttatgaagag catgacaagg cctgcgtccg aactttctat gagacacctc
 841 tccagttgct ggagaaggtc aagaatgtct ttaatgaaac aaagaatctc cttgacaagg
 901 actggaatat tttcagcaag aactgcaaca acagctttgc tgaatgctcc agccaagatg
 961 tggtgaccaa gcctgattgc aactgcctgt accccaaagc catccctagc agtgacccgg
1021 cctctgtctc ccctcatcag ccccctcgccc cctccatggc ccctgtggct ggcttgacct
1081 gggaggactc tgagggaact gagggcagct ccctcttgcc tggtgagcag cccctgcaca
1141 cagtggatcc aggcagtgcc aagcagcggc cacccaggag cacctgccag agctttgagc
1201 cgccagagac cccagttgtc aaggacagca ccatcggtgg ctcaccacag cctcgccccct
1261 ctgtcggggc cttcaacccc gggatggagg atattcttga ctctgcaatg ggcactaatt
1321 gggtcccaga agaagcctct ggagaggcca gtgagattcc cgtaccccaa gggacagagc
1381 tttccccctc caggccagga gggggcagca tgcagacaga gcccgccaga cccagcaact
1441 tcctctcagc atcttctcca ctccctgcat cagcaaaggg ccaacagccg gcagatgtaa
1501 ctggtaccgc cttgcccagg gtgggccccg tgaggcccac tggccaggac tggaatcaca
1561 ccccccagaa gacagaccat ccatctgccc tgctcagaga ccccccggag ccaggctctc
1621 ccaggatctc atcactgcgc ccccagggcc tcagcaaccc ctccaccctc tctgctcagc
1681 cacagctttc cagaagccac tcctcgggca gcgtgctgcc ccttggggag ctggagggca
1741 ggaggagcac cagggatcgg aggagccccg cagagccaga aggaggacca gcaagtgaag
1801 gggcagccag gcccctgccc cgttttaact ccgttccttt gactgacaca ggccatgaga
1861 ggcagtccga gggatcctcc agcccgcagc tccaggagtc tgtcttccac ctgctggtgc
1921 ccagtgtcat cctggtcttg ctggccgtcg gaggcctctt gttctacagg tggaggcggc
1981 ggagccatca agagcctcag agagcggatt ctcccttgga gcaaccagag ggcagccccc
2041 tgactcagga tgacagacag gtggaactgc cagtgtagag ggaattctaa gctggacgca
2101 cagaacagtc tctccgtggg aggagacatt atggggcgtc caccaccacc cctccctggc
2161 catcctcctg gaatgtggtc tgccctccac cagagctcct gcctgccagg actggaccag
2221 agcagccagg ctggggcccc tctgtctcaa cccgcagacc cttgactgaa tgagagaggc
2281 cagaggatgc tccccatgct gccactattt attgtgagcc ctggaggctc ccatgtgctt
2341 gaggaaggct ggtgagcccg gctcaggacc ctcttccctc aggggctgca ccctcctctc
2401 actcccttcc atgccggaac ccaggccagg gacccaccgg cctgtggttt gtgggaaagc
2461 agggtggacg ctgaggagtg aaagaaccct gcacccagag ggcctgcctg gtgccaaggt
2521 atcccagcct ggacaggcat ggacctgtct ccagagagag gagcctgaag ttcgtggggc
2581 gggacagcgt cggcctgatt tcccgtaaag gtgtgcagcc tgagagacgg gaagaggagg
2641 cctctggacc tgctggtctg cactgacagc ctgaagggtc tacaccctcg gctcacctaa
```

```
2701 gtgccctgtg ctggttgcca ggcgcagagg ggaggccagc cctgccctca ggacctgcct
2761 gacctgccag tgatgccaag agggggatca agcactggcc tctgcccctc ctccttccag
2821 cacctgccag agcttctcca ggaggccaag cagaggctcc cctcatgaag gaagccattg
2881 cactgtgaac actgtacctg cctgctgaac agcctgcccc cgtccatcca tgagccagca
2941 tccgtccgtc ctccactctc cagcctctcc ccagcctcct gcactgagct ggcctcacca
3001 gtcgactgag ggagcccctc agccctgacc ttctcctgac ctggcctttg actccccgga
3061 gtggagtggg gtgggagaac ctcctgggcc gccagccaga gccggtcttt aggctgtgtt
3121 gttcgcccag gtttctgcat cttgcacttt gacattccca agagggaagg gactagtggg
3181 agagagcaag ggaggggagg gcacagacag agaggctaca gggcgagctc tgactgaaga
3241 tgggcctttg aaatataggt atgcacctga ggttggggga gggtctgcac tcccaaaccc
3301 cagcgcagtg tcctttccct gctgccgaca ggaacctggg gctgaacagg ttatccctgt
3361 caggagccct ggactgggct gcatctcagc cccacctgca tggtatccag ctcccatcca
3421 cttctcaccc ttctttcctc ctgaccttgg tcagcagtga tgacctccaa ctctcaccca
3481 ccccctctac catcacctct aaccaggcaa gccagggtgg gagagcaatc aggagagcca
3541 ggcctcagct tccaatgcct ggagggcctc cactttgtgg ccagcctgtg gtggtggctc
3601 tgaggcctag gcaacgagcg acagggctgc cagttgcccc tgggttcctt tgtgctgctg
3661 tgtgcctcct ctcctgccgc cctttgtcct ccgctaagag accctgccct acctggccgc
3721 tgggccccgt gactttccct tcctgcccag gaaagtgagg gtcggctggc cccaccttcc
3781 ctgtcctgat gccgacagct tagggaaggg cagtgaactt gcatatgggg cttagccttc
3841 tagtcacagc ctctatattt gatgctagaa aacacatatt tttaaatgga agaaaaataa
3901 aaaggcattc ccccttcatc cccctacctt aaacatataa tattttaaag gtcaaaaaag
3961 caatccaacc cactgcagaa gctctttttg agcacttggt ggcatcagag caggaggagc
4021 cccagagcca cctctggtgt ccccccaggc tacctgctca ggaacccctt ctgttctctg
4081 agaagtcaag agaggacatt ggctcacgca ctgtgagatt ttgtttttat acttggaagt
4141 ggtgaattat tttatataaa gtcatttaaa tatctattta aaagatagga agctgcttat
4201 atatttaata ataaaagaag tgcacaagct gccaaaaaaa aaaaaaaaa
```

[0064] Nucleic acid and ribonucleic acid (RNA) molecules can be isolated from a particular biological sample using any of a number of procedures, which are well-known in the art, the particular isolation procedure chosen being appropriate for the particular biological sample. For example, freeze-thaw and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from solid materials; heat and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from urine; and proteinase K extraction can be used to obtain nucleic acid from blood (Roiff, A et al. PCR: Clinical Diagnostics and Research, Springer (1994)).

[0065] In some embodiments, one or more of the reagents (e.g. an antibody reagent and/or nucleic acid probe) described herein can comprise a detectable label and/or comprise the ability to generate a detectable signal (e.g. by catalyzing reaction converting a compound to a detectable product). Detectable labels can comprise, for example, a light-absorbing dye, a fluorescent dye, or a radioactive label. Detectable labels, methods of detecting them, and methods of incorporating them into reagents (e.g. antibodies and nucleic acid probes) are known in the art.

[0066] In some embodiments, detectable labels can include labels that can be detected by spectroscopic, photochemical, biochemical, immunochemical, electromagnetic,

radiochemical, or chemical means, such as fluorescence, chemifluoresence, or chemiluminescence, or any other appropriate means. The detectable labels used in the methods described herein can be primary labels (where the label comprises a moiety that is directly detectable or that produces a directly detectable moiety) or secondary labels (where the detectable label binds to another moiety to produce a detectable signal, e.g., as is common in immunological labeling using secondary and tertiary antibodies). The detectable label can be linked by covalent or non-covalent means to the reagent. Alternatively, a detectable label can be linked such as by directly labeling a molecule that achieves binding to the reagent via a ligand-receptor binding pair arrangement or other such specific recognition molecules. Detectable labels can include, but are not limited to radioisotopes, bioluminescent compounds, chromophores, antibodies, chemiluminescent compounds, fluorescent compounds, metal chelates, and enzymes.

[0067] In other embodiments, the detection reagent is labeled with a fluorescent compound. When the fluorescently labeled reagent is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. In some embodiments, a detectable label can be a fluorescent dye molecule, or fluorophore including, but not limited to fluorescein, phycoerythrin, phycocyanin, o-phthaldehyde, fluorescamine, Cy3™, Cy5™, allophycocyanine, Texas Red, peridenin chlorophyll, cyanine, tandem conjugates such as phycoerythrin-Cy5™, green fluorescent protein, rhodamine, fluorescein isothiocyanate (FITC) and Oregon Green™, rhodamine and derivatives (e.g., Texas red and tetrarhodimine isothiocynate (TRITC)), biotin, phycoerythrin, AMCA, CyDyes™, 6-carboxyfhiorescein (commonly known by the abbreviations FAM and F), 6-carboxy-2',4',7',4,7-hexachlorofiuorescein (HEX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfiuorescein (JOE or J), N,N,N',N'-tetramethyl-6carboxyrhodamine (TAMRA or T), 6-carboxy-X-rhodamine (ROX or R), 5-carboxyrhodamine-6G (R6G5 or G5), 6-carboxyrhodamine-6G (R6G6 or G6), and rhodamine 110; cyanine dyes, e.g. Cy3, Cy5 and Cy7 dyes; coumarins, e.g umbelliferone; benzimide dyes, e.g. Hoechst 33258; phenanthridine dyes, e.g. Texas Red; ethidium dyes; acridine dyes; carbazole dyes; phenoxazine dyes; porphyrin dyes; polymethine dyes, e.g. cyanine dyes such as Cy3, Cy5, etc; BODIPY dyes and quinoline dyes. In some embodiments, a detectable label can be a radiolabel including, but not limited to ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, and ^{33}P. In some embodiments, a detectable label can be an enzyme including, but not limited to horseradish peroxidase and alkaline phosphatase. An enzymatic label can produce, for example, a chemiluminescent signal, a color signal, or a fluorescent signal. Enzymes contemplated for use to detectably label an antibody reagent include, but are

not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. In some embodiments, a detectable label is a chemiluminescent label, including, but not limited to lucigenin, luminol, luciferin, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester. In some embodiments, a detectable label can be a spectral colorimetric label including, but not limited to colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, and latex) beads.

[0068] In some embodiments, detection reagents can also be labeled with a detectable tag, such as c-Myc, HA, VSV-G, HSV, FLAG, V5, HIS, or biotin. Other detection systems can also be used, for example, a biotin-streptavidin system. In this system, the antibodies immunoreactive (i. e. specific for) with the biomarker of interest is biotinylated. Quantity of biotinylated antibody bound to the biomarker is determined using a streptavidin-peroxidase conjugate and a chromagenic substrate. Such streptavidin peroxidase detection kits are commercially available, e. g. from DAKO; Carpinteria, CA. A reagent can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the reagent using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0069] In some embodiments, the methods, assays, and systems described herein can further comprise a step of obtaining a test sample from a subject. In some embodiments, the subject can be a human subject. In some embodiments, the subject can be a subject having LN or SLE. In some embodiments, the subject can be a subject in need of treatment for (e.g. having or diagnosed as having) LN or SLE. In some embodiments, the subject can be a subject undergoing treatment for LN or SLE. In some embodiments, the subject can be a subject in remission of LN.

[0070] In some embodiments, the method, assays, and systems described herein can further comprise a step of providing a treatment appropriate for treating LN. Treatment for LN includes, but is not limited to, CSF-1 inhibitors, corticosteroids, and immunosuppressive drugs.

[0071] In one aspect, described herein is a kit for performing any of the assays and/or methods described herein. In some embodiments, the kit can comprise a CSF-1-specific reagent.

[0072] A kit is any manufacture (e.g., a package or container) comprising at least one reagent, e.g., an antibody reagent(s) or nucleic acid probe, for specifically detecting, e.g., a CSF-1 expression product or fragment thereof, the manufacture being promoted, distributed, or sold as a unit for performing the methods or assays described herein. When the kits, and methods described herein are used for diagnosis and/or treatment of LN, the reagents (e.g., detection probes) or systems can be selected such that a positive result is obtained in at least about 80%, at least about 90%, at least about 95%, at least about 99% or in 100% of subjects having LN.

[0073] In some embodiments, described herein is a kit for the detection of a CSF-1 expression product in a sample, the kit comprising at least a first CSF-1-specific reagent as described herein which specifically binds the CSF-1 expression product, on a solid support. The reagent can optionally comprise a detectable label. The kits described herein include reagents and/or components that permit assaying the level of an expression product in a sample obtained from a subject (e.g., a biological sample obtained from a subject). The kits described herein can optionally comprise additional components useful for performing the methods and assays described herein.

[0074] A kit can further comprise devices and/or reagents for concentrating an expression product (e.g, a polypeptide) in a sample, e.g. a serum or urine sample. Thus, ultrafiltration devices permitting, e.g., protein concentration can also be included as a kit component.

[0075] Preferably, a diagnostic or prognostic kit for use with the methods and assays disclosed herein contains detection reagents for CSF-1 expression products. Such detection reagents comprise in addition to CSF-1-specific reagents, for example, buffer solutions, labels or washing liquids etc. Furthermore, the kit can comprise an amount of a known nucleic acid and/or polypeptide, which can be used for a calibration of the kit or as an internal control. A diagnostic kit for the detection of an expression product can also comprise accessory ingredients like secondary affinity ligands, e.g., secondary antibodies, detection dyes and any other suitable compound or liquid necessary for the performance of an expression product detection method known to the person skilled in the art. Such ingredients are known to the person skilled in the art and may vary depending on the detection method carried out. Additionally, the kit may comprise an instruction leaflet and/or may provide information as to the relevance of the obtained results.

[0076] In one aspect, the technology described herein is directed to systems (and computer readable media for causing computer systems) for obtaining data from at least one sample obtained from at least one subject, the system comprising 1) a measuring module

configured to receive the at least one sample and perform at least one analysis on the at least one sample to determine the level and/or activity of CSF-1 in the sample; 2) a storage device configured to store data output from the determination module; and 3) a display module for displaying a content based in part on the data output from the determination module, wherein the content comprises a signal indicative of the level and/or activity of CSF-1, and/or the slope of the temporal change in CSF-1 level.

[0077] In one embodiment, provided herein is a system comprising: (a) at least one memory containing at least one computer program adapted to control the operation of the computer system to implement a method that includes a measuring module configured to measure the level of CSF-1 in a test sample obtained from a subject; a storage module configured to store output data from the determination module; a analysis module adapted to process the data stored on the storage module to generate a slope, and to provide a retrieved content, and a display module for displaying the slope of the temporal change in CSF-1 level; and (b) at least one processor for executing the computer program (see **FIG. 7**).

[0078] The term "computer" can refer to any non-human apparatus that is capable of accepting a structured input, processing the structured input according to prescribed rules, and producing results of the processing as output. Examples of a computer include: a computer; a general purpose computer; a supercomputer; a mainframe; a super mini-computer; a mini-computer; a workstation; a micro-computer; a server; an interactive television; a hybrid combination of a computer and an interactive television; a tablet; and application-specific hardware to emulate a computer and/or software. A computer can have a single processor or multiple processors, which can operate in parallel and/or not in parallel. A computer also refers to two or more computers connected together via a network for transmitting or receiving information between the computers. An example of such a computer includes a distributed computer system for processing information via computers linked by a network.

[0079] The term "computer-readable medium" may refer to any storage device used for storing data accessible by a computer, as well as any other means for providing access to data by a computer. Examples of a storage-device-type computer-readable medium include: a magnetic hard disk; a floppy disk; an optical disk, such as a CD-ROM and a DVD; a magnetic tape; a memory chip. The term a "computer system" may refer to a system having a computer, where the computer comprises a computer-readable medium embodying software to operate the computer. The term “software" is used interchangeably herein with “program”

and refers to prescribed rules to operate a computer. Examples of software include: software; code segments; instructions; computer programs; and programmed logic.

[0080] The computer readable storage media can be any available tangible media that can be accessed by a computer. Computer readable storage media includes volatile and nonvolatile, removable and non-removable tangible media implemented in any method or technology for storage of information such as computer readable instructions, data structures, program modules or other data. Computer readable storage media includes, but is not limited to, RAM (random access memory), ROM (read only memory), EPROM (erasable programmable read only memory), EEPROM (electrically erasable programmable read only memory), flash memory or other memory technology, CD-ROM (compact disc read only memory), DVDs (digital versatile disks) or other optical storage media, magnetic cassettes, magnetic tape, magnetic disk storage or other magnetic storage media, other types of volatile and non-volatile memory, and any other tangible medium which can be used to store the desired information and which can accessed by a computer including and any suitable combination of the foregoing.

[0081] Computer-readable data embodied on one or more computer-readable media may define instructions, for example, as part of one or more programs that, as a result of being executed by a computer, instruct the computer to perform one or more of the functions described herein, and/or various embodiments, variations and combinations thereof. Such instructions may be written in any of a plurality of programming languages, for example, Java, J#, Visual Basic, C, C#, C++, Fortran, Pascal, Eiffel, Basic, COBOL assembly language, and the like, or any of a variety of combinations thereof. The computer-readable media on which such instructions are embodied may reside on one or more of the components of either of a system, or a computer readable storage medium described herein, may be distributed across one or more of such components.

[0082] The computer-readable media may be transportable such that the instructions stored thereon can be loaded onto any computer resource to implement the aspects of the present invention discussed herein. In addition, it should be appreciated that the instructions stored on the computer-readable medium, described above, are not limited to instructions embodied as part of an application program running on a host computer. Rather, the instructions may be embodied as any type of computer code (e.g., software or microcode) that can be employed to program a computer to implement aspects of the present invention. The computer executable instructions may be written in a suitable computer language or combination of several languages. Basic computational biology methods are known to those

of ordinary skill in the art and are described in, for example, Setubal and Meidanis et al., Introduction to Computational Biology Methods (PWS Publishing Company, Boston, 1997); Salzberg, Searles, Kasif, (Ed.), Computational Methods in Molecular Biology, (Elsevier, Amsterdam, 1998); Rashidi and Buehler, Bioinformatics Basics: Application in Biological Science and Medicine (CRC Press, London, 2000) and Ouelette and Bzevanis Bioinformatics: A Practical Guide for Analysis of Gene and Proteins (Wiley & Sons, Inc., 2nd ed., 2001).

[0083] Embodiments of the invention can be described through functional modules, which are defined by computer executable instructions recorded on computer readable media and which cause a computer to perform method steps when executed. The modules are segregated by function for the sake of clarity. However, it should be understood that the modules/systems need not correspond to discreet blocks of code and the described functions can be carried out by the execution of various code portions stored on various media and executed at various times. Furthermore, it should be appreciated that the modules can perform other functions, thus the modules are not limited to having any particular functions or set of functions.

[0084] The functional modules of certain embodiments of the invention include at minimum a measuring module, a storage module, a computing module, and a display module. The functional modules can be executed on one, or multiple, computers, or by using one, or multiple, computer networks. The measuring module has computer executable instructions to provide e.g., levels of expression products etc in computer readable form.

[0085] The measuring module can comprise any system for detecting a signal elicited from an assay to determine the level and/or activity of CSF-1 as described above herein. In some embodiments, such systems can include an instrument, e.g., AU2700 (Beckman Coulter Brea, CA) for quantitative measurement of polypeptides or e.g., a real time PCR machine, e.g. a LIGHTCYCLER™ (Roche). In some embodiments, the measuring module can measure the intensity of a detectable signal from an assay indicating the level of CSF-1 polypeptide in the test sample. In some embodiments, the assay can be an immunoassay. In some embodiments, the measuring module can measure the intensity of a detectable signal from a RT-PCR assay indicating the level of CSF-1 RNA transcript in the test sample.

[0086] The information determined in the determination system can be read by the storage module. As used herein the "storage module" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention

include stand-alone computing apparatus, data telecommunications networks, including local area networks (LAN), wide area networks (WAN), Internet, Intranet, and Extranet, and local and distributed computer processing systems. Storage modules also include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage media, magnetic tape, optical storage media such as CD-ROM, DVD, electronic storage media such as RAM, ROM, EPROM, EEPROM and the like, general hard disks and hybrids of these categories such as magnetic/optical storage media. The storage module is adapted or configured for having recorded thereon, for example, sample name, biomolecule assayed and the level of said biomolecule. Such information may be provided in digital form that can be transmitted and read electronically, e.g., via the Internet, on diskette, via USB (universal serial bus) or via any other suitable mode of communication.

[0087] As used herein, "stored" refers to a process for encoding information on the storage module. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising expression level information.

[0088] In some embodiments of any of the systems described herein, the storage module stores the output data from the determination module. In some embodiments, the storage module stores the CSF-1 level(s) measured at earlier time points in a sample or samples obtained from the same subject.

[0089] The “computing module” can use a variety of available software programs and formats for computing the level of CSF-1. Such algorithms are well established in the art. A skilled artisan is readily able to determine the appropriate algorithms based on the size and quality of the sample and type of data. The data analysis tools and equations described herein can be implemented in the computing module of the invention. In one embodiment, the computing module further comprises an analysis module (**FIG. 8**). By way of an example, when the value of CSF-1 in a sample obtained from a subject is measured, An analysis module can produce a slope that indicates the temporal change of CSF-1 levels in the subject. In certain embodiments, the previously measured CSF-1 levels of the subject can be pre-stored in the storage module. In some embodiments, the analysis module can provide a graph showing the temporal trend of the CSF-1 level measured at multiple time points. In various embodiments, the analysis module can be configured using existing commercially-available or freely-available software, and may be optimized for particular data analysis that is conducted.

[0090] The computing and/or analysis module, or any other module of the invention, can include an operating system (e.g., UNIX) on which runs a relational database management system, a World Wide Web application, and a World Wide Web server. World Wide Web application includes the executable code necessary for generation of database language statements (e.g., Structured Query Language (SQL) statements). Generally, the executables will include embedded SQL statements. In addition, the World Wide Web application may include a configuration file which contains pointers and addresses to the various software entities that comprise the server as well as the various external and internal databases which must be accessed to service user requests. The Configuration file also directs requests for server resources to the appropriate hardware--as may be necessary should the server be distributed over two or more separate computers. In one embodiment, the World Wide Web server supports a TCP/IP protocol. Local networks such as this are sometimes referred to as "Intranets." An advantage of such Intranets is that they allow easy communication with public domain databases residing on the World Wide Web (e.g., the GenBank or Swiss Pro World Wide Web site). In some embodiments users can directly access data (via Hypertext links for example) residing on Internet databases using a HTML interface provided by Web browsers and Web servers (**FIG. 9**).

[0091] The computing and/or analysis module provides a computer readable result that can be processed in computer readable form by predefined criteria, or criteria defined by a user, to provide content based in part on the analytical result that may be stored and output as requested by a user using an output module, e.g., a display module.

[0092] In some embodiments, the content displayed on the display module can be the level of CSF-1 in the sample obtained from a subject. In some embodiments, if the analysis module determines that the slope is positive and is above a threshold value, the display module displays a signal indicating that the subject is in need of treatment for LN. In some embodiments, the content displayed can be a graph showing the temporal trend of the CSF-1 level measured at multiple time points. In some embodiments, the content displayed on the display module can indicate whether the subject has an increased likelihood of having or developing LN. In some embodiments, the content displayed on the display module can be a numerical value indicating one of these risks or probabilities. In such embodiments, the probability can be expressed in percentages or a fraction. For example, higher percentage or a fraction closer to 1 indicates a higher likelihood of a subject having or developing LN. In some embodiments, the content displayed on the display module can be single word or phrases to qualitatively indicate a risk or probability. For example, a word “unlikely” can be

used to indicate a lower risk for having or developing LN, while "likely" can be used to indicate a high risk for having or developing LN.

[0093] In one embodiment of the invention, the content based on the computing and/or analytical result is displayed on a computer monitor. In one embodiment of the invention, the content based on the computing and/or analytical result is displayed through printable media. The display module can be any suitable device configured to receive from a computer and display computer readable information to a user. Non-limiting examples include, for example, general-purpose computers such as those based on Intel PENTIUM-type processor, Motorola PowerPC, Sun UltraSPARC, Hewlett-Packard PA-RISC processors, any of a variety of processors available from Advanced Micro Devices (AMD) of Sunnyvale, California, or any other type of processor, visual display devices such as flat panel displays, cathode ray tubes and the like, as well as computer printers of various types.

[0094] In one embodiment, a World Wide Web browser is used for providing a user interface for display of the content based on the computing/analytical result. It should be understood that other modules of the invention can be adapted to have a web browser interface. Through the Web browser, a user can construct requests for retrieving data from the computing/analysis module. Thus, the user will typically point and click to user interface elements such as buttons, pull down menus, scroll bars and the like conventionally employed in graphical user interfaces.

[0095] Systems and computer readable media described herein are merely illustrative embodiments of the invention for determining the level and/or activity of CSF-1 in a sample obtained from a subject, and therefore are not intended to limit the scope of the invention. Variations of the systems and computer readable media described herein are possible and are intended to fall within the scope of the invention.

[0096] The modules of the machine, or those used in the computer readable medium, may assume numerous configurations. For example, function may be provided on a single machine or distributed over multiple machines.

[0097] In another aspect, the technology described herein provides a method of treating LN in a subject, the method comprising administering a therapeutically-effective amount of a CSF-1 inhibitor to the subject.

[0098] A CSF-1 inhibitor can have an IC50 of less than 50 μM, e.g., a CSF-1 inhibitor can have an IC50 of from about 50 μM to about 5 nM, or less than 5 nM. For example, in some embodiments, a CSF-1 inhibitor has an IC50 of from about 50 μM to about 25 μM, from about 25 μM to about 10 μM, from about 10 μM to about 5 μM, from about 5 μM to

about 1 μM, from about 1 μM to about 500 nM, from about 500 nM to about 400 nM, from about 400 nM to about 300 nM, from about 300 nM to about 250 nM, from about 250 nM to about 200 nM, from about 200 nM to about 150 nM, from about 150 nM to about 100 nM, from about 100 nM to about 50 nM, from about 50 nM to about 30 nM, from about 30 nM to about 25 nM, from about 25 nM to about 20 nM, from about 20 nM to about 15 nM, from about 15 nM to about 10 nM, from about 10 nM to about 5 nM, or less than about 5 nM.

[0099] In some embodiments, a CSF-1 inhibitor can decrease the signaling cascade initiated by the CSF-1 protein. In some embodiments, a CSF-1 inhibitor can be an inhibitor for the CSF-1 receptor C-FMS. C-FMS is also identified as CSF1R, CD115, CSFR, FIM2, FMS, HDLS, or M-CSF-R. Examples of CSF-1 inhibitors that inhibit C-FMS include, but are not limited to, pexidartinib, JNJ-28312141, ARRY-382, GW 2580 (CAS 870483-87-7), Ki20227 (CAS 623142-96-1), AC708 by Ambit Siosciences, and antibodies such as those sold by Sigma Aldrich, Amgen (e.g., AMG-820), ImClone/Eli Lilly (e.g., IMC-CS4), and Roche (e.g., RG7155).

[00100] In some embodiments, the CSF-1 inhibitor is a small molecule. As used herein, the term "small molecule" refers to a natural or synthetic molecule having a molecular mass of less than about 5 kD, organic or inorganic compounds having a molecular mass of less than about 5 kD, less than about 2 kD, or less than about 1 kD.

[00101] In some embodiments, the CSF-1 inhibitor can be an anti-CSF-1 antibody molecule or an antigen-binding fragment thereof. Suitable antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, recombinant, single chain, Fab, Fab′, Fsc, Rv, and F(ab′)2 fragments. In some embodiments, neutralizing antibodies can be used as inhibitors of CSF-1. Antibodies are readily raised in animals such as rabbits or mice by immunization with the antigen. Immunized mice are particularly useful for providing sources of B cells for the manufacture of hybridomas, which in turn are cultured to produce large quantities of monoclonal antibodies. In general, an antibody molecule obtained from humans can be classified in one of the immunoglobulin classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

[00102] Antibodies provide high binding avidity and unique specificity to a wide range of target antigens and haptens. Monoclonal antibodies useful in the practice of the methods

disclosed herein include whole antibody and fragments thereof and are generated in accordance with conventional techniques, such as hybridoma synthesis, recombinant DNA techniques and protein synthesis.

[00103] The CSF-1 polypeptide, or a portion or fragment thereof, can serve as an antigen, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues.

[00104] Useful monoclonal antibodies and fragments can be derived from any species (including humans) or can be formed as chimeric proteins which employ sequences from more than one species. Human monoclonal antibodies or "humanized" murine antibody can also be used in accordance with the present invention. For example, murine monoclonal antibody can be "humanized" by genetically recombining the nucleotide sequence encoding the murine Fv region (i.e., containing the antigen binding sites) or the complementarily determining regions thereof with the nucleotide sequence encoding a human constant domain region and an Fc region. Humanized targeting moieties are recognized to decrease the immunoreactivity of the antibody or polypeptide in the host recipient, permitting an increase in the half-life and a reduction in the possibility of adverse immune reactions in a manner similar to that disclosed in European Patent Application No. 0,411,893 A2. The murine monoclonal antibodies should preferably be employed in humanized form. Antigen binding activity is determined by the sequences and conformation of the amino acids of the six complementarily determining regions (CDRs) that are located (three each) on the light and heavy chains of the variable portion (Fv) of the antibody. The 25-kDa single-chain Fv (scFv) molecule, composed of a variable region (VL) of the light chain and a variable region (VH) of the heavy chain joined via a short peptide spacer sequence, is one option for minimizing the size of an antibody agent. ScFvs provide additional options for preparing and screening a large number of different antibody fragments to identify those that specifically bind. Techniques have been developed to display scFv molecules on the surface of filamentous phage that contain the gene for the scFv. scFv molecules with a broad range orantigenic-specificities can be present in a single large pool of scFv-phage library.

[00105] Chimeric antibodies are immunoglobin molecules characterized by two or more segments or portions derived from different animal species. Generally, the variable region of the chimeric antibody is derived from a non-human mammalian antibody, such as murine

monoclonal antibody, and the immunoglobin constant region is derived from a human immunoglobin molecule. Preferably, both regions and the combination have low immunogenicity as routinely determined.

[00106] In some embodiments, the CSF-1 inhibitor is a nucleic acid or a nucleic acid analog or derivative thereof, also referred to as a nucleic acid agent herein. As will be appreciated by those skilled in the art, the depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand.

[00107] Without limitation, the nucleic acid agent can be single-stranded or double-stranded. A single-stranded nucleic acid agent can have double-stranded regions, e.g., where there is internal self-complementarity, and a double-stranded nucleic acid agent can have single-stranded regions. The nucleic acid can be of any desired length. In particular embodiments, nucleic acid can range from about 10 to 100 nucleotides in length. In various related embodiments, nucleic acid agents, single-stranded, double-stranded, and triple-stranded, can range in length from about 10 to about 50 nucleotides, from about 20 to about 50 nucleotides, from about 15 to about 30 nucleotides, from about 20 to about 30 nucleotides in length. In some embodiments, a nucleic acid agent is from about 9 to about 39 nucleotides in length. In some other embodiments, a nucleic acid agent is at least 30 nucleotides in length.

[00108] The nucleic acid agent can comprise modified nucleosides as known in the art. Modifications can alter, for example, the stability, solubility, or interaction of the nucleic acid agent with cellular or extracellular components that modify activity. In certain instances, it can be desirable to modify one or both strands of a double-stranded nucleic acid agent. In some cases, the two strands will include different modifications. In other instances, multiple different modifications can be included on each of the strands. The various modifications on a given strand can differ from each other, and can also differ from the various modifications on other strands. For example, one strand can have a modification, and a different strand can have a different modification. In other cases, one strand can have two or more different modifications, and the another strand can include a modification that differs from the at least two modifications on the first strand.

[00109] Single-stranded and double-stranded nucleic acid agents that are effective in inducing RNA interference are referred to as siRNA, RNAi agents, iRNA agents, or RNAi inhibitors herein. As used herein, the term "iRNA agent" refers to a nucleic acid agent which

can mediate the targeted cleavage of an RNA transcript via an RNA-induced silencing complex (RISC) pathway.

[00110] In some embodiments, the CSF-1 inhibitor is an antisense oligonucleotide. One of skill in the art is well aware that single-stranded oligonucleotides can hybridize to a complementary target sequence and prevent access of the translation machinery to the target RNA transcript, thereby preventing protein synthesis. The single-stranded oligonucleotide can also hybridize to a complementary RNA and the RNA target can be subsequently cleaved by an enzyme such as RNase H and thus preventing translation of target RNA. Alternatively, or in addition, the single-stranded oligonucleotide can modulate the expression of a target sequence via RISC mediated cleavage of the target sequence, i.e., the single-stranded oligonucleotide acts as a single-stranded RNAi agent. A "single-stranded RNAi agent" as used herein, is an RNAi agent which is made up of a single molecule. A single-stranded RNAi agent can include a duplexed region, formed by intra-strand pairing, *e.g.*, it can be, or include, a hairpin or pan-handle structure.

[00111] A small hairpin RNA or short hairpin RNA (shRNA) is a sequence of RNA that makes a tight hairpin turn that can be used to silence target gene expression via RNA interference (RNAi). shRNAs that can be used to inhibit CSF-1 are commercially available through vendors such as OriGene.

[00112] In general, any method of delivering a nucleic acid molecule can be adapted for use with the nucleic acid agents described herein.

[00113] Methods of delivering RNA interference agents, e.g., an siRNA, or vectors containing an RNA interference agent, to the target cells, for uptake include injection of a composition containing the RNA interference agent, e.g., an siRNA, or directly contacting the cell with a composition comprising an RNA interference agent, e.g., an siRNA. In another embodiment, RNA interference agent, e.g., an siRNA may be injected directly into any blood vessel, such as vein, artery, venule or arteriole, via, e.g., hydrodynamic injection or catheterization. Administration may be by a single injection or by two or more injections. The RNA interference agent is delivered in a pharmaceutically acceptable carrier. One or more RNA interference agents may be used simultaneously. In one embodiment, specific cells are targeted with RNA interference, limiting potential side effects. The method can use, for example, a complex or a fusion molecule comprising a cell targeting moiety and an RNA interference binding moiety that is used to deliver RNA interference effectively into cells. For example, an antibody-protamine fusion protein when mixed with siRNA, binds siRNA and selectively delivers the siRNA into cells expressing an antigen recognized by the antibody,

resulting in silencing of gene expression only in those cells that express the antigen. The siRNA or RNA interference-inducing molecule binding moiety is a protein or a nucleic acid binding domain or fragment of a protein, and the binding moiety is fused to a portion of the targeting moiety. The location of the targeting moiety can be either in the carboxyl-terminal or amino-terminal end of the construct or in the middle of the fusion protein. A viral-mediated delivery mechanism can also be employed to deliver siRNAs to cells in vitro and in vivo as described in Xia, H. et al. (2002) Nat Biotechnol 20(10):1006). Plasmid- or viral-mediated delivery mechanisms of shRNA may also be employed to deliver shRNAs to cells in vitro and in vivo as described in Rubinson, D.A., et al. ((2003) Nat. Genet. 33:401-406) and Stewart, S.A., et al. ((2003) RNA 9:493-501). The RNA interference agents, e.g., the siRNAs or shRNAs, can be introduced along with components that perform one or more of the following activities: enhance uptake of the RNA interfering agents, e.g., siRNA, by the cell, inhibit annealing of single strands, stabilize single strands, or otherwise facilitate delivery to the target cell and increase inhibition of the target gene, e.g., CSF-1. The dose of the particular RNA interfering agent will be in an amount necessary to effect RNA interference, e.g., post translational gene silencing (PTGS), of the particular target gene, thereby leading to inhibition of target gene expression or inhibition of activity or level of the protein encoded by the target gene.

[00114] In some embodiments, the CSF-1 inhibitor can also be a peptide, a peptidomimetic, a protein, a saccharide, a lipid, a glycosaminoglycan, an extract made from a biological material, or combinations thereof.

[00115] In some embodiments, the inhibitors described herein can be formulated as a pharmaceutically acceptable prodrug. As used herein, a “prodrug” refers to compounds that can be converted via some chemical or physiological process (e.g., enzymatic processes and metabolic hydrolysis) to a therapeutic agent. Thus, the term “prodrug" also refers to a precursor of a biologically active compound that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject, i.e. an ester, but is converted *in vivo* to an active compound, for example, by hydrolysis to the free carboxylic acid or free hydroxyl. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in an organism. The term “prodrug" is also meant to include any covalently bonded carriers, which release the active compound *in vivo* when such prodrug is administered to a subject. Prodrugs of an active compound may be prepared by modifying functional groups present in the active compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent active compound. Prodrugs include compounds

wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the active compound is administered to a subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of an alcohol or acetamide, formamide and benzamide derivatives of an amine functional group in the active compound and the like. See Harper, "Drug Latentiation" in Jucker, ed. *Progress in Drug Research* 4:221-294 (1962); Morozowich et al, "Application of Physical Organic Principles to Prodrug Design" in E. B. Roche ed. *Design of Biopharmaceutical Properties through Prodrugs and Analogs,* APHA Acad. Pharm. Sci. 40 (1977); *Bioreversible Carriers in Drug in Drug Design, Theory and Application,* E. B. Roche, ed., APHA Acad. Pharm. Sci. (1987); *Design of Prodrugs,* H. Bundgaard, Elsevier (1985); Wang et al. "Prodrug approaches to the improved delivery of peptide drug" in *Curr. Pharm. Design.* 5(4):265-287 (1999); Pauletti et al. (1997) Improvement in peptide bioavailability: Peptidomimetics and Prodrug Strategies, *Adv. Drug. Delivery Rev.* 27:235-256; Mizen et al. (1998) "The Use of Esters as Prodrugs for Oral Delivery of (3-Lactam antibiotics," *Pharm. Biotech.* ll,:345-365; Gaignault et al. (1996) "Designing Prodrugs and Bioprecursors I. Carrier Prodrugs," *Pract. Med. Chem.* 671-696; Asgharnejad, "Improving Oral Drug Transport", in *Transport Processes in Pharmaceutical Systems, G.* L. Amidon, P. I. Lee and E. M. Topp, Eds., Marcell Dekker, p. 185-218 (2000); Balant et al., "Prodrugs for the improvement of drug absorption via different routes of administration", *Eur. J. Drug Metab. Pharmacokinet.,* 15(2): 143-53 (1990); Balimane and Sinko, "Involvement of multiple transporters in the oral absorption of nucleoside analogues", *Adv. Drug Delivery Rev.,* 39(1-3): 183-209 (1999); Browne, "Fosphenytoin (Cerebyx)", *Clin. Neuropharmacol.* 20(1): 1-12 (1997); Bundgaard, "Bioreversible derivatization of drugs—principle and applicability to improve the therapeutic effects of drugs", *Arch. Pharm. Chemi* 86(1): 1-39 (1979); Bundgaard H. "Improved drug delivery by the prodrug approach", *Controlled Drug Delivery* 17: 179-96 (1987); Bundgaard H. "Prodrugs as a means to improve the delivery of peptide drugs",Arfv. *Drug Delivery Rev.* 8(1): 1-38 (1992); Fleisher et al. "Improved oral drug delivery: solubility limitations overcome by the use of prodrugs", Arfv. *Drug Delivery Rev.* 19(2): 115-130 (1996); Fleisher et al. "Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting", *Methods Enzymol.* 112 (Drug Enzyme Targeting, Pt. A): 360-81, (1985); Farquhar D, et al., "Biologically Reversible Phosphate-Protective Groups", *Pharm. Sci.,* 72(3): 324-325 (1983); Freeman S, et al., "Bioreversible Protection for the Phospho Group: Chemical Stability and Bioactivation of Di(4-acetoxy-benzyl) Methylphosphonate with Carboxyesterase," *Chem. Soc., Chem.*

Commun., 875-877 (1991); Friis and Bundgaard, "Prodrugs of phosphates and phosphonates: Novel lipophilic alphaacyloxyalkyl ester derivatives of phosphate- or phosphonate containing drugs masking the negative charges of these groups", *Eur. J. Pharm. Sci.* 4: 49-59 (1996); Gangwar et al., "Pro-drug, molecular structure and percutaneous delivery", *Des. Biopharm. Prop. Prodrugs Analogs, [Symp.]* Meeting Date 1976, 409-21. (1977); Nathwani and Wood, "Penicillins: a current review of their clinical pharmacology and therapeutic use", *Drugs* 45(6): 866-94 (1993); Sinhababu and Thakker, "Prodrugs of anticancer agents", *Adv. Drug Delivery Rev.* 19(2): 241-273 (1996); Stella et al., "Prodrugs. Do they have advantages in clinical practice?", *Drugs* 29(5): 455-73 (1985); Tan et al. "Development and optimization of anti-HIV nucleoside analogs and prodrugs: A review of their cellular pharmacology, structure-activity relationships and pharmacokinetics", *Adv. Drug Delivery Rev.* 39(1-3): 117-151 (1999); Taylor, "Improved passive oral drug delivery via prodrugs", *Adv. Drug Delivery Rev.,* 19(2): 131-148 (1996); Valentino and Borchardt, "Prodrug strategies to enhance the intestinal absorption of peptides", *Drug Discovery Today* 2(4): 148-155 (1997); Wiebe and Knaus, "Concepts for the design of anti-HIV nucleoside prodrugs for treating cephalic HIV infection", *Adv. Drug Delivery Rev.:* 39(l-3):63-80 (1999); Waller et al., "Prodrugs", *Br. J. Clin. Pharmac.* 28: 497-507 (1989), which are incorporated by reference herein in their entireties.

[00116] The CSF-1 inhibitor can be administered in a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises a pharmaceutically-acceptable carrier and/or diluent. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C_2-C_{12} alcohols,

such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used interchangeably herein.

[00117] The pharmaceutical compositions can be specially formulated for administration in solid, liquid or gel form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), lozenges, dragees, capsules, pills, tablets (e.g., those targeted for buccal, sublingual, and systemic absorption), boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; (8) transmucosally; or (9) nasally. Additionally the compounds described herein can be implanted into a patient or injected using a drug delivery system. See, for example, Urquhart, et al., Ann. Rev. Pharmacol. Toxicol. 24: 199-236 (1984); Lewis, ed. "Controlled Release of Pesticides and Pharmaceuticals" (Plenum Press, New York, 1981); and U.S. Pat. No. 3,773,919. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as hard gelatin capsules and soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquids such as suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or water-in-oil liquid emulsions), solutions, and elixirs; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms.

[00118] Parenteral dosage forms can be administered to patients by various routes, including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Since administration of parenteral dosage forms typically bypasses the patient's natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions. In addition, controlled-release

parenteral dosage forms can be prepared for administration of a patient, including, but not limited to, administration DUROS®-type dosage forms, and dose-dumping.

[00119] Suitable vehicles that can be used to provide parenteral dosage forms of the disclosure are well known to those skilled in the art. Examples include, without limitation: sterile water; water for injection USP; saline solution; glucose solution; aqueous vehicles such as but not limited to, sodium chloride injection, Ringer's injection, dextrose Injection, dextrose and sodium chloride injection, and lactated Ringer's injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and propylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

[00120] The pharmaceutical compositions may be administered in any dose or dosing regimen. With respect to the therapeutic methods of the invention, it is not intended that the administration be limited to a particular mode of administration, dosage, or frequency of dosing.

[00121] The compounds of the present invention can be administered by any appropriate route known in the art including, but not limited to, oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), pulmonary, nasal, rectal, and topical (including buccal and sublingual) administration.

[00122] In one embodiment, it may be desirable to administer the pharmaceutical compositions locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., by injection, by means of a catheter (e.g., a cardiac catheter, renal catheter, intrahepatic catheter, etc.), or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, fibers, or commercial skin substitutes.

[00123] In some embodiments, the pharmaceutical composition can be administered to a subject orally (e.g., in capsules, suspensions or tablets) or by parenteral administration. Conventional methods for oral administration include any one of the following; tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Parenteral administration can include, for example, intramuscular, intravenous, intraarticular, intraarterial, intrathecal, subcutaneous, or intraperitoneal administration. The pharmaceutical composition can also be administered orally, transdermally, topically, by inhalation (e.g., intrabronchial, intranasal, oral inhalation or intranasal drops) or rectally.

[00124] When administering the pharmaceutical composition parenterally, it will generally be formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils. The term "Dosage unit" form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00125] An effective amount, e.g., a therapeutically effective dose of the compound disclosed herein may be administered to the patient in a single dose or in multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one hour, three hours, six hours, eight hours, one day, two days, one week, two weeks, or one month. For example, a composition comprising the compound disclosed herein can be administered for, e.g., 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. For example, the dosage of the therapeutic can be increased if the lower dose does not provide sufficient therapeutic activity.

[00126] The term "effective amount" as used herein refers to the amount of a therapy needed to alleviate at least one or more symptoms of the disease or disorder (e.g., LN), and relates to a sufficient amount of pharmaceutical composition to provide the desired effect. The term "therapeutically effective amount" therefore refers to an amount of a therapy that is sufficient to cause a particular effect when administered to a typical subject. An effective amount as used herein, in various contexts, would also include an amount sufficient to delay the development of a symptom of the disease, alter the course of a symptom of the disease (for example but not limited to, slowing the progression of a symptom of the disease), or reverse a symptom of the disease. Thus, it is not generally practical to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" can be determined by one of ordinary skill in the art using only routine experimentation.

[00127] In some embodiments, an effective amount of a CSF-1 inhibitor can be an amount which causes the level of CSF-1 expression to decrease or, at least, to increase at a lower rate

than it would be expected to increase in a subject not receiving the CSF-1 inhibitor. In some embodiments, an effective amount can be an amount that decreases the amount of CSF-1 polypeptide present in the subject by a statistically significant amount. In some embodiments, an effective amount of a CSF-1 inhibitor can be an amount which reduces the activity of CSF-1 polypeptide.

[00128] A physician may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. The dose administered to a patient is sufficient to effect a beneficial therapeutic response in the patient over time, or, e.g., to reduce symptoms, or other appropriate activity, depending on the application. The dose is determined by the efficacy of the particular formulation, and the activity, stability or serum half-life of the composition being administered, and the condition of the patient, as well as the body weight or body surface area. The size of the dose is also determined by the existence, nature, and extent of any adverse side- effects that accompany the administration of a particular formulation, or the like in a particular subject. Therapeutic compositions are optionally tested in one or more appropriate *in vitro* and/or *in vivo* animal models of disease, and known to persons of ordinary skill in the art, to confirm efficacy, tissue metabolism, and to estimate dosages, according to methods well known in the art. In particular, dosages can be initially determined by activity, stability or other suitable measures of treatment vs. non-treatment (e.g., comparison of treated vs. untreated cells or animal models), in a relevant assay. Formulations are administered at a rate determined by the LD50 of the relevant formulation, and/or observation of any side-effects of the pharmaceutical composition at various concentrations, e.g., as applied to the mass and overall health of the patient.

[00129] The dosage can be determined by one of skill in the art and can also be adjusted by the individual physician in the event of any complication. Typically, the dosage of a composition comprising a CSF-1 inhibitor can range from 0.001mg/kg body weight to 5 g/kg body weight. In some embodiments, the dosage range is from 0.001 mg/kg body weight to 1g/kg body weight, from 0.001 mg/kg body weight to 0.5 g/kg body weight, from 0.001 mg/kg body weight to 0.1 g/kg body weight, from 0.001 mg/kg body weight to 50 mg/kg body weight, from 0.001 mg/kg body weight to 25 mg/kg body weight, from 0.001 mg/kg body weight to 10 mg/kg body weight, from 0.001 mg/kg body weight to 5 mg/kg body weight, from 0.001 mg/kg body weight to 1 mg/kg body weight, from 0.001 mg/kg body weight to 0.1 mg/kg body weight, or from 0.001 mg/kg body weight to 0.005 mg/kg body weight. Alternatively, in some embodiments the dosage range is from 0.1 g/kg body weight

to 5 g/kg body weight, from 0.5 g/kg body weight to 5 g/kg body weight, from 1 g/kg body weight to 5 g/kg body weight, from 1.5 g/kg body weight to 5 g/kg body weight, from 2 g/kg body weight to 5 g/kg body weight, from 2.5 g/kg body weight to 5 g/kg body weight, from 3 g/kg body weight to 5 g/kg body weight, from 3.5 g/kg body weight to 5 g/kg body weight, from 4 g/kg body weight to 5 g/kg body weight, or from 4.5 g/kg body weight to 5 g/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test bioassays or systems. The dosage should not be so large as to cause unacceptable adverse side effects.

[00130] LN can also be treated by mycophenolate mofetil, cyclophosphamide with corticosteroids, or azathioprine with corticosteroids.

[00131] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., disclosed herein and as such may vary. 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 is defined solely by the claims.

[00132] As used herein and in the claims, the singular forms include the plural reference and vice versa unless the context clearly indicates otherwise. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about."

[00133] Although any known methods, devices, and materials may be used in the practice or testing of the invention, the methods, devices, and materials in this regard are disclosed herein.

[00134] Some embodiments of the invention are listed in the following numbered paragraphs:

1. A method of monitoring disease activity in a subject with systemic lupus erythematosus, the method comprising:

(i) measuring, at each of two or more time points, a level of CSF-1 in a sample obtained from the subject;

(ii) determining a temporal trend of the level of CSF-1 as a function of the measurements of step (i); and

(iii) identifying the subject as (a) having an increased risk of developing lupus nephritis if the temporal trend is upward, or (b) having no or minimal risk of developing lupus nephritis if the temporal trend is flat or downward.

2. The method of paragraph 1, wherein the level of CSF-1 is measured at three or more time points.

3. The method of paragraph 1 or 2, wherein the level of CSF-1 is a protein level.

4. The method of any one of paragraphs 1-3, wherein the level of CSF-1 is measured by an immunoassay.

5. The method of paragraph 4, wherein the sample is contacted with an anti-CSF-1 antibody.

6. The method of paragraph 5, wherein the anti-CSF-1 antibody is detectably labeled or capable of generating a detectable signal.

7. The method of paragraph 5 or 6, wherein the antibody is fluorescently labeled.

8. The method of paragraph 1 or 2, wherein the level of CSF-1 is measured by measuring a nucleic acid encoding CSF-1.

9. The method of any one of paragraphs 1-8, wherein the sample is selected from the group consisting of blood, plasma, serum, and urine.

10. The method of any one of paragraphs 1-9, further comprising administering a treatment appropriate for treating lupus nephritis to the subject if the subject is identified as having an increased risk of developing lupus nephritis.

11. The method of paragraph 10, wherein the treatment comprises administering a CSF-1 inhibitor.

12. The method of any one of paragraphs 1-11, wherein the subject is identified as having an increased risk of developing lupus nephritis at least 30 days prior to symptoms of LN.

13. The method of any one of paragraphs 1-12, wherein the subject is a mammal.

14. The method of paragraph 13, wherein the mammal is a human.

15. A method of monitoring disease activity in a subject in remission of lupus nephritis (LN), the method comprising:

(i) measuring, at each of two or more time points, a level of CSF-1 in a sample obtained from the subject;

(ii) determining a temporal trend of the level of CSF-1 as a function of the measurements of step (i); and

(iii) identifying the subject as (a) having an increased risk of developing LN flares if the temporal trend is upward, or (b) having no or minimal risk of developing LN flares if the temporal trend is flat or downward.

16. The method of paragraph 15, wherein the level of CSF-1 is measured at three or more time points.

17. The method of paragraph 15 or 16, wherein the level of CSF-1 is a protein level.

18. The method of any one of paragraphs 15-17, wherein the level of CSF-1 is measured by an immunoassay.

19. The method of paragraph 18, wherein the sample is contacted with an anti-CSF-1 antibody.

20. The method of paragraph 19, wherein the anti-CSF-1 antibody is detectably labeled or capable of generating a detectable signal.

21. The method of paragraph 19 or 20, wherein the antibody is fluorescently labeled.

22. The method of paragraph 15 or 16, wherein the level of CSF-1 is measured by measuring a nucleic acid encoding CSF-1.

23. The method of any one of paragraphs 15-22, wherein the sample is selected from the group consisting of blood, plasma, serum, and urine.

24. The method of any one of paragraphs 15-23, further comprising administering a treatment appropriate for treating lupus nephritis to the subject if the subject is identified as having an increased risk of developing lupus nephritis.

25. The method of paragraph 24, wherein the treatment comprises administering a CSF-1 inhibitor.

26. The method of any one of paragraphs 15-25, wherein the subject is identified as having an increased risk of developing LN flares at least 30 days prior to symptoms of LN flares.

27. The method of any one of paragraphs 15-26, wherein the subject is a mammal.

28. The method of paragraph 27, wherein the mammal is a human.

29. A method of monitoring treatment progress in a subject having lupus nephritis, the method comprising:

(i) measuring, at a first time point, a first level of CSF-1 in a first sample obtained from the subject;

(ii) administering to the subject a therapeutic agent for treating lupus nephritis; and

(iii) measuring, at a second time point, a second level of CSF-1 in a second sample obtained from the subject, wherein the second time point is later than the first time point and after said administering, and wherein if the second level is significantly lower than the first level, then the treatment is considered to be effective.

30. The method of paragraph 29, wherein the first sample and the second sample are of the same type, each selected from the group consisting of blood, plasma, serum, and urine.

31. The method of paragraph 29 or 30, wherein the therapeutic agent is a CSF-1 inhibitor.

32. The method of any one of paragraphs 29-31, wherein the subject is a human.

33. Use of a CSF-1 inhibitor for the treatment of lupus nephritis.

Definitions

[00135] Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it pertains. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00136] As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not.

[00137] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00138] The terms "disease", "disorder", or "condition" are used interchangeably herein, refer to any alternation in state of the body or of some of the organs, interrupting or disturbing the performance of the functions and/or causing symptoms such as discomfort, dysfunction, distress, or even death to the person afflicted or those in contact with a person. A disease or disorder can also be related to a distemper, ailing, ailment, malady, disorder, sickness, illness, complaint, or affectation.

[00139] As used herein, the terms "colony stimulating factor 1", "CSF-1", "macrophage colony-stimulating factor", and "M-CSF" are used interchangeably to refer to a particular secreted cytokine which influences hematopoietic stem cells to differentiate into macrophages or other related cell types. Sequences for CSF-1 expression products are known for a number of species, e.g., human CSF-1 mRNA (NCBI Ref Seq: NM_000757) and polypeptide (NCBI Ref Seq: NP_000748). CSF-1 is found to be largely responsible for macrophage development, survival, proliferation, and activation.

[00140] As used herein, the term "inhibitor" refers to an agent which can decrease the expression and/or activity of the targeted expression product (e.g. mRNA encoding the target or a target polypeptide), e.g. by at least 10% or more, e.g. by at least 50% or more, 70% or more, 80% or more, 90% or more, 95% or more, or 98 % or more. The efficacy of an inhibitor of, for example, CSF-1, e.g. its ability to decrease the level and/or activity of CSF-1,

can be determined, e.g. by measuring the level of the CSF-1 expression product and/or the activity of CSF-1. Methods for measuring the level of a given mRNA and/or polypeptide are known to one of skill in the art, e.g. RT-PCR can be used to determine the level of RNA, and Western blotting or immunoassay with an antibody (e.g. an anti-CSF-1 antibody) can be used to determine the level of a polypeptide. The activity of CSF-1 can be determined using methods known in the art. In some embodiments, the inhibitor can be an inhibitory nucleic acid; an aptamer; an antibody reagent; an antibody; or a small molecule.

[00141] The terms “decrease”, “reduced”, “reduction”, or “inhibit” are all used herein to mean a decrease by a statistically significant amount. In some embodiments, “reduce,” “reduction" or “decrease" or “inhibit” typically means a decrease by at least 10% as compared to a reference level (e.g. the absence of a given treatment) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99% , or more. A decrease can be preferably down to a level accepted as within the range of normal for an individual without a given disorder.

[00142] The terms “increased”, “increase”, “enhance”, or “activate” are all used herein to mean an increase by a statically significant amount. In some embodiments, the terms “increased”, “increase”, “enhance”, or “activate” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level. In the context of a marker or symptom, an “increase” is a statistically significant increase in such level.

[00143] As used herein, a "subject" means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomologous monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g.,

trout, catfish and salmon. In some embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, "individual," "patient" and "subject" are used interchangeably herein.

[00144] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used as subjects that represent animal models of lupus nephritis. A subject can be male or female.

[00145] A subject can be one who has been previously diagnosed with or identified as suffering from or having a condition in need of treatment (e.g. lupus) or one or more complications related to such a condition, and optionally, have already undergone treatment for lupus nephritis or the one or more complications related to lupus nephritis. Alternatively, a subject can also be one who has lupus but who has not been previously diagnosed as having lupus nephritis or one or more complications related to lupus nephritis. For example, a subject can be one who exhibits one or more risk factors for lupus nephritis or one or more complications related to lupus nephritis.

[00146] A "subject in need" of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at elevated risk of developing that condition.

[00147] The term "sample", "biological sample", or "test sample" as used herein denotes a sample taken or isolated from a biological organism, e.g., a blood or plasma sample from a subject. Exemplary biological samples include, but are not limited to, a biofluid sample; serum; plasma; urine; saliva; and/or tissue sample etc. The term also includes a mixture of the above-mentioned samples. The term "test sample" also includes untreated or pretreated (or pre-processed) biological samples. In some embodiments, a test sample can comprise cells from subject. In some embodiments, the test sample can be a blood sample. In some embodiments, the test sample can be a plasma sample. In some embodiments, the test sample can be a serum sample. In some embodiments, the test sample can be a urine sample.

[00148] The test sample can be obtained by removing a sample from a subject, but can also be accomplished by using previously isolated sample (e.g. isolated at a prior time point and isolated by the same or another person). In addition, the test sample can be freshly collected or a previously collected sample.

[00149] In some embodiments, the test sample can be an untreated test sample. As used herein, the phrase "untreated test sample" refers to a test sample that has not had any prior sample pre-treatment except for dilution and/or suspension in a solution. Exemplary methods

for treating a test sample include, but are not limited to, centrifugation, filtration, sonication, homogenization, heating, freezing and thawing, and combinations thereof. In some embodiments, the test sample can be a frozen test sample, e.g., a frozen tissue. The frozen sample can be thawed before employing methods, assays and systems described herein. After thawing, a frozen sample can be centrifuged before being subjected to methods, assays and systems described herein. In some embodiments, the test sample is a clarified test sample, for example, by centrifugation and collection of a supernatant comprising the clarified test sample. In some embodiments, a test sample can be a pre-processed test sample, for example, supernatant or filtrate resulting from a treatment selected from the group consisting of centrifugation, filtration, thawing, purification, and any combinations thereof. In some embodiments, the test sample can be treated with a chemical and/or biological reagent. Chemical and/or biological reagents can be employed to protect and/or maintain the stability of the sample, including biomolecules (e.g., nucleic acid and protein) therein, during processing. One exemplary reagent is a protease inhibitor, which is generally used to protect or maintain the stability of protein during processing. The skilled artisan is well aware of methods and processes appropriate for pre-processing of biological samples required for determination of the level of an expression product as described herein.

[00150] As used herein, the term “nucleic acid” or “nucleic acid sequence” refers to any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double- stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA. In one aspect, the nucleic acid can be DNA. In another aspect, the nucleic acid can be RNA. Suitable nucleic acid molecules are DNA, including genomic DNA or cDNA. Other suitable nucleic acid molecules are RNA, including mRNA.

[00151] As used herein, the terms “protein" and “polypeptide" are used interchangeably herein to designate a series of amino acid residues, connected to each other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms "protein", and "polypeptide" refer to a polymer of amino acids, including modified amino acids (e.g., phosphorylated, glycated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. "Protein" and “polypeptide” are often used in reference to relatively large polypeptides, whereas the term "peptide" is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms "protein" and "polypeptide" are used interchangeably herein when referring to a gene product and fragments thereof. Thus,

exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[00152] As used herein an "antibody" refers to IgG, IgM, IgA, IgD or IgE molecules or antigen-specific antibody fragments thereof (including, but not limited to, a Fab, $F(ab')_2$, Fv, disulphide linked Fv, scFv, single domain antibody, closed conformation multispecific antibody, disulphide-linked scfv, diabody), whether derived from any species that naturally produces an antibody, or created by recombinant DNA technology; whether isolated from serum, B-cells, hybridomas, transfectomas, yeast or bacteria.

[00153] As described herein, an "antigen" is a molecule that is bound by a binding site comprising the complementarity determining regions (CDRs) of an antibody agent. Typically, antigens are bound by antibody ligands and are capable of raising an antibody response *in vivo*. An antigen can be a polypeptide, protein, nucleic acid or other molecule or portion thereof. The term "antigenic determinant" refers to an epitope on the antigen recognized by an antigen-binding molecule, and more particularly, by the antigen-binding site of said molecule.

[00154] As used herein, the term "antibody reagent" refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and which specifically binds a given antigen. An antibody reagent can comprise an antibody or a polypeptide comprising an antigen-binding domain of an antibody. In some embodiments, an antibody reagent can comprise a monoclonal antibody or a polypeptide comprising an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term "antibody reagent" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')2, Fd fragments, Fv fragments, scFv, and domain antibody (dAb) fragments (see, e.g. de Wildt et al., Eur J. Immunol. 1996; 26(3):629-39; which is incorporated by reference herein in its entirety)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and primatized antibodies. Antibodies also include midibodies, humanized antibodies, chimeric antibodies, and the like.

[00155] The VH and VL regions can be further subdivided into regions of

hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" ("FR"). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917; which are incorporated by reference herein in their entireties). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00156] The terms "antigen-binding fragment" or "antigen-binding domain", which are used interchangeably herein are used to refer to one or more fragments of a full length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term "antigen-binding fragment" of a full length antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546; which is incorporated by reference herein in its entirety), which consists of a VH or VL domain; and (vi) an isolated complementarity determining region (CDR) that retains specific antigen-binding functionality.

[00157] As used herein, the term "specific binding" refers to a chemical interaction between two molecules, compounds, cells and/or particles wherein the first entity binds to the second, target entity with greater specificity and affinity than it binds to a third entity which is a non-target. In some embodiments, specific binding can refer to an affinity of the first entity for the second target entity which is at least 10 times, at least 50 times, at least 100 times, at least 500 times, at least 1000 times or greater than the affinity for the third nontarget entity. A reagent specific for a given target is one that exhibits specific binding for that target under the conditions of the assay being utilized. In certain embodiments, specific binding is indicated by a dissociation constant on the order of $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M or below.

[00158] As used herein, "expression level" refers to the number of mRNA molecules and/or polypeptide molecules encoded by a given gene that are present in a cell or sample. Expression levels can be increased or decreased relative to a reference level.

[00159] As used herein, the terms "treat," "treatment," "treating," or "amelioration" refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow

down or stop the progression or severity of a condition associated with a disease or disorder, e.g. lupus nephritis. The term “treating" includes reducing or alleviating at least one adverse effect or symptom of a condition, disease or disorder, e.g. lupus nephritis. Treatment is generally “effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective" if the progression of a disease is reduced or halted. That is, “treatment" includes not just the improvement of symptoms or markers, but also a cessation of, or at least slowing of, progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, remission (whether partial or total), and/or decreased mortality, whether detectable or undetectable. The term "treatment" of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment).

[00160] As used herein, the term “pharmaceutical composition” refers to the active agent in combination with a pharmaceutically acceptable carrier e.g. a carrier commonly used in the pharmaceutical industry.

[00161] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00162] As used herein, the term "administering," refers to the placement of a compound as disclosed herein into a subject by a method or route which results in at least partial delivery of the agent at a desired site. Pharmaceutical compositions comprising the compounds disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[00163] Exemplary modes of administration include, but are not limited to, injection, infusion, instillation, inhalation, or ingestion. “Injection” includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intrahepatic, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion. The administration can be systemic or local.

[00164] The term “statistically significant" or “significantly" refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[00165] As used herein, the term “significantly” should be interpreted as if modified by the term “statistically”.

[00166] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art to which this disclosure belongs. It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. 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 is defined solely by the claims. Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter et al. (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties.

[00167] The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise.

[00168] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages may mean ±1% of the value being referred to. For example, about 100 means from 99 to 101.

[00169] Although methods and materials similar or equivalent to those disclosed herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term "comprises" means "includes." The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

[00170] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow. Further, to the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various embodiments herein described and illustrated can be further modified to incorporate features shown in any of the other embodiments disclosed herein.

[00171] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology disclosed herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00172] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and

examples for, the disclosure are disclosed herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments disclosed herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure.

[00173] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

EXAMPLES

[00174] The following examples illustrate some embodiments and aspects of the invention. It will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be performed without altering the spirit or scope of the invention, and such modifications and variations are encompassed within the scope of the invention as defined in the claims which follow. The technology disclosed herein is further illustrated by the following examples which in no way should be construed as being further limiting.

Example 1: Colony Stimulating Factor 1: A Predictive Biomarker for Lupus Nephritis

[00175] Colony-stimulating factor-1 (CSF-1) is expressed by kidney tubules at the onset of LN, increases with disease progression, and spills into the circulation in lupus-prone mice. We tested the hypothesis that amplified expression of CSF-1 detected in the serum or urine correlates with intrarenal CSF-1 expression and histopathology (increased macrophage accumulation, activity indices) and clinical kidney disease activity and predicts the onset and recurrence of nephritis in patients with systemic lupus erythematosus (SLE). We found increased serum or urine CSF-1 levels in patients with cutaneous, serositis, and musculoskeletal disease; however, the increase in CSF-1 levels was far greater (i.e., at least statistically significantly greater) in LN. Moreover, an elevation in serum or urine CSF-1

levels correlated with increasing intrarenal CSF-1 expression and histopathology. By longitudinally tracking patients, we found that elevated serum CSF-1 heralded the initial onset of disease, and a rise in serum or urine CSF-1 predicted recurrences of LN before clinical evidence of glomerular dysfunction and conventional serologic measures, even in patients with other manifestations of SLE. These findings indicate that serial monitoring for a rise in serum or urine CSF-1 levels in patients with SLE reflects kidney histopathology and may predict renal disease activity and the onset and recurrence of LN more accurately than conventional laboratory measures.

Materials and Methods

[00176] Serum, Urine, and Renal Biopsy Specimens

[00177] To diagnose LN (comporting with the ISN/RP 2004 classification)[16] and assess histopathology activity and chronicity indices,[32] human kidney sections from renal biopsy specimens were provided by the Department of Pathology, Johannes-Gutenberg University, Mainz and Friedrich-Alexander University Erlangen-Nuernberg, Germany. Renal pathologists, without access to the patient's clinical data, evaluated these biopsy specimens. We analyzed urine and serum samples that were collected from two cohorts: Mainz, Germany, and Pavia, Italy. Unless otherwise stated, the data reported are from Mainz. Specimens were taken from patients who fulfilled at least four of the American College of Rheumatology criteria for the classification of SLE, or noninflammatory kidney disease (minimal-change disease, amyloidosis, nephrosclerosis) after informed consent. Volunteers (age range, 18–70 years) were screened for health by exclusion of any prior kidney diseases, diabetes, hypertension, and autoimmune diseases. Serum and urine was collected at a single visit. Freshly voided urine and drawn blood samples were collected, centrifuged, divided into aliquots, and stored at –30°C before analysis. The use of these specimens was reviewed and approved by the Standing Committee for Clinical Studies of the Johannes-Gutenberg University and the University and IRCCS Policlinico S. Matteo Foundation, Pavia, Italy, in adherence to the Declaration of Helsinki. All samples were analyzed retrospectively in Mainz.

[00178] Disease Activity

[00179] Disease activity was evaluated by standard clinical serologic activity measures (C3c, C4, ANA, anti-dsDNA antibodies, creatinine, C-reactive protein, ESR [after 1 hour/2 hours]) and urine measures (proteinuria [24-hour collection] and active sediment). Activity indices were assessed in the same serum and urine samples as the CSF-1 measurements at

each time point. Clinical remission for patients with LN was defined as normal GFR and proteinuria <500 mg/24 hours according to the 2012 consensus recommendations from the European League Against Rheumatism/European Renal Association-European Dialysis and Transplant Association.[5] The criteria for renal flares was increasing proteinuria or serum creatinine, declining C3/C4 levels, or rising anti-dsDNA titers. The criteria for new-onset LN included newly developed proteinuria or increasing serum creatinine, declining C3/C4 levels or rising anti-dsDNA titers in patients with established SLE.[5] The following standard values of serologic activity markers were determined: C3 (0.9–1.8 g/L) and C4 (0.1–0.4 g/L) by enzyme immunoassay, ANA (1:80–1:5120) by immunofluorescence, dsDNA (30–200 IU/ml) by ELISA, C-reactive protein (<5 mg/dl) by nephelometry or turbidimetry in an automated analyzer, creatinine (0.5–0.8 mg/dl) by isotope dilution mass spectrometry, proteinuria (<150 mg/24 hours) by immunoturbidimetric assay, and active sediment (<5%) by microscopy.

[00180] CSF-1 ELISA

[00181] We measured human CSF-1 levels in serum and urine using a human CSF-1 ELISA kit according to the manufacturer's instructions. Samples were thawed and spun down and the supernatant fraction used for the ELISA. Undiluted serum and urine samples were analyzed. All measurements were made in duplicate. The laboratory personnel were blinded to clinical data. The ELISA antibodies and reagents were purchased from R&D Systems (McKinley Place, MN). The minimum detectable level of CSF-1 is <9 pg/ml.

[00182] Immunostaining

[00183] We evaluated serial sections (4 μm) of human kidney biopsy specimens. Antigens were retrieved by immersion in citrate buffer followed by blocking of endogenous peroxidase activity and nonspecific binding of avidin and biotin as previously described.[8] We incubated kidney sections with a primary antibody, goat anti-human CSF-1 antibody (N-16; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-human CD3 antibody (LabVision, Kalamazoo, MI), and mouse anti-human CD68 (C-20; Santa Cruz Biotechnology) and detected the primary antibody by incubation with biotinylated rabbit anti-goat antibody, goat anti-rabbit antibody, and goat anti-mouse antibody, respectively, followed by development with 3-3-diaminobenzidine (Vector Laboratories; Burlingame, CA). We verified staining specificity by replacing the primary antibody with goat IgG (for anti-CSF-1 antibody), rabbit IgG (for anti-CD3 antibody, anti-caspase-3 antibody), and mouse IgG (for anti-CD68 antibody) (eBio-science, San Diego, CA). CSF-1 specificity was verified by preabsorbing the anti-CSF-1 antibody with peptide (Santa Cruz Biotechnology). We determined the percentage positive TECs or positive infiltrating cells in 10 randomly selected high-power fields.

[00184] Statistical Analyses

[00185] Statistical analyses were based on the 161 patients and 162 controls from Mainz and 102 patients and 50 controls from Pavia. The data representing the mean±SEM were prepared using GraphPad PRISM, version 6.0. We used the nonparametric Mann-Whitney *U* test for comparison between two groups and the Kruskal-Wallis test for comparisons between three or more groups. For correlation analysis, we used the Spearman correlation coefficient. No correction for multiple testing was done, and we report *P* values for all tests. Because this is an explorative study, we have chosen not to use the term "statistically significant" but rather to discuss differences that cannot be explained by random variation alone. The area under the curve calculation of nonparametric receiver-operating characteristics was used for CSF-1 sensitivity and specificity. We calculated the intra-assay variability and interassay variability as defined elsewhere.[15] We calculated the PPV and NPV using the serum CSF-1 levels that were tracked in individual patients in FIGs. 4A-4C and 5A-5B. For each patient, the laboratory results nearest to the predefined time points 60 and 120 days before flare were used for this calculation. The difference in percentages between these values was then calculated and compared with the defined cutoff value (an increase of 25%). No other values between the defined time points were used for this calculation.

RESULTS

[00186] **Serum and Urine CSF-1 Levels Are Notably Elevated in Patients with LN**

[00187] We evaluated CSF-1 levels in two independent cohorts. The cohort from Mainz, Germany, included 161 patients with SLE with diverse disease manifestations and 162 healthy controls, and the cohort from Pavia, Italy, included 102 patients with SLE and 50 healthy controls. To determine whether serum and urine CSF-1 levels increase in patients with SLE, we compared serum and urine CSF-1 in patients with SLE and in healthy controls using these two large cohorts. Patients were included independent of disease duration, clinical activity, and treatment regimen (patient demographic characteristics and clinical profiles are shown in Table 1). We detected increased serum and urine CSF-1 levels in patients with SLE compared with healthy controls in both cohorts (FIGs. 1A-1D), although the range of CSF-1 levels varies between these cohorts. Because SLE is a diverse disease that targets many tissues for destruction, we stratified and compared CSF-1 levels according to the diseased tissue (FIGs. 1A-1D). While serum and urine CSF-1 are elevated in patients with SLE and LN (Table 1) and those with cutaneous lupus erythematosus (CLE), serositis, and musculoskeletal manifestations alone compared with healthy controls and noninflammatory

kidney disease (patient demographic characteristics and clinical profiles are shown in Table 2), CSF-1 levels are far higher in each cohort in patients with LN (FIGs. 1A-1D).

Table 1. Demographic and clinical characteristics according to study cohort

Variable	Overall		Biopsy-Proven LN	
	Mainz Cohort (*n*=161)	Pavia Cohort (*n*=102)	Mainz Cohort (*n*=50)	Pavia Cohort (*n*=15)
Race				
White	142	94	42	12
Asian	11	3	7	1
Others	8	5	1	2
Sex				
Female	150	95	41	13
Male	11	7	9	2
Age (yr)				
Mean	48.5±1.9	46.9±1.7		
Range	22-75	18-69		
Age at SLE diagnosis (yr)				
Mean	33.2±2.1	31.8±1.8	32±1.9	34±1.6
Range	11.1-72.3	12.5-71.4	11.5-69.1	13.1-73.2
Age at diagnosis of LN (yr)				
Mean			37.3±2.1	39.1±3.3
Range			19.5-69.3	17.1-67.2
Disease duration at time of LN onset (yr)				
Mean			5.0±1.1	6.6±131
Range			0-31.1	0-24.1
Overall duration of SLE (yr)				
Mean	13.9±1.6	15.7±1.4	11.1±1.1	12.1±0.9
Range	0.7-43.5	0.9-41.9	0.8-32	0.5-40.2
Clinical variables at the time of LN diagnosis				
ANA-positive patients (*n*)	157	100	48	15
Anti-dsDNA-positive patients (*n*)	126	78	39	6
Creatinine (mg/dl)			1.1±0.1	1.1±0.16
Proteinuria (24-hr collection)>0.5 g/d (g/d)				
Mean			2.9±0.4	1.4±0.4
Range			0.4-17.5	0.3-5.6
Depressed serum C3 level				
Patients (*n*)			38	12
Mean			0.6±0.5	0.7±0.1
Range			0.2-1.4	0.4-1.1
ISN/RPS class (*n*)				
Class II			11	2
Class III			10	4
Class IV			29	9
Class V			4	2
Medication				
Prednisolone dose (mg)	4.3±0.8	4.9±0.7	8.3±0.6	4.5±0.5
Prednisolone (*n*)	134	81	48	15
Mycophenolate mofetil (*n*)	78	36	40	11
Cyclophosphamide (only as induction therapy) (*n*)	31	12	23	10
Azathioprine (*n*)	42	13	4	4
Hydroxychloroquine (*n*)	92	67	30	12
Angiotensin-blocking agent (*n*)	72	34	42	10

Means are expressed with SDs.

Table 2. Demographic and Clinical Characteristics in Patients with Non-inflammatory Kidney Disease

Race (n)	White	45
	Asian	0
	Others	7
Sex (n)	Female	27
	Male	25
Disease type	Nephrosclerosis	32
	Amyloidosis	12
	Minimal Change Disease	10
Age at diagnosis (years)	Mean	48.6±2.8
	Range	24.9-74.1

[00188] CSF-1 levels in the sera and urine of patients with SLE and LN are sensitive and specific, as shown using receiver-operating characteristic curves (FIG. 6A). Confidence in our CSF-1 assay was supported by the CSF-1 interassay variability (8.5%) and intra-assay variability (7.2% for serum and 5.1% for urine).[15] Moreover, we found little variability in CSF-1 levels using serum and urine specimens that we froze and thawed four times (FIG. 6B). The difference (mean±SD) of the change of CSF-1 levels was 2.7±0.5 pg/ml in serum and 6.7±0.6 pg/ml in urine. In addition, we found little variability in serum CSF-1 levels (frozen and thawed once) of healthy volunteers and patients with LN in remission at multiple timed intervals (FIG. 6C). Finally, quantifying CSF-1 levels is both rapid and inexpensive.

[00189] These findings indicate that CSF-1 was elevated in patients with LN, although absolute CSF-1 levels differed in the two cohorts. Of note, there was wide diversity within and between the two cohorts. The patients were included irrespective of disease activity (active, flare, remission), severity, duration, and treatment (medications and regimens) (Table 1). These cross-sectional studies show that CSF-1 levels in the serum and urine are substantially higher in LN than in other manifestations of SLE in each cohort.

[00190] Serum and Urine CSF-1 Levels Reflect Intrarenal CSF-1 and Extent of Mø-Rich Inflammation in LN

[00191] On the basis of the data in FIGs. 1A-1D, we hypothesized that increased intrarenal CSF-1 spills into the serum or urine in patients with LN and thereby reflects the extent of Mø-rich intrarenal inflammation. Using immunostaining, we detected intrarenal CSF-1 expression in patients with LN, primarily within tubules (FIG. 2A), which was more

extensive in patients with class IV than class II disease. Because therapeutic intervention may skew our data, we restricted our initial CSF-1 analysis to patients with LN before initiation of therapy. We found that the magnitude of intrarenal CSF-1 expression correlated with serum and urine CSF-1 in LN before therapy (FIG. 2A). We hypothesized that increased CSF-1 in TEC reflects a rise in intrarenal Mø. We detected an increase in Mø ($CD68^+$) in LN compared with noninflammatory kidney diseases (minimal-change and thin basement membrane disease; data not shown). Moreover, the abundance of intrarenal Mø infiltration was correlated with CSF-1 expression by TEC (FIG. 2B) and serum or urine CSF-1 levels (FIG. 2B). Thus, elevated serum and urine CSF-1 reflect an increase in intrarenal CSF-1 expression and heightened Mø-mediated renal inflammation.

[00192] CSF-1 in Kidney, Serum, and Urine Levels Reflect Histopathology Activity and Severity, Not Chronicity, of LN

[00193] Because CSF-1 levels in the serum and urine reflect intrarenal CSF-1 expression, we hypothesized that the level of CSF-1 in the kidney, serum, and urine reflect renal disease activity in patients with LN, as reflected by histopathology. In fact, the magnitude of CSF-1 expression in the kidney, serum, and urine positively correlated with renal histopathology activity (glomerular proliferation, leukocyte exudation, karyorrhexis/fibrinoid necrosis, cellular crescents, hyaline deposits, and interstitial inflammation) (FIG. 2C). This finding is consistent with the hypothesis that amplified CSF-1 expression drives the initiation of LN. In contrast, the magnitude of CSF-1 expression in the kidney, serum, and urine did not correlate with histopathology indices of chronicity (glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis). We suggest that this lack of correlation results from a reduction of CSF-1 owing to destruction of the major source of CSF-1, tubules, during ESRD.[8]

[00194] Because LN is diverse, we determined whether the magnitude of CSF-1 in serum or urine was stratified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2004 classification.[16] Serum or urine CSF-1 levels were highest in type IV (diffuse GN), intermediate in type III (focal segmental GN), and lowest in type II (only mesangial alterations) (FIG. 2D). As the severity of inflammation increases from type II to Type IV, our findings are consistent with the enhanced expression of CSF-1 in tubules of patients with type IV compared with type II (FIG. 2A), as well as the hypothesis that serum and urine CSF-1 levels reflect the magnitude of renal inflammation.

[00195] Increased Serum or Urine CSF-1 Longitudinally Track with Elevated Clinical Disease Activity in LN

[00196] Our cross-sectional findings indicated that serum and urine CSF-1 were elevated in patients with LN and reflect the level of intrarenal infiltration by Møs. These findings prompted us to determine whether CSF-1 is a reliable biomarker reflecting disease activity in LN. Therefore, we longitudinally monitored CSF-1 in the serum and urine of patients with biopsy-proven LN to determine whether a change in CSF-1 levels tracks with clinical disease activity in the two cohorts (Mainz and Pavia) (demographic characteristics and patient profiles are shown in Table 1). In these studies, each patient served as his or her own control. Measurements were taken 4, 8, and 12 months after diagnosis of LN in the Mainz cohort and 3, 6, and 12 months after in the Pavia cohort. Serum and urine CSF-1 levels decreased at 4, 8, and 12 months and at 3, 6, and 12 months after diagnosis in the Mainz and Pavia cohorts, respectively. This finding correlated with improved indices of disease activity (rise in C3c and decrease in antinuclear antibody [ANA], anti-double-stranded DNA [dsDNA] antibodies, and erythrocyte sedimentation rate [ESR] in serum, proteinuria, active sediment, and Systemic Lupus Erythematosus Disease Activity Index [SLEDAI] (FIG. 3). Moreover, we calculated the decline of serum CSF-1 levels as a predictor of the response to therapy and remission (FIG. 3). This is of particular interest in daily clinical practice to minimize toxicity, especially in young patients. The decline in serum CSF-1 (≥25%) after initiation of therapy had a positive predictive value (PPV) of 88% and a negative predictive value (NPV) of 58%. The considerably higher PPV compared with NPV is understandable because more patients were in remission than patients who did not reach remission (40/10). Thus, serum or urinary CSF-1 levels tracked with LN clinical disease activity and may potentially predict the response to therapy.

[00197] Serum or Urine CSF-1 Decreases from Diagnosis to Remission and Increases in Advance of Glomerular Dysfunction and Standard Measures during LN Flares

[00198] Early diagnosis and therapy are essential to successful treatment of LN flares. We monitored serum and urine CSF-1 levels before, during, and after flares in LN alone without flares in other manifestations of SLE (FIGs. 4A-4C; patient demographic characteristics and clinical profiles are shown in Table 3). Serum and urine CSF-1 levels increased well in advance of LN flares (renal flare defined as an increase of urinary protein levels of ≥500 mg/24 hours, in accordance with the American College of Rheumatology criteria and SLEDAI) compared with other conventional clinical measures (FIG. 4A, composite). Of the clinical variables, proteinuria was the best indicator of an LN flare in this cohort. To determine whether a rise in CSF-1 is a better predictor of an LN flare than proteinuria in an

individual patient, we compared elevated CSF-1 (defined as 50% higher than normal) versus proteinuria at each point before the patient's renal biopsy. An elevation of serum CSF-1 heralded a LN flare in individual patients earlier (mean, 54 days) than did a rise in proteinuria (FIG. 4B, individual). Consistent with these data, serum and urine CSF-1 declined with LN remissions (FIG. 4C, individual) and remained stable in patients not in remission (data not shown). Thus, an increase (≥25%) in the CSF-1 level in serum or urine predicted an LN flare before overt glomerular dysfunction and standard clinical measures.

Table 3. Demographic and Clinical Characteristics in Patients with LN Flares

Race (n)	White	12
	Asian	0
	Others	2
Sex (n)	Female	9
	Male	5
Age at SLE diagnosis (years)	Mean	32.9±2.8
	Range	21.8-47.6
Age at diagnosis of LN (years)	Mean	38.1±2.7
	Range	23.8-50.7
Age at diagnosis of flare (years)	Mean	43.6±3.1
	Range	23.9-62.1
Time from diagnosis of LN to flare (years)	Mean	5.3±1.5
	Range	0.5-14.5
Overall duration of LN (years)	Mean	6.9±1.3
	Range	1.2-15.9
Clinical parameter at the time of LN flare		
Creatinine (mg/dl)		1.1±0.1
Proteinuria (24h collection) >0·5g	n	48
	Mean	1.7±0.2
	Range	1.1-3.1 g/d
ANA positive	n	12
anti-dsDNA positive	n	10
Depressed serum C3 level	n	9
	Mean	0.9±0.1
	Range	0.5-1.2
ISN/RPS class		
Class II	n	2
Class III	n	3
Class IV	n	8
Class V	n	1
Medication		
Prednisolone	n	12
Mycophenolatmofetile	n	10
Cyclophosphamide	n	0
Azathioprine	n	2
Hydroxychloroquine	n	8
Angiotensin-blocking agent	n	5

[00199] Increased Serum CSF-1 in SLE Heralds Onset of LN in Advance of Glomerular Dysfunction and Standard Clinical Measures

[00200] Identification of a biomarker in the serum or urine that predicts initiation of kidney injury would provide an opportunity to obviate irreversible intrarenal disease. We quantified serum and urine CSF-1 levels in longitudinally tracked patients (patient demographic and clinical characteristics are shown in Table 4) with SLE before, at, and after biopsy-proven diagnosis of LN. Serum CSF-1 increased several months in advance of proteinuria and other measures (active sediment and serum creatinine and alterations in serologic variables, including C-reactive protein, C3c, ANA, ESR, C4, anti-dsDNA antibodies, and SLEDAI) in LN (FIG. 5A, composite). To determine whether a rise in CSF-1 is an earlier predictor than proteinuria for the onset of LN in each individual patient, we compared the rise in CSF-1 (defined as 50% higher than normal) with proteinuria at each point before the patient's renal biopsy. An elevation of serum CSF-1 heralded LN flares in individual patients earlier (mean, 66 days) than proteinuria (FIG. 5B, individual). Moreover, serum and urine CSF-1 declined after diagnosis upon treatment, and this decline preceded a reduction of proteinuria and serologic measures (C3c, ESR, anti-dsDNA antibodies) (FIGs. 5A-5B, composite). The positive and negative predictors indicating that a rise in serum CSF-1 predicts new-onset LN (FIGs. 5A-5B) and an LN flare (FIGs. 4A-4C) were 83% (PPV) and 63% (NPV). Taken together, these findings indicate that a rise in serum CSF-1 is a biomarker for predicting the initial onset of LN in advance of glomerular dysfunction and standard clinical measures.

Table 4. Demographic and Clinical Characteristics in Patients with New Onset of LN

Race (n)	White	15
	Asian	0
	Others	2
Sex (n)	Female	12
	Male	5
Age at SLE diagnosis (years)	Mean	31±2.2
	Range	21.8-49.3
Age at diagnosis of LN (years)	Mean	37.3±2.4
	Range	22.1-55.5
Disease duration at the time of LN onset (years)	Mean	5.9±1.6
	Range	0.5-22.8
Clinical parameter at the time of LN onset		
Creatinine (mg/dl)		1.0±0.1
Proteinuria (24h collection) >0·5g	n	17
	Mean	2.1±0.3
	Range	0.6-6.3 g/d
ANA positive	n	16
anti-dsDNA positive	n	14
Depressed serum C3 level	n	11
	Mean	0.8±0.1
	Range	0.2-1.5
ISN/RPS class		
Class II	n	4
Class III	n	3
Class IV	n	9
Class V	n	1
Medication		
Prednisolone	n	16
Mycophenolatmofetile	n	12
Cyclophosphamide (only as induction therapy)	n	0
Azathioprine	n	2
Hydroxychloroquine	n	8
Angiotensin-blocking agent	n	5

DISCUSSION

[00201] On the basis of data from lupus-prone mice, amplified CSF-1 expression accurately predicted LN and correlated closely with disease activity. In a cross-sectional study using two cohorts, we show that serum and urine CSF-1 levels were increased in patients with SLE and CLE, serositis, and musculoskeletal manifestations. Within each cohort, CSF-1 levels were substantially greater in patients with LN. Using longitudinally tracked patients with SLE, we found that a rise in serum CSF-1 heralded the onset of LN and

that a rise in serum or urine CSF-1 predicted the recurrence of LN before glomerular dysfunction and conventional serologic measures. This pattern is evident even in patients with other manifestations of SLE. Moreover, serum and urine CSF-1 levels increase with progressing clinical disease activity and correlate with established renal histopathology variables. Taken together, these findings suggest that serial monitoring serum or urine CSF-1 levels in SLE patients can be a biomarker for predicting the onset, recurrence, and disease activity of LN that is more accurate than conventional laboratory measures.

[00202] Prior studies identifying biomarkers for LN analyzed an individual molecule or combinations of molecules (as reviewed in references 17–19 among cross-sectional studies), mainly in the urine.[20,21] Our current longitudinal analysis indicates that a rise in serial monitored serum CSF-1 levels in individual patients without documented LN heralds the onset of LN, while both serum and urine predict recurrences of biopsy-proven LN more accurately than do standard measures. Urinary, but not serum, neutrophil gelatinase-associated lipocalin predicted renal flares in patients with LN and is reportedly a more sensitive forecaster of flares in patients with a history of LN than are dsDNA antibody titers, but its predictive value was not superior to that of C3c and C4 in patients with LN.[21,24] By comparison, our longitudinal analysis of individual patients suggests that elevated serum and urine CSF-1 levels may predict LN recurrences, and elevated serum CSF-1 may herald the onset of LN in advance of glomerular dysfunction and multiple clinical LN measures, including dsDNA. Thus, our current study indicates that monitoring serum or urine CSF-1 offers a potential individualized approach to the management of patients with LN.

[00203] Although the current classification (ISN/RPS) of LN is largely based on glomerular,[16] rather than tubulointerstitial pathology, the magnitude of tubulointerstitial damage determines the fate of the kidney in LN and other renal diseases.[25,26] In fact, extensive Mø-rich interstitial infiltrates,[27,28] inflammation, tubular atrophy, and interstitial fibrosis are accurate morphologic predictors of a poor renal prognosis.[25,29,30] Thus, tracking molecules that mediate tubulointerstitial inflammation and injury are candidates for predicting renal disease outcomes in patients with SLE. We now show that increased serum or urine CSF-1 levels reflect the magnitude of CSF-1 expression in the tubules and Mø-rich inflammation in the interstitium and predict the onset, recurrence, and disease activity of LN. Our current findings suggest that Mø-mediated interstitial inflammation precedes and perhaps triggers glomerular disease in LN. This view is consistent with our experimental data in which transient kidney injury in lupus-prone hosts induces CSF-1; this, in turn, leads to Mø-mediated tubulointerstitial damage, followed by a rise in autoantibodies and immune

complex-mediated GN.[31] Because we now show that a rise in serum and urine CSF-1 heralds overt renal tubulointerstitial injury, tracking serum or urine CSF-1 potentially identifies a time-related opportunity to treat SLE before the onset of irreversible kidney damage.

[00204] The CSF-1–dependent Mø phenotype linked to lupus, rather than CSF-1 alone, is central to triggering LN. Our prior studies indicate that whereas increased CSF-1 drives Mø-mediated renal destructive inflammation in lupus-prone mice, CSF-1 fosters Mø-mediated renal repair in normal hosts.[31] This apparent dichotomy results from the time-related balance of M1 Mø destroyers, shifting to M2 Mø healers in normal kidneys, as opposed to the continuing dominance of M1 Mø in lupus-prone hosts. Thus, without wishing to be bound by theory, we suggest that CSF-1–driven aberrant Mø linked to lupus is the likely culprit that triggers LN in patients.

[00205] Although a molecule that is an accurate biomarker does not necessarily have to participate in the pathogenesis of disease, CSF-1 is central to initiating the pathogenesis of LN in mice. Because the ultimate goal of a therapeutic is to spare tissue destruction, the most promising therapeutic targets are initiators of tissue injury. As circulating CSF-1, largely generated by the kidney at the onset of inflammation, initiates a cascade of events culminating in kidney damage,[8] neutralizing of CSF-1 and blocking the CSF-1 receptor or signaling cascade may preserve renal structure and function. Thus, the CSF-1 pathway is a potential therapeutic target for LN. Similarly, the most valuable biomarkers are those that most accurately identify the inception or reactivation of a disease process before the advent of clinically detectable disease and immutable tissue injury.

[00206] Taken together, our findings suggest that quantifying CSF-1 in the serum or urine can be a reliable and inexpensive biomarker for managing the individualized treatment of patients with SLE.

References for Example 1:

1. Cervera R, et al., European Working Party on Systemic Lupus Erythematosus: Morbidity and mortality in systemic lupus erythematosus during a 10-year period: A comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore)* 82: 299-308, 2003

2. Fiehn C, HajjarY, Mueller K, Waldherr R, Ho AD, Andrassy K: Improved clinical outcome of lupus nephritis during the past decade: Importance of early diagnosis and treatment. *Ann Rheum Dis* 62: 435-439, 2003

3. Mok CC, Ying KY, Tang S, Leung CY, Lee KW, Ng WL, Wong RW, Lau CS: Predictors and outcome of renal flares after successful cyclophos-phamide treatment for diffuse proliferative lupus glomerulonephritis. *Arthritis Rheum* 50: 2559-2568, 2004

4. Sprangers B, Monahan M, Appel GB: Diagnosis and treatment of lupus nephritis flares—an update. *Nat Rev Nephrol* 8: 709-717, 2012

5. Bertsias GK, et al., European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association: Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 71: 1771¬1782, 2012

6. Pixley FJ, Stanley ER: CSF-1 regulation of the wandering macrophage: complexity in action. *Trends Cell Biol* 14: 628-638, 2004

7. Menke J, Iwata Y, Rabacal WA, Basu R, Yeung YG, Humphreys BD, Wada T, Schwarting A, Stanley ER, Kelley VR: CSF-1 signals directly to renal tubular epithelial cells to mediate repair in mice. *J Clin Invest* 119: 2330-2342, 2009

8. Menke J, Rabacal WA, Byrne KT, Iwata Y, Schwartz MM, Stanley ER, Schwarting A, Kelley VR: Circulating CSF-1 promotes monocyte and macrophage phenotypes that enhance lupus nephritis. *J Am Soc Nephrol* 20: 2581-2592, 2009

9. Yui MA, Brissette WH, Brennan DC, Wuthrich RP, Rubin-Kelley VE: Increased macrophage colony-stimulating factor in neonatal and adult autoimmune MRL-Ipr mice. *Am J Pathol* 139: 255-261, 1991

10. Lenda DM, Stanley ER, Kelley VR: Negative role of colony-stimulating factor-1 in macrophage, T cell, and B cell mediated autoimmune disease in MRL-Fas(lpr) mice. *J Immunol* 173: 4744-4754, 2004

11. Moore KJ, Naito T, Martin C, Kelley VR: Enhanced response of macrophages to CSF-1 in autoimmune mice: A gene transfer strategy. *J Immunol* 157: 433-440, 1996

12. Menke J, Iwata Y, Rabacal WA, Basu R, Stanley ER, Kelley VR: Distinct roles of CSF-1 isoforms in lupus nephritis. *J Am Soc Nephrol* 22: 1821¬1833, 2011

13. Lenda DM, Kikawada E, Stanley ER, Kelley VR: Reduced macrophage recruitment, proliferation, and activation in colony-stimulating factor-1-deficient mice results in decreased tubular apoptosis during renal inflammation. *J Immunol* 170: 3254-3262, 2003

14. Tesch GH, Schwarting A, Kinoshita K, Lan HY, Rollins BJ, Kelley VR: Monocyte chemoattractant protein-1 promotes macrophage-mediated tubular injury, but not glomerular injury, in nephrotoxic serum nephritis. *J Clin Invest* 103: 73-80, 1999

15. Ramljak S, Musholt PB, SchipperC, Flacke F, SieberJ, Borchert M, Forst T, Kutzner A: The precision study: Examining the inter- and intra-assay variability of replicate measurements of BGStar, iBGStar and 12 other blood glucose monitors. *Exp Opin Med Diagnostics* 7: 511-516, 2013

16. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, Nagata M: The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 15: 241-250, 2004

17. Brunner HI, Bennett MR, Mina R, Suzuki M, Petri M, Kiani AN, Pencil J, Witte D, Ying J, Rovin BH, Devarajan P: Association of noninvasively measured renal protein biomarkers with histologic features of lupus nephritis. *Arthritis Rheum* 64: 2687-2697, 2012

18. Reyes-Thomas J, Blanco I, Putterman C: Urinary biomarkers in lupus nephritis. *Clin Rev Allergy Immunol* 40: 138-150, 2011

19. Mok CC: Biomarkers for lupus nephritis: A critical appraisal. *J Biomed Biotechnol* 2010: 638413, 2010

20. Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, Hojaili B, Mackay M, Aranow C, Stohl W, Rovin BH, Michaelson JS, Putterman C: Urinary TWEAK as a biomarker of lupus nephritis: A multicenter cohort study. *Arthritis Res Ther* 11: R143, 2009

21. Rubinstein T, Pitashny M, Levine B, Schwartz N, Schwartzman J, Weinstein E, Pego-Reigosa JM, Lu TY, Isenberg D, Rahman A, Putterman C: Urinary neutrophil gelatinase-associated lipocalin as a novel biomarker for disease activity in lupus nephritis. *Rheumatology (Oxford)* 49: 960-971, 2010

22. Tian S, Li J, Wang L, Liu T, Liu H, Cheng G, Liu D, Deng Y, Gou R, Wan Y, Jia J, Chen C: Urinary levels of RANTES and M-CSF are predictors of lupus nephritis flare. *Inflam Res* 56: 304-310, 2007

23. Yang PT, Xiao WG, Zhao LJ, Lu J, He LM, Kasai H, Ito M: Increase in the level of macrophage colony-stimulating factor in patients with systemic lupus erythematosus. *Ann Rheum Dis* 67: 429-430, 2008

24. Hinze CH, Suzuki M, Klein-Gitelman M, Passo MH, Olson J, Singer NG, Haines KA, Onel K, O'Neil K, Silverman ED, Tucker L,Ying J, Devarajan P, Brunner HI: Neutrophil gelatinase-associated lipocalin is a predictor of the course of global and renal childhood-onset systemic lupus erythematosus disease activity. *Arthritis Rheum* 60: 2772-2781, 2009

25. Yu F, Wu LH, Tan Y, Li LH, Wang CL, Wang WK, Qu Z, Chen MH, GaoJJ, Li ZY, Zheng X, Ao J, Zhu SN, Wang SX, Zhao MH, Zou WZ, Liu G: Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system. *Kidney Int* 77: 820-829, 2010

26. Hunter MG, Hurwitz S, Bellamy CO, Duffield JS: Quantitative morphometry of lupus nephritis: The significance of collagen, tu¬bular space, and inflammatory infiltrate. *Kidney Int* 67: 94-102, 2005

27. Hill GS, Delahousse M, Nochy D, Remy P, Mignon F, Mery JP, Bariety J: Predictive power of the second renal biopsy in lupus nephritis: Significance of macrophages. *Kidney Int* 59: 304-316, 2001

28. Yang N, Isbel NM, Nikolic-Paterson DJ, Li Y, Ye R, Atkins RC, Lan HY: Local macrophage proliferation in human glomerulonephritis. *Kidney Int* 54: 143-151, 1998

29. Hill GS, Delahousse M, Nochy D, Mandet C, Bariety J: Proteinuria and tubulointerstitial lesions in lupus nephritis. *Kidney Int* 60: 1893-1903, 2001

30. Zhang X, Nagaraja HN, Nadasdy T, Song H, McKinley A, Prosek J, Kamadana S, Rovin BH: A composite urine biomarker reflects interstitial inflammation in lupus nephritis kidney biopsies. *Kidney Int* 81: 401–406, 2012

31. Iwata Y, Bostram EA, Menke J, Rabacal WA, Morel L, Wada T, Kelley VR: Aberrant macrophages mediate defective kidney repair that triggers nephritis in lupus-susceptible mice. *J Immunol* 188: 4568¬4580, 2012

32. Austin HA 3rd, Boumpas DT, Vaughan EM, Below JE: Predicting renal outcomes in severe lupus nephritis: Contributions of clinical and histologic data. *Kidney Int* 45: 544-550, 1994

CLAIMS

What is claimed is:

1. A method of monitoring disease activity in a subject with systemic lupus erythematosus, the method comprising:
   (i) measuring, at each of two or more time points, a level of CSF-1 in a sample obtained from the subject;
   (ii) determining a temporal trend of the level of CSF-1 as a function of the measurements of step (i); and
   (iii) identifying the subject as (a) having an increased risk of developing lupus nephritis if the temporal trend is upward, or (b) having no or minimal risk of developing lupus nephritis if the temporal trend is flat or downward.
2. The method of claim 1, wherein the level of CSF-1 is measured at three or more time points.
3. The method of claim 1 or 2, wherein the level of CSF-1 is a protein level.
4. The method of any one of claims 1-3, wherein the level of CSF-1 is measured by an immunoassay.
5. The method of claim 4, wherein the sample is contacted with an anti-CSF-1 antibody.
6. The method of claim 5, wherein the anti-CSF-1 antibody is detectably labeled or capable of generating a detectable signal.
7. The method of claim 5 or 6, wherein the antibody is fluorescently labeled.
8. The method of claim 1 or 2, wherein the level of CSF-1 is measured by measuring a nucleic acid encoding CSF-1.
9. The method of any one of claims 1-8, wherein the sample is selected from the group consisting of blood, plasma, serum, and urine.
10. The method of any one of claims 1-9, further comprising administering a treatment appropriate for treating lupus nephritis to the subject if the subject is identified as having an increased risk of developing lupus nephritis.
11. The method of claim 10, wherein the treatment comprises administering a CSF-1 inhibitor.
12. The method of any one of claims 1-11, wherein the subject is identified as having an increased risk of developing lupus nephritis at least 30 days prior to symptoms of LN.
13. The method of any one of claims 1-12, wherein the subject is a mammal.
14. The method of claim 13, wherein the mammal is a human.

15. A method of monitoring disease activity in a subject in remission of lupus nephritis (LN), the method comprising:
    (i) measuring, at each of two or more time points, a level of CSF-1 in a sample obtained from the subject;
    (ii) determining a temporal trend of the level of CSF-1 as a function of the measurements of step (i); and
    (iii) identifying the subject as (a) having an increased risk of developing LN flares if the temporal trend is upward, or (b) having no or minimal risk of developing LN flares if the temporal trend is flat or downward.
16. The method of claim 15, wherein the level of CSF-1 is measured at three or more time points.
17. The method of claim 15 or 16, wherein the level of CSF-1 is a protein level.
18. The method of any one of claims 15-17, wherein the level of CSF-1 is measured by an immunoassay.
19. The method of claim 18, wherein the sample is contacted with an anti-CSF-1 antibody.
20. The method of claim 19, wherein the anti-CSF-1 antibody is detectably labeled or capable of generating a detectable signal.
21. The method of claim 19 or 20, wherein the antibody is fluorescently labeled.
22. The method of claim 15 or 16, wherein the level of CSF-1 is measured by measuring a nucleic acid encoding CSF-1.
23. The method of any one of claims 15-22, wherein the sample is selected from the group consisting of blood, plasma, serum, and urine.
24. The method of any one of claims 15-23, further comprising administering a treatment appropriate for treating lupus nephritis to the subject if the subject is identified as having an increased risk of developing lupus nephritis.
25. The method of claim 24, wherein the treatment comprises administering a CSF-1 inhibitor.
26. The method of any one of claims 15-25, wherein the subject is identified as having an increased risk of developing LN flares at least 30 days prior to symptoms of LN flares.
27. The method of any one of claims 15-26, wherein the subject is a mammal.
28. The method of claim 27, wherein the mammal is a human.

29. A method of monitoring treatment progress in a subject having lupus nephritis, the method comprising:

(i) measuring, at a first time point, a first level of CSF-1 in a first sample obtained from the subject;

(ii) administering to the subject a therapeutic agent for treating lupus nephritis; and

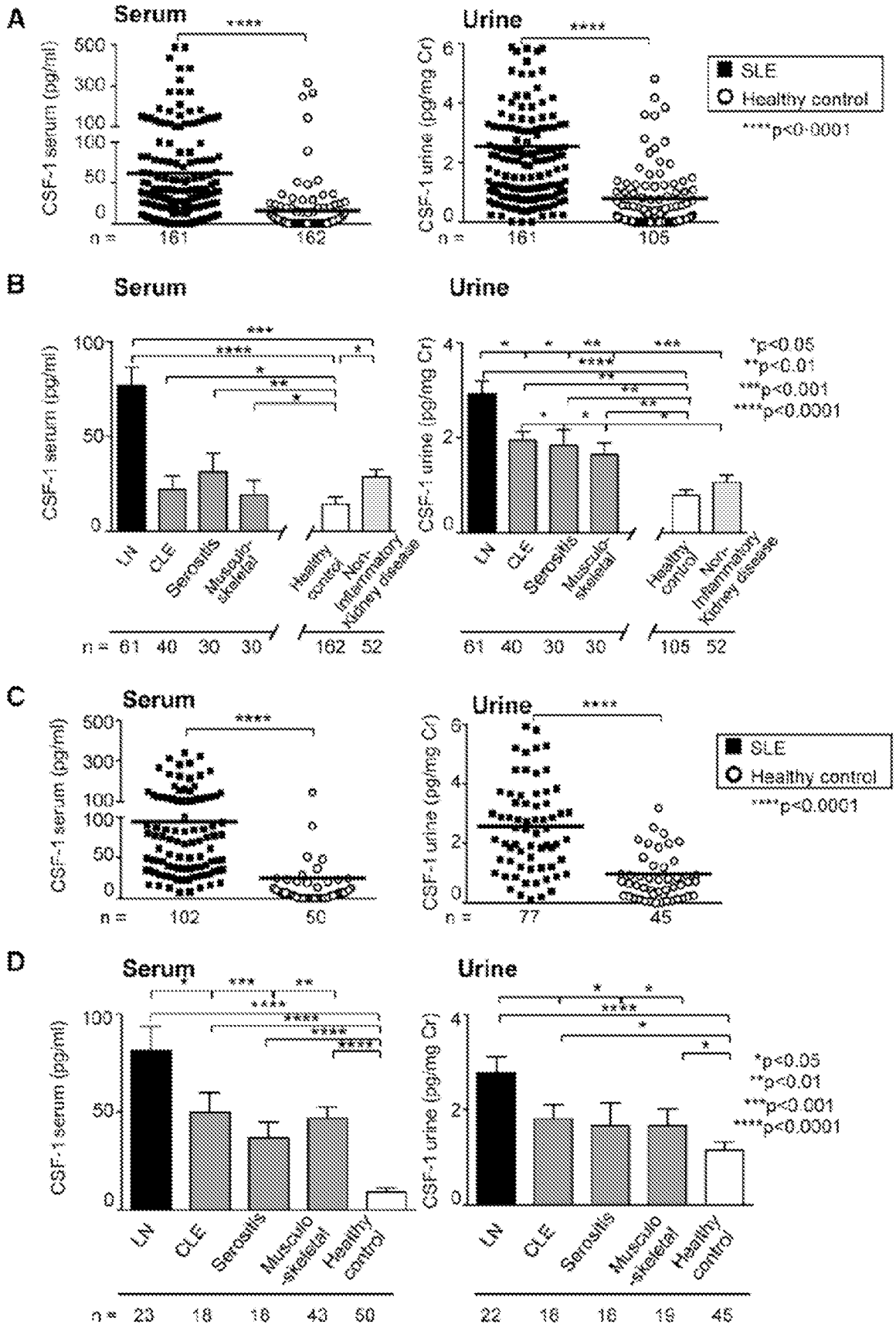
(iii) measuring, at a second time point, a second level of CSF-1 in a second sample obtained from the subject, wherein the second time point is later than the first time point and after said administering, and wherein if the second level is significantly lower than the first level, then the treatment is considered to be effective.

30. The method of claim 29, wherein the first sample and the second sample are of the same type, each selected from the group consisting of blood, plasma, serum, and urine.

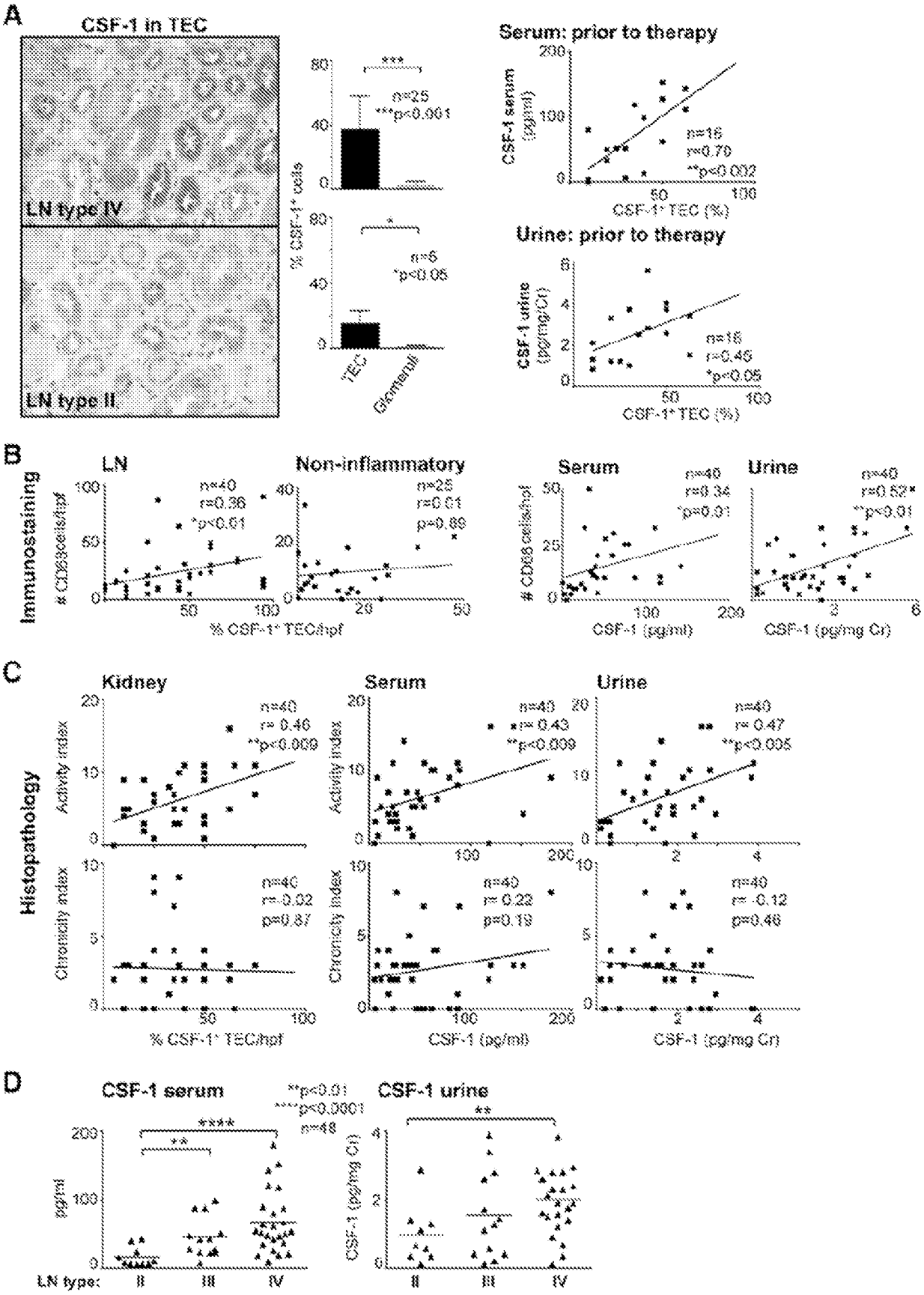
31. The method of claim 29 or 30, wherein the therapeutic agent is a CSF-1 inhibitor.

32. The method of any one of claims 29-31, wherein the subject is a human.

33. Use of a CSF-1 inhibitor for the treatment of lupus nephritis.


A
Serum
Urine
CSF-1 serum (pg/ml)
CSF-1 urine (pg/mg Cr)
SLE
Healthy control
****p<0.0001
n = 161 162
n = 161 105
B
Serum
Urine
CSF-1 serum (pg/ml)
CSF-1 urine (pg/mg Cr)
*p<0.05
**p<0.01
***p<0.001
****p<0.0001
LN
CLE
Serositis
Musculo-skeletal
Healthy control
Non-inflammatory kidney disease
n = 61 40 30 30 162 52
61 40 30 30 105 52
C
Serum
Urine
CSF-1 serum (pg/ml)
CSF-1 urine (pg/mg Cr)
SLE
Healthy control
****p<0.0001
n = 102 50
n = 77 45
D
Serum
Urine
CSF-1 serum (pg/ml)
CSF-1 urine (pg/mg Cr)
*p<0.05
**p<0.01
***p<0.001
****p<0.0001
LN
CLE
Serositis
Musculo-skeletal
Healthy control
n = 23 18 16 43 50
22 16 16 19 45

FIGs. 1A-1D


A
CSF-1 in TEC
LN type IV
LN type II
% CSF-1+ cells
n=25
***p<0.001
n=5
*p<0.05
TEC
Glomeruli
Serum: prior to therapy
CSF-1 serum (pg/ml)
n=16
r=0.76
**p<0.002
CSF-1+ TEC (%)
Urine: prior to therapy
CSF-1 urine (pg/mg/Cr)
n=16
r=0.45
*p<0.05
CSF-1+ TEC (%)
B
Immunostaining
LN
Non-inflammatory
# CD68cells/hpf
n=40
r=0.36
*p<0.01
n=25
r=0.01
p=0.89
% CSF-1+ TEC/hpf
Serum
n=40
r=0.34
*p=0.01
Urine
n=40
r=0.52*
**p<0.01
CSF-1 (pg/ml)
CSF-1 (pg/mg Cr)
C
Histopathology
Kidney
Serum
Urine
Activity index
n=40
r= 0.46
**p<0.009
n=40
r= 0.43
**p<0.009
n=40
r= 0.47
**p<0.005
Chronicity index
n=40
r=-0.02
p=0.87
n=40
r= 0.22
p=0.19
n=40
r= -0.12
p=0.46
% CSF-1+ TEC/hpf
CSF-1 (pg/ml)
CSF-1 (pg/mg Cr)
D
CSF-1 serum
**p<0.01
****p<0.0001
n=48
CSF-1 urine
pg/ml
CSF-1 (pg/mg Cr)
LN type:
II
III
IV

FIGs. 2A-2D

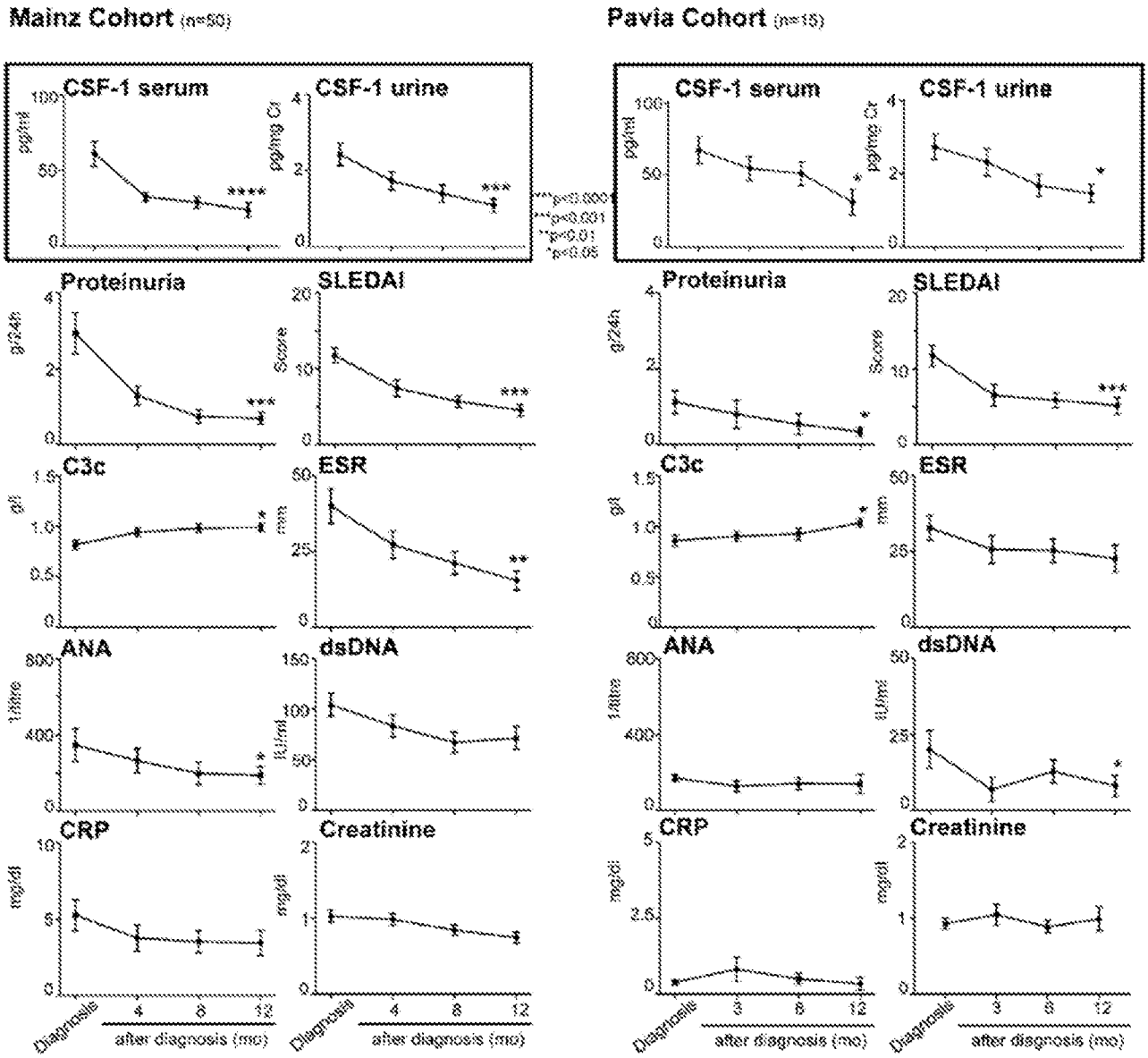
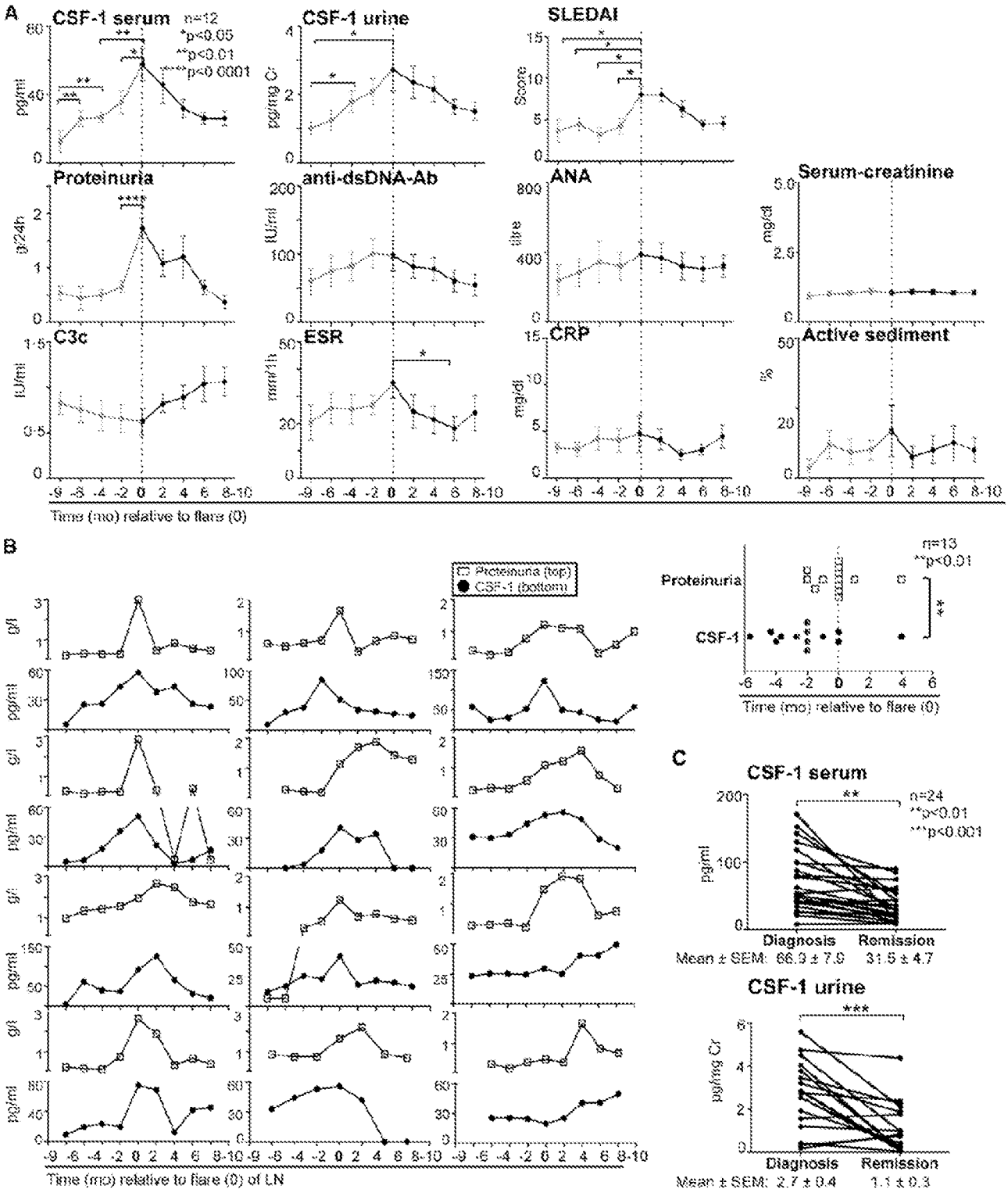
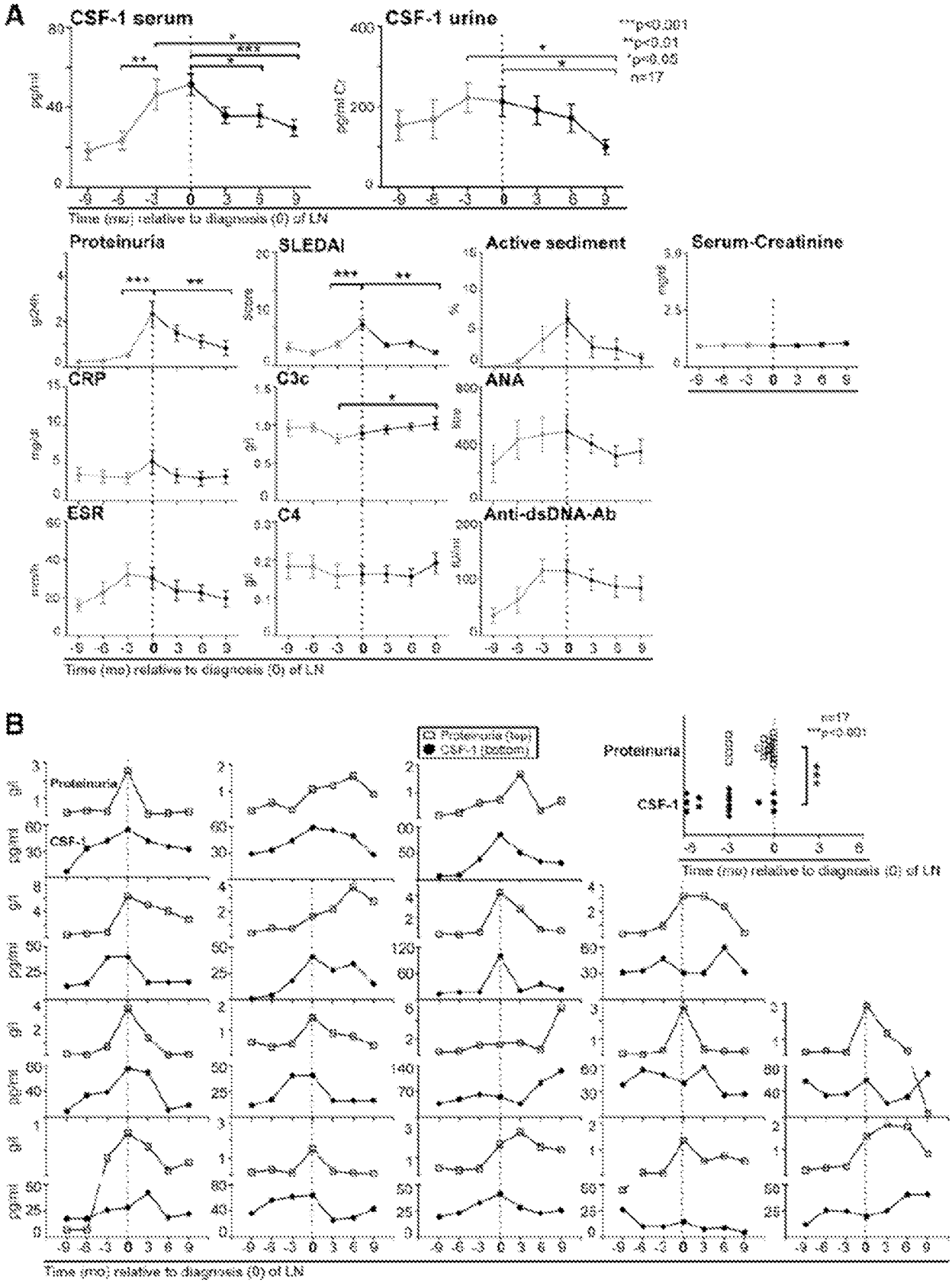

Mainz Cohort (n=50)
Pavia Cohort (n=15)
CSF-1 serum
CSF-1 urine
Proteinuria
SLEDAI
C3c
ESR
ANA
dsDNA
CRP
Creatinine
Diagnosis
after diagnosis (mo)

FIG. 3


A
CSF-1 serum
n=12
*p<0.05
**p<0.01
***p<0.0001
pg/ml
CSF-1 urine
pg/mg Cr
SLEDAI
Score
Proteinuria
g/24h
anti-dsDNA-Ab
IU/ml
ANA
titre
Serum-creatinine
mg/dl
C3c
IU/ml
ESR
mm/1h
CRP
mg/dl
Active sediment
%
-9 -6 -4 -2 0 2 4 6 8-10
Time (mo) relative to flare (0)
B
Proteinuria (top)
CSF-1 (bottom)
g/l
pg/ml
Time (mo) relative to flare (0) of LN
Proteinuria
CSF-1
n=13
**p<0.01
Time (mo) relative to flare (0)
C
CSF-1 serum
n=24
**p<0.01
***p<0.001
pg/ml
Diagnosis
Remission
Mean ± SEM: 66.9 ± 7.9 31.5 ± 4.7
CSF-1 urine
pg/mg Cr
Diagnosis
Remission
Mean ± SEM: 2.7 ± 0.4 1.1 ± 0.3

FIGs. 4A-4C


A
CSF-1 serum
CSF-1 urine
***p<0.001
**p<0.01
*p<0.05
n=17
Time (mo) relative to diagnosis (0) of LN
Proteinuria
SLEDAI
Active sediment
Serum-Creatinine
CRP
C3c
ANA
ESR
C4
Anti-dsDNA-Ab
Time (mo) relative to diagnosis (0) of LN
B
Proteinuria (top)
CSF-1 (bottom)
Proteinuria
CSF-1
n=17
***p<0.001
Time (mo) relative to diagnosis (0) of LN

FIGs. 5A-5B

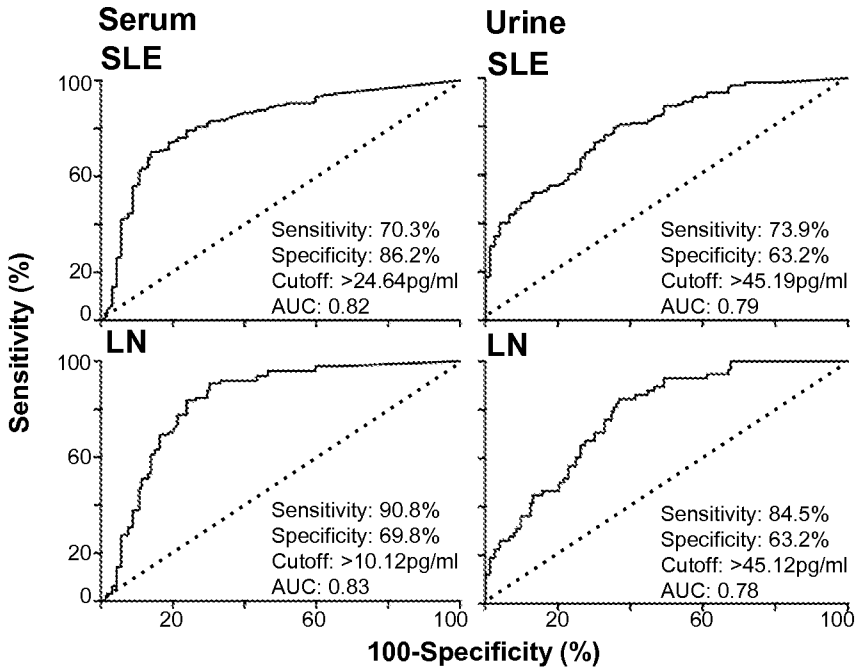

Serum
SLE
Urine
SLE
LN
LN
Sensitivity: 70.3%
Specificity: 86.2%
Cutoff: >24.64pg/ml
AUC: 0.82
Sensitivity: 73.9%
Specificity: 63.2%
Cutoff: >45.19pg/ml
AUC: 0.79
Sensitivity: 90.8%
Specificity: 69.8%
Cutoff: >10.12pg/ml
AUC: 0.83
Sensitivity: 84.5%
Specificity: 63.2%
Cutoff: >45.12pg/ml
AUC: 0.78
Sensitivity (%)
100-Specificity (%)
0
20
60
100

FIG. 6A

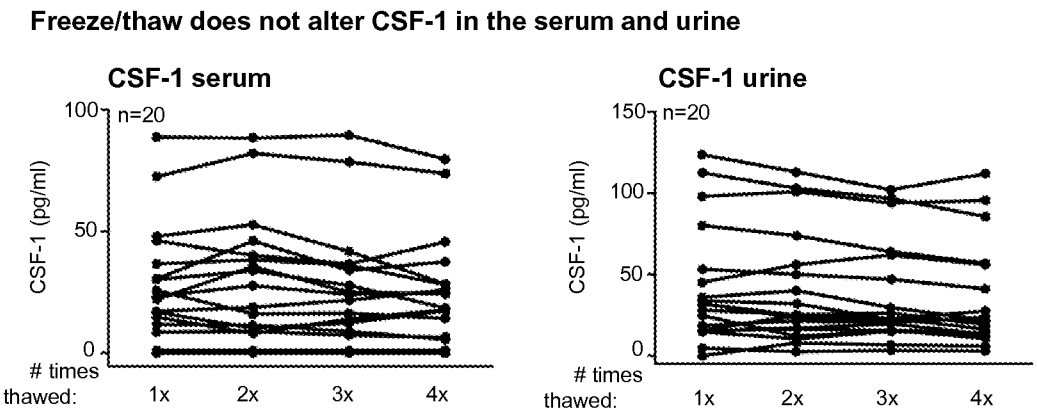

Freeze/thaw does not alter CSF-1 in the serum and urine
CSF-1 serum
CSF-1 urine
n=20
n=20
CSF-1 (pg/ml)
times thawed:
1x
2x
3x
4x
0
50
100
150

FIG. 6B

CSF-1 (serum) variability measured at different time-points

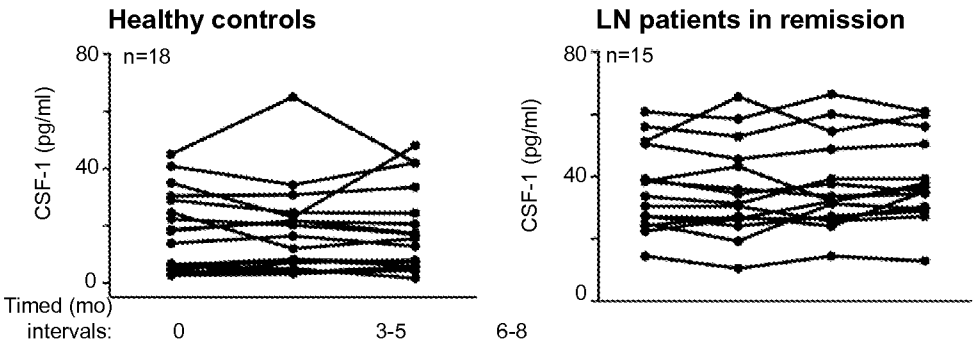


FIG. 6C

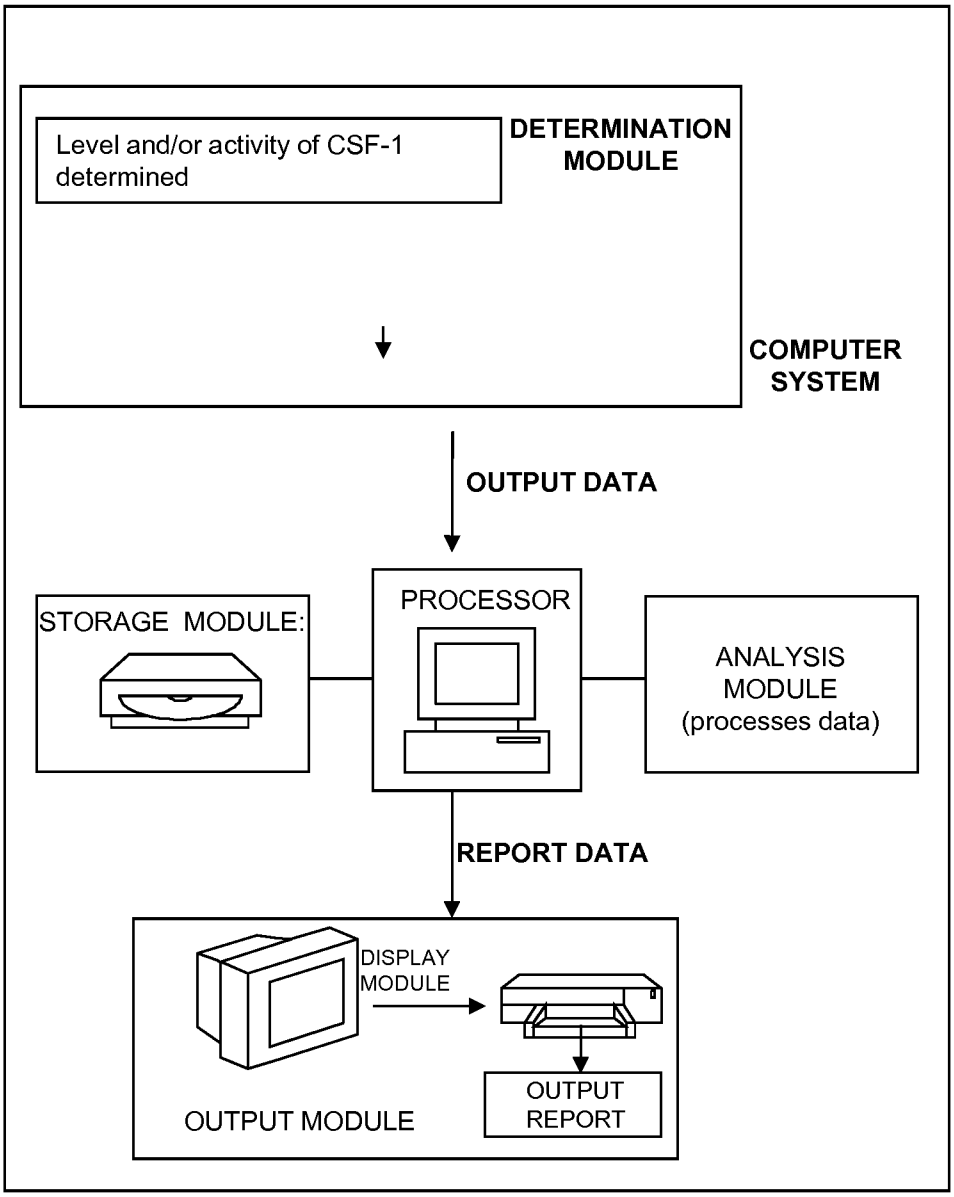

Level and/or activity of CSF-1 determined
DETERMINATION MODULE
COMPUTER SYSTEM
OUTPUT DATA
STORAGE MODULE:
PROCESSOR
ANALYSIS MODULE (processes data)
REPORT DATA
DISPLAY MODULE
OUTPUT REPORT
OUTPUT MODULE

FIG. 7

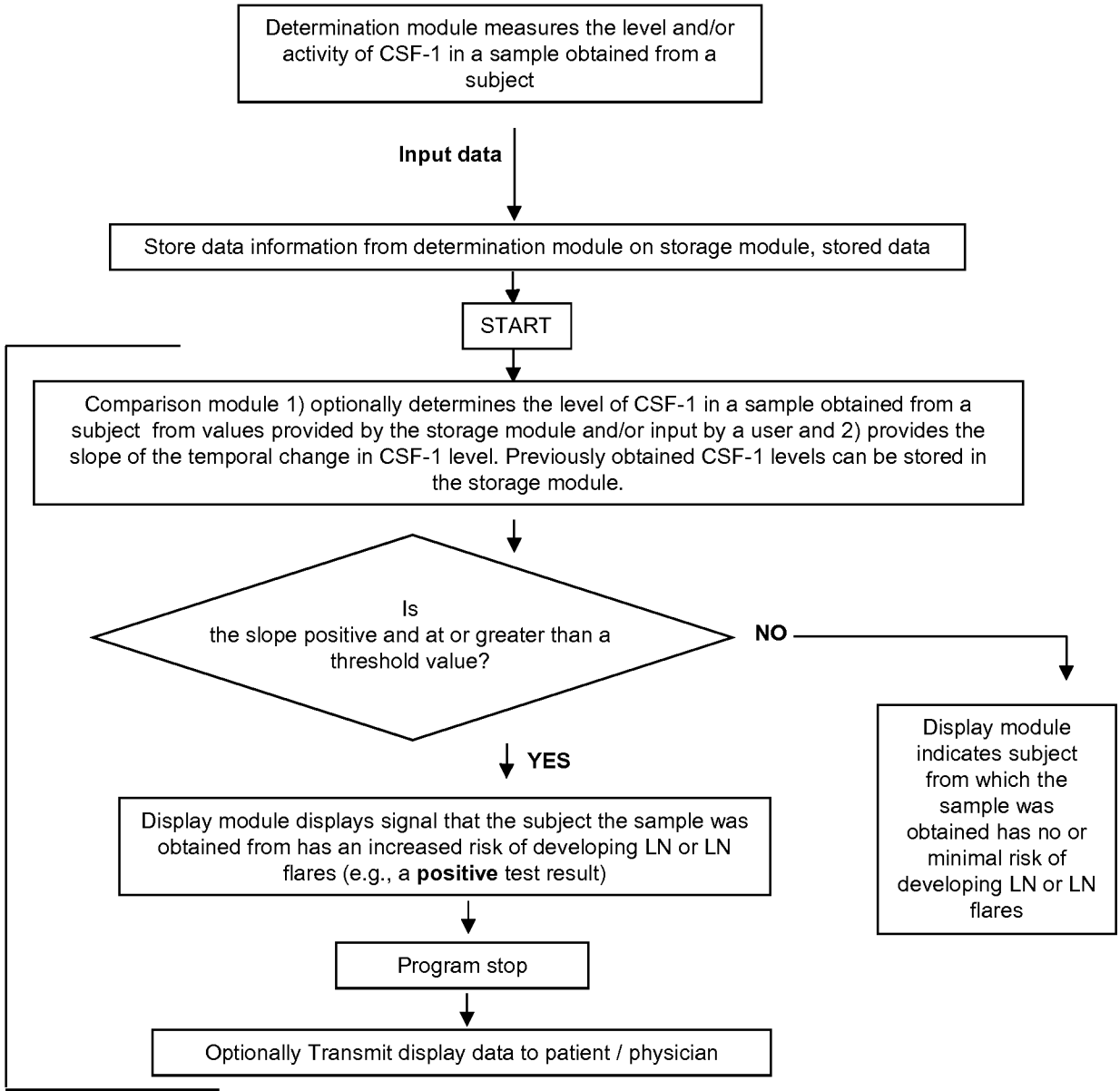

Determination module measures the level and/or activity of CSF-1 in a sample obtained from a subject
Input data
Store data information from determination module on storage module, stored data
START
Comparison module 1) optionally determines the level of CSF-1 in a sample obtained from a subject from values provided by the storage module and/or input by a user and 2) provides the slope of the temporal change in CSF-1 level. Previously obtained CSF-1 levels can be stored in the storage module.
Is the slope positive and at or greater than a threshold value?
NO
YES
Display module indicates subject from which the sample was obtained has no or minimal risk of developing LN or LN flares
Display module displays signal that the subject the sample was obtained from has an increased risk of developing LN or LN flares (e.g., a positive test result)
Program stop
Optionally Transmit display data to patient / physician

FIG. 8

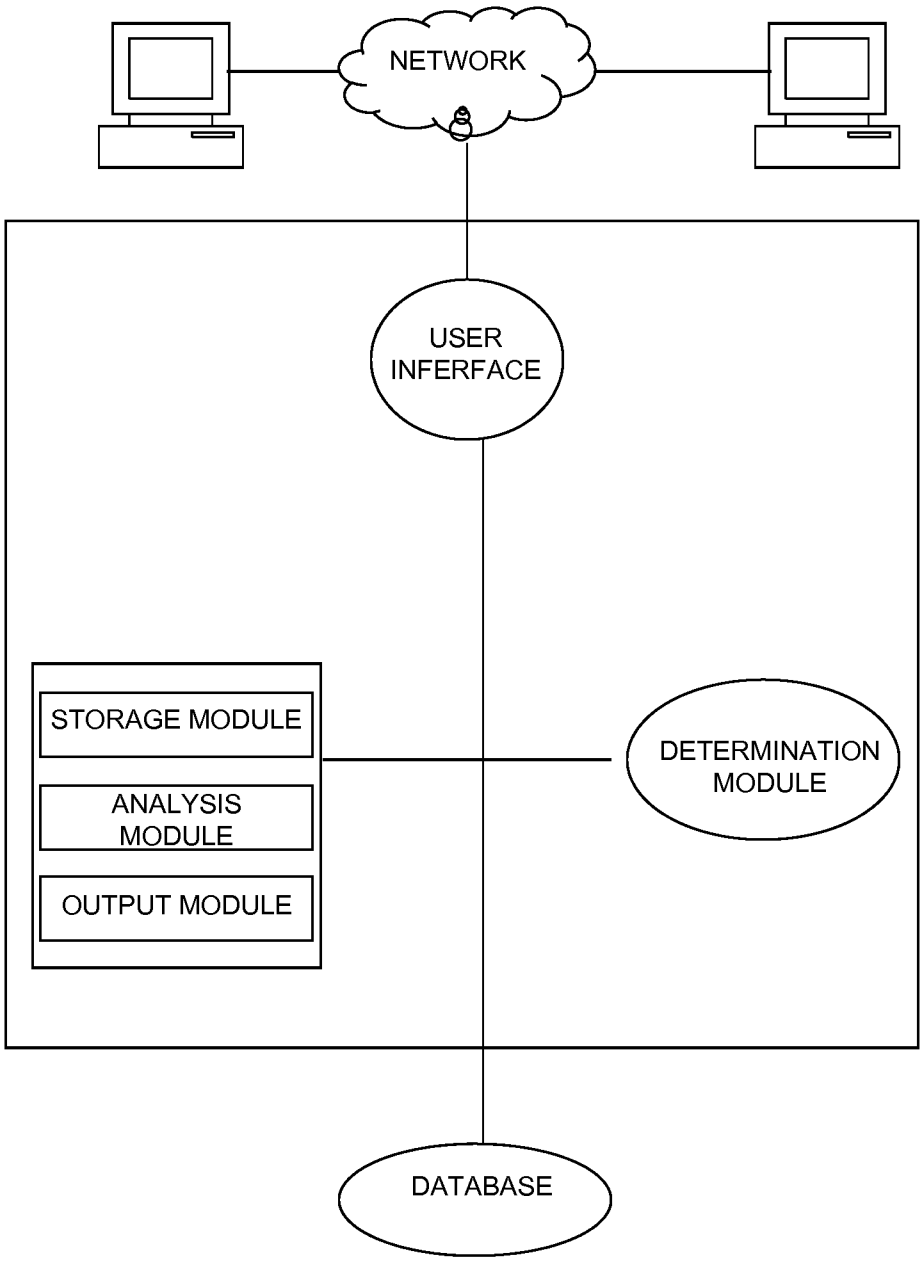

NETWORK
USER INFERFACE
STORAGE MODULE
ANALYSIS MODULE
OUTPUT MODULE
DETERMINATION MODULE
DATABASE

FIG. 9

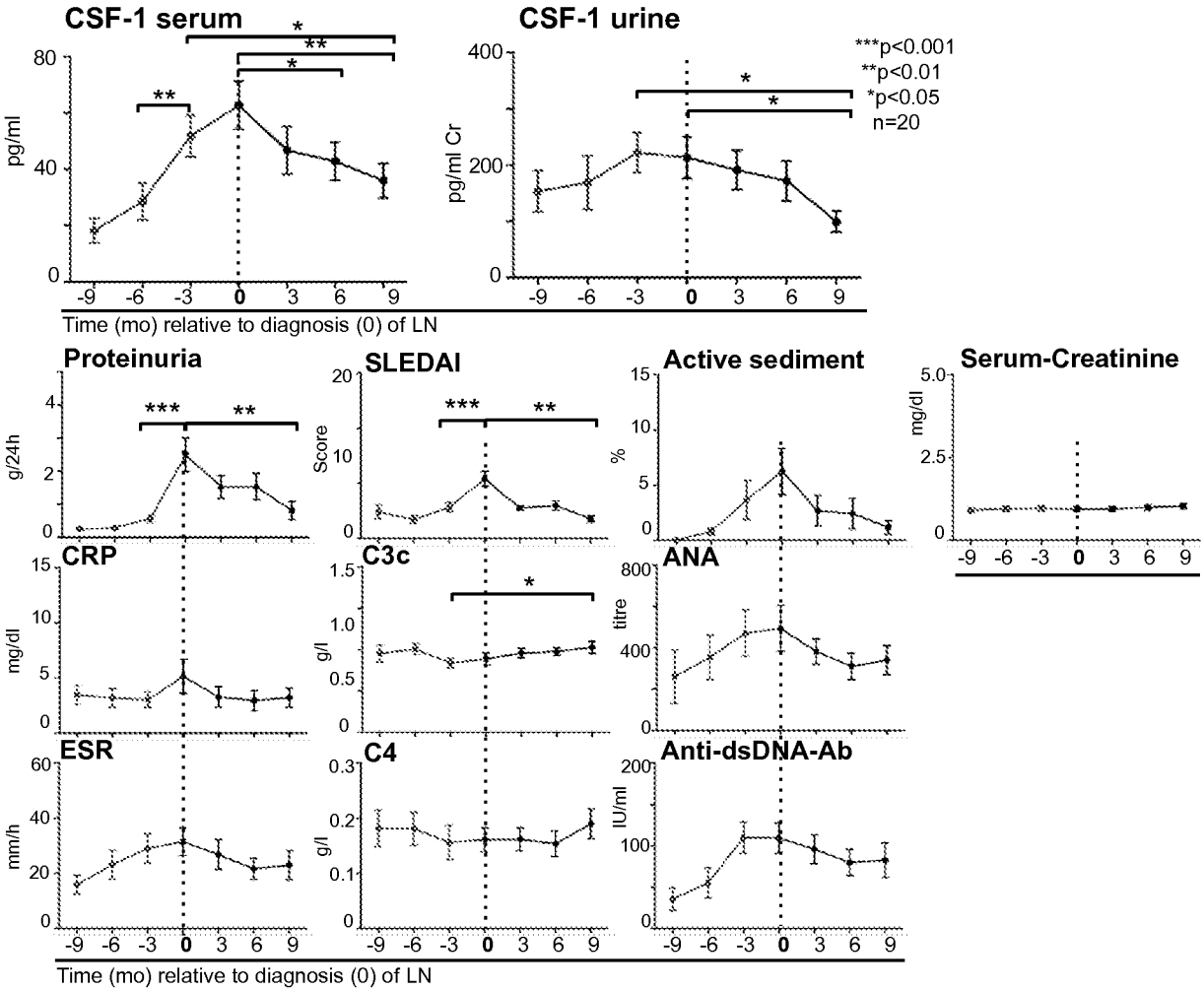

CSF-1 serum
CSF-1 urine
pg/ml
pg/ml Cr
***p<0.001
**p<0.01
*p<0.05
n=20
Time (mo) relative to diagnosis (0) of LN
Proteinuria
SLEDAI
Active sediment
Serum-Creatinine
g/24h
Score
%
mg/dl
CRP
C3c
ANA
mg/dl
g/l
titre
ESR
C4
Anti-dsDNA-Ab
mm/h
g/l
IU/ml
Time (mo) relative to diagnosis (0) of LN

FIG. 10A

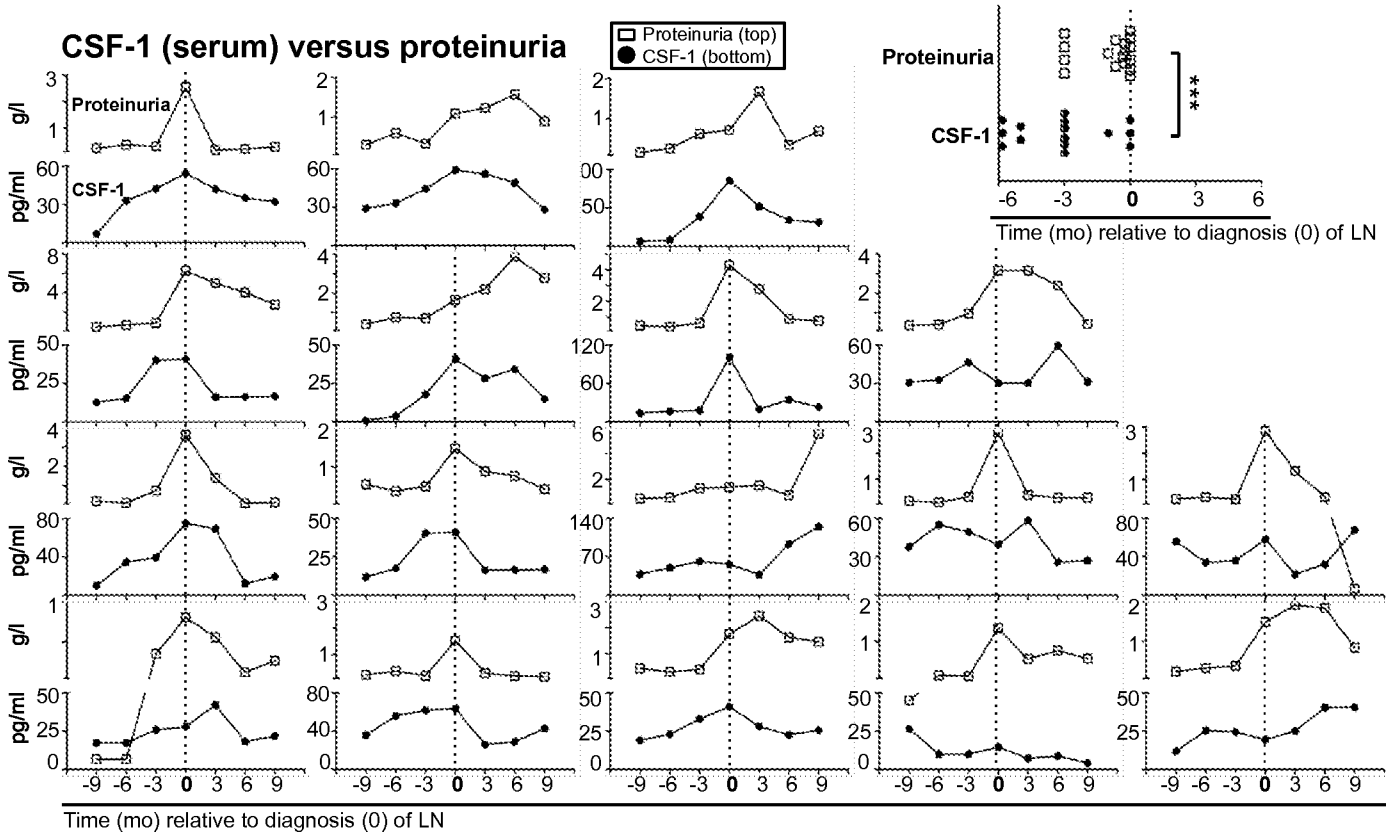

CSF-1 (serum) versus proteinuria
Proteinuria (top)
CSF-1 (bottom)
Proteinuria
CSF-1
g/l
pg/ml
***
Time (mo) relative to diagnosis (0) of LN
-9 -6 -3 0 3 6 9
Time (mo) relative to diagnosis (0) of LN

FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/25719

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/564; C07K 16/28 (2015.01)
CPC - C07K 16/2866; G01N 2800/52
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G01N 33/564; A61P 19/02, 29/00; C07K 16/28; A61K 39/395, C12Q 1/48 (2015.01)
CPC: A61K 39/3955, 45/06; C07K 16/2866; G01N 2333/52, 2333/96494

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google/Google Scholar; NCBI/PubMed; ProQuest; Search terms used: subject, patient, CSF-1, level, expression, marker, biomarker, lupus, risk, sample

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/036357 A1 (FIVE PRIME THERAPEUTICS, INC.); March 6, 2014; paragraphs [007], [010], [026], [027], [075], [088], [0197], [0214]	1-3, 8/1, 8/2, 15-17, 22/15, 22/16, 29-31, 33
A	WO 2013/068902 A1 (PFIZER INC.) May 16, 2013; abstract	1-3, 8/1, 8/2, 15-17, 22/15, 22/1, 29-31, 33

☐ 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	Date of mailing of the international search report
27 July 2015 (27.07.2015)	14 AUG 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/25719

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-7, 9-14, 18-21, 23-28, 32
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:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

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.