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(54) Title: METHOD AND COMPOSITION FOR TREATING CANCER, KILLING METASTATIC CANCER CELLS AND PREVENTING CANCER METASTASIS USING ANTIBODY TO ADVANCED GLYCATION END PRODUCTS (AGE)

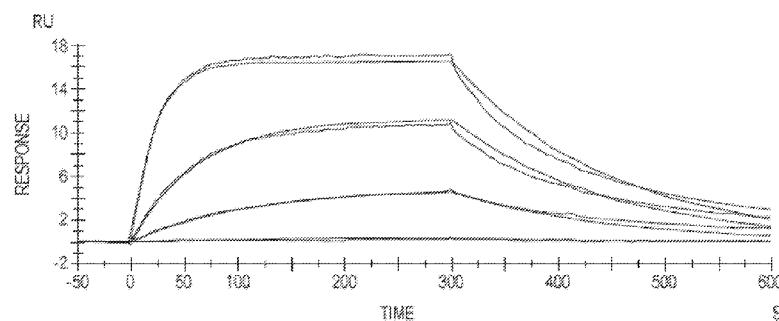


FIG. 1

(57) Abstract: A method of treating cancer, killing metastatic cancer cells, killing potentially- malignant neoplasm cells and/or preventing cancer metastasis comprises administering to a subject a composition comprising an anti-AGE antibody. A method of diagnosing metastatic cancer comprises detecting an immune complex comprising an anti-AGE antibody bound to a cell expressing an AGE modification.

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METHOD AND COMPOSITION FOR TREATING CANCER, KILLING  
METASTATIC CANCER CELLS AND PREVENTING CANCER METASTASIS  
USING ANTIBODY TO ADVANCED GLYCATION END PRODUCTS (AGE)

## BACKGROUND

[01] Senescent cells are cells that are partially-functional or non-functional and are in a state of proliferative arrest. Senescence is a distinct state of a cell, and is associated with biomarkers, such as activation of the biomarker p16<sup>Ink4a</sup> ("p16"), and expression of β-galactosidase. Senescence begins with damage or stress (such as overstimulation by growth factors) of cells. The damage or stress negatively impacts mitochondrial DNA in the cells to cause them to produce free radicals which react with sugars in the cell to form methyl glyoxal (MG). MG in turn reacts with proteins or lipids to generate advanced glycation end products (AGEs). In the case of the protein component lysine, MG reacts to form carboxymethyllysine, which is an AGE. AGEs also form from non-enzymatic reaction of sugars in the blood with external cell proteins.

[02] Damage or stress to mitochondrial DNA also sets off a DNA damage response which induces the cell to produce cell cycle blocking proteins. These blocking proteins prevent the cell from dividing. Continued damage or stress causes mTOR production, which in turn activates protein synthesis and inactivates protein breakdown. Further stimulation of the cells leads to programmed cell death (apoptosis).

[03] p16 is a protein involved in regulation of the cell cycle, by inhibiting the S phase. It can be activated during ageing or in response to various stresses, such as DNA damage, oxidative stress or exposure to drugs. p16 is typically considered a tumor suppressor protein, causing a cell to become senescent in response to DNA damage and irreversibly preventing the cell from entering a hyperproliferative state. However, there has been some ambiguity in this regard, as some tumors show overexpression of p16, while other show downregulated expression. Evidence suggests that overexpression of p16 in some tumors results from a defective

retinoblastoma protein (“Rb”). p16 acts on Rb to inhibit the S phase, and Rb downregulates p16, creating negative feedback. Defective Rb fails to both inhibit the S phase and downregulate p16, thus resulting in overexpression of p16 in hyperproliferating cells. Romagosa, C. *et al.*, p16<sup>Ink4a</sup> overexpression in cancer: a tumor suppressor gene associated with senescence and high-grade tumors, *Oncogene*, Vol. 30, 2087-2097 (2011).

**[04]** Senescent cells are known to fuel the growth of cancer cells. Senescent cells are associated with secretion of many factors involved in intercellular signaling, including pro-inflammatory factors; secretion of these factors has been termed the senescence-associated secretory phenotype, or SASP. One study showed that senescent mesenchymal stem cells promote proliferation and migration of breast cancer cells by the secretion of IL-6 (Di, G-h. *et al.* IL-6 Secreted from Senescent Mesenchymal Stem Cells Promotes Proliferation and migration of Breast Cancer Cells, *PLOS One*, Vol. 9, 11, e113572 (2014)). Another study showed that senescent human fibroblasts increase the growth of tumors by the secretion of matrix metalloproteinase (Liu, D. *et al.* Senescent Human Fibroblasts Increase the Early Growth of Xenograft Tumors via Matrix Metalloproteinase Secretion, *Cancer Res*, Vol. 67, 3117-3126 (2007)).

**[05]** Senescent cells secrete reactive oxygen species (“ROS”) as part of the SASP. ROS is believed to play an important role in maintaining senescence of cells. The secretion of ROS creates a bystander effect, where senescent cells induce senescence in neighboring cells: ROS create the very cellular damage known to activate p16 expression, leading to senescence (Nelson, G., A senescent cell bystander effect: senescence-induced senescence, *Aging Cell*, Vo. 11, 345-349 (2012)). The p16/Rb pathway leads to the induction of ROS, which in turn activates the protein kinase C delta creating a positive feedback loop that further enhance ROS, helping maintain the irreversible cell cycle arrest; it has even been suggested that exposing cancer cells to ROS might be effective to treat cancer by inducing cell phase arrest in hyperproliferating cells (Rayess, H. *et al.*, Cellular senescence and tumor suppressor gene p16, *Int J Cancer*, Vol. 130, 1715-1725 (2012)).

[06] Advanced glycation end-products (AGEs; also referred to as AGE-modified proteins, or glycation end-products) arise from a non-enzymatic reaction of sugars with protein side-chains (Ando, K. et al., Membrane Proteins of Human Erythrocytes Are Modified by Advanced Glycation End Products during Aging in the Circulation, *Biochem Biophys Res Commun.*, Vol. 258, 123, 125 (1999)). This process begins with a reversible reaction between the reducing sugar and the amino group to form a Schiff base, which proceeds to form a covalently-bonded Amadori rearrangement product. Once formed, the Amadori product undergoes further rearrangement to produce AGEs. Hyperglycemia, caused by diabetes mellitus (DM), and oxidative stress promote this post-translational modification of membrane proteins (Lindsey JB, et al., "Receptor For Advanced Glycation End-Products (RAGE) and soluble RAGE (sRAGE): Cardiovascular Implications," *Diabetes Vascular Disease Research*, Vol. 6(1), 7-14, (2009)). AGEs have been associated with several pathological conditions including diabetic complications, inflammation, retinopathy, nephropathy, atherosclerosis, stroke, endothelial cell dysfunction, and neurodegenerative disorders (Bierhaus A, "AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept," *Cardiovasc Res*, Vol. 37(3), 586-600 (1998)).

[07] AGE-modified proteins are also a marker of senescent cells. This association between glycation end-product and senescence is well known in the art. See, for example, Gruber, L. (WO 2009/143411, 26 Nov. 2009), Ando, K. et al. (Membrane Proteins of Human Erythrocytes Are Modified by Advanced Glycation End Products during Aging in the Circulation, *Biochem Biophys Res Commun.*, Vol. 258, 123, 125 (1999)), Ahmed, E.K. et al. ("Protein Modification and Replicative Senescence of WI-38 Human Embryonic Fibroblasts" *Aging Cells*, vol. 9, 252, 260 (2010)), Vlassara, H. et al. (Advanced Glycosylation Endproducts on Erythrocyte Cell Surface Induce Receptor-Mediated Phagocytosis by Macrophages, *J. Exp. Med.*, Vol. 166, 539, 545 (1987)) and Vlassara et al. ("High-affinity-receptor-mediated Uptake and Degradation of Glucose-modified Proteins: A Potential Mechanism for the Removal of Senescent Macromolecules" *Proc. Natl. Acad. Sci. USAI*, Vol. 82, 5588, 5591 (1985)). Furthermore, Ahmed, E.K. et al. indicates that glycation end-products are "one of the

major causes of spontaneous damage to cellular and extracellular proteins" (Ahmed, E.K. *et al.*, see above, page 353). Accordingly, the accumulation of glycation end-products is associated with senescence. Since the formation of glycation end-products is associated with oxidation, the accumulation of glycation end-products may be a result of the formation of ROS in the senescent cells (Fu, M.-X., *et al.*, The Advanced Glycation End Product, *N<sup>E</sup>-(Carboxymethyl)lysine*, Is a Product of both Lipid Peroxidation and Glycoxidation Reactions, *J. Biol. Chem.*, Vol. 271, 9982-9986 (1996)).

## SUMMARY

[08] In a first aspect, the invention is a method of treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis comprising administering to a subject a composition comprising an anti-AGE antibody.

[09] In a second aspect, the invention is a method of treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis comprising administering a composition comprising a first anti-AGE antibody and a second anti-AGE antibody. The second anti-AGE antibody is different from the first anti-AGE antibody.

[10] In a third aspect, the invention is a method of treating a subject with cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis comprising a first administering of an anti-AGE antibody; followed by testing the subject for effectiveness of the first administration at treating the cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis; followed by a second administering of the anti-AGE antibody.

[11] In a fourth aspect, the invention is use of an anti-AGE antibody for the manufacture of a medicament for treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis.

[12] In a fifth aspect, the invention is a composition comprising an anti-AGE antibody for use in treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis.

[13] In a sixth aspect, the invention is a composition for treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis comprising a first anti-AGE antibody, a second anti-AGE antibody and a pharmaceutically acceptable carrier. The first anti-AGE antibody is different from the second anti-AGE antibody.

[14] In a seventh aspect, the invention is a method of diagnosing metastatic cancer comprising detecting an immune complex comprising an anti-AGE antibody bound to a cell expressing an AGE modification.

[15] In an eighth aspect, the invention is an immune complex comprising an anti-AGE antibody bound to a metastatic cancer cell. The metastatic cancer cell expresses an AGE modification.

[16] In a ninth aspect, the invention is a kit for diagnosing metastatic cancer comprising an anti-AGE antibody, a control sample and, optionally, a reagent that binds to the anti-AGE antibody.

[17] **DEFINITIONS**

[18] The term "peptide" means a molecule composed of 2-50 amino acids.

[19] The term "protein" means a molecule composed of more than 50 amino acids.

[20] The terms "advanced glycation end-product," "AGE," "AGE-modified protein or peptide," "glycation end-product" and "AGE antigen" refer to modified proteins or peptides that are formed as the result of the reaction of sugars with protein side chains that further rearrange and form irreversible cross-links. This process begins with a reversible reaction between a reducing sugar and an amino group to form a Schiff base, which proceeds to form a covalently-bonded Amadori rearrangement product. Once formed, the Amadori product undergoes further rearrangement to

produce AGEs. AGE-modified proteins and antibodies to AGE-modified proteins are described in U.S. 5,702,704 to Bucala ("Bucala") and U.S. 6,380,165 to Al-Abed *et al.* ("Al-Abed"). Glycated proteins or peptides that have not undergone the necessary rearrangement to form AGEs, such as N-deoxyfructosyllysine found on glycated albumin, are not AGEs. AGEs may be identified by the presence of AGE modifications (also referred to as AGE epitopes or AGE moieties) such as 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole ("FFI"); 5-hydroxymethyl-1-alkylpyrrole-2-carbaldehyde ("Pyrraline"); 1-alkyl-2-formyl-3,4-diglycosyl pyrrole ("AFGP"), a non-fluorescent model AGE; carboxymethyllysine; and pentosidine. ALI, another AGE, is described in Al-Abed.

[21] "An antibody that binds to an AGE-modified protein on a cell", "anti-AGE antibody" or "AGE antibody" means an antibody or other protein that binds to an AGE-modified protein or peptide and includes a constant region of an antibody, where the protein or peptide which has been AGE-modified is a protein or peptide normally found bound on the surface of a cell, preferably a mammalian cell, more preferably a human, cat, dog, horse, camelid (for example, camel or alpaca), cattle, sheep, or goat cell. "An antibody that binds to an AGE-modified protein on a cell", "anti-AGE antibody" or "AGE antibody" does not include an antibody or other protein which binds with the same specificity and selectivity to both the AGE-modified protein or peptide, and the same non-AGE-modified protein or peptide (that is, the presence of the AGE modification does not increase binding). AGE-modified albumin is not an AGE-modified protein on a cell, because albumin is not a protein normally found bound on the surface of cells. "An antibody that binds to an AGE-modified protein on a cell", "anti-AGE antibody" or "AGE antibody" only includes those antibodies which lead to removal, destruction, or death of the cell. Also included are antibodies which are conjugated, for example to a toxin, drug, or other chemical or particle. Preferably, the antibodies are monoclonal antibodies, but polyclonal antibodies are also permissible.

[22] The term "senescent cell" means a cell which is in a state of proliferative arrest and expresses one or more biomarkers of senescence, such as activation of p16<sup>Ink4a</sup> or expression of senescence-associated  $\beta$ -galactosidase.

**[23]** The term "variant" means a nucleotide, protein or amino acid sequence different from the specifically identified sequences, wherein one or more nucleotides, proteins or amino acid residues is deleted, substituted or added. Variants may be naturally-occurring allelic variants, or non-naturally-occurring variants. Variants of the identified sequences may retain some or all of the functional characteristics of the identified sequences.

**[24]** The term "percent (%) sequence identity" is defined as the percentage of amino acid residues in a candidate sequence that are identical to the amino acid residues in a reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Preferably, % sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program is publicly available from Genentech, Inc. (South San Francisco, CA), or may be compiled from the source code, which has been filed with user documentation in the U.S. Copyright Office and is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

**[25]** In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. Where the length of amino acid sequence A is not equal to the length

of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained using the ALIGN-2 computer program.

[26] The term "immune complex" means the combination of an antibody bound to an antigen. An immune complex may also be referred to as an "antibody-antigen complex."

#### **BRIEF DESCRIPTION OF THE DRAWING**

[27] FIG. 1 is a graph of the response versus time in an antibody binding experiment.

[28] FIG. 2 illustrates a kit for diagnosing cancer metastases.

[29] FIG. 3 illustrates a graph of the normalized tumor volume over the course of an *in vivo* study investigating the effect of an anti-AGE antibody on tumor growth, metastatic potential and cachexia.

[30] FIG. 4 illustrates a graph of the normalized body weight of the mice over the course of an *in vivo* study investigating the effect of an anti-AGE antibody on tumor growth, metastatic potential and cachexia.

#### **DETAILED DESCRIPTION**

[31] Recent research in *C. elegans* suggests that cells which are actively proliferating are not invasive, and that invasive cells such as metastasizing cancer cells are not proliferating and must be in cell-cycle arrest (Matus *et al.*, Invasive Cell Fate Requires G1 Cell-Cycle arrest and Histone Deacetylase-Mediated Changes in Gene Expression, *Developmental Cell*, Vol. 35, 162-174 (2015)). Other researchers have found that formation of ROS can induce cancer cells to metastasize (Porporato, P. E., *et al.* A Mitochondrial Switch Promotes Tumor Metastasis, *Cell Reports*, Vol. 8, 754-766 (2014)).

[32] Cell phase arrest and the production of ROS gives metastasizing cancer cells many characteristics of senescent cells, which would be expected to include the presence of AGE-modified proteins on the cell surface. AGE-modified proteins therefore provide an antigen which can be targeted using antibodies, to seek out and destroy metastasizing cancer cells. Administration of anti-AGE antibodies would kill metastasizing cancer cells, thereby treating cancer. Administration of anti-AGE antibodies would prevent metastasis in a cancer patient and could be used to prevent the spread of cancer, in a prophylactic manner.

[33] Potentially-malignant neoplasms, such as seborrheic keratosis, actinic keratosis and carcinoma *in situ*, have many characteristics of senescent cells, such as expression of p16, which would be expected to include the presence of AGE-modified proteins on the cell surface. AGE-modified proteins therefore provide an antigen which can be targeted using antibodies, to seek out and destroy potentially-malignant neoplasm cells. Administration of anti-AGE antibodies would kill potentially-malignant neoplasm cells, thereby preventing cancer. Administration of anti-AGE antibodies would prevent cancer in a patient, in a prophylactic manner.

[34] An antibody that binds to an AGE-modified protein on a cell (“anti-AGE antibody” or “AGE antibody”) is known in the art. Examples include those described in U.S. 5,702,704 (Bucala) and U.S. 6,380,165 (Al-Abed *et al.*). Examples include an antibody that binds to one or more AGE-modified proteins having an AGE modification such as FFI, pyrraline, AFGP, ALI, carboxymethyllysine, carboxyethyllysine and pentosidine, and mixtures of such antibodies. Preferably, the antibody binds carboxymethyllysine-modified proteins. Preferably, the antibody is non-immunogenic to the animal in which it will be used, such as non-immunogenic to humans; companion animals including cats, dogs and horses; and commercially important animals, such as camels (or alpaca), cattle (bovine), sheep, and goats. More preferably, the antibody has the same species constant region as antibodies of the animal to reduce the immune response against the antibody, such as being humanized (for humans), feline (for cats), caninized (for dogs), equinized (for horses), camelized (for camels or alpaca), bovinized (for cattle), ovinized (for sheep), or caprine (for goats). Most preferably, the antibody is identical to that of the

animal in which it will be used (except for the variable region), such as a human antibody, a cat antibody, a dog antibody, a horse antibody, a camel antibody, a bovine antibody, a sheep antibody or a goat antibody. Details of the constant regions and other parts of antibodies for these animals are described below. Preferably, the antibody is a monoclonal antibody, but polyclonal antibodies are also permissible.

[35] Particularly preferred anti-AGE antibodies include those which bind to proteins or peptides that exhibit a carboxymethyllysine or carboxyethyllysine AGE modification. Carboxymethyllysine (also known as N(epsilon)-(carboxymethyl)lysine, N(6)-carboxymethyllysine, or 2-Amino-6-(carboxymethylamino)hexanoic acid) and carboxyethyllysine (also known as N-epsilon-(carboxyethyl)lysine) are found on proteins or peptides and lipids as a result of oxidative stress and chemical glycation. CML- and CEL-modified proteins or peptides are recognized by the receptor RAGE which is expressed on a variety of cells. CML and CEL have been well-studied and CML- and CEL-related products are commercially available. For example, Cell Biolabs, Inc. sells CML-BSA antigens, CML polyclonal antibodies, CML immunoblot kits, and CML competitive ELISA kits ([www.cellbiolabs.com/cml-assays](http://www.cellbiolabs.com/cml-assays)) as well as CEL-BSA antigens and CEL competitive ELISA kits ([www.cellbiolabs.com/cel-n-epsilon-carboxyethyl-lysine-assays-and-reagents](http://www.cellbiolabs.com/cel-n-epsilon-carboxyethyl-lysine-assays-and-reagents)). A particularly preferred antibody includes the variable region of the commercially available mouse anti-glycation end-product antibody raised against carboxymethyl lysine conjugated with keyhole limpet hemocyanin, the carboxymethyl lysine MAb (Clone 318003) available from R&D Systems, Inc. (Minneapolis, MN; catalog no. MAB3247), modified to have a human constant region (or the constant region of the animal into which it will be administered). Commercially-available antibodies, such as the carboxymethyl lysine antibody corresponding to catalog no. MAB3247 from R&D Systems, Inc., may be intended for diagnostic purposes and may contain material that is not suited for use in animals or humans. Preferably, commercially-available antibodies are purified and/or isolated prior to use in animals or humans to remove toxins or other potentially-harmful material.

[36] The anti-AGE antibody has low rate of dissociation from the antibody-antigen complex, or  $k_d$  (also referred to as  $k_{back}$  or off-rate), preferably at most  $9 \times 10^{-3}$ ,  $8 \times 10^{-3}$ ,  $7 \times 10^{-3}$  or  $6 \times 10^{-3}$  (sec $^{-1}$ ). The anti-AGE antibody has a high affinity for the AGE-modified protein of a cell, which may be expressed as a low dissociation constant  $K_D$  of at most  $9 \times 10^{-6}$ ,  $8 \times 10^{-6}$ ,  $7 \times 10^{-6}$ ,  $6 \times 10^{-6}$ ,  $5 \times 10^{-6}$ ,  $4 \times 10^{-6}$  or  $3 \times 10^{-6}$  (M). Preferably, the binding properties of the anti-AGE antibody are similar to, the same as, or superior to the carboxymethyl lysine MAb (Clone 318003) available from R&D Systems, Inc. (Minneapolis, MN; catalog no. MAB3247), illustrated in FIG. 1.

[37] The anti-AGE antibody may destroy AGE-modified cells through antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is a mechanism of cell-mediated immune defense in which an effector cell of the immune system actively lyses a target cell whose membrane-surface antigens have been bound by specific antibodies. ADCC may be mediated by natural killer (NK) cells, macrophages, neutrophils or eosinophils. The effector cells bind to the Fc portion of the bound antibody. Administration of NK cells, such as NK92 cells (a cell line available from NantKwest, Culver City, CA), together with, or subsequent to, administration of anti-AGE antibodies, can enhance the compliment activity and therefore the effectiveness of the anti-AGE antibodies to kill metastasizing cancer cells. The anti-AGE antibody may also destroy AGE-modified cells through complement-dependent cytotoxicity (CDC). In CDC, the complement cascade of the immune system is triggered by an antibody binding to a target antigen.

[38] The anti-AGE antibody may be conjugated to an agent that causes the destruction of AGE-modified cells. Such agents may be a toxin, a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

[39] A toxin, such as pore-forming toxins (PFT) (Aroian R. *et al.*, "Pore-Forming Toxins and Cellular Non-Immune Defenses (CNIDs)," *Current Opinion in Microbiology*, 10:57-61 (2007)), conjugated to an anti-AGE antibody may be injected into a patient to selectively target and remove AGE-modified cells. The anti-AGE antibody recognizes and binds to AGE-modified cells. Then, the toxin causes pore formation at the cell surface and subsequent cell removal through osmotic lysis.

[40] Magnetic nanoparticles conjugated to the anti-AGE antibody may be injected into a patient to target and remove AGE-modified cells. The magnetic nanoparticles can be heated by applying a magnetic field in order to selectively remove the AGE-modified cells.

[41] As an alternative, magnetic spin-vortex discs, which are magnetized only when a magnetic field is applied to avoid self-aggregation that can block blood vessels, begin to spin when a magnetic field is applied, causing membrane disruption of target cells. Magnetic spin-vortex discs, conjugated to anti-AGE antibodies specifically target AGE-modified cell types, without removing other cells.

[42] Antibodies typically comprise two heavy chains and two light chains of polypeptides joined to form a "Y" shaped molecule. The constant region determines the mechanism used to target the antigen. The amino acid sequence in the tips of the "Y" (the variable region) varies among different antibodies. This variation gives the antibody its specificity for binding antigen. The variable region, which includes the ends of the light and heavy chains, is further subdivided into hypervariable (HV - also sometimes referred to as complementarity determining regions, or CDRs) and framework (FR) regions. When antibodies are prepared recombinantly, it is also possible to have a single antibody with variable regions (or complementary determining regions) that bind to two different antigens, with each tip of the "Y" being specific to one of the antigens; these are referred to as bi-specific antibodies.

[43] A humanized anti-AGE antibody according to the present invention may have the human constant region sequence of amino acids shown in SEQ ID NO: 22. The heavy chain complementarity determining regions of the humanized anti-AGE antibody may have one or more of the protein sequences shown in SEQ ID NO: 23 (CDR1H), SEQ ID NO: 24 (CDR2H) and SEQ ID NO: 25 (CDR3H). The light chain complementarity determining regions of the humanized anti-AGE antibody may have one or more of the protein sequences shown in SEQ ID NO: 26 (CDR1L), SEQ ID NO: 27 (CDR2L) and SEQ ID NO: 28 (CDR3L).

**[44]** The heavy chain of human (*Homo sapiens*) antibody immunoglobulin G1 may have or may include the protein sequence of SEQ ID NO: 1. The variable domain of the heavy chain may have or may include the protein sequence of SEQ ID NO: 2. The complementarity determining regions of the variable domain of the heavy chain (SEQ ID NO: 2) are shown in SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43. The kappa light chain of human (*Homo sapiens*) antibody immunoglobulin G1 may have or may include the protein sequence of SEQ ID NO: 3. The variable domain of the kappa light chain may have or may include the protein sequence of SEQ ID NO: 4. Optionally, the arginine (Arg or R) residue at position 128 of SEQ ID NO: 4 may be omitted. The complementarity determining regions of the variable domain of the light chain (SEQ ID NO: 4) are shown in SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46. The variable regions may be codon-optimized, synthesized and cloned into expression vectors containing human immunoglobulin G1 constant regions. In addition, the variable regions may be used in the humanization of non-human antibodies.

**[45]** The antibody heavy chain may be encoded by the DNA sequence of SEQ ID NO: 12, a murine anti-AGE immunoglobulin G2b heavy chain. The protein sequence of the murine anti-AGE immunoglobulin G2b heavy chain encoded by SEQ ID NO: 12 is shown in SEQ ID NO: 16. The variable region of the murine antibody is shown in SEQ ID NO: 20, which corresponds to positions 25-142 of SEQ ID NO: 16. The antibody heavy chain may alternatively be encoded by the DNA sequence of SEQ ID NO: 13, a chimeric anti-AGE human immunoglobulin G1 heavy chain. The protein sequence of the chimeric anti-AGE human immunoglobulin G1 heavy chain encoded by SEQ ID NO: 13 is shown in SEQ ID NO: 17. The chimeric anti-AGE human immunoglobulin includes the murine variable region of SEQ ID NO: 20 in positions 25-142. The antibody light chain may be encoded by the DNA sequence of SEQ ID NO: 14, a murine anti-AGE kappa light chain. The protein sequence of the murine anti-AGE kappa light chain encoded by SEQ ID NO: 14 is shown in SEQ ID NO: 18. The variable region of the murine antibody is shown in SEQ ID NO: 21, which corresponds to positions 21-132 of SEQ ID NO: 18. The antibody light chain may alternatively be encoded by the DNA sequence of SEQ ID NO: 15, a chimeric anti-

AGE human kappa light chain. The protein sequence of the chimeric anti-AGE human kappa light chain encoded by SEQ ID NO: 15 is shown in SEQ ID NO: 19. The chimeric anti-AGE human immunoglobulin includes the murine variable region of SEQ ID NO: 21 in positions 21-132.

**[46]** A humanized anti-AGE antibody according to the present invention may have or may include one or more humanized heavy chains or humanized light chains. A humanized heavy chain may be encoded by the DNA sequence of SEQ ID NO: 30, 32 or 34. The protein sequences of the humanized heavy chains encoded by SEQ ID NOs: 30, 32 and 34 are shown in SEQ ID NOs: 29, 31 and 33, respectively. A humanized light chain may be encoded by the DNA sequence of SEQ ID NO: 36, 38 or 40. The protein sequences of the humanized light chains encoded by SEQ ID NOs: 36, 38 and 40 are shown in SEQ ID NOs: 35, 37 and 39, respectively. Preferably, the humanized anti-AGE antibody maximizes the amount of human sequence while retaining the original antibody specificity. A complete humanized antibody may be constructed that contains a heavy chain having a protein sequence chosen from SEQ ID NOs: 29, 31 and 33 and a light chain having a protein sequence chosen from SEQ ID NOs: 35, 37 and 39.

**[47]** Particularly preferred anti-AGE antibodies may be obtained by humanizing murine monoclonal anti-AGE antibodies. Murine monoclonal anti-AGE antibodies have the heavy chain protein sequence shown in SEQ ID NO: 47 (the protein sequence of the variable domain is shown in SEQ ID NO: 52) and the light chain protein sequence shown in SEQ ID NO: 57 (the protein sequence of the variable domain is shown in SEQ ID NO: 62). A preferred humanized heavy chain may have the protein sequence shown in SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50 or SEQ ID NO: 51 (the protein sequences of the variable domains of the humanized heavy chains are shown in SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, respectively). A preferred humanized light chain may have the protein sequence shown in SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61 (the protein sequences of the variable domains of the humanized light chains are shown in SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, respectively). Preferably, a humanized anti-AGE monoclonal antibody is

composed a heavy chain having a protein sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50 and SEQ ID NO: 51 and a light chain having a protein sequence selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60 and SEQ ID NO: 61. Humanized monoclonal anti-AGE antibodies composed of these protein sequences may have better binding and/or improved activation of the immune system, resulting in greater efficacy.

**[48]** The protein sequence of an antibody from a non-human species may be modified to include the variable domain of the heavy chain having the sequence shown in SEQ ID NO: 2 or the kappa light chain having the sequence shown in SEQ ID NO: 4. The non-human species may be a companion animal, such as the domestic cat or domestic dog, or livestock, such as cattle, the horse or the camel. Preferably, the non-human species is not the mouse. The heavy chain of the horse (*Equus caballus*) antibody immunoglobulin gamma 4 may have or may include the protein sequence of SEQ ID NO: 5 (EMBL/GenBank accession number AY445518). The heavy chain of the horse (*Equus caballus*) antibody immunoglobulin delta may have or may include the protein sequence of SEQ ID NO: 6 (EMBL/GenBank accession number AY631942). The heavy chain of the dog (*Canis familiaris*) antibody immunoglobulin A may have or may include the protein sequence of SEQ ID NO: 7 (GenBank accession number L36871). The heavy chain of the dog (*Canis familiaris*) antibody immunoglobulin E may have or may include the protein sequence of SEQ ID NO: 8 (GenBank accession number L36872). The heavy chain of the cat (*Felis catus*) antibody immunoglobulin G2 may have or may include the protein sequence of SEQ ID NO: 9 (DDBJ/EMBL/GenBank accession number KF811175).

**[49]** Animals of the camelid family, such as camels (*Camelus dromedarius* and *Camelus bactrianus*), llamas (*Lama glama*, *Lama pacos* and *Lama vicugna*), alpacas (*Vicugna pacos*) and guanacos (*Lama guanicoe*), have a unique antibody that is not found in other mammals. In addition to conventional immunoglobulin G antibodies composed of heavy and light chain tetramers, camelids also have heavy chain immunoglobulin G antibodies that do not contain light chains and exist as heavy chain dimers. These antibodies are known as heavy chain antibodies, HCabs,

single-domain antibodies or sdAbs, and the variable domain of a camelid heavy chain antibody is known as the VHH. The camelid heavy chain antibodies lack the heavy chain CH1 domain and have a hinge region that is not found in other species. The variable region of the Arabian camel (*Camelus dromedarius*) single-domain antibody may have or may include the protein sequence of SEQ ID NO: 10 (GenBank accession number AJ245148). The variable region of the heavy chain of the Arabian camel (*Camelus dromedarius*) tetrameric immunoglobulin may have or may include the protein sequence of SEQ ID NO: 11 (GenBank accession number AJ245184).

**[50]** In addition to camelids, heavy chain antibodies are also found in cartilaginous fishes, such as sharks, skates and rays. This type of antibody is known as an immunoglobulin new antigen receptor or IgNAR, and the variable domain of an IgNAR is known as the VNAR. The IgNAR exists as two identical heavy chain dimers composed of one variable domain and five constant domains each. Like camelids, there is no light chain.

**[51]** The protein sequences of additional non-human species may be readily found in online databases, such as the International ImMunoGeneTics Information System ([www.imgt.org](http://www.imgt.org)), the European Bioinformatics Institute ([www.ebi.ac.uk](http://www.ebi.ac.uk)), the DNA Databank of Japan ([ddbj.nig.ac.jp/arsa](http://ddbj.nig.ac.jp/arsa)) or the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

**[52]** An anti-AGE antibody or a variant thereof may include a heavy chain variable region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 20, including post-translational modifications thereof. A variable region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity may contain substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-AGE antibody including that sequence retains the ability to bind to AGE. The substitutions, insertions, or deletions may occur in regions outside the variable region.

**[53]** An anti-AGE antibody or a variant thereof may include a light chain variable region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 21, including post-translational modifications thereof. A variable region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity may contain substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-AGE antibody including that sequence retains the ability to bind to AGE. The substitutions, insertions, or deletions may occur in regions outside the variable region.

**[54]** Alternatively, the antibody may have the complementarity determining regions of commercially available mouse anti-glycation end-product antibody raised against carboxymethyl lysine conjugated with keyhole limpet hemocyanin (CML-KLH), the carboxymethyl lysine MAb (Clone 318003) available from R&D Systems, Inc. (Minneapolis, MN; catalog no. MAB3247).

**[55]** The antibody may have or may include constant regions which permit destruction of targeted cells by a subject's immune system.

**[56]** Mixtures of antibodies that bind to more than one type AGE or AGE-modified proteins may also be used.

**[57]** Bi-specific antibodies, which are anti-AGE antibodies directed to two different epitopes, may also be used. Such antibodies will have a variable region (or complementary determining region) from those of one anti-AGE antibody, and a variable region (or complementary determining region) from a different antibody.

**[58]** Antibody fragments may be used in place of whole antibodies. For example, immunoglobulin G may be broken down into smaller fragments by digestion with enzymes. Papain digestion cleaves the N-terminal side of inter-heavy chain disulfide bridges to produce Fab fragments. Fab fragments include the light chain and one of the two N-terminal domains of the heavy chain (also known as the Fd fragment). Pepsin digestion cleaves the C-terminal side of the inter-heavy chain disulfide bridges to produce F(ab')<sub>2</sub> fragments. F(ab')<sub>2</sub> fragments include both light chains

and the two N-terminal domains linked by disulfide bridges. Pepsin digestion may also form the Fv (fragment variable) and Fc (fragment crystallizable) fragments. The Fv fragment contains the two N-terminal variable domains. The Fc fragment contains the domains which interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Pepsin may also cleave immunoglobulin G before the third constant domain of the heavy chain (CH3) to produce a large fragment F(abc) and a small fragment pFc'. Antibody fragments may alternatively be produced recombinantly.

[59] If additional antibodies are desired, they can be produced using well-known methods. For example, polyclonal antibodies (pAbs) can be raised in a mammalian host by one or more injections of an immunogen, and if desired, an adjuvant. Typically, the immunogen (and adjuvant) is injected in a mammal by a subcutaneous or intraperitoneal injection. The immunogen may be an AGE-modified protein of a cell, such as AGE-antithrombin III, AGE-calmodulin, AGE-insulin, AGE-  
ceruloplasmin, AGE-collagen, AGE-cathepsin B, AGE-albumin such as AGE-bovine serum albumin (AGE-BSA), AGE-human serum albumin and ovalbumin, AGE-crystallin, AGE-plasminogen activator, AGE-endothelial plasma membrane protein, AGE-aldehyde reductase, AGE-transferrin, AGE-fibrin, AGE-copper/zinc SOD, AGE-apo B, AGE-fibronectin, AGE-pancreatic ribose, AGE-apo A-I and II, AGE-hemoglobin, AGE-Na<sup>+</sup>/K<sup>+</sup>-ATPase, AGE-plasminogen, AGE-myelin, AGE-lysozyme, AGE-immunoglobulin, AGE-red cell Glu transport protein, AGE-β-N-acetyl hexokinase, AGE-apo E, AGE-red cell membrane protein, AGE-aldo reductase, AGE-ferritin, AGE-red cell spectrin, AGE-alcohol dehydrogenase, AGE-haptoglobin, AGE-tubulin, AGE-thyroid hormone, AGE-fibrinogen, AGE-β<sub>2</sub>-microglobulin, AGE-sorbitol dehydrogenase, AGE-α<sub>1</sub>-antitrypsin, AGE-carbonate dehydratase, AGE-RNAse, AGE-low density lipoprotein, AGE-hexokinase, AGE-apo C-I, AGE-RNAse, AGE-hemoglobin such as AGE-human hemoglobin, AGE-albumin such as AGE-bovine serum albumin (AGE-BSA) and AGE-human serum albumin, AGE-low density lipoprotein (AGE-LDL) and AGE-collagen IV. AGE-modified cells, such as AGE-modified erythrocytes, whole, lysed, or partially digested, may also be used as AGE antigens. Examples of adjuvants include Freund's complete, monophosphoryl

Lipid A synthetic-trehalose dicorynomycolate, aluminum hydroxide (alum), heat shock proteins HSP 70 or HSP96, squalene emulsion containing monophosphoryl lipid A,  $\alpha$ 2-macroglobulin and surface active substances, including oil emulsions, pleuronic polyols, polyanions and dinitrophenol. To improve the immune response, an immunogen may be conjugated to a polypeptide that is immunogenic in the host, such as keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, cholera toxin, labile enterotoxin, silica particles or soybean trypsin inhibitor. A preferred immunogen conjugate is AGE-KLH. Alternatively, pAbs may be made in chickens, producing IgY molecules.

[60] Monoclonal antibodies (mAbs) may also be made by immunizing a host or lymphocytes from a host, harvesting the mAb-secreting (or potentially secreting) lymphocytes, fusing those lymphocytes to immortalized cells (for example, myeloma cells), and selecting those cells that secrete the desired mAb. Other techniques may be used, such as the EBV-hybridoma technique. Techniques for the generation of chimeric antibodies by splicing genes encoding the variable domains of antibodies to genes of the constant domains of human (or other animal) immunoglobulin result in "chimeric antibodies" that are substantially human (humanized) or substantially "ized" to another animal (such as cat, dog, horse, camel or alpaca, cattle, sheep, or goat) at the amino acid level. If desired, the mAbs may be purified from the culture medium or ascites fluid by conventional procedures, such as protein A-sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, ammonium sulfate precipitation or affinity chromatography. Additionally, human monoclonal antibodies can be generated by immunization of transgenic mice containing a third copy IgG human trans-loci and silenced endogenous mouse Ig loci or using human-transgenic mice. Production of humanized monoclonal antibodies and fragments thereof can also be generated through phage display technologies.

[61] A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Preferred examples of such carriers or diluents include water, saline, Ringer's solutions and dextrose solution. Supplementary active compounds can also

be incorporated into the compositions. Solutions and suspensions used for parenteral administration can include a sterile diluent, such as water for injection, saline solution, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

**[62]** Pharmaceutical compositions suitable for injection include sterile aqueous solutions or dispersions for the extemporaneous preparation of sterile injectable solutions or dispersion. Various excipients may be included in pharmaceutical compositions of antibodies suitable for injection. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CREMOPHOR EL® (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid so as to be administered using a syringe. Such compositions should be stable during manufacture and storage and must be preserved against contamination from microorganisms such as bacteria and fungi. Various antibacterial and anti-fungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, and thimerosal, can contain microorganism contamination. Isotonic agents such as sugars, polyalcohols, such as manitol, sorbitol, and sodium chloride can be included in the composition. Compositions that can delay absorption include agents such as aluminum monostearate and gelatin. Sterile injectable solutions can be prepared by incorporating antibodies, and optionally other therapeutic components, in the required amount in an appropriate solvent with one or a combination of ingredients as required, followed by sterilization. Methods of preparation of sterile solids for the preparation of sterile injectable solutions include vacuum drying and freeze-drying to yield a solid.

**[63]** For administration by inhalation, the antibodies may be delivered as an aerosol spray from a nebulizer or a pressurized container that contains a suitable propellant, for example, a gas such as carbon dioxide. Antibodies may also be

delivered via inhalation as a dry powder, for example using the iSPERSE™ inhaled drug deliver platform (PULMATRIX, Lexington, Mass.). The use of anti-AGE antibodies which are chicken antibodies (IgY) may be non-immunogenic in a variety of animals, including humans, when administered by inhalation.

[64] Topical application may be effective for cancers and potentially-malignant neoplasms present in the skin, for example melanomas, seborrheic keratosis and actinic keratosis. Compositions for topical administration may be in the form of creams or lotions.

[65] An appropriate dosage level of each type of antibody will generally be about 0.01 to 500 mg per kg patient body weight. Preferably, the dosage level will be about 0.1 to about 250 mg/kg; more preferably about 0.5 to about 100 mg/kg. A suitable dosage level may be about 0.01 to 250 mg/kg, about 0.05 to 100 mg/kg, or about 0.1 to 50 mg/kg. Within this range the dosage may be 0.05 to 0.5, 0.5 to 5 or 5 to 50 mg/kg. Although each type of antibody may be administered on a regimen of 1 to 4 times per day, such as once or twice per day, antibodies typically have a long half-life *in vivo*. Accordingly, each type of antibody may be administered once a day, once a week, once every two or three weeks, once a month, or once every 60 to 90 days.

[66] A subject that receives administration of an anti-AGE antibody may be tested to determine if it has been effective to treat the cancer, by examining the patient for the spread of the cancer to different parts of the body, particularly in lymph nodes. Any suitable diagnostic test may be used, such as a biopsy, endoscopy, blood test or diagnostic imaging test such as an X-ray or CT scan. The diagnostic test may also include anti-AGE antibodies for detection. Administration of antibody and subsequent testing may be repeated until the desired therapeutic result is achieved. Similarly, a subject may be tested to determine if a potentially-malignant neoplasm has been effectively treated by a reduction in size, or disappearance, of the neoplasm.

**[67]** Unit dosage forms can be created to facilitate administration and dosage uniformity. Unit dosage form refers to physically discrete units suited as single dosages for the subject to be treated, containing a therapeutically effective quantity of one or more types of antibodies in association with the required pharmaceutical carrier. Preferably, the unit dosage form is in a sealed container and is sterile.

**[68]** Any mammal that could develop metastatic cancer may be treated by the methods herein described. Humans are a preferred mammal for treatment. Other mammals that may be treated include mice, rats, goats, sheep, cows, horses and companion animals, such as dogs or cats. A subject in need of treatment may be identified by the diagnosis of a cancer. Cancers which are particularly subject to metastasis include lung cancer, melanoma, colon cancer, renal cell carcinoma, prostate cancer, cancer of the cervix, bladder cancer, rectal cancer, esophageal cancer, liver cancer, mouth and throat cancer, multiple myeloma, ovarian cancer, and stomach cancer. Treatment may be of patients experiencing metastatic cancer. Treatment may also be administered to patients who have cancer, but prior to any identified metastasis, in order to prevent metastasis. Similarly, any mammal that could develop potentially-malignant neoplasms may be treated by the methods herein described. Humans are a preferred mammal for treatment. Other mammals that may be treated include mice, rats, goats, sheep, cows, horses and companion animals, such as dogs or cats. A subject in need of treatment may be identified by the diagnosis of a potentially-malignant neoplasm.

**[69]** A particularly preferred treatment group includes subjects who are unable to receive conventional cancer treatments such as surgery, radiation therapy or chemotherapy. A patient with metastatic cancer or at risk for cancer metastasis may not be able to undergo certain cancer treatments due to other diagnoses, physical conditions or complications. For example, pregnant women cannot receive radiation therapy due to a risk of harm to the fetus. Aged or weakened patients, such as those experiencing cancer cachexia, may not be good candidates for surgery due to a risk of not surviving an invasive procedure. Patients who already have a compromised immune system or a chronic infection may not be able to receive chemotherapy since many chemotherapy drugs harm the immune system.

[70] The anti-AGE antibodies may be used in cellular purification processes, such as immunopanning and immunoadsorption. Purification processes are useful in isolating desirable or unwanted cells from tissue cultures, cell cultures or blood. Cellular purification may be used in transplantations, such as a bone marrow transplant, or transfusions, such as a blood transfusion. Cellular purification is especially useful in autologous stem cell transplantation during chemotherapy to remove metastasizing malignant cells and concentrate beneficial stem cells. Immunopanning or immunoadsorption using an anti-AGE antibody may isolate metastasizing cancer, from a tissue culture, cell culture or blood sample.

[71] The anti-AGE antibodies may also be used to diagnose cancer metastases. An immune complex (also known as an antibody-antigen complex) including an anti-AGE antibody bound to a metastatic cancer cell expressing AGE-modified proteins is a unique analyte that may be predictive or indicative of metastatic cancer. The specific binding of anti-AGE antibodies to metastasizing cancer cells may allow for the detection of cancer metastases at subclinical levels. Diagnostic anti-AGE antibodies may be used to detect circulating metastatic cancer cells that pose a risk of metastasizing in a new location. Alternatively, diagnostic anti-AGE antibodies may be used to test cells obtained from a specific location for the presence of metastatic cancer cells. A biopsy may involve collecting cells from a specific part of the body that is a known risk for accumulation of metastatic cancer cells, such as the lymph nodes, lungs, liver, brain or bones, or from a part of the body where metastasis is suspected due to other symptoms, such as a suspicious lump. Anti-AGE antibodies may be used in any diagnostic method that employs antibodies for detection of an analyte of interest. For example, an immune complex may be detected using a suitable imaging technique after attaching a label to the antibodies, such as a fluorescent label or radiolabel; using cytological techniques such as immunofluorescence, flow cytometry or fluorescence-activated cell sorting (FACS); using biochemical techniques such as immunoassays, especially enzyme-linked immunosorbent arrays (ELISA), Western blotting or immunoprecipitation; or using cellular purification techniques such as immunopanning.

[72] FIG. 2 illustrates a kit **200** for diagnosing cancer metastases. The kit may include an anti-AGE antibody **210**, a control **220** and, optionally, a reagent **230** for detecting the anti-AGE antibody. The anti-AGE antibody, the control and the optional reagent may be supplied in any suitable container, such as bottles, ampules, envelopes, test tubes, vials, flasks or syringes. The anti-AGE antibody and/or the reagent may optionally be labelled, such as with a fluorescent label, radiolabel or a gold particle. The control may be normal serum from an animal in which a secondary antibody was made, a solution containing a known amount of an AGE-modified protein or peptide or fixed or preserved cells that exhibit an AGE modification. Examples of reagents for detecting the anti-AGE antibody include secondary antibodies, such as an anti-human polyclonal antibody made in donkey and labelled with rhodamine. The kit may optionally be housed in a container **240**. The kit may optionally include printed instructions **250**. Preferably, the contents of the kit are sterile and ready for use.

[73] The kit may optionally include a container for housing the kit ingredients. The container may be formed of a rigid, durable material, such as plastic, or may be flexible, such as a bag or soft-sided box.

[74] The kit may optionally include instructions for use. The instructions may be provided as printed instructions or in electronic format, such as on a universal serial bus (USB) drive, on a secure digital (SD) card, or hosted over the internet and accessible through a quick response (QR) code.

[75] Kits may optionally contain additional diagnostic materials or equipment such as buffers, fixatives, blocking solutions, protease inhibitors, substrates for analysis such as microscope slides and/or cover slips, microtiter plates and cell extraction reagents such as detergents and detergent solutions.

[76] The one-letter amino acid sequence that corresponds to SEQ ID NO: 1 is shown below:

10	20	30	40	50
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MNLLLILTFV AAAVAQVQLL QPGAELVKPG ASVKLACKAS GYLFTTYWMH

	60	70	80	90	
	WLKQRPGQGL	EWIGEISPTN	GRAYYNARFK	SEATLTVDKS	
	100	110	120	130	
	SNTAYMQLSS	LTSEASAVYY	CARAYGNYEF	AYWGQGTLVT	
	140	150	160	170	
	VSVASTKGPS	VFPLAPSSKS	TSGGTAALGC	LVKDYFPEPV	
	180	190	200	210	220
	TVSWNSGALT	SGVHTFPAVL	QSSGLYSLSS	VVTVPSSSLG	TQTYICNVNH
	230	240	250	260	
	KPSNTKVDKK	VEPKSCDKTH	TCPPCPAPEL	LGGPSVFLFP	
	270	280	290	300	
	PKPKDTLMIS	RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	
	310	320	330	340	
	HNAKTKPREE	QYNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS	
	350	360	370	380	390
	NKALPAPIEK	TISKAKGQPR	EPQVYTLPPS	REEMTKNQVS	LTCLVKGFYP
	400	410	420	430	
	SDIAVEWESN	GQPENNYKTT	PPVLDSDGSF	FLYSKLTVDK	
	440	450	460		
	SRWQQGNVFS	CSVMHEALHN	HYTQKSLSL	PGK	

**[77]** Positions 16-133 of the above amino acid sequence correspond to SEQ ID NO: 2. Positions 46-50 of the above amino acid sequence correspond to SEQ ID NO: 41. Positions 65-81 of the above amino acid sequence correspond to SEQ ID NO: 42. Positions 114-122 of the above amino acid sequence correspond to SEQ ID NO: 43.

**[78]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 3 is shown below:

	10	20	30	40	50	
	MNLLLILTFV	AAAVADVVM	QTPLSLPVSL	GDQASISCRS	RQSLVNSNGN	

60	70	80	90	100
TFLQWYLQKP GQSPKLLIYK VSLRFSGVPD RFSGSGSGTD FTLKISRVEA				
110	120	130	140	150
EDLGLYFCSQ STHVPPTFGG GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA				
160	170	180	190	
SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD				
200	210	220	230	
STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC				

[79] Positions 16-128 of the above amino acid sequence correspond to SEQ ID NO: 4. Optionally, the arginine (Arg or R) residue at position 128 of SEQ ID NO: 4 may be omitted. Positions 39-54 of the above amino acid sequence correspond to SEQ ID NO: 44. Positions 70-76 of the above amino acid sequence correspond to SEQ ID NO: 45. Positions 109-117 of the above amino acid sequence correspond to SEQ ID NO: 46.

[80] The DNA sequence that corresponds to SEQ ID NO: 12 is shown below:

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAACTCTGCTGTTCTGAGCCTGGC  
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGCTGCAGCCAGGTGCCAGAGCTC  
GTGAAACCTGGCGCCTCTGTGAAGCTGGCCTGCAAGGCTTCCGGCTACCTGTT  
CACCAACCTACTGGATGCACTGGCTGAAGCAGAGGCCAGGCCAGGGCCTGGAA  
TGGATCGCGAGATCTCCCCACCAACGGCAGAGCCTACTACAACGCCCGGTT  
CAAGTCCGAGGCCACCCCTGACCGTGGACAAGTCCTCCAACACCGCCTACATGC  
AGCTGTCCTCCCTGACCTCTGAGGCCTCCGCCGTGTACTACTGCGCCAGAGCT  
TACGGCAACTACGAGTTGCCTACTGGGCCAGGGCACCCCTCGTACAGTGTC  
TGTGGCTAAGACCACCCCTCCCTCCGTGTACCCCTCTGGCTCCTGGCTGTGGCG  
ACACCACCGGATCCTCTGTGACCCCTGGCTGCCCTCGTGAAGGGCTACTCCCT  
GAGTCCGTACCGTGACCTGGAACCTCCGGCTCCCTGTCCTCCCGTGCACAC  
CTTCCAGCCCTGCTGCAGTCCGGCTGTACACCATGTCCTCAGCGTGACAG  
TGCCCTCCTCCACCTGGCTTCCCAGACCGTGACATGCTCTGTGGCCCACCC  
GCCTCTTCCACCAACCGTGGACAAGAAGCTGGAACCCCTCCGGCCCCATCTCCAC  
CATCAACCCTTGCCTCCCTGCAAAGAATGCCACAAAGTGCCCTGCCCTGCCCAACC

TGGAAGGCGGCCCTCCGTGTTCATCTTCCCACCCAACATCAAGGACGTGCTG  
ATGATCTCCCTGACCCCCAAAGTGACCTGCGTGGTGGACGTGTCCGAGGA  
CGACCCCTGACGTGCAGATCAGTTGGTCGTGAACAAACGTGGAAGTGCACACCG  
CCCAGACCCAGACACACAGAGAGGACTACAACAGCACCATCAGAGTGGTGTCT  
ACCCTGCCCATCCAGCACCAGGACTGGATGTCCGGCAAAGAATTCAAGTGCAA  
AGTGAACAACAAGGACCTGCCAGCCCCATCGAGCGGACCATCTCCAAGATCA  
AGGCCTCGTGCAGGCTCCCCAGGTGTACATTCTGCCTCCACCAGCCGAGCA  
GCTGTCCCAGGAAGGATGTGTCTGTACATGTCTGGTCGTGGCTCAACCCCG  
GCGACATCTCCGTGGAATGGACCTCCAACGGCCACACCGAGGAAAACATACAAG  
GACACCGCCCCCTGTGCTGGACTCCGACGGCTCCTACTTCATCTACTCCAAGCT  
GAACATGAAGACCTCCAAGTGGAAAAGACCGACTCCTCTCTGCAACGTGC  
GGCACGAGGGCCTGAAGAACTACTACCTGAAGAAAACCATCTCCGGTCCCCC  
GGCTAG

[81] The DNA sequence that corresponds to SEQ ID NO: 13 is shown below:

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCTGAGCCTGGC  
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGCTGCAGCCAGGTGCCAGCTC  
GTGAAACCTGGCGCCTCTGTGAAGCTGGCTGCAAGGCTCCGGTACCTGTT  
CACCACTACTGGATGCACTGGCTGAAGCAGAGGCCAGGCCAGGGCCTGGAA  
TGGATCGCGAGATCTCCCCACCAACGGCAGAGCCTACTACAACGCCGGTT  
CAAGTCCGAGGCCACCCGTACCGTGGACAAGTCCTCCAACACCGCCTACATGC  
AGCTGTCCTCCCTGACCTCTGAGGCCTCCGCGTGTACTACTGCGCCAGAGCT  
TACGGCAACTACGAGTTCGCCTACTGGGCCAGGGCACCCCTGTGACAGTGTC  
TGTGGCTAGCACCAAGGGCCCCAGCGTGTCCCTCTGGCCCCCAGCAGCAAG  
AGCACCAGCGCGGAACCGCCGCCCCGGCTGCCTGGTAAGGACTACTTCC  
CCGAGCCCGTACCGTGTCCCTGGAACAGCGGCCGCTCTGACCGAGCGAGTGCA  
CACCTCCCTGCCGTGCTGCAGAGCAGCGCCGTACTCCCTGAGCAGCGTG  
GTGACCGTCCCCAGCAGCAGCCTGGCACCCAGACCTACATCTGCAACGTGAA  
CCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGC  
GACAAGACCCACACCTGCCCTCCCTGCCCGCCCCCGAGCTGCTGGCGGAC  
CCAGCGTGTCCCTGTTCCCTCCAAGCCCAAGGACACCCGTGATGATCAGCCGC  
ACCCCGAGGTGACCTGCGTGGTGGACGTGAGCCACGAGGACCCCGAGG

TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG  
CCTCGGGAGGAGCAGTACAACCTCCACCTACCGCGTGGTGAGCGTGCTGACCG  
TGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAA  
CAAGGCCCTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAG  
CCCCGGGAGCCTCAGGTGTACACCCTGCCCTGGTGAGGGCTTCTACCCCTCCGACATC  
AGAACCCAGGTGAGCCTGACCTGCCTGGTGAGGGCTTCTACCCCTCCGACATC  
GCCGTGGAGTGGGAGAGCAACGCCAGCCTGAGAACAACTACAAGACCACCC  
CTCCCGTGTGGACAGCGACGGCAGCTTCTCCTGTACAGCAAGCTGACCGTG  
GACAAGTCCCCGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACG  
AGGCCCTGCACAACCACACACCCAGAAGAGCCTGAGCCTGAGCCCCGGATA  
G

**[82]** The DNA sequence that corresponds to SEQ ID NO: 14 is shown below:

ATGGAGACCGACACCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT  
CCACCGGAGACGTCGTGATGACCCAGACCCCTCTGCCCTGCCCTGTGTCTCTG  
GGCGACCAGGCCTCCATCTCCTGCCGGTAGACAGTCCCTCGTGAACCTCAA  
CGGCAACACCTTCCTGCAGTGGTATCTGCAGAAGGCCGGCCAGTCCCCAAGC  
TGCTGATCTACAAGGTGTCCCTGCCGGTCTCCGGCGTGCACAGATTTCC  
GGCTCTGGCTCTGGCACCGACTTCACCCCTGAAGATCTCCCGGGTGGAAAGCCGA  
GGACCTGGCCTGTACTTCTGCAGCCAGTCCACCCACGTGCCCTACATTG  
GCGGAGGCACCAAGCTGGAAATCAAACGGGAGATGCTGCACCAACTGTATCC  
ATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGC  
TTCTTGAACAACCTCTACCCCAAAGACATCAATGTCAAGTGGAAAGATTGATGGC  
AGTGAACGACAAATGGCGTCTGAACAGTTGGACTGATCAGGACAGCAAAGA  
CAGCACCTACAGCATGAGCAGCACCCCTACGTTGACCAAGGACGAGTATGAAC  
GACATAACAGCTATAACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG  
TCAAGAGCTTCAACAGGAATGAGTGTGA

**[83]** The DNA sequence that corresponds to SEQ ID NO: 15 is shown below:

ATGGAGACCGACACCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT  
CCACCGGAGACGTCGTGATGACCCAGACCCCTCTGCCCTGCCCTGTGTCTCTG

GGCGACCAGGCCTCCATCTCCTGCCGGTAGACAGTCCCTCGTAACCTCAA  
CGGCAACACCTCCTGCAGTGGTATCTGCAGAAGCCCGGCCAGTCCCCAAGC  
TGCTGATCTACAAGGTGTCCTGCAGGTTCTCCGGCGTGCCTGACAGATTTCC  
GGCTCTGGCTCTGGCACCGACTTCACCCCTGAAGATCTCCCGGGTGGAAAGCCGA  
GGACCTGGGCCTGTACTTCTGCAGCCAGTCCACCCACGTGCCCTACATTG  
GCGGAGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCCAGCGTGT  
CATCTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGT  
GCCTGCTGAACAACCTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAAGGTGGA  
CAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACTCC  
AAGGACAGCACCTACAGCCTGAGCAGCACCTGACCCCTGAGCAAGGCCGACTA  
CGAGAACAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGC  
CCCGTGACCAAGAGCTTCAACCGGGCGAGTGCTAA

**[84]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 16 is shown below:

MDPKGSLSWRILLFLSLAFELSYGQVQLLQPGAEVLVKPGASVKLACKASGYLF  
WMHWLKQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLT  
SEASAVYYCARAYGNYEFAYWGQGTLTVSVAKTPPSVYPLAPGCGD  
TGSSVT  
LGCLVKGYFPESVTWTNSGSLSSSVHTFPALLQSGLYTMSSSV  
TVP  
SSTWPSQT  
VTC  
SVAHPASSTTV  
DKKLEPSGP  
I  
STINPCPP  
CKE  
CHKCP  
APN  
LEGGPSV  
F  
IFPPNI  
DK  
V  
L  
M  
ISL  
TPK  
V  
TC  
V  
V  
D  
V  
SE  
DD  
P  
DV  
Q  
IS  
WF  
V  
NN  
V  
EV  
HTA  
QT  
Q  
T  
H  
RE  
D  
Y  
N  
S  
T  
I  
R  
V  
V  
S  
T  
L  
C  
L  
V  
V  
G  
F  
N  
P  
G  
D  
I  
S  
V  
E  
W  
T  
S  
N  
G  
H  
T  
E  
E  
N  
Y  
K  
D  
T  
A  
P  
V  
L  
D  
S  
G  
S  
Y  
F  
I  
Y  
S  
K  
L  
N  
M  
K  
T  
K  
W  
E  
K  
T  
D  
S  
F  
C  
N  
V  
R  
H  
E  
G  
L  
K  
N  
Y  
Y  
L  
K  
K  
T  
I  
R  
S  
P  
G  
\*

**[85]** The alanine residue at position 123 of the above amino acid sequence may optionally be replaced with a serine residue. The tyrosine residue at position 124 of the above amino acid sequence may optionally be replaced with a phenylalanine residue. Positions 25-142 of the above amino acid sequence correspond to SEQ ID NO: 20. SEQ ID NO: 20 may optionally include the substitutions at positions 123 and 124. SEQ ID NO: 20 may optionally contain one additional lysine residue after the terminal valine residue.

**[86]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 17 is shown below:

MDPKGSLSWRILLFLSLAFELSYGQVQLLQPGAEVLKPGASVKLACKASGYLFTTY  
WMHWLKQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLT  
SEASAVYYCARAYGNYEFAYWGQGTLTVSVASTKGPSVFLAPSSKSTSGGTAA  
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQT  
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLPPKPKDTLMIS  
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVVSVLTVL  
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPVYTLPPSRDELTKNQVSL  
TCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDGSFFLYSKLTVDKSRWQQG  
NVFSCSVMHEALHNHYTQKSLSLSPG\*

**[87]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 18 is shown below:

METDTLLLWVLLWVPGSTGDVVMQTPLSLPVSLGDQASISCRSRQSLVNSNGN  
TFLQWYLQKPGQSPKLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDGLYF  
CSQSTHVPPTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI  
NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTQKDEYERHNSYTCEATHK  
TSTSPIVKSFNRNEC\*

**[88]** Positions 21-132 of the above amino acid sequence correspond to SEQ ID NO: 21.

**[89]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 19 is shown below:

METDTLLLWVLLWVPGSTGDVVMQTPLSLPVSLGDQASISCRSRQSLVNSNGN  
TFLQWYLQKPGQSPKLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDGLYF  
CSQSTHVPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCFLNNFYPREA  
KVQWKVDNALQSGNSQESVTEQDSKDSTYSMSSTLTQKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC\*

[90] The one-letter amino acid sequence that corresponds to SEQ ID NO: 22 is shown below:

10	20	30	40	50
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV				
60	70	80	90	100
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVER				
110	120	130	140	150
KCCVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP				
160	170	180	190	
EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ				
200	210	220	230	240
DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN				
250	260	270	280	290
QVSLTCLVKKG FYPSDISVEW ESNGQPENNY KTPPPMLDSD GSFFLYSKLT				
300	310	320		
VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK				

[91] The one-letter amino acid sequence that corresponds to SEQ ID NO: 23 is SYTMGV.

[92] The one-letter amino acid sequence that corresponds to SEQ ID NO: 24 is TISSGGGSTYYPDSVKG.

[93] The one-letter amino acid sequence that corresponds to SEQ ID NO: 25 is QGGWLPPFAX, where X may be any naturally occurring amino acid.

[94] The one-letter amino acid sequence that corresponds to SEQ ID NO: 26 is RASKSVSTSSRGYSYMH.

[95] The one-letter amino acid sequence that corresponds to SEQ ID NO: 27 is LVSNLES.

[96] The one-letter amino acid sequence that corresponds to SEQ ID NO: 28 is QHIRELTRS.

[97] The one-letter amino acid sequence that corresponds to SEQ ID NO: 29 is MDPKGSLSWRILLFLSLAFELSYGQVQLVQSGAEVKPGASVKVSCKASGYLFTTY WMHWVRQAPGQGLEWMGEISPNTNGRAYYNQKFQGRVTMTVDKSTNTVYMELOSS LRSEDTAVYYCARAYGNYFAYWGQGTLTVSSASTKGPSVPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKHTCPPCPPELLGGPSVFLPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTIASKAGQPREPQVYTLPPSRDELKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG.

[98] The DNA sequence that corresponds to SEQ ID NO: 30 is ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCTGAGCCTGGC CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAACCTGGCGCCTCCGTAGAGTGTCCCTGCAAGGCTCCGGCTACCTGTT ACCACCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAGGGCCTGGAAT GGATGGCGAGATCTCCCCTACCAACGGCAGAGCCTACTACAACAGAAATTCC AGGGCAGAGTGACCATGACCGTGGACAAGTCCACCAACACCGTGTACATGGAA CTGTCCTCCCTGCGGAGCGAGGACACCGCCGTGTACTACTGCGCTAGAGCCTA CGGCAACTACGATTGCCTACTGGGCCAGGGCACCCCTCGTACAGTGTCTC TGCTAGCACCAAGGGCCCCAGCGTGTCCCTCTGGCCCCCAGCAGCAAGAGC ACCAGCGCGGAACCGCCGCCCTGGCTGCCCTGGAAAGGACTACTTCCCCGA GCCCGTGACCGTGTCCCTGGAACAGCGCGCTCTGACCGAGCGAGTGCACACC TTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGA CCGTGCCAGCAGCAGCCTGGCACCCAGACCTACATCTGCAACGTGAACCACA AGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAACAGAGCTGCGACAA GACCCACACCTGCCCTCCCTGCCCCGCCCGAGCTGCTGGCGGACCCAGCG TGTTCCCTGTTCCCTCCAAGCCAAGGACACCCCTGATGATCAGCCGACCCCC GAGGTGACCTGCGTGGTGGACGTGAGCCACGAGGACCCGAGGTGAGTT CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCTCGG

GAGGAGCAGTACAACCTCCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGC  
ACCAGGACTGGCTGAACGGCAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC  
CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGG  
AGCCTCAGGTGTACACCCCTGCCCGAGCCGACGAGCTGACAAGAACAG  
GTGAGCCTGACCTGCCTGGTGAAGGGCTCTACCCCTCCGACATGCCGTGGA  
GTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCGTG  
CTGGACAGCGACGCAGCTTCTCCTGTACAGCAAGCTGACCGTGGACAAGTCC  
CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGTGCACGAGGCCCTGC  
ACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCGGATAGTAA.

**[99]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 31 is  
MDPKGSLSWRILLFLSLAFELSYGQVQLVQSGAEVKKPGASVKVSCKASGYLF  
WMHWVRQAPGQGLEWMGEISPTNGRAYYNAKFQGRVTMTVDKSTNTAYMELSS  
LRSEDTAVYYCARAYGNYFAYWGQGTLTVSSASTKGPSVFLAPSSKSTSGGTA  
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTVPSQLGTQ  
TYICNVNHKPSNTKVDKKVEPKSCDKTHCPPCPPELLGGPSVFLPPKPKDTLMIS  
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVVSVLTVL  
HQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSRDELKNQVSLT  
CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN  
VFSCSVMHEALHNHYTQKSLSLSPG.

**[100]** The DNA sequence that corresponds to SEQ ID NO: 32 is  
ATGGACCCCAAGGGCAGCCTGAGCTGGAGAACCTGCTGTTCTGAGCCTGGC  
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG  
AAGAAACCTGGCGCCTCCGTGAGGTGTCCTGCAAGGCTCCGGTACCTGTT  
ACCACCTACTGGATGCACTGGGTGCGACAGGCCCTGGACAGGGCCTGGAAT  
GGATGGCGAGATCTCCCTACCAACGGCAGAGCCTACTACAACCAAAATTCC  
AGGGCAGAGTGACCATGACCGTGGACAAGTCCACCAACACCGCTTACATGGAA  
CTGTCCTCCCTGCGGAGCGAGGACACCGCCGTGTACTACTGCGCTAGAGCCTA  
CGGCAACTACGATTGCCACTGGGCCAGGGCACCCCTCGTACAGTGTCTC  
TGCTAGCACCAAGGGCCCCAGCGTGTCCCTCTGGCCCCCAGCAGCAAGAGC  
ACCAGCGCGGAACCGCCGCCCTGGCTGCCTGGGAAGGACTACTTCCCCGA  
GCCCGTGACCGTGTCCGGAACAGCGCGCTGTACCGAGCGAGTGCACACC

TTCCCTGCCGTGCTGCAGAGCAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGA  
 CCGTGCCAGCAGCAGCAGCTGGGCACCCAGACCTACATCTGCAACGTGAACCACA  
 AGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGCGACAA  
 GACCCACACCTGCCCTCCCTGCCCGCCCCGAGCTGCTGGCGGACCCAGCG  
 TGTTCCCTGTTCCCTCCCAAGCCCAAGGACACCCCTGATGATCAGCCGCACCCCC  
 GAGGTGACCTGCGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAGTT  
 CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGG  
 GAGGAGCAGTACAACCTCCACCTACCGCGTGGTGAGCGTGTGACCGTGCTGC  
 ACCAGGACTGGCTGAACGGCAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC  
 CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGG  
 AGCCTCAGGTGTACACCCCTGCCCGCCAGCCGCGACGAGCTGACAAGAACAG  
 GTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATGCCGTGGA  
 GTGGGAGAGCAACGCCAGCCTGAGAACAACTACAAGACCAACCCCTCCGTG  
 CTGGACAGCGACGCAGCTTCTCCTGTACAGCAAGCTGACCGTGGACAAGTCC  
 CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGTGACCGAGGCCCCTGC  
 ACAACCAACTACACCCAGAAGAGCCTGAGCCTGAGCCCAGATAGTAA.

**[101]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 33 is  
 MDPKGSLSWRILLFLSLAFELSYGQVQLVQSGAEVKKPGASVKVSCKASGYLFTTY  
 WMHWVRQAPGQGLEWMGEISPTNGRAYYNAKFQGRVTMTVDKSINTAYMELSRL  
 RSDDTAVYYCARAYGNYFAYWGQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAA  
 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTVPSQLGTQT  
 YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPPELGGPSVFLFPPKPKDTLMISR  
 TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNNAKTPREEQYNSTYRVVSVLTVLH  
 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELKNQVSLTC  
 LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV  
 FSCSVMHEALHNHYTQKSLSLSPG.

**[102]** The DNA sequence that corresponds to SEQ ID NO: 34 is  
 ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCCTGAGCCTGGC  
 CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG  
 AAGAAACCTGGCGCCTCCGTGAGGTGCCTGCAAGGCTCCGGTACCTGTT  
 ACCACCTACTGGATGCACGGTGCACAGGCCCCCTGGACAGGGCCTGGAAT

GGATGGCGAGATCTCCCTACCAACGGCAGAGCCTACTACAACCAAAATTCC  
AGGGCAGAGTGACCATGACCGTGGACAAGTCATCAACACCGCTTACATGGAA  
CTGTCCAGACTGCGGAGCGATGACACCGCCGTGTACTACTGCGCTAGAGCCTA  
CGGCAACTACGATTGCCTACTGGGCCAGGGCACCCCTCGTGACAGTGTCTC  
TGCTAGCACCAAGGGCCCCAGCGTGTCCCTCTGGCCCCCAGCAGCAAGAGC  
ACCAGCGCGGAACCGCCGCCCTGGCTGCCTGGGAAGGACTACTTCCCCGA  
GCCCGTGACCGTGTCCCTGGAACAGCGCGCTCTGACCAGCGAGTGCACACC  
TTCCCTGCCGTGCTGCAGAGCAGCGGCTGTACTCCCTGAGCAGCGTGGTGA  
CCGTGCCAGCAGCAGCCTGGCACCCAGACCTACATCTGCAACGTGAACCACA  
AGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGCGACAA  
GACCCACACCTGCCCTCCCTGCCCGCCCCGAGCTGCTGGCGGACCCAGCG  
TGTTCCCTGTTCCCTCCCAAGCCCAAGGACACCCCTGATGATCAGCCGCACCCCC  
GAGGTGACCTGCGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAGTT  
CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGG  
GAGGAGCAGTACAACCTCCACCTACCGCGTGGTAGCGTGTGACCGTGCTGC  
ACCAGGACTGGCTGAACGGCAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC  
CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGG  
AGCCTCAGGTGTACACCCCTGCCCGCCAGCCGCGACGAGCTGACAAGAACAG  
GTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATGCCGTGGA  
GTGGGAGAGCAACGCCAGCCTGAGAACAACTACAAGACCACCCCTCCGTG  
CTGGACAGCGACGCAGCTTCTCCTGTACAGCAAGCTGACCGTGGACAAGTCC  
CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGTGACCGAGGCCCTGC  
ACAACCAACTACACCCAGAAGAGCCTGAGCCTGAGCCCGGATAGTAA.

**[103]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 35 is  
METDTLLWVLLWVPGSTGDVVMTQSPLSLPVTLGQPASISCRSSQSLVNSNGNT  
FLQWYQQRPGQSPRLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY  
CSQSTHVPPTFGGGTVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAK  
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLKADYEKHKVYACEVTHQG  
LSSPVTKSFNRGEC.

**[104]** The DNA sequence that corresponds to SEQ ID NO: 36 is  
ATGGAGACCGACACCCCTGCTGCTCTGGGTGCTGCTCTGGGTGCCCGGCT

CCACCGGAGACGTCGTGATGACCCAGTCCCTCTGTCCCTGCCTGTGACCCCTG  
GGACAGCCTGCCTCCATCTCCTCAGATCCTCCAGTCCCTCGTGAACCTCAAC  
GGCAACACCTCCTGCAGTGGTATCAGCAGCGGCCTGCCAGAGCCCCAGAC  
TGCTGATCTACAAGGTGTCCTGCGGTTCTCCGGCGTGCCGACGATTTCG  
GCTCTGGCTCTGGCACCGACTTCACCCCTGAAGATCTCCCGGGTGGAGCCGAG  
GACGTGGCGTGTACTACTGCTCCCAGAGCACCCACGTGCCCTACATTGG  
CGGAGGCACCAAGTGGAAATCAAGCGGACCGTGGCCGCCCCAGCGTGTCA  
TCTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGTG  
CCTGCTGAACAACTTCTACCCCCCGCGAGGCCAAGGGCAGTGGAGGTGGACA  
ACGCCCTGCAGAGCGAACAGCCAGGAGAGCGTACCGAGCAGGACTCAA  
GGACAGCACCTACAGCCTGAGCAGCACCCCTGACCTGAGCAAGGCCACTAC  
GAGAAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGCCC  
CGTGACCAAGAGCTCAACCGGGGCGAGTGCTAA.

**[105]** The one-letter amino acid sequence that corresponds to SEQ ID NO: 37 is  
METDTLLWVLLWVPGSTGDVVMQSPLSLPVTLGQPASISCRSRQSLVNSNGN  
TFLQWYQQRPGQSPRLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY  
YCSQSTHVPPTFGGGTVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREA  
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTTLSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC.

**[106]** The DNA sequence that corresponds to SEQ ID NO: 38 is  
ATGGAGACCGACACCCCTGCTGCTCTGGTGCTGCTGCTCTGGGTGCCCGGCT  
CCACCGGAGACGTCGTGATGACCCAGTCCCTCTGTCCCTGCCTGTGACCCCTG  
GGACAGCCTGCCTCCATCTCCTCAGATCCAGGCAGTCCCTCGTGAACCTCAAC  
GGCAACACCTCCTGCAGTGGTATCAGCAGCGGCCTGCCAGAGCCCCAGAC  
TGCTGATCTACAAGGTGTCCTGCGGTTCTCCGGCGTGCCGACGATTTCG  
GCTCTGGCTCTGGCACCGACTTCACCCCTGAAGATCTCCCGGGTGGAGCCGAG  
GACGTGGCGTGTACTACTGCTCCCAGAGCACCCACGTGCCCTACATTGG  
CGGAGGCACCAAGTGGAAATCAAGCGGACCGTGGCCGCCCCAGCGTGTCA  
TCTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGTG  
CCTGCTGAACAACTTCTACCCCCCGCGAGGCCAAGGGCAGTGGAGGTGGACA  
ACGCCCTGCAGAGCGAACAGCCAGGAGAGCGTACCGAGCAGGACTCAA

GGACAGCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGCAAGGCCGACTAC  
GAGAAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGCCC  
CGTGACCAAGAGCTTCAACCAGGGCGAGTGCTAA.

[107] The one-letter amino acid sequence that corresponds to SEQ ID NO: 39 is  
METDTLLWVLLWVPGSTGDVVMTQSPLSSPVTLGQPASISCRSSQSLVNSNGN  
TFLQWYHQRPGQPPRLLIYKVSLRFSGVPDFSGSGAGKDFTLKISRVEADVGVY  
YCSQSTHVPPTFGQGTLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREA  
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC.

[108] The DNA sequence that corresponds to SEQ ID NO: 40 is  
ATGGAGACCGACACCCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT  
CCACCGGAGACGTCGTGATGACCCAGTCCCTCTGTCCAGTCCTCGTGAACCTCAAC  
GGACAGCCTGCCTCCATCTCCTCAGATCCTCCAGTCCCTCGTGAACCTCAAC  
GGCAACACCTTCCTGCAGTGGTATCACCAGCGGCCTGCCAGCCTCCCAGACT  
GCTGATCTACAAGGTGTCCTGCGGTTCTCCGGCGTCCCCGACGATTTCCGG  
CTCTGGCGCTGGCAAGGACTTCACCCCTGAAGATCTCCGGGTGGAAGCCGAG  
GACGTGGCGTGTACTACTGCTCCCAGAGCACCCACGTCCCCCTACATTGG  
CCAGGGCACCAACTGGAATCAAGCGGACCGTGGCCGCCAGCGTGGTCA  
TCTTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGTG  
CCTGCTGAACAACCTCTACCCCCCGCGAGGCCAAGGGAGTGGAAAGGTGGACA  
ACGCCCTGCAGAGCGGAAACAGCCAGGAGAGCGTGACCGAGCAGGACTCAA  
GGACAGCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGCAAGGCCGACTAC  
GAGAAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGCCC  
CGTGACCAAGAGCTTCAACCAGGGCGAGTGCTAA.

[109] The one-letter amino acid sequence that corresponds to SEQ ID NO: 47 is  
MGWTLVFLFLLSVTAGVHSQVQLLQPGAEVLKPGASVKLACKASGYLFTTYWMHW  
LKQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLTSEASA  
VYYCARSFGNYEFAYWQGTLTVSVASTKGPSVFLAPSSKSTSGGTAALGCLVK  
DYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVN  
HKPSNTKVDKKVEPKSCDKHTCPPCPAPELLGPSVFLFPPKPKDTLMISRTPEVT

CVVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDWL  
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPVYTLPPSRDELTKNQVLTCLVKGF  
YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSV  
MHEALHNHYTQKSLSLSPGK.

[110] The one-letter amino acid sequence that corresponds to SEQ ID NO: 48 is  
MGWTLVFLFLLSVTAGVHSEVQLLESGAEAKPGASVKLSCKASGYLFTTYWMHW  
VHQAPGQRLEWMGEISPTNGRAYYNARFKSRVTITVDKSASTAYMELSSLRSEDT  
AVYYCARSFGNYEFAYWQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV  
KDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTPSSSLGTQTYICNV  
NHKPSNTKVDKKVEPKSCDKTHCPCPAPELLGPSVFLPPKPKDTLMISRTPEV  
TCVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDW  
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPVYTLPPSRDELTKNQVLTCLVKG  
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS  
VMHEALHNHYTQKSLSLSPGK.

[111] The one-letter amino acid sequence that corresponds to SEQ ID NO: 49 is  
MGWTLVFLFLLSVTAGVHSQVQLVQSGAEVKKPGASVKVSCKASGYLFTTYWMH  
WVRQAPGQRLEWIGEISPTNGRAYYNARFKSRVTITRDTASTAYMELSSLRSEDT  
AVYYCARSFGNYEFAYWQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV  
KDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTPSSSLGTQTYICNV  
NHKPSNTKVDKKVEPKSCDKTHCPCPAPELLGPSVFLPPKPKDTLMISRTPEV  
TCVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDW  
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPVYTLPPSRDELTKNQVLTCLVKG  
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS  
VMHEALHNHYTQKSLSLSPGK.

[112] The one-letter amino acid sequence that corresponds to SEQ ID NO: 50 is  
MGWTLVFLFLLSVTAGVHSQVQLVQSGAEVKKPGSSVKVSCKASGYLFTTYWMH  
WVRQAPGQGLEWMGEISPTNGRAYYNARFKSRVTITADKSTSTAYMELSSLRSED  
TAVYYCARSFGNYEFAYWQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
VKDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTPSSSLGTQTYICNV  
VNHKPSNTKVDKKVEPKSCDKTHCPCPAPELLGPSVFLPPKPKDTLMISRTPE

VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIASKAKGQPREPQVYTLPPSRDELTKNQVLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCCSVMEALHNHYTQKSLSLSPGK.

[113] The one-letter amino acid sequence that corresponds to SEQ ID NO: 51 is MGWTLVFLFLLSVTAGVHSQVQLVQSGAEVKKPGASVKVSCEASGYLFTTYWMHWVRQAPGQGLEWMGEISPTNGRAYYNARFKSRVTITRDTINTAYMELSRLRSDDTAVYYCARSFGNYEFAYWQGTLTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPCPAPELLGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIASKAKGQPREPQVYTLPPSRDELTKNQVLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCCSVMEALHNHYTQKSLSLSPGK.

[114] The one-letter amino acid sequence that corresponds to SEQ ID NO: 52 is QVQLLQPGAEVLKPGASVKLACKASGYLFTTYWMHWLQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLTSEASAVYYCARSFGNYEFAYWGQGTLTVSV.

[115] The one-letter amino acid sequence that corresponds to SEQ ID NO: 53 is EVQLLESGAEAKKPGASVKLSCKASGYLFTTYWMHWVHQAPGQRLEWMGEISPTNGRAYYNARFKSRVTITVDKSASTAYMELSSLRSEDTAVYYCARSFGNYEFAYWGQGTLTVSS.

[116] The one-letter amino acid sequence that corresponds to SEQ ID NO: 54 is QVQLVQSGAEVKKPGASVKVSCKASGYLFTTYWMHWVRQAPGQRLEWIGEISPTNGRAYYNARFKSRVTITRDTASTAYMELSSLRSEDTAVYYCARSFGNYEFAYWGQGTLTVSS.

[117] The one-letter amino acid sequence that corresponds to SEQ ID NO: 55 is QVQLVQSGAEVKKPGSSVKVSCKASGYLFTTYWMHWVRQAPGQGLEWMGEISPT

TNGRAYYNARFKSRVTITADKSTSTAYMELSSLRSEDTAVYYCARSFGNYEFAYW  
GQGTLTVSS.

[118] The one-letter amino acid sequence that corresponds to SEQ ID NO: 56 is  
QVQLVQSGAEVKPGASVKVSCEASGYLFTTYWMHWVRQAPGQGLEWMGEISP  
TNGRAYYNARFKSRVTITRDTINTAYMELSRLRSDDTAVYYCARSFGNYEFAYWG  
QGTLTVSS.

[119] The one-letter amino acid sequence that corresponds to SEQ ID NO: 57 is  
MVSSAQFLGLLLLCFQGTRCDVVMTQTPLSLPVSLGDQASISCRSRQSLVNSNGNT  
FLQWYLQKPGQSPKLLIYKVSLRFSGVPDFSGSGSGTDFTLKISRVEAEDLGLYF  
CSQSTHVPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREA  
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTTLSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC.

[120] The one-letter amino acid sequence that corresponds to SEQ ID NO: 58 is  
MVSSAQFLGLLLLCFQGTRCDIVMTQTPLSLPVTLGQPASISCRSRQSLVNSNGNT  
FLQWLQQRPGQPPRLLIYKVSLRFSGVPDFSGSGAGTDFTLTISRVEAEDVGIYF  
CSQSTHVPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREA  
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTTLSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC.

[121] The one-letter amino acid sequence that corresponds to SEQ ID NO: 59 is  
MVSSAQFLGLLLLCFQGTRCDIVMTQTPLSLSVTPGQPASISCRSRQSLVNSNGNT  
FLQWYLQKPGQSPQLLIYKVSLRFSGVPDFSGSGSGTDFTLKISRVEPEDVGVYY  
CSQSTHVPPTFGGGTKVEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPRE  
AKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTTLSKADYEKHKVYACEVTH  
QGLSSPVTKSFNRGEC.

[122] The one-letter amino acid sequence that corresponds to SEQ ID NO: 60 is  
MVSSAQFLGLLLLCFQGTRCDVVMTQSPLSLPVTLGQPASISCRSRQSLVNSNGNT  
FLQWFQQRPGQSPRRIYKVSLRFSGVPDFSGSGSDTDFTLRISRVEAEDVGLYY  
CSQSTHVPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREA

KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTTLSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC.

[123] The one-letter amino acid sequence that corresponds to SEQ ID NO: 61 is  
MVSSAQFLGLLLLCFQGTRCDIVMTQTPLSLVTPGQPASISCRSRQSLVNSNGNT  
FLQWLLQKPGQPPQQLIYKVSLRFSGVNPNRFSGSGSGTDFTLKISRVEAEDVGLYY  
CSQSTHVPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCNNNFYPREA  
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTTLSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGEC.

[124] The one-letter amino acid sequence that corresponds to SEQ ID NO: 62 is  
DVVMTQTPLSLPVSLGQDQASISCRSRQSLVNSNGNTFLQWYLQKPGQSPKLLIYKV  
SLRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYFCSQSTHVPPTFGGGTKLEIK.

[125] The one-letter amino acid sequence that corresponds to SEQ ID NO: 63 is  
DIVMTQTPLSLPVTLGQPASISCRSRQSLVNSNGNTFLQWYLQKPGQPPRLLIYKV  
SLRFSGVPDRFSGSGAGTDFTLTISRVEAEDVGIYFCSQSTHVPPTFGQGTKVEIK.

[126] The one-letter amino acid sequence that corresponds to SEQ ID NO: 64 is  
DIVMTQTPLSLSVTPGQPASISCRSRQSLVNSNGNTFLQWYLQKPGQSPQQLIYKV  
SLRFSGVPDRFSGSGSGTDFTLKISRVEPEDVGVYYCSQSTHVPPTFGGGTKVEV  
K.

[127] The one-letter amino acid sequence that corresponds to SEQ ID NO: 65 is  
DVVMTQSPLSLPVTLGQPASISCRSRQSLVNSNGNTFLQWFQQRPGQSPRRLIYKV  
VSLRFSGVPDRFSGSGSDFTLRISRVEAEDVGLYYCSQSTHVPPTFGQGTKLEI  
K.

[128] The one-letter amino acid sequence that corresponds to SEQ ID NO: 66 is  
DIVMTQTPLSLSVTPGQPASISCRSRQSLVNSNGNTFLQWLLQKPGQPPQQLIYKV  
SLRFSGVPNRFSFGSGSGTDFTLKISRVEAEDVGLYYCSQSTHVPPTFGGGTKVEIK.

[129] **EXAMPLES**

[130] Example 1: *In vivo* study of the administration of anti-glycation end-product antibody. This example show that the anti-AGE antibody can target cell have AGE-modified proteins on the cell surface. Although the cells considered in this study are senescent cells, they may be considered a model of metastasizing cancer cells.

[131] To examine the effects of an anti-glycation end-product antibody, the antibody was administered to the aged CD1(ICR) mouse (Charles River Laboratories), twice daily by intravenous injection, once a week, for three weeks (Days 1, 8 and 15), followed by a 10 week treatment-free period. The test antibody was a commercially available mouse anti-glycation end-product antibody raised against carboxymethyl lysine conjugated with keyhole limpet hemocyanin, the carboxymethyl lysine MAb (Clone 318003) available from R&D Systems, Inc. (Minneapolis, MN; catalog no. MAB3247). A control reference of physiological saline was used in the control animals.

[132] Mice referred to as "young" were 8 weeks old, while mice referred to as "old" were 88 weeks ( $\pm 2$  days) old. No adverse events were noted from the administration of the antibody. The different groups of animals used in the study are shown in Table 1.

[133]

Table 1: The different groups of animals used in the study

Group No.	Test Material	Mice	Dose Level (µg/gm/BID/ week)	Number of Animals	
				Main Study	Treatment-Free
				Females	Females
1	Saline	young	0	20	-
2	Saline	old	0	20	20
3	Antibody	old	2.5	20	20
4	None	old	0	20	pre
5	Antibody	old	5.0	20	20

- = Not Applicable, Pre = Subset of animals euthanized prior to treatment start for collection of adipose tissue.

[134]

$P16^{INK4a}$  mRNA, a marker for senescent cells, was quantified in adipose tissue of the groups by Real Time-qPCR. The results are shown in Table 2. In the table  $\Delta\Delta Ct = \Delta Ct$  mean control Group (2) –  $\Delta Ct$  mean experimental Group (1 or 3 or 5); Fold Expression =  $2^{-\Delta\Delta Ct}$ .

[135]

Table 2:  $P16^{INK4a}$  mRNA quantified in adipose tissue

Calculation (unadjusted to Group 4: 5.59)	Group 2 vs Group 1		Group 2 vs Group 3		Group 2 vs Group 5	
	Group 2	Group 1	Group 2	Group 3	Group 2	Group 5
Mean $\Delta Ct$	5.79	7.14	5.79	6.09	5.79	7.39
$\Delta\Delta Ct$	-1.35		-0.30		-1.60	
Fold Expression	<b>2.55</b>		<b>1.23</b>		<b>3.03</b>	

[136]

The table above indicates that untreated old mice (Control Group 2) express 2.55-fold more  $p16^{INK4a}$  mRNA than the untreated young mice (Control Group 1), as expected. This was observed when comparing Group 2 untreated old mice euthanized at end of recovery Day 85 to Group 1 untreated young mice euthanized at end of treatment Day 22. When results from Group 2 untreated old mice were compared to results from Group 3 treated old mice euthanized Day 85, it was observed that  $p16^{INK4a}$  mRNA was 1.23-fold higher in Group 2 than in Group 3. Therefore, the level of  $p16^{INK4a}$  mRNA expression was lower when the old mice were treated with 2.5 µg/gram/BID/week of antibody.

[137] When results from Group 2 (Control) untreated old mice were compared to results from Group 5 (5  $\mu$ g/gram) treated old mice euthanized Day 22, it was observed that p16<sup>Ink4a</sup> mRNA was 3.03-fold higher in Group 2 (controls) than in Group 5 (5  $\mu$ g/gram). This comparison indicated that the Group 5 animals had lower levels of p16<sup>Ink4a</sup> mRNA expression when they were treated with 5.0  $\mu$ g/gram/BID/week, providing p16<sup>Ink4a</sup> mRNA expression levels comparable to that of the young untreated mice (i.e. Group 1). Unlike Group 3 (2.5  $\mu$ g/gram) mice that were euthanized at end of recovery Day 85, Group 5 mice were euthanized at end of treatment Day 22.

[138] These results indicate the antibody administration resulted in the killing of senescent cells.

[139] The mass of the gastrocnemius muscle was also measured, to determine the effect of antibody administration on sarcopenia. The results are provided in Table 3. The results indicate that administration of the antibody increased muscle mass as compared to controls, but only at the higher dosage of 5.0  $\mu$ g/gm/BID/ week.

[140] Table 3: Effect of antibody administration on mass of the gastrocnemius muscle

Group	Summary Information	Absolute weight of Gastrocnemius Muscle	Weight relative to body mass of Gastrocnemius Muscle
1	Mean	0.3291	1.1037
	SD	0.0412	0.1473
	N	20	20
2	Mean	0.3304	0.7671
	SD	0.0371	0.1246
	N	20	20
3	Mean	0.3410	0.7706
	SD	0.0439	0.0971
	N	19	19

5	Mean	0.4074	0.9480
	SD	0.0508	0.2049
	N	9	9

[141] These results demonstrate that administration of antibodies that bind to AGEs of a cell resulted in a reduction of cells expressing p16<sup>Ink4a</sup>, a biomarker of senescence. The data show that reducing senescent cells leads directly to an increase in muscle mass in aged mice. These results indicate that the loss of muscle mass, a classic sign of sarcopenia, can be treated by administration of antibodies that bind to AGEs of a cell. The results suggest that administration of the antibodies would be effective in treating cancer metastases by removing senescent cells.

[142] Example 2: Affinity and kinetics of test antibody

[143] The affinity and kinetics of the test antibody used in Example 1 were analyzed using Na<sub>2</sub>Na-bis(carboxymethyl)-L-lysine trifluoroacetate salt (Sigma-Aldrich, St. Louis, MO) as a model substrate for an AGE-modified protein of a cell. Label-free interaction analysis was carried out on a BIACORE™ T200 (GE Healthcare, Pittsburgh, PA), using a Series S sensor chip CM5 (GE Healthcare, Pittsburgh, PA), with Fc1 set as blank, and Fc2 immobilized with the test antibody (molecular weight of 150,000 Da). The running buffer was a HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05% P-20, pH of 7.4), at a temperature of 25 °C. Software was BIACORE™ T200 evaluation software, version 2.0. A double reference (Fc2-1 and only buffer injection), was used in the analysis, and the data was fitted to a Langmuir 1:1 binding model.

[144] Table 4: Experimental set-up of affinity and kinetics analysis

Association and dissociation	
Flow path	Fc1 and Fc2

Flow rate ( $\mu$ l/min.)	30
Association time (s)	300
Dissociation time (s)	300
Sample concentration ( $\mu$ M)	20 – 5 – 1.25 (x2) – 0.3125 – 0.078 - 0

[145] A graph of the response versus time is illustrated in FIG. 1. The following values were determined from the analysis:  $k_a$  (1/Ms) =  $1.857 \times 10^3$ ;  $k_d$  (1/s) =  $6.781 \times 10^{-3}$ ;  $K_D$  (M) =  $3.651 \times 10^{-6}$ ;  $R_{max}$  (RU) = 19.52; and  $\text{Chi}^2$  = 0.114. Because the  $\text{Chi}^2$  value of the fitting is less than 10% of  $R_{max}$ , the fit is reliable.

[146] Example 3: Construction and production of murine anti-AGE IgG2b antibody and chimeric anti-AGE IgG1 antibody

[147] Murine and chimeric human anti-AGE antibodies were prepared. The DNA sequence of murine anti-AGE antibody IgG2b heavy chain is shown in SEQ ID NO: 12. The DNA sequence of chimeric human anti-AGE antibody IgG1 heavy chain is shown in SEQ ID NO: 13. The DNA sequence of murine anti-AGE antibody kappa light chain is shown in SEQ ID NO: 14. The DNA sequence of chimeric human anti-AGE antibody kappa light chain is shown in SEQ ID NO: 15. The gene sequences were synthesized and cloned into high expression mammalian vectors. The sequences were codon optimized. Completed constructs were sequence confirmed before proceeding to transfection.

[148] HEK293 cells were seeded in a shake flask one day before transfection, and were grown using serum-free chemically defined media. The DNA expression constructs were transiently transfected into 0.03 liters of suspension HEK293 cells. After 20 hours, cells were sampled to obtain the viabilities and viable cell counts, and titers were measured (Octet QKe, ForteBio). Additional readings were taken throughout the transient transfection production runs. The cultures were harvested

on day 5, and an additional sample for each was measured for cell density, viability and titer.

**[149]** The conditioned media for murine and chimeric anti-AGE antibodies were harvested and clarified from the transient transfection production runs by centrifugation and filtration. The supernatants were run over a Protein A column and eluted with a low pH buffer. Filtration using a 0.2 µm membrane filter was performed before aliquoting. After purification and filtration, the protein concentrations were calculated from the OD280 and the extinction coefficient. A summary of yields and aliquots is shown in Table 5:

**[150]** Table 5: Yields and Aliquots

Protein	Concentration (mg/mL)	Volume (mL)	No. of vials	Total Yield (mg)
Murine anti-AGE	0.08	1.00	3	0.24
Chimeric anti-AGE	0.23	1.00	3	0.69

**[151]** Antibody purity was evaluated by capillary electrophoresis sodium-dodecyl sulfate (CE-SDS) analysis using LabChip® GXII (PerkinElmer).

**[152]** Example 4: Binding of murine (parental) and chimeric anti-AGE antibodies

**[153]** The binding of the murine (parental) and chimeric anti-AGE antibodies described in Example 3 was investigated by a direct binding ELISA. An anti-carboxymethyl lysine (CML) antibody (R&D Systems, MAB3247) was used as a control. CML was conjugated to KLH (CML-KLH) and both CML and CML-KLH were coated overnight onto an ELISA plate. HRP-goat anti-mouse Fc was used to detect the control and murine (parental) anti-AGE antibodies. HRP-goat anti-human Fc was used to detect the chimeric anti-AGE antibody.

**[154]** The antigens were diluted to 1  $\mu$ g/mL in 1x phosphate buffer at pH 6.5. A 96-well microtiter ELISA plate was coated with 100  $\mu$ L/well of the diluted antigen and let sit at 4°C overnight. The plate was blocked with 1x PBS, 2.5% BSA and allowed to sit for 1-2 hours the next morning at room temperature. The antibody samples were prepared in serial dilutions with 1x PBS, 1% BSA with the starting concentration of 50  $\mu$ g/mL. Secondary antibodies were diluted 1:5,000. 100  $\mu$ L of the antibody dilutions was applied to each well. The plate was incubated at room temperature for 0.5-1 hour on a microplate shaker. The plate was washed 3 times with 1x PBS. 100  $\mu$ L/well diluted HRP-conjugated goat anti-human Fc secondary antibody was applied to the wells. The plate was incubated for 1 hour on a microplate shaker. The plate was then washed 3 times with 1x PBS. 100  $\mu$ L HRP substrate TMB was added to each well to develop the plate. After 3-5 minutes elapsed, the reaction was terminated by adding 100  $\mu$ L of 1N HCl. A second direct binding ELISA was performed with only CML coating. The absorbance at OD450 was read using a microplate reader.

**[155]** The OD450 absorbance raw data for the CML and CML-KLH ELISA is shown in the plate map below. 48 of the 96 wells in the well plate were used. Blank wells in the plate map indicate unused wells.

## [156] Plate map of CML and CML-KLH ELISA:

Conc. (ug/mL)	1	2	3	4	5	6	7
50	0.462	0.092	0.42		1.199	0.142	1.852
16.67	0.312	0.067	0.185		0.31	0.13	0.383
5.56	0.165	0.063	0.123		0.19	0.115	0.425
1.85	0.092	0.063	0.088		0.146	0.099	0.414
0.62	0.083	0.072	0.066		0.108	0.085	0.248
0.21	0.075	0.066	0.09		0.096	0.096	0.12
0.07	0.086	0.086	0.082		0.098	0.096	0.098
0	0.09	0.085	0.12		0.111	0.083	0.582

R&D      Parental      Chimeric
R&D      Parental      Chimeric

Positive      Anti-
Anti-
Anti-

Control      AGE
AGE
AGE

CML-KLH Coat
CML Coat

[157] The OD450 absorbance raw data for the CML-only ELISA is shown in the plate map below. 24 of the 96 wells in the well plate were used. Blank wells in the plate map indicate unused wells.

## [158] Plate map of CML-only ELISA:

Conc. (ug/mL)	1	2	3	4	5	6	7
50	1.913	0.165	0.992				
16.66667	1.113	0.226	0.541				
5.555556	0.549	0.166	0.356				
1.851852	0.199	0.078	0.248				
0.617284	0.128	0.103	0.159				
0.205761	0.116	0.056	0.097				
0.068587	0.073	0.055	0.071				
0	0.053	0.057	0.06				

R&D      Parental      Chimeric

Positive      Anti-
Anti-
Anti-

Control      AGE
AGE
AGE

[159] The control and chimeric anti-AGE antibodies showed binding to both CML and CML-KLH. The murine (parental) anti-AGE antibody showed very weak to no binding to either CML or CML-KLH. Data from repeated ELISA confirms binding of the control and chimeric anti-AGE to CML. All buffer control showed negative signal.

[160] Example 5: Humanized antibodies

[161] Humanized antibodies were designed by creating multiple hybrid sequences that fuse select parts of the parental (mouse) antibody sequence with the human framework sequences. Acceptor frameworks were identified based on the overall sequence identity across the framework, matching interface position, similarly classed CDR canonical positions, and presence of N-glycosylation sites that would have to be removed. Three humanized light chains and three humanized heavy chains were designed based on two different heavy and light chain human acceptor frameworks. The amino acid sequences of the heavy chains are shown in SEQ ID NO: 29, 31 and 33, which are encoded by the DNA sequences shown in SEQ ID NO: 30, 32 and 34, respectively. The amino acid sequences of the light chains are shown in SEQ ID NO: 35, 37 and 39, which are encoded by the DNA sequences shown in SEQ ID NO: 36, 38 and 40, respectively. The humanized sequences were methodically analyzed by eye and computer modeling to isolate the sequences that would most likely retain antigen binding. The goal was to maximize the amount of human sequence in the final humanized antibodies while retaining the original antibody specificity. The light and heavy humanized chains could be combined to create nine variant fully humanized antibodies.

[162] The three heavy chains and three light chains were analyzed to determine their humanness. Antibody humanness scores were calculated according to the method described in Gao, S. H., *et al.*, "Monoclonal antibody humanness score and its applications", BMC Biotechnology, 13:55 (July 5, 2013). The humanness score represents how human-like an antibody variable region sequence looks. For heavy chains a score of 79 or above is indicative of looking human-like; for light chains a score of 86 or above is indicative of looking human-like. The humanness of the

three heavy chains, three light chains, a parental (mouse) heavy chain and a parental (mouse) light chain are shown below in Table 6:

[163] Table 6: Antibody humanness

Antibody	Humanness (Framework + CDR)
Parental (mouse) heavy chain	63.60
Heavy chain 1 (SEQ ID NO: 29)	82.20
Heavy chain 2 (SEQ ID NO: 31)	80.76
Heavy chain 3 (SEQ ID NO: 33)	81.10
Parental (mouse) light chain	77.87
Light chain 1 (SEQ ID NO: 35)	86.74
Light chain 2 (SEQ ID NO: 37)	86.04
Light chain 3 (SEQ IN NO: 39)	83.57

[164] Full-length antibody genes were constructed by first synthesizing the variable region sequences. The sequences were optimized for expression in mammalian cells. These variable region sequences were then cloned into expression vectors that already contain human Fc domains; for the heavy chain, the IgG1 was used.

[165] Small scale production of humanized antibodies was carried out by transfecting plasmids for the heavy and light chains into suspension HEK293 cells using chemically defined media in the absence of serum. Whole antibodies in the conditioned media were purified using MabSelect SuRe Protein A medium (GE Healthcare).

[166] Nine humanized antibodies were produced from each combination of the three heavy chains having the amino acid sequences shown in SEQ ID NO: 29, 31 and 33 and three light chains having the amino acid sequences shown in SEQ ID NO: 35, 37 and 39. A comparative chimeric parental antibody was also prepared. The antibodies and their respective titers are shown below in Table 7:

[167] Table 7: Antibody titers

Antibody	Titer (mg/L)
Chimeric parental	23.00
SEQ ID NO: 29 + SEQ ID NO: 35	24.67
SEQ ID NO: 29 + SEQ ID NO: 37	41.67
SEQ ID NO: 29 + SEQ ID NO: 39	29.67
SEQ ID NO: 31 + SEQ ID NO: 35	26.00
SEQ ID NO: 31 + SEQ ID NO: 37	27.33
SEQ ID NO: 31 + SEQ ID NO: 39	35.33
SEQ ID NO: 33 + SEQ ID NO: 35	44.00
SEQ ID NO: 33 + SEQ ID NO: 37	30.33
SEQ ID NO: 33 + SEQ ID NO: 39	37.33

[168] The binding of the humanized antibodies may be evaluated, for example, by dose-dependent binding ELISA or cell-based binding assay.

[169] Example 6 (prophetic): killing of metastatic cancer cells, and treating metastatic cancer

[170] Aggregates of human ovarian cancer cells (Creative BioArray, Shirley, NY) are inoculated i.p. into two groups (A and B) of T and B cell -deficient prkdcscid (SCID) mice, specifically NSG mice available from Jackson Laboratories (Farmington, CT). Group A is a control group injected intravenously with physiological saline and Group B is injected intravenously with 5  $\mu$ g per gram per mouse of any of the anti-AGE monoclonal antibodies described.

[171] 80 days post inoculation with cancer cells, mice from both Groups A and B undergo gross and histological examination. The antibody-treated group B mice have significantly fewer metastatic foci than Group A control mice.

[172] Example 6: *In vivo* study of the administration of a carboxymethyl lysine monoclonal antibody

[173] The effect of a carboxymethyl lysine antibody on tumor growth, metastatic potential and cachexia was investigated. *In vivo* studies were carried out in mice using a murine breast cancer tumor model. Female BALB/c mice (BALB/cAnNCrl, Charles River) were eleven weeks old on Day 1 of the study.

[174] 4T1 murine breast tumor cells (ATCC CRL-2539) were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 2 mM glutamine, 25  $\mu$ g/mL gentamicin, 100 units/mL penicillin G Na and 100  $\mu$ g/mL streptomycin sulfate. Tumor cells were maintained in tissue culture flasks in a humidified incubator at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95% air.

[175] The cultured breast cancer cells were then implanted in the mice. 4T1 cells were harvested during log phase growth and re-suspended in phosphate buffered saline (PBS) at a concentration of 1 x 10<sup>6</sup> cells/mL on the day of implant. Tumors were initiated by subcutaneously implanting 1 x 10<sup>5</sup> 4T1 cells (0.1 mL suspension) into the right flank of each test animal. Tumors were monitored as their volumes approached a target range of 80–120 mm<sup>3</sup>. Tumor volume was determined using the formula: tumor volume = (tumor width)<sup>2</sup>(tumor length)/2. Tumor weight was approximated using the assumption that 1 mm<sup>3</sup> of tumor volume has a weight of 1 mg. Thirteen days after implantation, designated as Day 1 of the study, mice were

sorted into four groups (n=15/group) with individual tumor volumes ranging from 108 to 126 mm<sup>3</sup> and a group mean tumor volume of 112 mm<sup>3</sup>. The four treatment groups are shown in Table 8 below:

[176] Table 8: Treatment groups

Group	Description	Agent	Dosing (µg/g)
1	Control	phosphate buffered saline (PBS)	N/A
2	Low-dose	carboxymethyl lysine monoclonal antibody	5
3	High-dose	carboxymethyl lysine monoclonal antibody	10
4	Observation only	None	N/A

[177] An anti-carboxymethyl lysine monoclonal antibody was used as a therapeutic agent. 250 mg of carboxymethyl lysine monoclonal antibody was obtained from R&D Systems (Minneapolis, MN). Dosing solutions of the carboxymethyl lysine monoclonal antibody were prepared at 1 and 0.5 mg/mL in a vehicle (PBS) to provide the active dosages of 10 and 5 µg/g, respectively, in a dosing volume of 10 mL/kg. Dosing solutions were stored at 4 °C protected from light.

[178] All treatments were administered intravenously (i.v.) twice daily for 21 days, except on Day 1 of the study where the mice were administered one dose. On Day 19 of the study, i.v. dosing was changed to intraperitoneal (i.p.) dosing for those animals that could not be dosed i.v. due to tail vein degradation. The dosing volume was 0.200 mL per 20 grams of body weight (10 mL/kg), and was scaled to the body weight of each individual animal.

**[179]** The study continued for 23 days. Tumors were measured using calipers twice per week. Animals were weighed daily on Days 1–5, then twice per week until the completion of the study. Mice were also observed for any side effects. Acceptable toxicity was defined as a group mean body weight loss of less than 20% during the study and not more than 10% treatment-related deaths. Treatment efficacy was determined using data from the final day of the study (Day 23).

**[180]** The ability of the anti-carboxymethyl lysine antibody to inhibit tumor growth was determined by comparing the median tumor volume (MTV) for Groups 1-3. Tumor volume was measured as described above. Percent tumor growth inhibition (%TGI) was defined as the difference between the MTV of the control group (Group 1) and the MTV of the drug-treated group, expressed as a percentage of the MTV of the control group. %TGI may be calculated according to the formula: %TGI = (1- $MTV_{treated}/MTV_{control}$ ) x 100. FIG. 3 illustrates a graph of the normalized tumor volume over the course of an *in vivo* study investigating the effect of an anti-AGE antibody on tumor growth, metastatic potential and cachexia.

**[181]** The ability of the anti-carboxymethyl lysine antibody to inhibit cancer metastasis was determined by comparing lung cancer foci for Groups 1-3. Percent inhibition (%Inhibition) was defined as the difference between the mean count of metastatic foci of the control group and the mean count of metastatic foci of a drug-treated group, expressed as a percentage of the mean count of metastatic foci of the control group. %Inhibition may be calculated according to the following formula: %Inhibition = (1-Mean Count of Foci<sub>treated</sub>/Mean Count of Foci<sub>control</sub>) x 100.

**[182]** The ability of the anti-carboxymethyl lysine antibody to inhibit cachexia was determined by comparing the weights of the lungs and gastrocnemius muscles for Groups 1-3. Tissue weights were also normalized to 100 g body weight. FIG. 4 illustrates a graph of the normalized body weight of the mice over the course of an *in vivo* study investigating the effect of an anti-AGE antibody on tumor growth, metastatic potential and cachexia.

[183] Treatment efficacy was also evaluated by the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume was 50% or less of its Day 1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm<sup>3</sup> for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 mm<sup>3</sup> for three consecutive measurements during the course of the study.

[184] Statistical analysis was carried out using Prism (GraphPad) for Windows 6.07. Statistical analyses of the differences between Day 23 mean tumor volumes (MTVs) of two groups were accomplished using the Mann-Whitney *U* test. Comparisons of metastatic foci were assessed by ANOVA-Dunnett. Normalized tissue weights were compared by ANOVA. Two-tailed statistical analyses were conducted at significance level *P* = 0.05. Results were classified as statistically significant or not statistically significant.

[185] The results of the study are shown below in Table 9:

[186] Table 9: Results

Group	MTV (mm <sup>3</sup> )	%TGI	Lung foci	%Inhibition	PR	CR	Gastroc. weight/ normalized (mg)	Lung weight/ normalized (mg)
1	1800	N/A	70.4	N/A	0	0	353.4/19.68	2799.4/292.98
2	1568	13%	60.3	14%	0	0	330.4/21.62	2388.9/179.75
3	1688	6%	49.0	30%	0	0	398.6/24.91	2191.6/214.90

[187] All treatment regimens were acceptably tolerated with no treatment-related deaths. The only animal deaths were non-treatment-related deaths due to metastasis. The %TGI trended towards significance ( $P > 0.05$ , Mann-Whitney) for the 5  $\mu\text{g/g}$  (Group 2) and 10  $\mu\text{g/g}$  treatment group (Group 3). The %Inhibition trended towards significance ( $P > 0.05$ , ANOVA-Dunnett) for the 5  $\mu\text{g/g}$  treatment group. The %Inhibition was statistically significant ( $P \leq 0.01$ , ANOVA-Dunnett) for the 10  $\mu\text{g/g}$  treatment group. The ability of the carboxymethyl lysine antibody to treat cachexia trended towards significance ( $P > 0.05$ , ANOVA) based on a comparison of the organ weights of the lung and gastrocnemius between treatment groups and the control group. The results indicate that administration of an anti-carboxymethyl lysine monoclonal antibody is able to reduce cancer metastases.

[188] Example 7: Diagnosis of metastatic cancer (prophetic)

[189] A patient with breast cancer exhibits enlarged lymph nodes. An oncologist suspects that the breast cancer has metastasized to her lymph nodes. The oncologist obtains a blood sample as well as a biopsy from one of her enlarged lymph nodes. Cells from the blood sample and the biopsy are tested for the presence of AGE-modified cancer cells using a kit containing a fluorescent-labelled anti-AGE antibody and a control. The diagnostic test indicates the presence of circulating AGE-modified cancer cells in the patient's blood as well as the presence of metastatic breast cancer cells in the lymph nodes. A second staining of the cells for cell-surface nucleolin, a well-known cancer marker, as described in U.S. Pat. No. 7,541,150 to Miller *et al.* confirms the presence of cancerous cells.

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**WHAT IS CLAIMED IS:**

1. A method of treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis, comprising administering to a subject a composition comprising an anti-AGE antibody.
  
2. A method of treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis, comprising administering a composition comprising a first anti-AGE antibody and a second anti-AGE antibody;  
wherein the second anti-AGE antibody is different from the first anti-AGE antibody.
  
3. A method of treating a subject with cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis, comprising:  
a first administering of an anti-AGE antibody; followed by  
testing the subject for effectiveness of the first administration at treating the cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis; followed by  
a second administering of the anti-AGE antibody.
  
4. Use of an anti-AGE antibody for the manufacture of a medicament for treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis.
  
5. A composition comprising an anti-AGE antibody for use in treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis.

6. A composition for treating cancer, killing metastatic cancer cells, killing potentially-malignant neoplasm cells and/or preventing cancer metastasis, comprising

- (a) a first anti-AGE antibody,
- (b) a second anti-AGE antibody, and
- (c) a pharmaceutically acceptable carrier,

wherein the first anti-AGE antibody is different from the second anti-AGE antibody.

7. A method of diagnosing metastatic cancer, comprising detecting an immune complex comprising an anti-AGE antibody bound to a cell expressing an AGE modification.

8. An immune complex, comprising an anti-AGE antibody bound to a metastatic cancer cell,

wherein the metastatic cancer cell expresses an AGE modification.

9. A kit for diagnosing metastatic cancer, comprising  
an anti-AGE antibody,  
a control sample, and  
optionally, a reagent that binds to the anti-AGE antibody.

10. The method, use or composition of any of the preceding claims, wherein the composition further comprises a pharmaceutically acceptable carrier.

11. The method of any of the preceding claims, wherein the subject is selected from the group consisting of humans, mice, rats, goats, sheep, cows, horses, dogs and cats.

12. The method of any of the preceding claims, wherein the subject is a human.

13. The method, use or composition of any of the preceding claims, wherein the anti-AGE antibody is non-immunogenic to a species selected from the group consisting of humans, cats, dogs, horses, camels, alpaca, cattle, sheep, and goats

14. The method of any of the preceding claims, wherein the subject has metastatic cancer.

15. The method of any of the preceding claims, wherein the subject does not have metastatic cancer.

16. The method, use or composition of any of the preceding claims, wherein the anti-AGE antibody binds an AGE antigen comprises at least one protein or peptide that exhibits AGE modifications selected from the group consisting of FFI, pyrraline, AFGP, ALI, carboxymethyllysine, carboxyethyllysine and pentosidine.

17. The method, use or composition of any of the preceding claims, wherein the anti-AGE antibody binds a carboxymethyllysine-modified protein.

18. The method, use or composition of any of the preceding claims, wherein the anti-AGE antibody binds a carboxyethyllysine-modified protein.

19. The method or composition of any of the preceding claims, wherein the first anti-AGE antibody and the second anti-AGE antibody bind AGE antigens comprising at least one protein or peptide that exhibit different AGE modifications selected from the group consisting of FFI, pyrraline, AFGP, ALI, carboxymethyllysine, carboxyethyllysine and pentosidine.

20. The method or composition of any of the preceding claims, wherein the composition is in unit dosage form.

21. The method or composition of any of the preceding claims, wherein the composition is in multidosage form

22. The method or composition of any of the preceding claims, wherein the composition is sterile.

23. The method, use or composition of any of the previous claims, wherein the anti-AGE antibody comprises a protein or peptide comprising at least one amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, and SEQ ID NO: 39.

24. The method, use or composition of any of the previous claims, wherein the antibody comprises a protein or peptide comprising at least one amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group consisting of SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28.

25. The method, use or composition of any of the previous claims, wherein the antibody comprises

a heavy chain, and

a light chain,

wherein the heavy chain comprises an amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 17, SEQ ID NO: 29, SEQ ID NO: 31, and SEQ ID NO: 33, or

the light chain comprises an amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group

consisting of SEQ ID NO: 3, SEQ ID NO: 19, SEQ ID NO: 35, SEQ ID NO: 37, and SEQ ID NO: 39.

26. The method, use or composition of any of the previous claims, wherein the antibody comprises

a heavy chain, and

a light chain,

wherein the heavy chain comprises an amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 17, SEQ ID NO: 29, SEQ ID NO: 31, and SEQ ID NO: 33, and

the light chain comprises an amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 19, SEQ ID NO: 35, SEQ ID NO: 37, and SEQ ID NO: 39.

27. The method, use or composition of any of the previous claims, wherein the antibody comprises complementary determining region comprising at least one amino acid sequence having at least 90% sequence identity, preferably at least 95% sequence identity, more preferably at least 98% sequence identity, with an amino acid sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28, and

the antibody is substantially non-immunogenic to a species selected from the group consisting of mice, rats, goats, sheep, cows, horses, dogs and cats.

28. The kit of any of the preceding claims, further comprising a reagent that binds to the anti-AGE antibody.

29. The kit of any of the preceding claims, wherein the reagent comprises a secondary antibody.

30. The kit of any of the preceding claims, wherein the anti-AGE antibody, the control sample, and the reagent are sterile.

31. The kit of any of the preceding claims, further comprising a container, wherein the anti-AGE antibody, the control sample, and the reagent are in the container.

32. The kit of any of the preceding claims, wherein the anti-AGE antibody and/or the reagent are labelled.

33. The kit of any of the preceding claims, wherein the label comprises at least one label selected from the group consisting of a fluorescent label, a radiolabel, and a gold particle.

34. The method or immune complex of any of the preceding claims, wherein the cell expressing an AGE modification is a metastatic cancer cell.

35. The method or immune complex of any of the preceding claims, wherein the cell expressing an AGE modification is a circulating cell.

36. The method or immune complex of any of the preceding claims, wherein the cell expressing an AGE modification is obtained from a part of the body selected from the group consisting of the lymph nodes, the lungs, the liver, the brain or the bones.

37. The method of any of the preceding claims, wherein the subject is pregnant.

38. The method of any of the preceding claims, wherein the subject has been previously diagnosed with cancer cachexia.

39. The method of any of the preceding claims, wherein the subject has a compromised immune system.

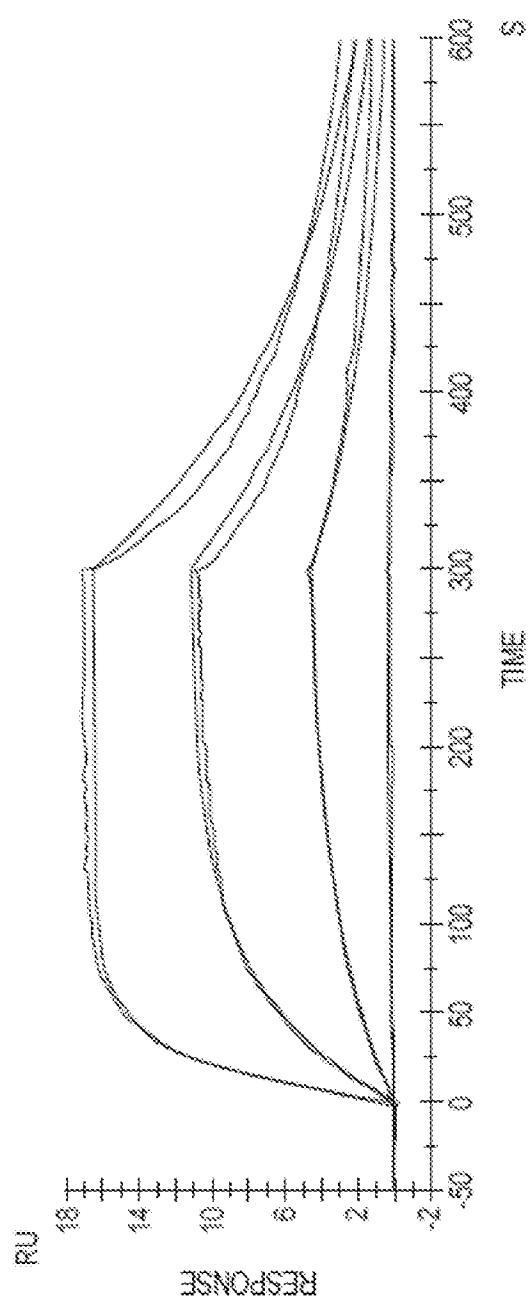


FIG. 1

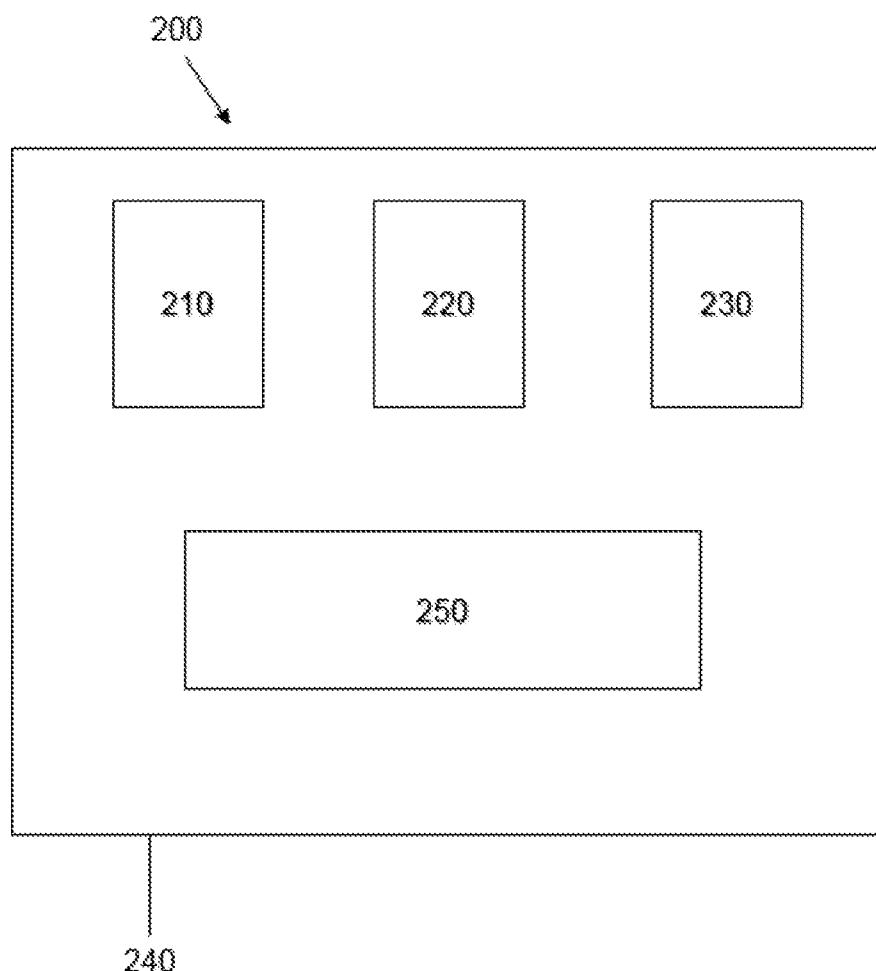


FIG. 2

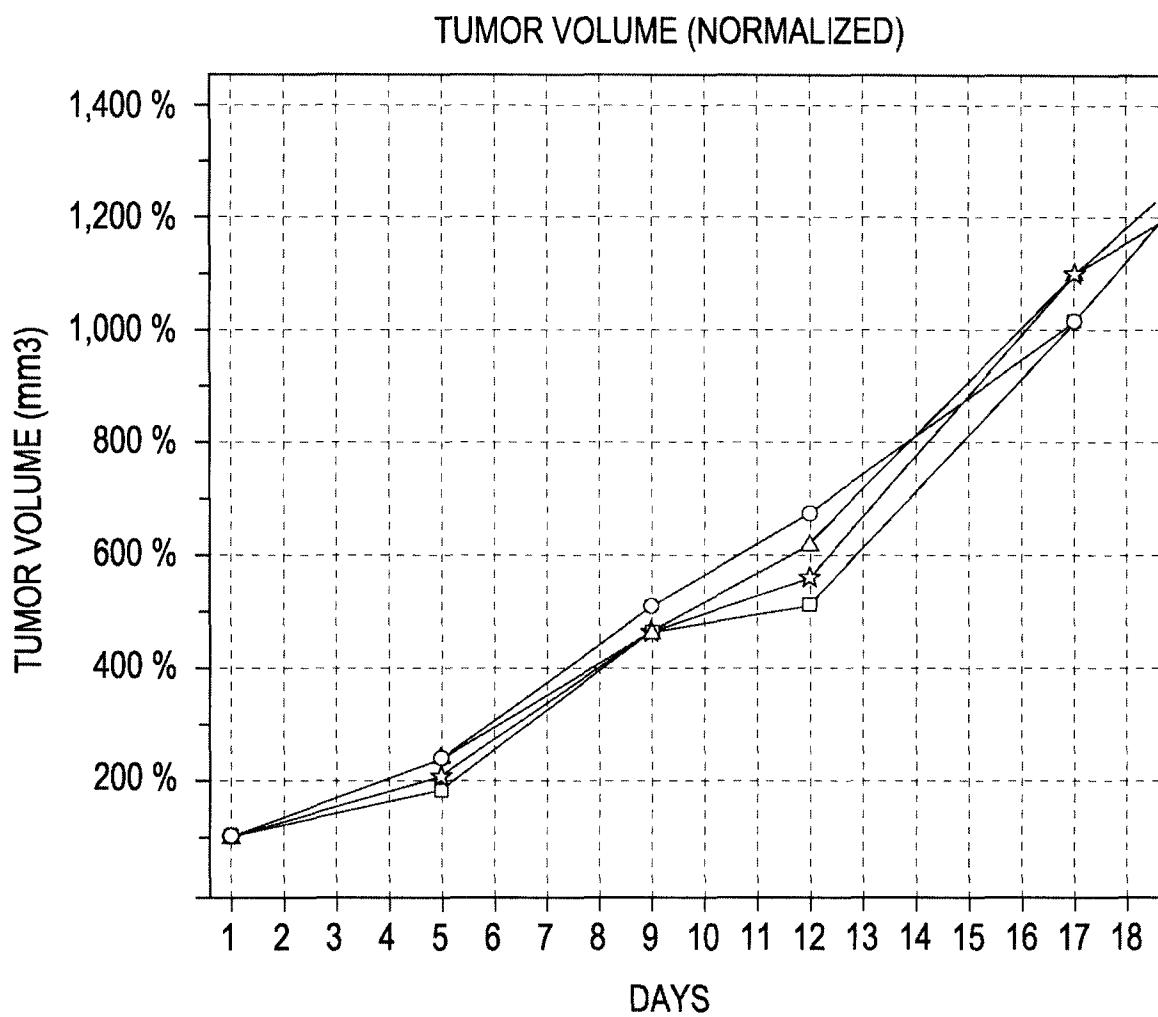


FIG. 3

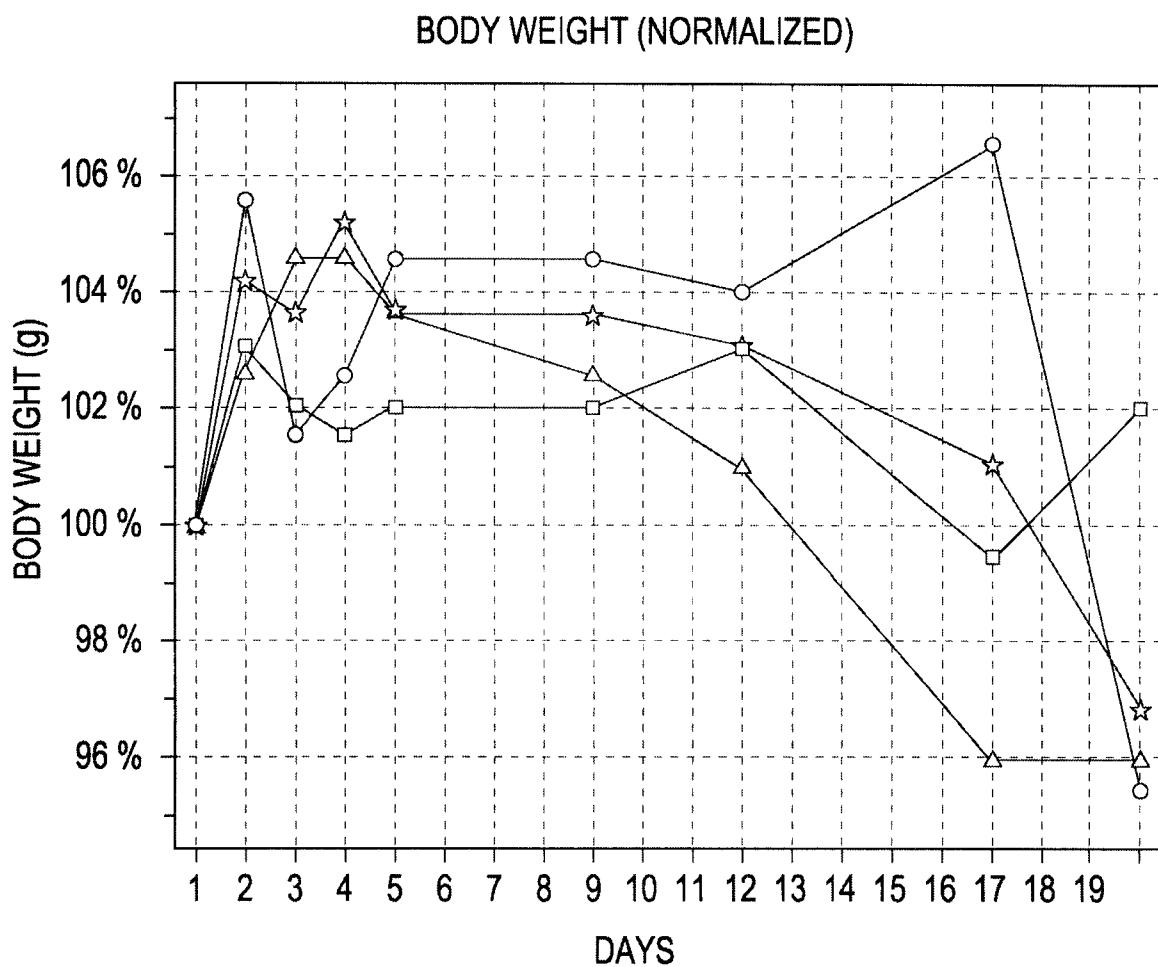


FIG. 4

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2017/018185

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C07K16/44 A61K39/395  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>VAN HEIJST JEROEN W J ET AL: "Advanced glycation end products in human cancer tissues: detection of Nepsilon-(carboxymethyl)lysine and argypyrimidine.", June 2005 (2005-06), ANNALS OF THE NEW YORK ACADEMY OF SCIENCES JUN 2005, VOL. 1043, PAGE(S) 725 - 733, XP008184426, ISSN: 0077-8923 the whole document</p> <p>-----</p> <p style="text-align: center;">-/-</p>	1-39

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
10 May 2017	17/05/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Hix, Rebecca

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International application No
PCT/US2017/018185

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