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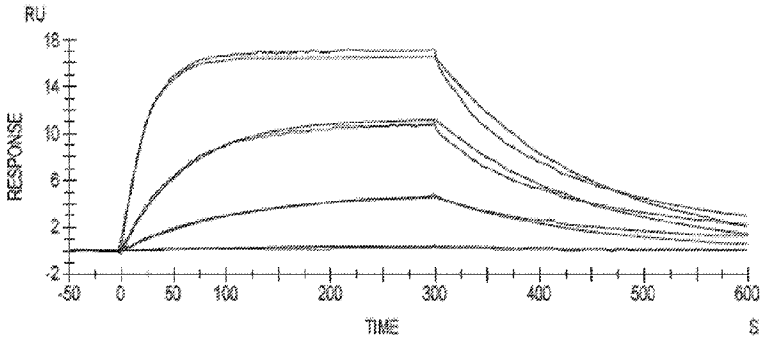
(54) Titre : PROCEDE ET COMPOSITION POUR TRAITER LE CANCER, DETRUIRE LES CELLULES CANCEREUSES METASTATIQUES ET PREVENIR LA METASTASE CANCEREUSE EN UTILISANT DES ANTICORPS CONTRE LES PRODUITS TERMINAUX DE GLYCATION AVANCEE (AGE)

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



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

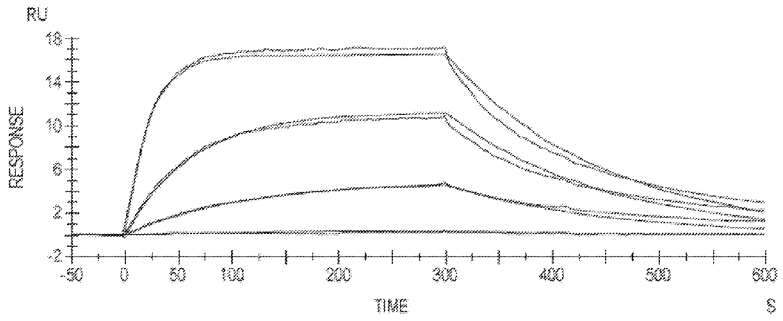

FIG. 1

(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.

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 $p16^{Ink4a}$ ("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.*, $p16^{Ink4a}$ 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 associate 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^E-(Carboxymehtyl)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 $p16^{Ink4a}$ or expression of senescence-associated β-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 speared 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 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), felinized (for cats), caninized (for dogs), equuinized (for horses), camelized (for camels or alpaca), bovinized (for cattle), ovinized (for sheep), or caperized (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) as well as CEL-BSA antigens and CEL competitive ELISA kits (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 9×10^{-3}, 8×10^{-3}, 7×10^{-3} or 6×10^{-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 9×10^{-6}, 8×10^{-6}, 7×10^{-6}, 6×10^{-6}, 5×10^{-6}, 4×10^{-6} or 3×10^{-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), the European Bioinformatics Institute (www.ebi.ac.uk), the DNA Databank of Japan (ddbj.nig.ac.jp/arsa) or the National Center for Biotechnology Information (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 of 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 $F(ab')_2$ fragments. $F(ab')_2$ 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 (C_H3) 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^+/K^+-ATPase, AGE-plasminogen, AGE-myelin, AGE-lysozyme, AGE-immunoglobulin, AGE-red cell Glu transport protein, AGE-β-N-acetyl hexominase, AGE-apo E, AGE-red cell membrane protein, AGE-aldose reductase, AGE-ferritin, AGE-red cell spectrin, AGE-alcohol dehydrogenase, AGE-haptoglobin, AGE-tubulin, AGE-thyroid hormone, AGE-fibrinogen, AGE-β_2-microglobulin, AGE-sorbitol dehydrogenase, AGE-α_1-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, α2-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 and 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
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 HYTQKSLSLS 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 AAAVADVVMT 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:

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGC
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGCTGCAGCCAGGTGCCGAGCTC
GTGAAACCTGGCGCCTCTGTGAAGCTGGCCTGCAAGGCTTCCGGCTACCTGTT
CACCACCTACTGGATGCACTGGCTGAAGCAGAGGCCAGGCCAGGGCCTGGAA
TGGATCGGCGAGATCTCCCCCACCAACGGCAGAGCCTACTACAACGCCCGGTT
CAAGTCCGAGGCCACCCTGACCGTGGACAAGTCCTCCAACACCGCCTACATGC
AGCTGTCCTCCCTGACCTCTGAGGCCTCCGCCGTGTACTACTGCGCCAGAGCT
TACGGCAACTACGAGTTCGCCTACTGGGGCCAGGGCACCCTCGTGACAGTGTC
TGTGGCTAAGACCACCCCTCCCTCCGTGTACCCTCTGGCTCCTGGCTGTGGCG
ACACCACCGGATCCTCTGTGACCCTGGGCTGCCTCGTGAAGGGCTACTTCCCT
GAGTCCGTGACCGTGACCTGGAACTCCGGCTCCCTGTCCTCCTCCGTGCACAC
CTTTCCAGCCCTGCTGCAGTCCGGCCTGTACACCATGTCCTCCAGCGTGACAG
TGCCCTCCTCCACCTGGCCTTCCCAGACCGTGACATGCTCTGTGGCCCACCCT
GCCTCTTCCACCACCGTGGACAAGAAGCTGGAACCCTCCGGCCCCATCTCCAC
CATCAACCCTTGCCCTCCCTGCAAAGAATGCCACAAGTGCCCTGCCCCCAACC

TGGAAGGCGGCCCTTCCGTGTTCATCTTCCCACCCAACATCAAGGACGTGCTG
ATGATCTCCCTGACCCCCAAAGTGACCTGCGTGGTGGTGGACGTGTCCGAGGA
CGACCCTGACGTGCAGATCAGTTGGTTCGTGAACAACGTGGAAGTGCACACCG
CCCAGACCCAGACACACAGAGAGGACTACAACAGCACCATCAGAGTGGTGTCT
ACCCTGCCCATCCAGCACCAGGACTGGATGTCCGGCAAAGAATTCAAGTGCAA
AGTGAACAACAAGGACCTGCCCAGCCCCATCGAGCGGACCATCTCCAAGATCA
AGGGCCTCGTGCGGGCTCCCCAGGTGTACATTCTGCCTCCACCAGCCGAGCA
GCTGTCCCGGAAGGATGTGTCTCTGACATGTCTGGTCGTGGGCTTCAACCCCG
GCGACATCTCCGTGGAATGGACCTCCAACGGCCACACCGAGGAAAACTACAAG
GACACCGCCCCTGTGCTGGACTCCGACGGCTCCTACTTCATCTACTCCAAGCT
GAACATGAAGACCTCCAAGTGGGAAAAGACCGACTCCTTCTCCTGCAACGTGC
GGCACGAGGGCCTGAAGAACTACTACCTGAAGAAAACCATCTCCCGGTCCCCC
GGCTAG

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

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGC
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGCTGCAGCCAGGTGCCGAGCTC
GTGAAACCTGGCGCCTCTGTGAAGCTGGCCTGCAAGGCTTCCGGCTACCTGTT
CACCACCTACTGGATGCACTGGCTGAAGCAGAGGCCAGGCCAGGGCCTGGAA
TGGATCGGCGAGATCTCCCCCACCAACGGCAGAGCCTACTACAACGCCCGGTT
CAAGTCCGAGGCCACCCTGACCGTGGACAAGTCCTCCAACACCGCCTACATGC
AGCTGTCCTCCCTGACCTCTGAGGCCTCCGCCGTGTACTACTGCGCCAGAGCT
TACGGCAACTACGAGTTCGCCTACTGGGGCCAGGGCACCCTCGTGACAGTGTC
TGTGGCTAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAG
AGCACCAGCGGCGGAACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCC
CCGAGCCCGTGACCGTGTCCTGGAACAGCGGCGCTCTGACCAGCGGAGTGCA
CACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTG
GTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA
CCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGC
GACAAGACCCACACCTGCCCTCCCTGCCCCGCCCCCGAGCTGCTGGGCGGAC
CCAGCGTGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGC
ACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGG

TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG
CCTCGGGAGGAGCAGTACAACTCCACCTACCGCGTGGTGAGCGTGCTGACCG
TGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAA
CAAGGCCCTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAG
CCCCGGGAGCCTCAGGTGTACACCCTGCCCCCCAGCCGCGACGAGCTGACCA
AGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATC
GCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCC
CTCCCGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTG
GACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACG
AGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGATA
G

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

ATGGAGACCGACACCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT
CCACCGGAGACGTCGTGATGACCCAGACCCCTCTGTCCCTGCCTGTGTCTCTG
GGCGACCAGGCCTCCATCTCCTGCCGGTCTAGACAGTCCCTCGTGAACTCCAA
CGGCAACACCTTCCTGCAGTGGTATCTGCAGAAGCCCGGCCAGTCCCCCAAGC
TGCTGATCTACAAGGTGTCCCTGCGGTTCTCCGGCGTGCCCGACAGATTTTCC
GGCTCTGGCTCTGGCACCGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGA
GGACCTGGGCCTGTACTTCTGCAGCCAGTCCACCCACGTGCCCCCTACATTTG
GCGGAGGCACCAAGCTGGAAATCAAACGGGCAGATGCTGCACCAACTGTATCC
ATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGC
TTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGC
AGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGA
CAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAAC
GACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGTTGA

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

ATGGAGACCGACACCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT
CCACCGGAGACGTCGTGATGACCCAGACCCCTCTGTCCCTGCCTGTGTCTCTG

GGCGACCAGGCCTCCATCTCCTGCCGGTCTAGACAGTCCCTCGTGAACTCCAA
CGGCAACACCTTCCTGCAGTGGTATCTGCAGAAGCCCGGCCAGTCCCCCAAGC
TGCTGATCTACAAGGTGTCCCTGCGGTTCTCCGGCGTGCCCGACAGATTTTCC
GGCTCTGGCTCTGGCACCGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGA
GGACCTGGGCCTGTACTTCTGCAGCCAGTCCACCCACGTGCCCCCTACATTTG
GCGGAGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCCCCCAGCGTGTT
CATCTTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGT
GCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGA
CAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACTCC
AAGGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTA
CGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGC
CCCGTGACCAAGAGCTTCAACCGGGGCGAGTGCTAA

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

MDPKGSLSWRILLFLSLAFELSYGQVQLLQPGAELVKPGASVKLACKASGYLFTTY
WMHWLKQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLT
SEASAVYYCARAYGNYEFAYWGQGTLVTVSVAKTTPPSVYPLAPGCGDTTGSSVT
LGCLVKGYFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQT
VTCSVAHPASSTTVDKKLEPSGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIK
DVLMISLTPKVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTIRVVS
TLPIQHQDWMSGKEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSRK
DVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLNMKTSKW
EKTDSFSCNVRHEGLKNYYLKKTISRSPG*

[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:

MDPKGSLSWRILLFLSLAFELSYGQVQLLQPGAELVKPGASVKLACKASGYLFTTY
WMHWLKQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLT
SEASAVYYCARAYGNYEFAYWGQGTLVTVSVASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPG*

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

METDTLLLWVLLLWVPGSTGDVVMTQTPLSLPVSLGDQASISCRSRQSLVNSNGN
TFLQWYLQKPGQSPKLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYF
CSQSTHVPPTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI
NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHK
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:

METDTLLLWVLLLWVPGSTGDVVMTQTPLSLPVSLGDQASISCRSRQSLVNSNGN
TFLQWYLQKPGQSPKLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYF
CSQSTHVPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
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
QVSLTCLVKG FYPSDISVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT
300 310 320
VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK

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

[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 MDPKGSLSWRILLFLSLAFELSYGQVQLVQSGAEVKKPGASVKVSCKASGYLFTTY WMHWVRQAPGQGLEWMGEISPTNGRAYYNQKFQGRVTMTVDKSTNTVYMELSS LRSEDTAVYYCARAYGNYFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG.

[98] The DNA sequence that corresponds to SEQ ID NO: 30 is ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGC CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG AAGAAACCTGGCGCCTCCGTGAGGTGTCCTGCAAGGCTTCCGGCTACCTGTTC ACCACCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAGGGCCTGGAAT GGATGGGCGAGATCTCCCCTACCAACGGCAGAGCCTACTACAACAGAAATTCC AGGGCAGAGTGACCATGACCGTGGACAAGTCCACCAACACCGTGTACATGGAA CTGTCCTCCCTGCGGAGCGAGGACACCGCCGTGTACTACTGCGCTAGAGCCTA CGGCAACTACGATTCGCCTACTGGGGCCAGGGCACCCTCGTGACAGTGTCCTC TGCTAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGC ACCAGCGGCGGAACCGCCGCCCTGGGCTGCCTGGGAAGGACTACTTCCCCGA GCCCGTGACCGTGTCCTGGAACAGCGGCGCTCTGACCAGCGGAGTGCACACC TTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGA CCGTGCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACA AGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGCGACAA GACCCACACCTGCCCTCCCTGCCCCGCCCCGAGCTGCTGGGCGGACCCAGCG TGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCC GAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAGTT CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGG

GAGGAGCAGTACAACTCCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGC
ACCAGGACTGGCTGAACGGCAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC
CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGG
AGCCTCAGGTGTACACCCTGCCCCCCAGCCGCGACGAGCTGACAAGAACCAG
GTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGA
GTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTG
CTGGACAGCGACGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCC
CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGC
ACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCGGATAGTAA.

[99] The one-letter amino acid sequence that corresponds to SEQ ID NO: 31 is
MDPKGSLSWRILLFLSLAFELSYGQVQLVQSGAEVKKPGASVKVSCKASGYLFTTY
WMHWVRQAPGQGLEWMGEISPTNGRAYYNAKFQGRVTMTVDKSTNTAYMELSS
LRSEDTAVYYCARAYGNYFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPG.

[100] The DNA sequence that corresponds to SEQ ID NO: 32 is
ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGC
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG
AAGAAACCTGGCGCCTCCGTGAGGTGTCCTGCAAGGCTTCCGGCTACCTGTTC
ACCACCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAGGGCCTGGAAT
GGATGGGCGAGATCTCCCCTACCAACGGCAGAGCCTACTACAACCAAAATTCC
AGGGCAGAGTGACCATGACCGTGGACAAGTCCACCAACACCGCTTACATGGAA
CTGTCCTCCCTGCGGAGCGAGGACACCGCCGTGTACTACTGCGCTAGAGCCTA
CGGCAACTACGATTCGCCTACTGGGGCCAGGGCACCCTCGTGACAGTGTCCTC
TGCTAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGC
ACCAGCGGCGGAACCGCCGCCCTGGGCTGCCTGGGAAGGACTACTTCCCCGA
GCCCGTGACCGTGTCCTGGAACAGCGGCGCTCTGACCAGCGGAGTGCACACC

TTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGA
CCGTGCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACA
AGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGCGACAA
GACCCACACCTGCCCTCCCTGCCCCGCCCCGAGCTGCTGGGCGGACCCAGCG
TGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCC
GAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAGTT
CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGG
GAGGAGCAGTACAACTCCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGC
ACCAGGACTGGCTGAACGGCAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC
CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGG
AGCCTCAGGTGTACACCCTGCCCCCCAGCCGCGACGAGCTGACAAGAACCAG
GTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGA
GTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTG
CTGGACAGCGACGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCC
CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGC
ACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCGGATAGTAA.

[101] The one-letter amino acid sequence that corresponds to SEQ ID NO: 33 is
MDPKGSLSWRILLFLSLAFELSYGQVQLVQSGAEVKKPGASVKVSCKASGYLFTTY
WMHWVRQAPGQGLEWMGEISPTNGRAYYNAKFQGRVTMTVDKSINTAYMELSRL
RSDDTAVYYCARAYGNYFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPG.

[102] The DNA sequence that corresponds to SEQ ID NO: 34 is
ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGC
CTTCGAGCTGAGCTACGGCCAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTG
AAGAAACCTGGCGCCTCCGTGAGGTGTCCTGCAAGGCTTCCGGCTACCTGTTC
ACCACCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAGGGCCTGGAAT

GGATGGGCGAGATCTCCCCTACCAACGGCAGAGCCTACTACAACCAAAATTCC
AGGGCAGAGTGACCATGACCGTGGACAAGTCCATCAACACCGCTTACATGGAA
CTGTCCAGACTGCGGAGCGATGACACCGCCGTGTACTACTGCGCTAGAGCCTA
CGGCAACTACGATTCGCCTACTGGGGCCAGGGCACCCTCGTGACAGTGTCCTC
TGCTAGCACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGC
ACCAGCGGCGGAACCGCCGCCCTGGGCTGCCTGGGAAGGACTACTTCCCCGA
GCCCGTGACCGTGTCCTGGAACAGCGGCGCTCTGACCAGCGGAGTGCACACC
TTCCCTGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGA
CCGTGCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACA
AGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGCGACAA
GACCCACACCTGCCCTCCCTGCCCCGCCCCGAGCTGCTGGGCGGACCCAGCG
TGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCC
GAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAGTT
CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGG
GAGGAGCAGTACAACTCCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGC
ACCAGGACTGGCTGAACGGCAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC
CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGG
AGCCTCAGGTGTACACCCTGCCCCCCAGCCGCGACGAGCTGACAAGAACCAG
GTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGA
GTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTG
CTGGACAGCGACGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCC
CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGC
ACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCGGATAGTAA.

[103] The one-letter amino acid sequence that corresponds to SEQ ID NO: 35 is
METDTLLLWVLLLWVPGSTGDVVMTQSPLSLPVTLGQPASISCRSSQSLVNSNGNT
FLQWYQQRPGQSPRLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY
CSQSTHVPPTFGGGTVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC.

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

CCACCGGAGACGTCGTGATGACCCAGTCCCCTCTGTCCCTGCCTGTGACCCTG
GGACAGCCTGCCTCCATCTCCTCAGATCCTCCCAGTCCCTCGTGAACTCCAAC
GGCAACACCTTCCTGCAGTGGTATCAGCAGCGGCCTGGCCAGAGCCCCAGAC
TGCTGATCTACAAGGTGTCCCTGCGGTTCTCCGGCGTGCCCGACGATTTTCCG
GCTCTGGCTCTGGCACCGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAG
GACGTGGGCGTGTACTACTGCTCCCAGAGCACCCACGTGCCCCCTACATTTGG
CGGAGGCACCAAGTGGAAATCAAGCGGACCGTGGCCGCCCCCAGCGTGTTCA
TCTTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGTG
CCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGGCAGTGGAAGGTGGACA
ACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACTCCAA
GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTAC
GAGAAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGCCC
CGTGACCAAGAGCTTCAACCGGGGCGAGTGCTAA.

[105] The one-letter amino acid sequence that corresponds to SEQ ID NO: 37 is
METDTLLLWVLLLWVPGSTGDVVMTQSPLSLPVTLGQPASISCRSRQSLVNSNGN
TFLQWYQQRPGQSPRLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY
YCSQSTHVPPTFGGGTVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC.

[106] The DNA sequence that corresponds to SEQ ID NO: 38 is
ATGGAGACCGACACCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT
CCACCGGAGACGTCGTGATGACCCAGTCCCCTCTGTCCCTGCCTGTGACCCTG
GGACAGCCTGCCTCCATCTCCTCAGATCCAGGCAGTCCCTCGTGAACTCCAAC
GGCAACACCTTCCTGCAGTGGTATCAGCAGCGGCCTGGCCAGAGCCCCAGAC
TGCTGATCTACAAGGTGTCCCTGCGGTTCTCCGGCGTGCCCGACGATTTTCCG
GCTCTGGCTCTGGCACCGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAG
GACGTGGGCGTGTACTACTGCTCCCAGAGCACCCACGTGCCCCCTACATTTGG
CGGAGGCACCAAGTGGAAATCAAGCGGACCGTGGCCGCCCCCAGCGTGTTCA
TCTTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGTG
CCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGGCAGTGGAAGGTGGACA
ACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACTCCAA

GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTAC
GAGAAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGCCC
CGTGACCAAGAGCTTCAACCGGGGCGAGTGCTAA.

[107] The one-letter amino acid sequence that corresponds to SEQ ID NO: 39 is
METDTLLLWVLLLWVPGSTGDVVMTQSPLSSPVTLGQPASISCRSSQSLVNSNGN
TFLQWYHQRPGQPPRLLIYKVSLRFSGVPDRFSGSGAGKDFTLKISRVEAEDVGVY
YCSQSTHVPPTFGQGTLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC.

[108] The DNA sequence that corresponds to SEQ ID NO: 40 is
ATGGAGACCGACACCCTGCTGCTCTGGGTGCTGCTGCTCTGGGTGCCCGGCT
CCACCGGAGACGTCGTGATGACCCAGTCCCCTCTGTCCAGTCCTGTGACCCTG
GGACAGCCTGCCTCCATCTCCTCAGATCCTCCCAGTCCCTCGTGAACTCCAAC
GGCAACACCTTCCTGCAGTGGTATCACCAGCGGCCTGGCCAGCCTCCCAGACT
GCTGATCTACAAGGTGTCCCTGCGGTTCTCCGGCGTGCCCGACGATTTTCCGG
CTCTGGCGCTGGCAAGGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAG
GACGTGGGCGTGTACTACTGCTCCCAGAGCACCCACGTGCCCCCTACATTTGG
CCAGGGCACCAACTGGAAATCAAGCGGACCGTGGCCGCCCCCAGCGTGTTCA
TCTTCCCTCCCAGCGACGAGCAGCTGAAGTCTGGCACCGCCAGCGTGGTGTG
CCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGGCAGTGGAAGGTGGACA
ACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACTCCAA
GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTAC
GAGAAGACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGACTGTCTAGCCC
CGTGACCAAGAGCTTCAACCGGGGCGAGTGCTAA.

[109] The one-letter amino acid sequence that corresponds to SEQ ID NO: 47 is
MGWTLVFLFLLSVTAGVHSQVQLLQPGAELVKPGASVKLACKASGYLFTTYWMHW
LKQRPGQGLEWIGEISPTNGRAYYNARFKSEATLTVDKSSNTAYMQLSSLTSEASA
VYYCARSFGNYEFAYWQGTLVTVSVASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGPSVFLFPPKPKDTLMISRTPEVT

CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK.

[110] The one-letter amino acid sequence that corresponds to SEQ ID NO: 48 is
MGWTLVFLFLLSVTAGVHSEVQLLESGAEAKKPGASVKLSCKASGYLFTTYWMHW
VHQAPGQRLEWMGEISPTNGRAYYNARFKSRVTITVDKSASTAYMELSSLRSEDT
AVYYCARSFGNYEFAYWQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK.

[111] The one-letter amino acid sequence that corresponds to SEQ ID NO: 49 is
MGWTLVFLFLLSVTAGVHSQVQLVQSGAEVKKPGASVKVSCKASGYLFTTYWMH
WVRQAPGQRLEWIGEISPTNGRAYYNARFKSRVTITRDTSASTAYMELSSLRSEDT
AVYYCARSFGNYEFAYWQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK.

[112] The one-letter amino acid sequence that corresponds to SEQ ID NO: 50 is
MGWTLVFLFLLSVTAGVHSQVQLVQSGAEVKKPGSSVKVSCKASGYLFTTYWMH
WVRQAPGQGLEWMGEISPTNGRAYYNARFKSRVTITADKSTSTAYMELSSLRSED
TAVYYCARSFGNYEFAYWQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGPSVFLFPPKPKDTLMISRTPE

VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK.

[113] The one-letter amino acid sequence that corresponds to SEQ ID NO: 51 is
MGWTLVFLFLLSVTAGVHSQVQLVQSGAEVKKPGASVKVSCEASGYLFTTYWMH
WVRQAPGQGLEWMGEISPTNGRAYYNARFKSRVTITRDTSINTAYMELSRLRSDD
TAVYYCARSFGNYEFAYWQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK.

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

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

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

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

TNGRAYYNARFKSRVTITADKSTSTAYMELSSLRSEDTAVYYCARSFGNYEFAYW
GQGTLVTVSS.

[118] The one-letter amino acid sequence that corresponds to SEQ ID NO: 56 is
QVQLVQSGAEVKKPGASVKVSCEASGYLFTTYWMHWVRQAPGQGLEWMGEISP
TNGRAYYNARFKSRVTITRDTSINTAYMELSRLRSDDTAVYYCARSFGNYEFAYWG
QGTLVTVSS.

[119] The one-letter amino acid sequence that corresponds to SEQ ID NO: 57 is
MVSSAQFLGLLLLCFQGTRCDVVMTQTPLSLPVSLGDQASISCRSRQSLVNSNGNT
FLQWYLQKPGQSPKLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYF
CSQSTHVPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC.

[120] The one-letter amino acid sequence that corresponds to SEQ ID NO: 58 is
MVSSAQFLGLLLLCFQGTRCDIVMTQTPLSLPVTLGQPASISCRSRQSLVNSNGNT
FLQWLQQRPGQPPRLLIYKVSLRFSGVPDRFSGSGAGTDFTLTISRVEAEDVGIYF
CSQSTHVPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC.

[121] The one-letter amino acid sequence that corresponds to SEQ ID NO: 59 is
MVSSAQFLGLLLLCFQGTRCDIVMTQTPLSLSVTPGQPASISCRSRQSLVNSNGNT
FLQWYLQKPGQSPQLLIYKVSLRFSGVPDRFSGSGSGTDFTLKISRVEPEDVGVYY
CSQSTHVPPTFGGGTKVEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

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

KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC.

[123] The one-letter amino acid sequence that corresponds to SEQ ID NO: 61 is MVSSAQFLGLLLLCFQGTRCDIVMTQTPLSLSVTPGQPASISCRSRQSLVNSNGNT FLQWLLQKPGQPPQLLIYKVSLRFSGVPNRFSGSGSGTDFTLKISRVEAEDVGLYY CSQSTHVPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC.

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

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

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

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

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

[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 (±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) – ΔCt 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 ΔCt	5.79	7.14	5.79	6.09	5.79	7.39
ΔΔCt	-1.35		-0.30		-1.60	
Fold Expression	**2.55**		**1.23**		**3.03**	

[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 µg/gram) treated old mice euthanized Day 22, it was observed that $p16^{Ink4a}$ mRNA was 3.03-fold higher in Group 2 (controls) than in Group 5 (5 µg/gram). This comparison indicated that the Group 5 animals had lower levels of $p16^{Ink4a}$ mRNA expression when they were treated with 5.0 µg/gram/BID/week, providing $p16^{Ink4a}$ mRNA expression levels comparable to that of the young untreated mice (i.e. Group 1). Unlike Group 3 (2.5 µg/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 µg/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 $p16^{Ink4a}$, 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 Nα,Nα-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 immodilized with the test antibody (molecular weigh 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 (µl/min.)	30
Association time (s)	300
Dissociation time (s)	300
Sample concentration (µM)	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 x 10^3; k_d (1/s) = 6.781 x 10^{-3}; K_D (M) = 3.651 x 10^{-6}; R_{max} (RU) = 19.52; and Chi^2 = 0.114. Because the 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

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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 µg/mL in 1x phosphate buffer at pH 6.5. A 96-well microtiter ELISA plate was coated with 100 µL/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 µg/mL. Secondary antibodies were diluted 1:5,000. 100 µL 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 µL/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 µL HRP substrate TMB was added to each well to develop the plate. After 3-5 minutes elapsed, the reaction was terminated by adding 100 µL 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 Positive Control	Parental Anti-AGE	Chimeric Anti-AGE		R&D Positive Control	Parental Anti-AGE	Chimeric Anti-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 Positive Control	Parental Anti-AGE	Chimeric Anti-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).

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[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 µg 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 µg/mL gentamicin, 100 units/mL penicillin G Na and 100 µg/mL streptomycin sulfate. Tumor cells were maintained in tissue culture flasks in a humidified incubator at 37 °C in an atmosphere of 5% CO_2 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 10^6 cells/mL on the day of implant. Tumors were initiated by subcutaneously implanting 1 x 10^5 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^3. Tumor volume was determined using the formula: tumor volume = $(\text{tumor width})^2(\text{tumor length})/2$. Tumor weight was approximated using the assumption that 1 mm^3 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^3 and a group mean tumor volume of 112 mm^3. 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: $\%TGI = (1-MTV_{treated}/MTV_{control}) \times 100$. 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: $\%Inhibition = (1-\text{Mean Count of } Foci_{treated}/\text{Mean Count of } Foci_{control}) \times 100$.

[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^3 for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 mm^3 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^3)	%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 µg/g (Group 2) and 10 µg/g treatment group (Group 3). The %Inhibition trended towards significance ($P > 0.05$, ANOVA-Dunnett) for the 5 µg/g treatment group. The %Inhibition was statistically significant ($P \leq 0.01$, ANOVA-Dunnett) for the 10 µg/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 composition comprising an anti-advanced glycation end-product (anti-AGE) antibody and a pharmaceutically acceptable carrier for use in treating metastatic cancer, and/or preventing cancer metastasis in a subject, wherein the anti-AGE antibody binds a carboxymethyllysine-modified protein.

2. The composition for use according to claim 1, wherein the subject is selected from the group consisting of humans, mice, rats, goats, sheep, cows, horses, dogs and cats.

3. The composition for use according to any one of claims 1 and 2, wherein the subject is a human.

4. The composition for use according to any one of claims 1-3, 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.

5. The composition for use according to any one of claims 1-4, wherein the subject has metastatic cancer.

6. The composition for use according to any one of claims 1-4, wherein the subject does not have metastatic cancer.

7. The composition for use according to any one of claims 1-6, wherein the composition is in unit dosage form.

8. The composition for use according to any one of claims 1-5 and 7, wherein:

(a) the subject is a human;

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(b) 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;

(c) the subject has metastatic cancer; and

(d) the composition is in unit dosage form.

9. The composition for use according to any one of claims 1-6, wherein the composition is in multidosage form.

10. The composition for use according to any one of claims 1-9, wherein the composition is sterile.

11. The composition for use according to any one of claims 1-10, wherein the anti-AGE antibody binds a metastatic cancer cell expressing an AGE modification.

12. The composition for use according to any one of claims 1-11, wherein the anti-AGE antibody binds a circulating cell expressing an AGE modification.

13. The composition for use according to any one of claims 1-12, wherein the subject is pregnant.

14. The composition for use according to any one of claims 1-13, wherein the subject has been previously diagnosed with cancer cachexia.

15. The composition for use according to any one of claims 1-14, wherein the subject has a compromised immune system.

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16. The composition for use according to any one of claims 1-15, wherein the metastatic cancer is metastatic breast cancer.

17. A composition comprising an anti-advanced glycation end-products (anti-AGE) antibody and a pharmaceutically acceptable carrier for use in treating breast cancer, and/or preventing breast cancer in a subject, wherein the anti-AGE antibody binds a carboxymethyllysine-modified protein.

18. The composition for use according to claim 17, wherein the subject is selected from the group consisting of humans, goats, sheep, cows, horses, dogs and cats.

19. The composition for use according to any one of claims 17 and 18, wherein the subject is a human.

20. The composition for use according to any one of claims 17-19, 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.

21. The composition for use according to any one of claims 17-20, wherein the subject has breast cancer.

22. The composition for use according to any one of claims 17-20, wherein the subject does not have breast cancer.

23. The composition for use according to any one of claims 17-22, wherein the composition is in unit dosage form.

24. The composition for use according to any one of claims17-22, wherein the composition is in multidosage form.

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25. The composition for use according to any one of claims 17-24, wherein the composition is sterile.

26. The composition for use according to any one of claims 17-25, wherein the anti-AGE antibody binds a breast cancer cell expressing an AGE modification.

27. The composition for use according to any one of claims 17-26, wherein the subject is pregnant.

28. The composition for use according to any one of claims 17-27, wherein the subject has been previously diagnosed with cancer cachexia.

29. The composition for use according to any one of claims 17-28, wherein the subject has a compromised immune system.

30. The composition for use according to any one of claims 17-21, 23 and 25-29, wherein:

(a) the subject is a human;
(b) the anti-AGE antibody is non-immunogenic to humans;
(c) the subject has breast cancer; and
(d) the composition is in unit dosage form.

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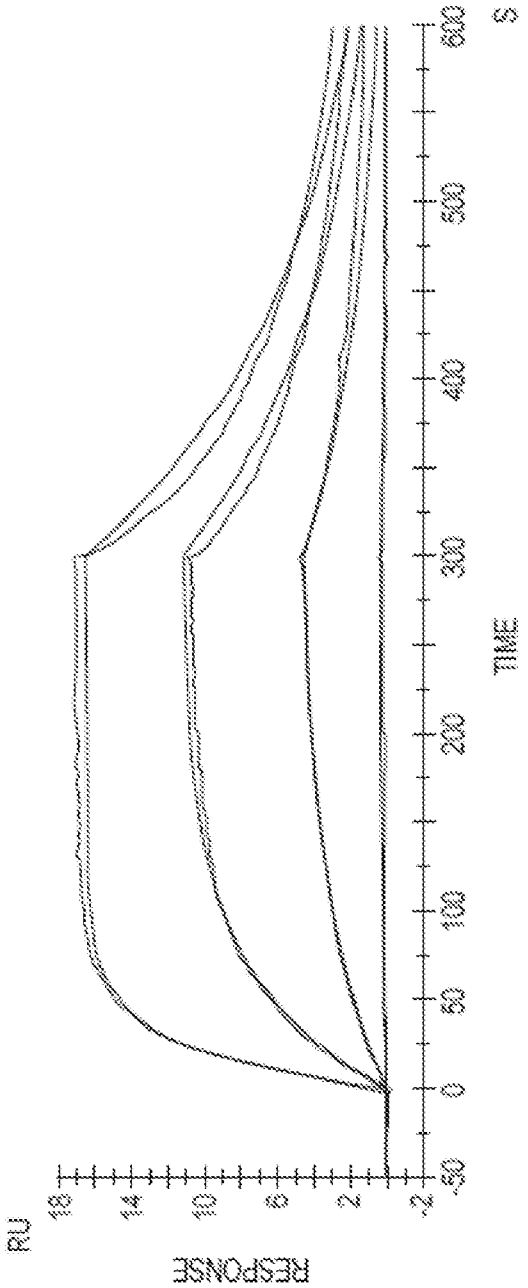

FIG. 1

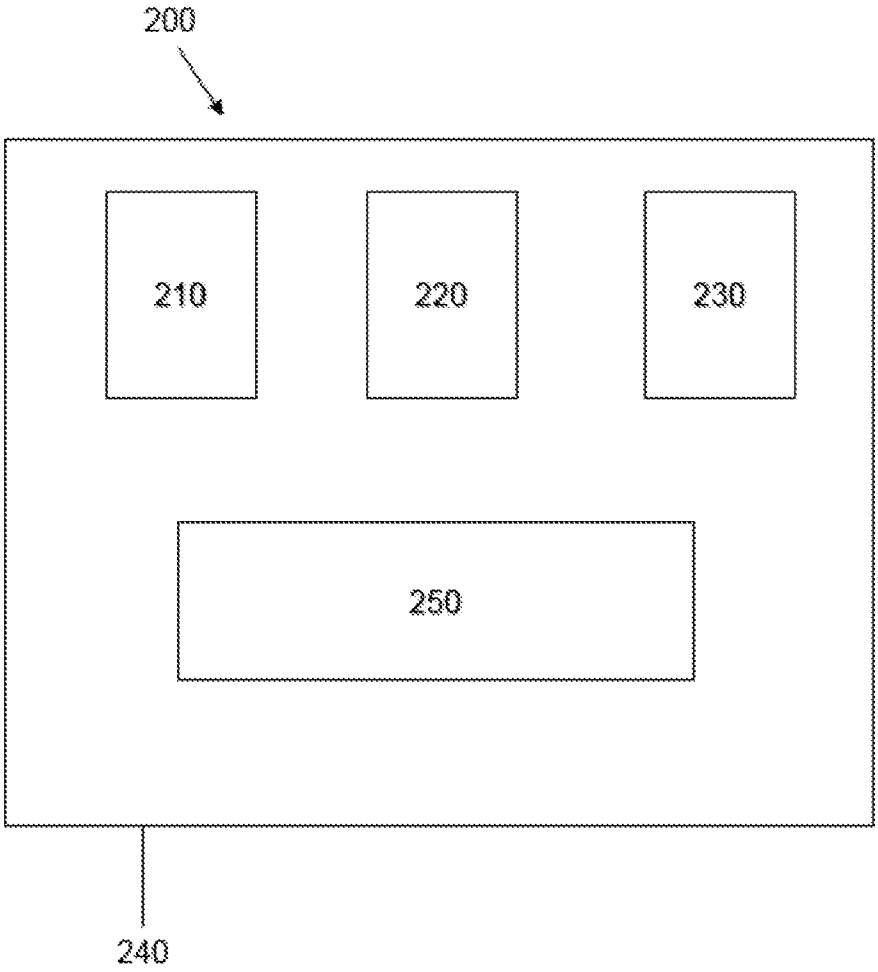

FIG. 2

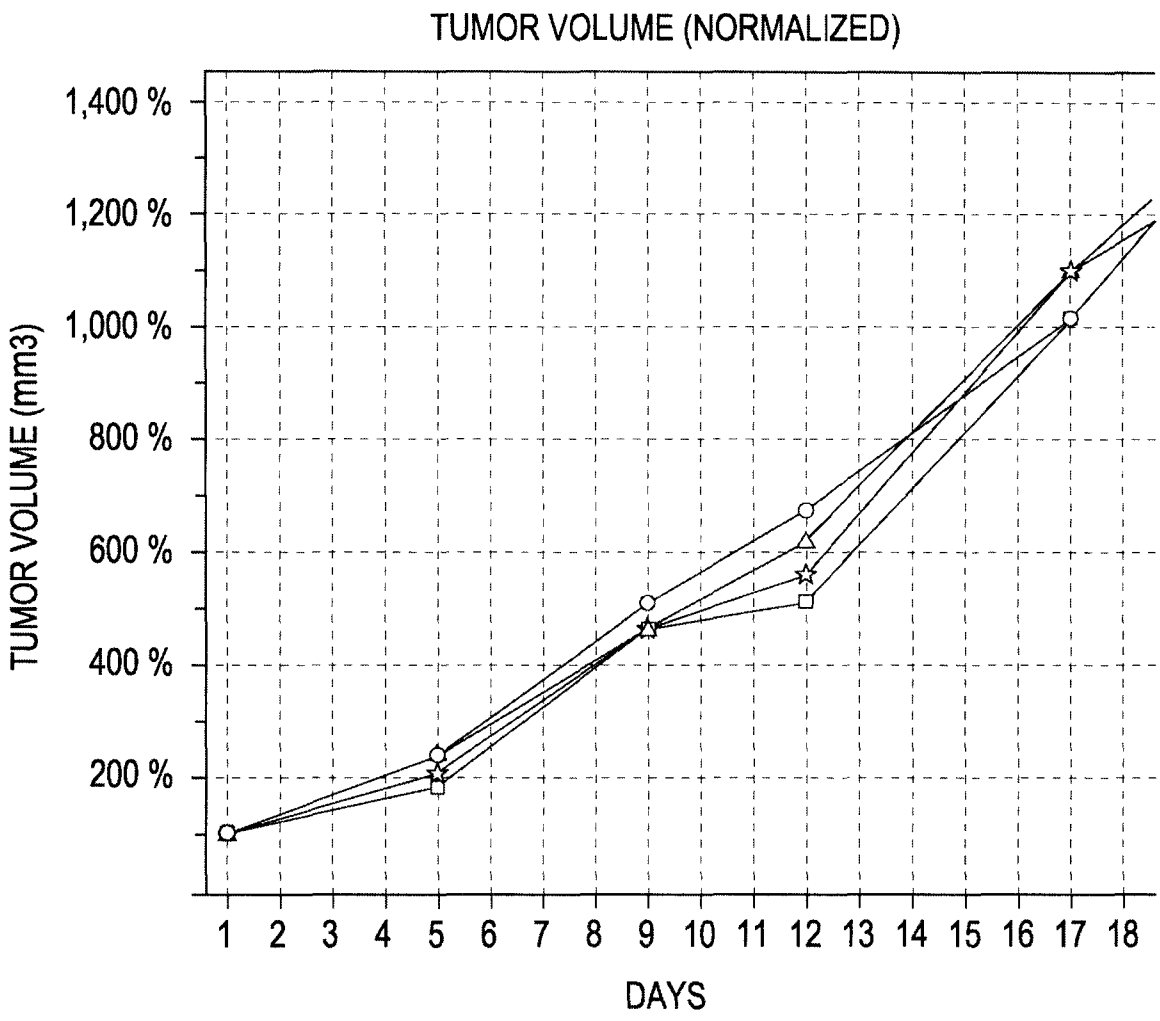
TUMOR VOLUME (NORMALIZED)
TUMOR VOLUME (mm3)
1,400 %
1,200 %
1,000 %
800 %
600 %
400 %
200 %
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
DAYS

FIG. 3

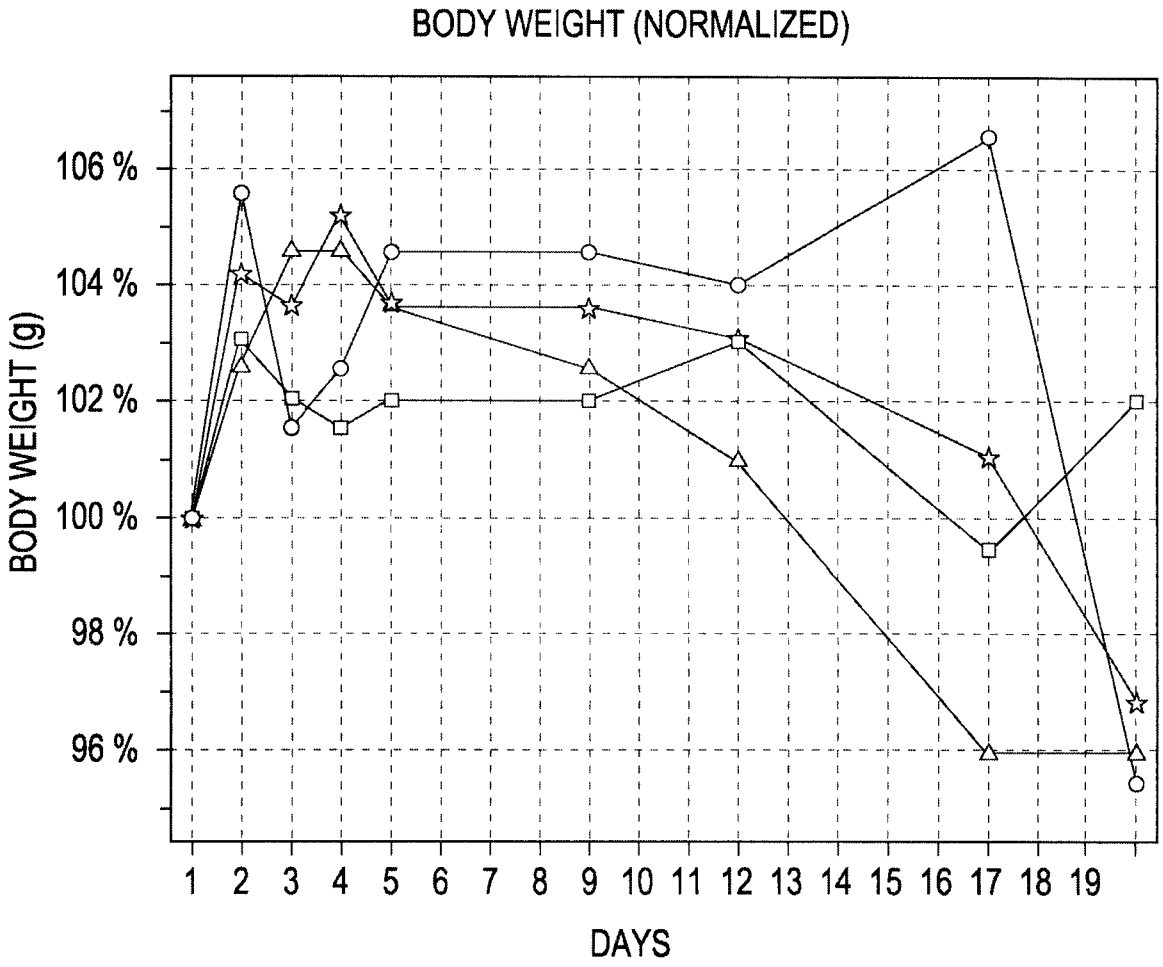
BODY WEIGHT (NORMALIZED)
BODY WEIGHT (g)
106 %
104 %
102 %
100 %
98 %
96 %
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
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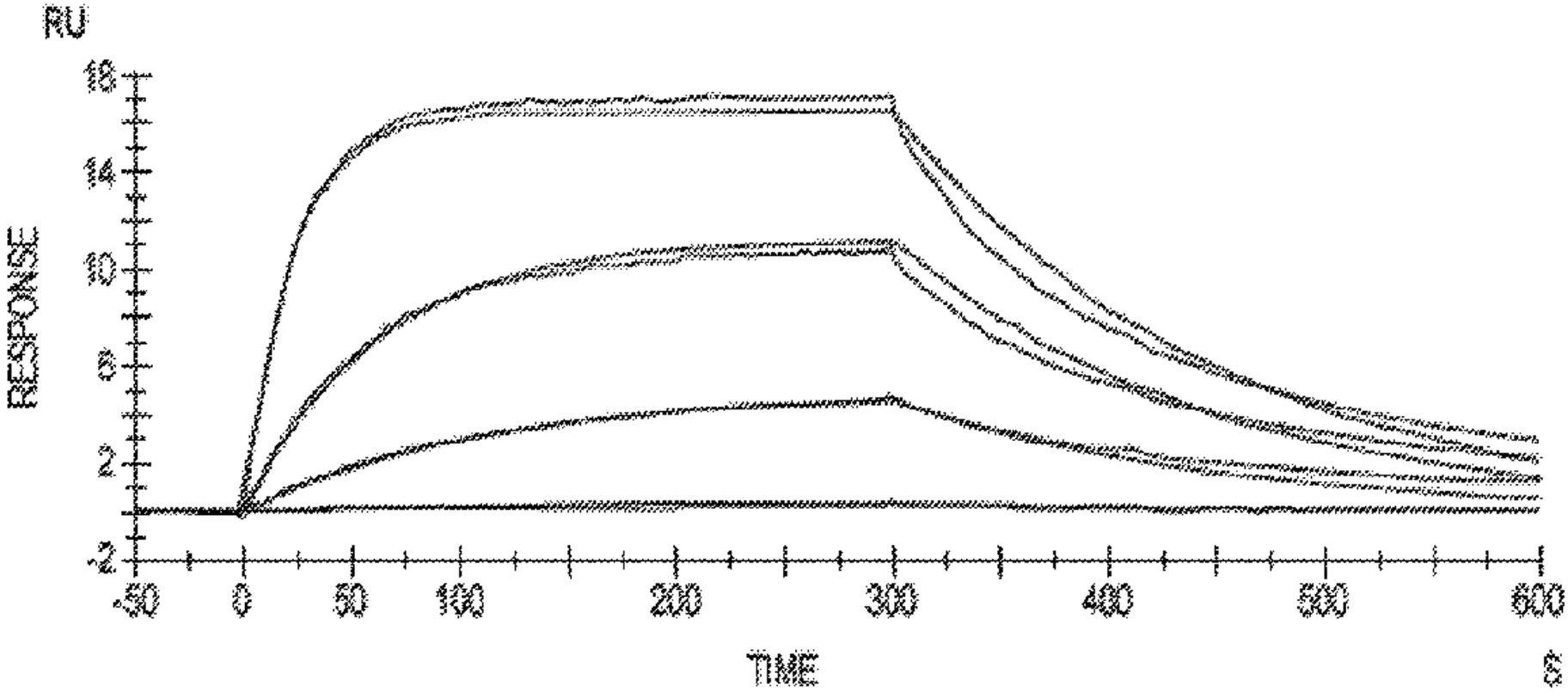
FIG. 4


RU
18
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