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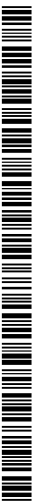
(54) Title: METHODS FOR DIAGNOSIS USING ANTI-CYTOKINE RECEPTOR ANTIBODIES

(57) Abstract: Labeled antibodies, antibody fragments or peptides binding to soluble cytokines or cytokine receptors are used to diagnose whether a patient has cancer or an autoimmune disease. In a preferred embodiment, a radiolabelled tag that is chemically bound to a peptide, antibody, or antibody fragment specific for sTNFR-1 and/or sTNFR2 is injected into a patient with a tumor, or suspected tumor, or with any disease associated with STNF1/STNF-2. The patient is then imaged using standard nuclear imaging equipment to detect areas or sites of concentration of the radiolabel and/or receptor/inhibitor and/or antigen. By screening for cancer by the substances it produces, using an injected antibody to that substance with a tracer attached to it, one can detect cancer at a very early stage, potentially even microscopically.

METHODS FOR DIAGNOSIS USING
ANTI-CYTOKINE RECEPTOR ANTIBODIES

Background of the Invention

This application claims priority to U.S. Serial No. 60/590,400 filed July 22,
5   2004.

The present invention is generally in the field of diagnosing areas in the body
that contain antigenic, diseased, transformed or neoplastic tissue that is associated with
an inflammatory response. This is accomplished by the administration and imaging of
radiolabelled antibodies to tumor necrosis factor receptor. This diagnostic test is
10   intended for early diagnosis of disease in mammals.

The two main causes of death in this country are heart disease and cancer.
Researchers today are finding more and more evidence that these diseases start with an
inflammatory response involving the immune system. The various response organs,
such as the gut, skin, lungs, bone marrow, thymus, and spleen, react with the
15   production of special messenger substances that activate/create macrophages, T-cells,
B-cells and NK-cells, all with specific functions to protect the body from invasion of
other living organisms as well as toxins. Some of these messenger substances include
cytokines such as TNF and C-reactive protein, lymphotoxins, and leukotrienes.

Tumor Necrosis Factor (TNF) is a pirotrophic pro-inflammatory cytokine
20   produced by macrophages. The amount of TNF is crucial; too much can cause
cachexia and septic shock, while too little can allow infection and cancer. The body's
white cells will recognize a cancer cell, bind to it (if not blocked/inhibited) and kill it
with TNF. This reaction is inhibited by immunosuppressants, either from outside the
body (medications, radiation, environmental toxins) or from within (tumor production
25   of cell surface TNF receptors (sTNF-R1 and sTNF-R2). If the inhibitors/blockers of
the normal white cell response can be removed, then the immune system can attack and
kill the affected cells.

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The TNF receptors (sTNF-R1 and sTNF-R2) are present on all mammalian cells. Over production and shedding of these receptors into body fluids causal to acquired immune tolerance. These soluble shed receptors are found in increased quantities in the immediate cellular microenvironment of antigenic tissues and cells. (Lentz MR. The

5 phylogeny of oncology. *Mol Biotherm.* 2:137-144 (1990)). The result of this over-production and shedding is to protect the antigenic cell or an antigen from an otherwise normal inflammatory/immune response.

The most crucial aspect in the successful treatment of any cancer is early detection. Likewise, it is crucial to properly diagnose chronic and acute inflammatory

10 conditions, i.e, autoimmune diseases, before tissue and organ destruction occurs. PET, MRI, and CT scans are limited in their sensitivity.

In an embodiment the invention provides a method and system for early detection of tumors and other types of diseased tissue.

Summary of the Invention

15 Labeled antibodies, antibody fragments or peptides binding to soluble cytokines or cytokine receptors are used to diagnose whether a patient has cancer or an autoimmune disease. In a preferred embodiment, a radiolabelled tag that is chemically bound to a peptide, antibody, or antibody fragment specific for sTNFR-1 and/or sTNFR2 is injected into a patient with a tumor, or suspected tumor, or with any disease

20 associated with STNF-1/STNF-2. The patient is then imaged using standard nuclear imaging equipment to detect areas or sites of concentration of the radiolabel and/or receptor/inhibitor and/or antigen. By screening for cancer by the substances it produces, using an injected antibody to that substance with a tracer attached to it, one can detect cancer at a very early stage, potentially even microscopically.

25 According to the invention there is also provided a method for diagnosing inflammation or cancer comprising:

administering to a patient having an elevated blood level of a soluble cytokine receptor an effective amount of a labeled antibody preferentially binding to the cytokine receptor on the surface of tumor cells; and

30 imaging the patient to detect bound labelled antibody.

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According to the invention there is also provided a method for diagnosing inflammation or cancer comprising:

administering to a patient unlabelled antibody or antibody binding to soluble cytokine receptor produced by tumor cells to bind to background soluble receptor, then

5   administering an effective amount of a labelled antibody binding to the cytokine receptor on the surface of tumor cells; and

imaging the patient to detect bound labelled antibody.

According to the invention there is also provided a kit comprising an effective dosage amount of labeled humanized monoclonal antibodies or labeled binding ligand

10   to a cytokine or cytokine receptor to image cancer or inflammation in a patient, in a pharmaceutically acceptable carrier for intravenous administration, when used in the method according to the invention.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated

15   element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of

20   these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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Detailed Description of the Invention

I.   **Compositions**

        Antibodies

                The composition for administration to a patient for early detection of
5   cancer consists of antibodies, or antibody fragments, to cytokines or cytokine
        receptors. In the preferred embodiment, the antibodies will typically be
        reactive with both the soluble and immobilized forms of the receptor. These
        include soluble tumor necrosis factor receptor ("sTNF-R"), either sTNFI or
        sTNR2, soluble interleukin-2 receptor ("sIL-2R"), soluble interleukin-1
10   receptor ("sIL-1R"), soluble interleukin-6 receptor ("sIL-6R"), soluble
        interleukin-12 (IL-12R)
        or soluble interferon-gamma receptor ("sIFN-gammaR"). These materials
        are all commercially available and described in detail in the literature.
                As used herein, "antibody" refers to antibody, antibody fragments or
15   binding peptides (single chain, recombinant, or humanized), that are
        immunoreactive with the receptor molecules. In the most preferred
        embodiment, the antibody is reactive with the carboxy-terminus of the shed
        receptor molecules, thereby avoiding concerns with signal transduction by
        the receptor that is still present on the cell surface.
20   Antibodies can be obtained from various commercial sources such as
        Genzyme Pharmaceuticals. Alternatively, antibodies to the receptor proteins
        can be generated by standard techniques, typically immunization using
        human receptor proteins. Antibodies are typically generated by
        immunization of an animal using an adjuvant such as Freund's adjuvant in
25   combination with an immunogenic amount of the protein administered over a
        period of weeks in two to three week intervals, then isolated from the serum,
        or from hybridomas made from immunoglobulin producing cells from the
        immunized animals, which express the antibodies in culture.
                Because the methods for immunizing animals yield antibody which is
30   not of human origin, the antibodies could elicit an adverse effect if
        administered to humans. Methods for "humanizing" antibodies, or
        generating less immunogenic fragments of non-human antibodies, are well

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known. A humanized antibody is one in which only the antigen-recognized sites, or complementarily-determining hypervariable regions (CDRs) are of non-human origin, whereas all framework regions (FR) of variable domains are products of human genes. These "humanized" antibodies have less

5   potential for a xenographic rejection stimulus when introduced to a human recipient. Humanized antibodies are defined as a monoclonal antibody ("mAb") constructed with only the antigen-binding regions (also called complementary-determining regions or CDRs) derived from a mouse, and the remainder of the variable regions, and constant regions, derived from a

10 human source (Reichert *Nature Biotechnology* 19: 819-822 (2004)). The procedure for constructing humanized antibodies is as follows. A mouse hybridoma cell line expressing the desired antibody is grown in an appropriate culture medium. Cells are harvested and total RNA is isolated. Complementary DNA (cDNA) is generated that codes for the variable

15 domains of the mouse antibody to be humanized. This is accomplished using polymerase chain reaction (PCR) primers that hybridize to the 5' ends of the mouse leader sequences and to the 5' ends of the mouse constant regions. The light chain and heavy chain variable regions are cloned. PCR amplification of the cDNA is accomplished using light and heavy chain

20 specific primers. The PCR product is cloned directly into a vector. This vector is transformed into bacteria. The bacteria are selected for colonies containing the vector with the mouse variable regions.

Construction of the humanized antibody involves modifying the mouse variable regions at the 5' and 3' ends using PCR primers to create

25 restriction enzyme sites for convenient insertion into expression vectors, and to incorporate splice-donor sites for RNA splicing of the variable and constant regions. The modified mouse variable regions are then inserted into the framework regions of a human antibody. The final vector encodes the CDRs "grafted" or "humanized" into the framework regions of the human

30 variable region and the human constant region (Penichet et al. *Drug Development Research* 61: 121-136 (2004)). These vectors often contain human cytomegalovirus (CMV) enhancer and promoter for transcription, a

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gene for selection of transformed cells (often neomycin), and the simian
virus 40 origin of replication for COS cells. Preliminary expression and
analysis of the humanized antibody is accomplished by transfection of
mammalian cells. The concentration of the antibody produced can be
5   analyzed by using enzyme-linked immunosorbent assay (ELISA). The
binding activity of the antibody can be determined by competitive ELISA
and/or equilibrium dialysis.

        Antibodies humanized this way have been shown to have up to one-
third greater binding affinity than the corresponding murine antibody (Adair
10   *Immunol. Rev.* 130: 5-40 (1992). Allergenicity is also reduced with
humanized antibodies. It has been demonstrated that 20 – 40% of patient
exhibit HAMA reactions to murine antibodies, while only 7% have a HAMA
reaction to humanized antibodies (Vaughan et al. *Nature Biotechnology* 16:
535-539 (1998); Maloney In: Monoclonal antibody-based therapy of cancer.
15   New York: Marcel Dekker p. 53-79 (1998); Berkower *Curr. Opin.*
Biotechnology 7: 622 (1996); Brumley et al. *AORN J.* 62: 343-355 (1995);
Esteva et al In: Monoclonal antibody-based therapy of cancer. New York:
Marcel Dekker p. 309-338 (1998). *See also* Daugherty, et al., *Nucl. Acids*
Res., 19:2471-2476 (1991); Clackson, T., et al., Nature, 352:624-688 (1991);
20   and Kabat, H.A., et al., Sequences of Proteins of Immunological Interest, 4th
Ed. (U.S. Dept. Health and Human Services, Bethesda, MD, 1987). Methods
to make chimeric monoclonal antibodies by DNA recombinant technology,
as described by Velders, et al. Cancer Res., *54:* 1753-1760 (1994), are also
described in Vaquero, Appl. Biol. Sci. 96: 20, 11128-11133 (1999, and
25   Jaakkola, Amer.J. Pathol. *157:463-471 (2000).*

        Alternatively, the immunogenic stimulus presented by the
monoclonal antibodies may be decreased by the use of Pharmacia's
(Pharmacia LKB Biotechnology, Sweden) "Recombinant Phage Antibody
System" (RPAS), which generates a single-chain Fv fragment (ScFv) which
30   incorporates the complete antigen-binding domain of the antibody. In the
RPAS, antibody variable heavy and light chain genes are separately
amplified from the hybridoma mRNA and cloned into an expression vector.

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The heavy and light chain domains are co-expressed on the same polypeptide chain after joining with a short linker DNA which codes for a flexible peptide. This assembly generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody.

5   Compared to the intact monoclonal antibody, the recombinant ScFv includes a considerably lower number of epitopes, and thereby presents a much weaker immunogenic stimulus when injected into humans.

Human antibody-producing XenoMouse strains can be used to generate potent fully human anti-TNFR monoclonal antibodies. These mouse

10   strains are engineered to be deficient in mouse antibody production and to contain integrated megabase-sized fragments from the human heavy and κ light chain loci with the majority of the human antibody gene repertoire. The human immunoglobulin loci provide the XenoMouse strains with the ability to produce high-affinity human MAbs to a broad spectrum of antigens

15   including human antigens, as described by Mendez, et al., Nat. Genet., *15:* 146-156 (1997). See also Jakobovits, Exp. Opin. Invest. Drugs, *7:* 607-614 (1998).

Antibodies (monoclonal, chimeric and chimeric-mutated, or single-chain antibody (scAb) can be purified from ascitic fluid by affinity

20   chromatography. They are precipitated from ascite (Amersham Pharmacia Biotech) by ammonium sulfate and purified on protein-A Sepharose™. IgG is eluted with citrate buffer, pH 3.5, neutralized, and loaded onto an HSA-Sepharose™ column, which separates the chimeric antibodies (HSA-specific) from mouse host IgGs. $F(ab')_2$ fragments are obtained by pepsin

25   digestion of the corresponding IgG in acetate buffer, pH 4.6, followed by chromatography on a Superdex™ 75 column (fast protein liquid chromatography) and affinity chromatography on protein A-Sepharose™. Purity of antibodies and $F(ab')_2$ fragments is verified by SDS-PAGE under reduced and non-reduced conditions and visualized by staining with

30   Coomassie Blue R-250 (Sigma).

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Radiolabels

     The humanized antibody is labeled with a detectable substance. The tracer can be anything that can be detected by conventional nuclear medicine scanning devices such as ^{131}I or ^{125}I. ^{125}I is mostly used for immunochemical

5   analyses due to its low-energy gamma and X-ray radiation for easier detection. Iodination of antibodies or other proteins is a straightforward and effective method of labeling. This tracer is attached to the Fc tail end of the antibody so that it will not interfere with the subsequent *in vivo* antigen (R1/R2) to antibody response.

10   For example, monoclonal antibodies can be radiolabeled according to the iodogen method of Fraker, et al., Biochem. Biophys. Res. Commun., *80:* 849-857 (1978). Briefly, 1.0 ml of antibody (5 mg/ml) and 100 µl 0.5 M sodium phosphate (pH 7.2) are added to a iodogen-coated tube (50 µg). Subsequently, 200 µCi of Na^{125}I (Amersham-Cygne, Hertogenbosch, The

15   Netherlands) or 8 mCi of Na^{131}I (Nordion, Fleurus, Belgium) are added. After 15 min of incubation at room temperature, the reaction mixture is applied on a PD-10 column (Pharmacia, Woerden, The Netherlands) and eluted with a phosphate buffered NaCl solution (pH 7.4; 8.2 g/liter NaCl, 1.9 g/liter Na$_2$HPO$_4$·2H$_2$O, and 0.3 g/liter NaH$_2$PO$_4$·2H$_2$O). The first activity

20   peak eluted from the PD-10 column is collected, and cold antibody added to obtain a monoclonal antibody solution with a specific activity of 10 µCi ^{125}I/mg monoclonal antibody or a specific activity of 0.7 mCi ^{131}I/mg monoclonal antibody. ITLC is used to determine the presence of free radioiodine using Gelman ITLC-SG strips (Gelman Sciences, Inc., Ann

25   Arbor, MI) and 0.15 M sodium citrate (pH 5.5) as the mobile phase (release criterion: <5% free radioiodine).

     The antibodies can also be labeled with I^{123} and I^{131}, respectively, using the standard chloramine-T method. Briefly, an adequate amount of I^{123} or I^{131} in 100 to 150 µl of 0.18 mol/L phosphate buffer at pH 7.5 and 100 µg

30   of antibody were mixed with 0.15 µg chloramine-T. After 5 minutes, the radiolabeled antibody was purified using PD-10 Sephadex^{TM} G-25 size exclusion column (Pharmacia Biotech, Uppsala, Sweden) with 2%

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albumin/0.9% sodium chloride mobile phase. The purity of the radiolabeled immunoconjugate is determined by instant thin layer chromatography with 20% trichloro acetic acid as a solvent.

Carriers for Administration to Patients

5   The antibodies can be formulated in standard pharmaceutical carriers for administration to patients in need thereof. These include saline, phosphate buffered saline, and other aqueous carriers, and liposomes, polymeric microspheres and other controlled release deliver devices, as are well known in the art. The antibodies can also be administered with

10   adjuvant, such as muramyl dipeptide or other materials approved for use in humans (Freund's adjuvant can be used for administration of antibody to animals).

II.   Method of Administration and Detection

15   The antibody is administered to a patient in need thereof in an effective amount to bind to the tumors or areas of inflammation. For example, on day 0, patients received a first i.v. infusion of 5 mg of monoclonal antibody tracer labeled with 50 µCi of [125]I. Later, a second i.v. infusion of 5 mg of monoclonal antibody tracer labeled with 3.5 mCi of [131]I

20   is administered. Whole-body images are recorded 1 h, 2 days, and 4 days after the second infusion, using a dual-headed gamma camera equipped with a high-energy collimator (Multispect 2; Siemens Inc., Hoffman Estates, IL.)

        In the most preferred embodiment, unlabelled antibody is administered first to bind to "background" soluble receptor and increase the

25   contrast with bound antibody. Alternatively, the antibody is first injected without a tracer to absorb the soluble R1/R2 (normal levels known to be 750-1750 pcg/mL for R1 and 1500-3100 pcg/mL for R2), then another dose of the antibody injected with the tracer for a cleaner scan and to assure attraction to the R1/R2 cloud around the cancer. For the imaging

30   experiments a nonbinding human chimeric antibody can also be used as a negative control.

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In the preferred embodiment, the scan is similar to a bone scan, although PET or other methods of detection could also be used. Nuclear imaging uses low, relatively non-toxic doses of radioactive substances that are linked to compounds used by the body's cells or compounds that

5   recognize tumor cells. Using special detection equipment, the radioactive substances can be traced in the body to see where and when they concentrate. In a preferred embodiment, the device is a Magnetic Resonance Imaging (MRI) device that is equipped with a "zoom" lens and a magnetic tracer such that a definitive pattern will be observed allowing differentiation between

10   areas of cancer and inflammation or infection. Two main areas of nuclear imaging are radiolabeled antibodies and PET scans. A SPECT (single photon emission computed tomography) transmission scan can be done to detect the radioactive substance and reveal where the tumor is located. Using radiolabeled monoclonal antibodies in this way is sometimes called

15   immunoscintigraphy.

It is preferable to have data from administration of the antibodies to normal subjects to determine any "background noise" since there is soluble R1/R2 in the blood without the presence of cancer. The amount of noise also depends on age and renal function of the individual (greater clearance in

20   younger patients, so a lower threshold of R1/R2 values would be used). Removing this "noise" increases the sensitivity of the test. In addition, scanning with the antibody in all stages of cancer will provide more accurate information for staging and to prove the validity/efficacy of this testing technique.

25   All of the costs with scanning are less than $1000/person. This testing will help diagnose and locate the cancer in its infancy. Routine optimization can be used to increase imaging of the patient, and determining how long to wait before the scan is performed to allow the best antigen-antibody response to occur; alternatively one can also scan at set intervals

30   from maximum flush to complete washout). Standard care is used to address any potential anaphylactoid or anaphylactic reaction to the tracer and/or antibody (risk reduced by identifying those at risk through skin testing), and

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knowing the amount of antibody to inject to elicit a measurable response radiologically, but not cause enough of an immune response to result in patient distress.

In addition to detection of tumors, the compositions can be used to
5   detect areas of any tissue inflammation releasing pro-inflammatory cytokines and chemokines as well as specific anti-cytokines and chemokines. By locating the anatomical site of this process early in the natural history of the disease, one can intervene early and stop or inhibit the disease process before it becomes clinically manifest. For example, one of these substances, C-
10   reactive protein, is known to be a very sensitive risk indicator to stroke and heart disease. Unfortunately, C-reactive protein is a pentameric protein made by the liver in response to dead or dying tissue, which release cationic protein. By the time this molecule is elevated in blood, tissue is already dead and dying. By tagging cytokine and anti-cytokine antibodies and tracer,
15   areas of disease in the earliest stages can be identified and treated before tissues die. Atherosclerotic plaque is thought to be deposited in an artery in response to tissue invasion of cytomegalovirus ("CMV"), *C. pneumoniae*, or *H. pylori*. An antigen-antibody ("Ag-Ab") test for these organisms or for the localization of the consequent inflammatory response, should also allow
20   early definitive treatment and provide for subsequent follow-up testing to identify any association with an infective agent and evaluate the adequacy of therapy for prognosis of the disease. This type of testing can be used to test for other autoimmune diseases, including rheumatoid arthritis (RA), multiple sclerosis (MS), and systemic lupus erythematosus (SLE).
25   The present invention will be further understood with reference to the following non-limiting examples.

Example 1. Measurement of sTNFR-1 and sTNFR-2 Levels Allow Early Diagnosis of Cancer

A patient in her 50's was diagnosed with Hashimoto's Thyroiditis.
30   Hashimoto's Thyroiditis is a type of autoimmune thyroid disease in which the immune system attacks and destroys the thyroid gland. The thyroid helps set the rate of metabolism - the rate at which the body uses energy.

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Hashimoto's prevents the gland from producing enough thyroid hormones
for the body to work correctly. Common symptoms of Hashimoto's
Thyroiditis are fatigue, depression, sensitivity to cold, weight gain, muscle
weakness, coarsening of the skin, dry or brittle hair, constipation, muscle

5   cramps, increased menstrual flow, and goiter. Several years later the patient
was diagnosed with Type I Diabetes Mellitus (Type I DM). Type 1 DM is
considered to be a T-lymphocyte-dependent autoimmune disease
characterized by infiltration and destruction of the islets of Langerhans, the
endocrine unit of the pancreas. Symptoms of type 1 diabetes are often

10   polyuria (frequent urination) and polydipsia (increased thirst and consequent
increased fluid intake). There may also be weight loss (despite normal or
increased eating), increased appetite, and irreducible fatigue. Another
common presenting symptom is altered vision. Especially dangerous
symptoms in diabetics include the smell of acetone on the patient's breath (a

15   sign of ketoacidosis), Kussmaul breathing (a rapid, deep breathing), and any
altered state of consciousness or arousal (hostility and mania are both
possible, as is confusion and lethargy). Type I DM can lead to diabetic coma,
which causes unconsciousness, early symptoms include polyuria, nausea,
vomiting and abdominal pain, with lethargy and somnolence a later

20   development, progressing to unconsciousness and death if untreated. Unlike
other Type I DM patients undergoing insulin therapy, the blood glucose
levels of the patient described herein were extremely difficult to manage.
The patient's glucose levels ranged from 500 mmol/L to 28 mmol/L within a
15 minute span and could not be controlled. The observed fluctuation in

25   glucose levels and the difficulty in managing these glucose levels are very
unusual. The patient was fitted with an insulin pump, but this did not resolve
the problem. The patient was then analyzed for levels of C-Reactive Protein
(CRP) and erythrocyte sedimentation rate (ESR). The CRP test is a test
which measures the concentration in blood serum of a special type of protein

30   produced in the liver that is present during episodes of acute inflammation or
infection. High levels of CRP may be an indicator of several conditions,
including rheumatoid arthritis, rheumatic fever, cancer, tuberculosis,

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pneumonia, heart attack, and lupus.. The erythrocyte sedimentation rate

(ESR) is another test which gives information about non-specific

inflammation. The ESR test involves placing a blood sample in a tube and

determining how fast the red blood cells settle to the bottom in one hour. The

5   patient had normal levels of CRP and ESR. Despite the normal levels of

CRP and ESR, the patient was still experiencing fluctuations in glucose

levels. A cancer cell has 6 times more insulin receptors and 10 times more

Growth Hormone (GH) receptors than a normal cell. Therefore, a cancer cell

will take up more insulin and more GH than a normal cell. This is why

10   cancer cells grow at the expense of the host's body. This would also explain

the patient's uncontrollable fluctuation in glucose levels. Therefore, the

patient was then analyzed by the method described above for sTNFR-1 and

sTNFR-2 levels in order to determine if the patient had cancer. Levels of

TNF-alpha were high. Levels of TNF-alpha receptor I and II were also high.

15   The high levels of sTNFR-1 and sTNFR-2 suggested that the patient had

cancer. However, the patient's PET scan was normal. By analysis of

sTNFR-1 and sTNFR-2 levels, the patient was diagnosed with cancer. The

patient had vulvar sclerosis (VS) with increased p53 expression.

Keratinocytes affected by VS show a proliferative phenotype and can exhibit

20   markers of neoplastic progression such as increased p53 expression and

DNA aneuploidy. As a chronic scarring inflammatory dermatosis, VS could

act as both "initiator and promoter" of carcinogenesis. Because keratinocytes

of VS significantly express tumor suppressor gene p53 protein, the p53 gene

may be involved early in this proposed pathway of carcinogenesis. Prior to

25   analysis of the sTNFR-1 and sTNFR-2 levels the patient was not being

treated for cancer. The VS had been diagnosed "precancerous" and

controlled and therefore, was not being treated. The patient is now being

treated for cancer. These results demonstrate that the methods described

herein for early detection of tumors and other types of diseased tissue are

30   sensitive and efficacious.

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Example 2. sTNFR-1 and sTNFR-2 is a Highly Sensitive Test for Cancer

A 62 year old patient was found to have a posterior uterine mass that had developed sometime over a period of 3 years. The patient was

5   diagnosed with cancer and was told that it would be necessary to have exploratory laparoscopic surgery and to remove everything that looked like potential cancer for biopsy. Cancer can be diagnosed by taking a sample of the tumor (biopsy). During a biopsy, the tumor material is examined by a pathologist, a physician who specializes in diagnosing diseases by looking at

10   the cells under a microscope. Laparoscopy is the usual first step in confirming the presence of a mass and obtaining a tissue sample for biopsy. Laparoscopic surgery uses small incisions and specially designed instruments to enter the abdomen or pelvis. In exploratory laparoscopy, a larger incision is made in the skin and abdominal muscles to gain access to

15   the pelvic region. Exploratory laparoscopy is a thorough attempt to find the exact extent of cancer spread. To identify possible invasion by the cancer, samples are taken of structures in the pelvis and abdomen including the diaphragm (the muscle that separates the organs of the chest from the organs of the abdomen), the peritoneum (the membrane that lines the abdomen), the

20   omentum (a fatty membrane that covers the organs of the abdomen), lymph nodes, bladder, and bowel. The goal is to remove as much cancerous tissue as possible (debulking). This may involve removing one or both ovaries (oophorectomy), the uterus (hysterectomy), fallopian tubes (salpingectomy), and other organs. Typically, the surgeon does not know ahead of time

25   exactly which organs and structures will require removal. Therefore, it is recommended that the full extent of the operation be discussed with a surgeon before consenting to the procedure. The procedure carries a slight risk of puncturing a blood vessel or organ, which could cause blood to seep into the abdominal cavity. Puncturing the intestines could allow intestinal

30   contents to seep into the cavity. These are serious complications and major surgery may be required to correct the problem. There is also the possibility that it may become apparent that open surgery is required. Rare

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complications include hemorrhage, inflammation of the abdominal cavity lining, abscess, and problems related to general anesthesia. Therefore, less invasive methods than surgery as tools for diagnosing cancer would be useful to prevent unnecessary surgeries.

5   In this case, the patient did not undergo surgery and obtained a second opinion. The patient was analyzed as described by the method above for sTNFR-1 and sTNFR-2 levels in order to determine if the patient had cancer. Both sTNFR-1 and sTNFR-2 levels were in the normal range. These results indicate that the uterine mass was benign and that the patient did not

10   have cancer. The patient was examined by a third physician who indicated that the patient did not cancer. The patient elected to wait for three months and undergo a repeat PET scan. The patient did not have cancer and therefore, a painful surgery procedure was avoided. These results demonstrate that the methods described herein for detection of tumors and

15   other types of diseased tissue are sensitive, efficacious, and a viable alternative to exploratory surgery.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1.   A method for diagnosing inflammation or cancer comprising:
        administering to a patient having an elevated blood level of a soluble cytokine
receptor an effective amount of a labeled antibody preferentially binding to the
cytokine receptor on the surface of tumor cells; and
        imaging the patient to detect bound labelled antibody.

2.   The method according to claim 1 wherein the antibody is a humanized
monoclonal antibody.

3.   The method according to either claim 1 or claim 2 wherein the cytokine receptor
is selected from the group consisting of soluble tumor necrosis factor receptor ("sTNF-
R"), soluble interleukin-2 receptor ("sIL-2R"), soluble interleukin-1 receptor ("sIL-
1R"), soluble interleukin-6 receptor ("sIL-6R"), soluble interferon- gamma receptor
("sIFN-gammaR"), and soluble interleukin-12 receptor(sIL-12R).

4.   The method according to any one of claims 1 to 3 wherein the antibodies are
labeled with a label selected from the group consisting of I^{125}, I^{123} and I^{131}.

5.   The method according to any one of claims 1 to 4 wherein the patient has
cancer.

6.   The method according to any one of claims 1 to 5 wherein the patient has an
autoimmune disease.

7.   The method according to any one of claims 1 to 6 wherein the patient has a
chronic or acute infectious disease.

8.   A method for diagnosing inflammation or cancer comprising:
        administering to a patient unlabelled antibody or antibody binding to soluble
cytokine receptor produced by tumor cells to bind to background soluble receptor, then
        administering an effective amount of a labelled antibody binding to the cytokine
receptor on the surface of tumor cells; and
        imaging the patient to detect bound labelled antibody.

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9.   The method according to claim 8 wherein the cytokine receptor is TNFR1 or
TNFR2.

10.   The method according to either claim 8 or claim 9 wherein 5 mg of radiolabeled
5   antibody is intravenously administered on day zero, then a second infusion of 5 mg of
labeled antibody is administered, and whole-body images recorded the same day, 2
days, and 4 days after the second infusion.

11.   The method according to any one of claims 1 to 10 wherein the patient is
10   imaged one, two or four days after administration of labeled antibody.

12.   The method according to any one of claims 1 to 11 wherein the labeled antibody
is detected using PET.

15   13.   The method according to any one of claims 1 to 12 wherein the labeled antibody
is detected using MRI.

14.   A kit comprising an effective dosage amount of labeled humanized monoclonal
antibodies or labeled binding ligand to a cytokine or cytokine receptor to image cancer
20   or inflammation in a patient, in a pharmaceutically acceptable carrier for intravenous
administration, when used in the method according to any one of claims 1 to 13.

15.   The kit according to claim 14 further comprising unlabelled antibodies or
ligands for use as controls.
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16.   The kit according to either claim 14 or claim 15 further comprising directions in
a package insert.

17.   A method for diagnosing inflammation or cancer according to claim 1 or claim
30   8, substantially as herein described with reference to any one or more of the Examples.

18.   A kit according to claim 14, substantially as herein described with reference to
any one or more of the Examples.

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