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(54) Title: VACCINES FOR USE IN TREATING VARIOUS DISEASES AND DISORDERS

(57) Abstract: Various diseases and disorders associated with cellular senescence may be treated by immunizing a subject in need thereof against AGE-modified proteins or peptides of a cell. Immunizing a subject includes administering a vaccine that comprises an AGE antigen. Vaccines against AGE-modified proteins or peptides contain an AGE antigen, an adjuvant, optional preservatives and optional excipients.

VACCINES FOR USE IN TREATING VARIOUS DISEASES AND DISORDERS

BACKGROUND

[01] Sarcopenia is the loss of muscle mass, quality and strength associated with aging. Humans begin to lose muscle mass and function at some point in the third decade of life. This loss of muscle mass typically accelerates around age 75. Sarcopenia develops in both physically active and physically inactive people. As the average human lifespan continues to increase, sarcopenia is becoming a significant health concern. The loss of muscle mass from sarcopenia may lead to poor balance, reduced gait speed and frailty. Individuals suffering from sarcopenia are more susceptible to injury and disability, and may be unable to live independently as a result. The spread of sarcopenia will likely result in increases in health care and assisted living expenses.

[02] Sarcopenia has been considered to be an inevitable result of aging and the natural deterioration of the body over time. The primary treatment for sarcopenia is exercise. Physical exercise, particularly resistance training or strength training, can reduce the impact of sarcopenia. Testosterone, anabolic steroids, ghrelin, vitamin D, angiotensin converting enzyme inhibitors (ACE inhibitors), eicosapentaenoic acid (EPA), myostatin, selective androgen receptor modulators (SARMs), urocortin II (Ucn2) and hormone replacement therapy have been investigated or are being studied as potential treatments for sarcopenia. Despite this research, there are currently no U.S. Food and Drug Administration (FDA)-approved agents for treating sarcopenia.

[03] A recent study has identified a causal link between cellular senescence and age-related disorders, such as sarcopenia. A research team at the Mayo Clinic in Rochester, Minnesota, demonstrated that effects of aging in mice could be delayed by eliminating senescent cells in their fat and muscle tissues without overt side effects (Baker, D. J. *et al.*, "Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-

associated disorders", Nature, Vol. 479, pp. 232-236, (2011)). Elimination of senescent cells in transgenic mice was shown to substantially delay the onset of sarcopenia and cataracts, and to reduce senescence indicators in skeletal muscle and the eye. The study established that life-long and late-life treatment of transgenic mice for removal of senescent cells has no negative side effects and selectively delays age-related phenotypes that depend on cells (*Id.*, page 234, col. 2, line 16 through page 235, col. 1, line 2). The authors theorized that removal of senescent cells may represent an avenue for treating or delaying age-related diseases in humans and improving healthy human lifespan (*Id.*, page 235, col. 2, lines 38-51).

[04] Senescent cells are cells that are partially-functional or non-functional and are in a state of irreversible proliferative arrest. Senescence is a distinct state of a cell, and is associated with biomarkers, such as activation of the biomarker $p16^{Ink4a}$, and expression of β-galactosidase. Senescence begins with damage or stress (such as overstimulation by growth factors) of cells.

[05] Advanced glycation end-products (AGEs; also referred to AGE-modified proteins, or glycation end-products) arise from a non-enzymatic reaction of sugars with protein side-chains in aging cells (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 may also be formed from other processes. For example, the advanced glycation end product, N^{ε}-(carboxymethyl)lysine, is a product of both lipid peroxidation

and glycoxidation reactions. 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)).

[06] 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 and lack of function.

[07] Cellular senescence and the accumulation of AGEs have been implicated in a number of diseases and disorders in addition to sarcopenia and age-related disorders. Senescence of cells in the central nervous system such as glial cells, astrocytes and microglial cells has been associated with neurodegenerative disorders. Abnormal accumulation of senescent astrocytes has been associated with Alzheimer's disease (AD) (Bhat, R. *et al.*, "Astrocyte Senescence as a Component of Alzheimer's Disease", *PLOS ONE*, Vol. 7(9), e45069, pp. 1-10 (Sept. 2012)). Microglial cell senescence

associated with normal aging is exacerbated by the presence of the amyloid plaques indicative of AD (Flanary, B. E. *et al.*, "Evidence That Aging And Amyloid Promote Microglial Cell Senescence", *Rejuvenation Research*, Vol. 10(1), pp. 61-74 (March 2007)). The presence of AGEs with astrocytes and microglial cells in AD is further evidence of the presence of senescent cells (Takeda, A., *et al.* "Advanced glycation end products co-localize with astrocytes and microglial cells in Alzheimer's disease brain", *Acta Neuropathologica*, Vol. 95, pp. 555-558 (1998)). On the basis of recently reported findings, Chinta *et al.* proposed that environmental stressors associated with Parkinson's disease (PD) may act in part by eliciting senescence within non-neuronal glial cells, contributing to the characteristic decline in neuronal integrity that occurs in this disorder (Chinta, S. J. *et al.* "Environmental stress, ageing and glial cell senescence: a novel mechanistic link to Parkinson's disease?", *J Intern Med*, Vol. 273, pp. 429-436 (2013)). Astrocyte senescence is also associated with PD (M. Mori, "The Parkinsonian Brain: Cellular Senescence and Neurodegeneration, *SAGE* (June 30, 2015) (sage.buckinstitute.org/the-parkinsonian-brain-cellular-senescence-and-neurodegeneration/). In a rodent model of familial amyotrophic lateral sclerosis (ALS) overexpressing mutant superoxide dismutase-1 (m-SOD1), the rate of astrocytes acquiring a senescent phenotype is accelerated (Das, M. M. and Svendsen, C. N., "Astrocytes show reduced support of motor neurons with aging that is accelerated in a rodent model of ALS", *Neurobiology of Aging*, Vol. 36, pp. 1130-1139 (2015)). Even in multiple sclerosis (MS), microglia and macrophages are shifted toward a strongly proinflammatory phenotype, reminiscent of SASP, and may potentiate neuronal damage by releasing proinflammatory cytokines and molecules (Luessi, F., *et al.* "Neurodegeneration in multiple sclerosis: novel treatment strategies" *Expert Rev. Neurother.*, Vol 9, pp.1061-1077 (2012)).

[08] Some neurodegenerative disorders are associated with abnormal cellular senescence outside the central nervous system. Most satellite cells, also known as myosatellite cells, present in the muscle tissue of ALS patients exhibit an abnormal senescent-like morphology, although they may be capable of proliferating *in vitro*

(Pradat, P.-F. *et al.*, "Abnormalities of satellite cells function in amyotrophic lateral sclerosis" *Amyotrophic Lateral Sclerosis*, Vol. 12, pp. 264-271 (2011)). Satellite cells are small multipotent cells found in mature muscle, which are able to give rise to additional satellite cells, or differentiate into myoblasts as well as provide additional myonuclei. In an animal model of Duchenne muscular dystrophy (MD), reduced proliferative capacity and premature senescence of myoblasts was observed (Wright, W. E., "Myoblast Senescence in Muscular Dystrophy" *Exp Cell Res*, Vol. 157, pp. 343-354 (1985)). Myoblasts are precursor cells which differentiate into myocytes (also referred to as muscle cells).

[09] Neurodegenerative disorders are also associated with abnormal protein accumulations (King, O.D., *et al.*, "The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease" *Brain Res.* Vol.1462, pp. 61–80 (2012)). A characteristic of PD and Lewy body dementia is the formation of Lewy bodies that form inside nerve cells. The primary structural component of the Lewy bodies is alpha-synuclein protein, in the form of fibrils. The presence of tangles and plaques are a characteristic of AD, the presence of which is used to definitively diagnose the condition. Plaques, composed of beta-amyloid protein (also referred to as amyloid beta, Aβ or Abeta), accumulate between nerve cells. Tangles, composed of tau protein, form twisted fibers within cells. Prion diseases (also known as transmissible spongiform encephalopathies (TSEs)), include a variety of human and animal disorder such as Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease, bovine spongiform encephalopathy ("mad cow" disease), scrapie (in sheep and goats), chronic wasting disease (in deer and elk), kuru and fatal familial insomnia. Prion protein is a misfolded protein molecule which may propagate by transmitting a misfolded protein state, resulting in the accumulation of the misfolded protein and causing tissue damage and cell death (Dobson, D.M., "The structural basis of protein folding and its links with human disease" *Phil. Trans. R. Soc. Lond.* B, Vol. 356, pp. 133-145 (2001)). In these diseases, it is believed the protein is a normal protein which misfolds or forms an abnormal aggregate. In the case of some patients with familial ALS, a mutated

superoxide dismutase-1 (SOD1) forms inclusions and accumulates (Kato, S., *et al.* "Advanced glycation endproduct-modified superoxide dismutase-1 (SOD1)-positive inclusions are common to familial amyotrophic lateral sclerosis patients with SOD1 gene mutations and transgenic mice expressing human SOD1 with a G85R mutation" *Acta Neuropathol*, Vol. 100, pp. 490-505 (2000)).

[10] The damage or stress that causes cellular senescence also 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. In the case of the protein component lysine, MG reacts to form carboxymethyllysine, which is an AGE.

[11] 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).

[12] 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 is 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).

[13] Senescent cells are also 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)).

[14] Vaccines have been widely used since their introduction by Edward Jenner in the 1770s to confer immunity against a wide range of diseases and afflictions. Vaccine preparations contain a selected immunogenic agent capable of stimulating immunity to an antigen. Typically, antigens are used as the immunogenic agent in vaccines, such as, for example, viruses, either killed or attenuated, and purified viral components. Antigens used in the production of cancer vaccines include, for example, tumor-associated carbohydrate antigens (TACAs), dendritic cells, whole cells and viral vectors. Different techniques are employed to produce the desired amount and type of antigen being sought. For example, pathogenic viruses are grown either in eggs or cells. Recombinant DNA technology is often utilized to generate attenuated viruses for vaccines.

[15] Immunity is a long-term immune response, either cellular or humoral. A cellular immune response is activated when an antigen is presented, preferably with a co-stimulator to a T-cell which causes it to differentiate and produce cytokines. The cells involved in the generation of the cellular immune response are two classes of T-helper (Th) cells, Th1 and Th2. Th1 cells stimulate B cells to produce predominantly antibodies of the IgG2A isotype, which activates the complement cascade and binds the

Fc receptors of macrophages, while Th2 cells stimulate B cells to produce IgG1 isotype antibodies in mice, IgG4 isotype antibodies in humans, and IgE isotype antibodies. The human body also contains "professional" antigen-presenting cells such as dendritic cells, macrophages, and B cells.

[16] A humoral immune response is triggered when a B cell selectively binds to an antigen and begins to proliferate, leading to the production of a clonal population of cells that produce antibodies that specifically recognize that antigen and which may differentiate into antibody-secreting cells, referred to as plasma-cells or memory-B cells. Antibodies are molecules produced by B-cells that bind a specific antigen. The antigen-antibody complex triggers several responses, either cell-mediated, for example by natural killers (NK) or macrophages, or serum-mediated, for example by activating the complement system, a complex of several serum proteins that act sequentially in a cascade that result in the lysis of the target cell.

[17] Immunological adjuvants (also referred to simply as "adjuvants") are the component(s) of a vaccine which augment the immune response to the immunogenic agent. Adjuvants function by attracting macrophages to the immunogenic agent and then presenting the agent to the regional lymph nodes to initiate an effective antigenic response. Adjuvants may also act as carriers themselves for the immunogenic agent. Adjuvants may induce an inflammatory response, which may play an important role in initiating the immune response. Adjuvants include mineral compounds such as aluminum salts, oil emulsions, bacterial products, liposomes, immunostimulating complexes and squalene.

[18] Other components of vaccines include pharmaceutically acceptable excipients, preservatives, diluents and pH adjusters. A variety of these components of vaccines, as well as adjuvants, are described in www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/B/excipient-table-2.pdf and Vogel, F. R. *et al.*, "A compendium of vaccine adjuvants and excipients", *Pharmaceutical Biotechnology*, Vol. 6, pp. 141-228 (1995).

[19] Vaccines may therefore be used to stimulate the production of antibodies in the body and provide immunity against antigens. When an antigen is introduced to a subject that has been vaccinated and developed immunity to that antigen, the immune system may destroy or remove cells that express the antigen.

SUMMARY

[20] In a first aspect, the invention is a method of treating or preventing a disease or disorder associated with cellular senescence comprising immunizing a subject in need thereof against AGE-modified proteins or peptides of a cell.

[21] In a second aspect, the invention is a method of treating a subject with a disease or disorder associated with cellular senescence comprising administering a first vaccine comprising a first AGE antigen and administering a second vaccine comprising a second AGE antigen. The second AGE antigen is different from the first AGE antigen.

[22] In a third aspect, the invention is use of an AGE antigen for the manufacture of a medicament for treating or preventing a disease or disorder associated with cellular senescence.

[23] In a fourth aspect, the invention is a composition comprising an AGE antigen for use in treating or preventing a disease or disorder associated with cellular senescence.

[24] In a fifth aspect, the invention is a composition comprising an AGE antigen for use in treating sarcopenia.

[25] In a sixth aspect, the invention is a composition comprising an AGE antigen for use in promoting tissue or organ regeneration.

[26] In a seventh aspect, the invention is a composition comprising an AGE antigen for use in promoting regenerative processes or overcoming aging effects.

[27] In an eighth aspect, the invention is a composition comprising an AGE antigen for use in treating atherosclerosis.

[28] In an ninth aspect, the invention is a composition comprising an AGE antigen for use in preventing or delaying the onset of cataracts.

[29] In a tenth aspect, the invention is a composition comprising an AGE antigen for use in preventing or delaying the onset of loss of adipose tissue.

[30] In an eleventh aspect, the invention is a composition comprising an AGE antigen for use in preventing or delaying the onset of lordokyphosis.

[31] In a twelfth aspect, the invention is a composition comprising an AGE antigen for use in treating inflammation or auto-immune disorders.

[32] In a thirteenth aspect, the invention is a composition comprising an AGE antigen for use in treating neurodegenerative disorders.

[33] In a fourteenth aspect, the invention is a composition comprising an AGE antigen for use in treating cancer or cancer metastases.

[34] In a fifteenth aspect, the invention is a composition comprising an AGE antigen for use in increasing health span.

[35] In a sixteenth aspect, the invention is a method of reducing the number of AGE-modified cells in a patient, comprising administering a vaccine comprising an AGE antigen.

DEFINITIONS

[36] The term “peptide” means a molecule composed of 2-50 amino acids.

[37] The term “protein” means a molecule composed of more than 50 amino acids.

[38] The term "sarcopenia" means the syndrome characterized by the presence of (1) low muscle mass and (2) low muscle function (low muscle strength or reduced physical performance). Muscle mass may be measured by body imaging techniques, such as computed tomography scanning (CT scan), magnetic resonance imaging (MRI) or dual energy X-ray absorptiometry (DXA or DEXA); bioimpedance analysis (BIA); body potassium measurement, such as total body potassium (TBK) or partial body potassium (PBK); or anthropometric measurements, such as mid-upper arm circumference, skin fold thickness or calf circumference. Preferably, muscle mass is measured by CT scan, MRI or DXA. Muscle strength may be measured by handgrip strength, knee flexion/extension or peak expiratory flow. Preferably, muscle strength is measured by handgrip strength. Physical performance may be measured by the Short Physical Performance Battery, gait speed measurement, timed get-up-and-go (TGUG) or the stair climb power test. Preferably, physical performance is measured by gait speed measurement. A subject may be identified as having sarcopenia or in need of treatment if (1) the subject is at least 25 years old and (2) his or her measured muscle mass and measured muscle function are two standard deviations or more below the mean value for healthy 25 year olds of the same gender and no alternative pathology has been identified to account for the reduced muscle mass and reduced muscle function. Preferably, a subject being treated for sarcopenia is at least 40 years old. More preferably, a subject being treated for sarcopenia is at least 50 years old. Most preferably, a subject being treated for sarcopenia is at least 60 years old. Alternatively, a subject may be identified as having sarcopenia or in need of treatment if (1) his or her gait speed is less than 1.0 m/s across a 4 m course and (2) he or she has an objectively measured low muscle mass, such as, for example, an appendicular mass relative to the square of height less than or equal to 7.23 kg/m^2 for male subjects or less than or equal to 5.67 kg/m^2 for female subjects (Fielding, R. A., *et al.*, "Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences", *Journal of the American Medical Directors Association*, Vol. 12(4), pp. 249-256 (May 2011).

[39] The term "neurodegenerative disorder" means disorders which result in neurons loosing function and/or dying, in the central nervous system including the brain. Such disorders included central nervous system neurodegenerative disorders such as AD, PD, Lewy body dementia, MS, prion diseases (also known as transmissible spongiform encephalopathies (TSEs), including Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease, bovine spongiform encephalopathy ("mad cow" disease), scrapie (in sheep and goats), chronic wasting disease (in deer and elk), kuru and fatal familial insomnia), and ALS.

[40] "Neurodegenerative proteins" are proteins which accumulate in a patient having a neurodegenerative disorders and which are associated with the neurodegenerative disorder. Examples include, beta-amyloid protein plaques (associated with AD), tau protein tangles (associated with AD), mutated superoxide dismutase-1 (associated with ALS), prion protein aggregates (associated with TSEs) and alpha-synuclein protein fibrils (associated with PD and Lewy Body dementia). A "neurodegenerative protein" is the form of the protein which accumulates during the neurodegenerative disorder, typically a mutant or mis-folded form.

[41] The terms "advanced glycation end-product," "AGE," "AGE-modified protein or peptide," and "glycation end-product" 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.

[42] The term "AGE antigen" means a substance that elicits an immune response against an AGE-modified protein or peptide of a cell. The immune response against an AGE-modified protein or peptide of a cell does not include the production of antibodies to the non-AGE-modified protein or peptide.

[43] The term "AGE antibody" means an antibody specific for an AGE-modified protein or peptide of a cell.

[44] The term "senescent cell" means a cell which is in a state of irreversible proliferative arrest and expresses one or more biomarkers of senescence, such as activation of $p16^{Ink4a}$ or expression of β-galactosidase. Also included are cells which express one or more biomarkers of senescence, do not proliferate *in vivo*, but may proliferate *in vitro* under certain conditions, such as some satellite cells found in the muscles of ALS patients.

[45] The term "increasing health span" means reducing age-related phenotypes. Age-related phenotypes include, for example, sarcopenia, cataracts, loss of adipose tissue and lordokyphosis.

DETAILED DESCRIPTION

[46] The identification of a link between cellular senescence and sarcopenia allows for new treatment possibilities. For example, if the immunogenic agent of a vaccine is an AGE-modified protein or peptide, the immune system of an immunized subject may kill or induce apoptosis in cells expressing the AGE-modified protein or peptide.

[47] The present invention uses enhanced clearance of cells expressing AGE-modified proteins or peptides (AGE-modified cells) to treat or ameliorate sarcopenia. Vaccination against AGE-modified proteins or peptides of a cell produces the desired result of controlling the presence of AGE-modified cells in a subject in need thereof. The continuous and virtually ubiquitous surveillance exercised by the immune system in the body in response to a vaccination allows maintaining low levels of AGE-modified cells in the body. Vaccination against AGE-modified proteins or peptides of a cell can help remove or kill senescent cells. The process of senescent cell removal or destruction allows vaccination against AGE-modified proteins or peptides of a cell to be used to treat sarcopenia.

[48] Vaccination against AGE-modified proteins or peptides of a cell may also be used for increasing health span. Health span may be increased by reducing age-related phenotypes. The vaccine may be used, for example, to prevent or delay the onset of cataracts, lordokyphosis or loss of adipose tissue.

[49] Other diseases or disorders that are associated with cellular senescence may also be treated or ameliorated by vaccination against AGE-modified proteins or peptides of a cell. For example, the vaccine may be used to treat neurodegenerative disorders, cancer, cancer metastases or atherosclerosis.

[50] Vaccines against AGE-modified proteins or peptides contain an AGE antigen, an adjuvant, optional preservatives and optional excipients. Examples of AGE antigens include AGE-modified proteins or peptides such as AGE-antithrombin III, AGE-calmodulin, AGE-insulin, AGE-ceruloplasmin, AGE-collagen, AGE-cathepsin B, AGE-albumin, 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. Suitable AGE antigens also include proteins or peptides that exhibit AGE modifications (also referred to as AGE epitopes or AGE moieties) such as carboxymethyllysine, carboxyethyllysine, pentosidine, pyrraline, FFI, AFGP and ALI. Further details of some of these AGE-modified proteins or peptides and their preparation are described in Bucala.

[51] A particularly preferred AGE antigen is a protein or peptide that exhibits a carboxymethyllysine AGE modification. Carboxymethyllysine (also known as CML, N(epsilon)-(carboxymethyl)lysine, N(6)-carboxymethyllysine, or 2-Amino-6-(carboxymethylamino)hexanoic acid) is found on proteins or peptides and lipids as a result of oxidative stress and chemical glycation, and has been correlated with aging. CML-modified proteins or peptides are recognized by the receptor RAGE which is expressed on a variety of cells. CML has been well-studied and CML-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).

[52] AGE antigens may be conjugated to carrier proteins to enhance antibody production in a subject. Antigens that are not sufficiently immunogenic alone may require a suitable carrier protein to stimulate a response from the immune system. Examples of suitable carrier proteins include keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, cholera toxin, labile enterotoxin, silica particles and

soybean trypsin inhibitor. Preferably, the carrier protein is KLH. KLH has been extensively studied and has been identified as an effective carrier protein in experimental cancer vaccines. A preferred AGE antigen-carrier protein conjugate is CML-KLH.

[53] Adjuvants include mineral compounds such as aluminum salts, oil emulsions, bacterial products, liposomes, immunostimulating complexes and squalene. Aluminum compounds are the most widely used adjuvants in human and veterinary vaccines. These aluminum compounds include aluminum salts such as aluminum phosphate ($AlPO_4$) and aluminum hydroxide ($Al(OH)_3$) compounds, typically in the form of gels, and are generically referred to in the field of vaccine immunological adjuvants as "alum." Aluminum hydroxide is a poorly crystalline aluminum oxyhydroxide having the structure of the mineral boehmite. Aluminum phosphate is an amorphous aluminum hydroxyphosphate. Negatively charged species (for example, negatively charged antigens) can absorb onto aluminum hydroxide gels at neutral pH, whereas positively charged species (for example, positively charged antigens) can absorb onto aluminum phosphate gels at neutral pH. It is believed that these aluminum compounds provide a depot of antigen at the site of administration, thereby providing a gradual and continuous release of antigen to stimulate antibody production. Aluminum compounds tend to more effectively stimulate a cellular response mediated by Th2, rather than Th1 cells.

[54] Emulsion adjuvants include water-in-oil emulsions (for example, Freund's adjuvants, such as killed mycobacteria in oil emulsion) and oil-in-water emulsions (for example, MF-59). Emulsion adjuvants include an immunogenic component, for example squalene (MF-59) or mannide oleate (Incomplete Freund's Adjuvants), which can induce an elevated humoral response, increased T cell proliferation, cytotoxic lymphocytes and cell-mediated immunity.

[55] Liposomal or vesicular adjuvants (including paucilamellar lipid vesicles) have lipophilic bilayer domains and an aqueous milieu which can be used to encapsulate and

transport a variety of materials, for example an antigen. Paucilamellar vesicles (for example, those described in U.S. Pat. No. 6,387,373) can be prepared by mixing, under high pressure or shear conditions, a lipid phase comprising a non-phospholipid material (for example, an amphiphile surfactant; see U.S. Pat. Nos. 4,217,344; 4,917,951; and 4,911,928), optionally a sterol, and any water-immiscible oily material to be encapsulated in the vesicles (for example, an oil such as squalene oil and an oil-soluble or oil-suspended antigen); and an aqueous phase such as water, saline, buffer or any other aqueous solution used to hydrate the lipids. Liposomal or vesicular adjuvants are believed to promote contact of the antigen with immune cells, for example by fusion of the vesicle to the immune cell membrane, and preferentially stimulate the Th1 sub-population of T-helper cells.

[56] Other types of adjuvants include *Mycobacterium bovis* bacillus Calmette–Guérin (BCG), quill-saponin and unmethylated CpG dinucleotides (CpG motifs). Additional adjuvants are described in U.S. Patent Application Publication Pub. No. US 2010/0226932 (September 9, 2010) and Jiang, Z-H. *et al.* "Synthetic vaccines: the role of adjuvants in immune targeting", *Current Medicinal Chemistry*, Vol. 10(15), pp. 1423-39 (2003). Preferable adjuvants include Freund's complete adjuvant and Freund's incomplete adjuvant.

[57] The vaccine may optionally include one or more preservatives, such as antioxidants, antibacterial and antimicrobial agents, as well as combinations thereof. Examples include benzethonium chloride, ethylenediamine-tetraacetic acid sodium (EDTA), thimerosal, phenol, 2-phenoxyethanol, formaldehyde and formalin; antibacterial agents such as amphotericin B, chlortetracycline, gentamicin, neomycin, polymyxin B and streptomycin; antimicrobial surfactants such as polyoxyethylene-9, 10-nonyl phenol (Triton N-101, octoxynol-9), sodium deoxycholate and polyoxyethylated octyl phenol (Triton X-l00). The production and packaging of the vaccine may eliminate the need for a preservative. For example, a vaccine that has been sterilized and stored in a sealed container may not require a preservative.

[58] Other components of vaccines include pharmaceutically acceptable excipients, such as stabilizers, thickening agents, toxin detoxifiers, diluents, pH adjusters, tonicity adjustors, surfactants, antifoaming agents, protein stabilizers, dyes and solvents. Examples of such excipients include hydrochloric acid, phosphate buffers, sodium acetate, sodium bicarbonate, sodium borate, sodium citrate, sodium hydroxide, potassium chloride, potassium chloride, sodium chloride, polydimethylsilozone, brilliant green, phenol red (phenolsulfon-phthalein), glycine, glycerin, sorbitol, histidine, monosodium glutamate, potassium glutamate, sucrose, urea, lactose, gelatin, sorbitol, polysorbate 20, polysorbate 80 and glutaraldehyde.

[59] The vaccine may be provided in unit dosage form or in multidosage form, such as 2-100 or 2-10 doses. The unit dosages may be provided in a vial with a septum, or in a syringe with or without a needle. The vaccine may be administered intravenously, subdermally or intraperitoneally. Preferably, the vaccine is sterile.

[60] The vaccine may be administered one or more times, such as 1 to 10 times, including 2, 3, 4, 5, 6, 7, 8 or 9 times, and may be administered over a period of time ranging from 1 week to 1 year, 2-10 weeks or 2-10 months. Furthermore, booster vaccinations may be desirable, over the course of 1 year to 20 years, including 2, 5, 10 and 15 years.

[61] A subject that receives a vaccine for AGE-modified proteins or peptides of a cell may be tested to determine if he or she has developed an immunity to the AGE-modified proteins or peptides. Suitable tests may include blood tests for detecting the presence of an antibody, such as immunoassays or antibody titers. Alternatively, an immunity to AGE-modified proteins or peptides may be determined by measuring changes in muscle mass over time. For example, a baseline muscle mass in a subject may be measured followed by administration of the vaccine for AGE-modified proteins or peptides of a cell. Immunity to AGE-modified proteins or peptides may be determined by periodically measuring muscle mass in the subject and comparing the subsequent measurements to the baseline measurement. A subject may be considered

to have developed an immunity to AGE-modified proteins or peptides if he or she does not demonstrate loss of muscle mass between subsequent measurements or over time. Alternatively, the concentration and/or number of senescent cells in fat or muscle tissue may also be monitored. Vaccination and subsequent testing may be repeated until the desired therapeutic result is achieved.

[62] The vaccination process may be designed to provide immunity against multiple AGE moieties. A single AGE antigen may induce the production of AGE antibodies which are capable of binding to multiple AGE moieties. Alternatively, the vaccine may contain multiple AGE antigens. In addition, a subject may receive multiple vaccines, where each vaccine contains a different AGE antigen.

[63] Any mammal that could develop sarcopenia or other diseases or disorders associated with cellular senescence 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 disease or disorder that is known to cause elevated levels of AGEs such as, for example, diabetes (both Type 1 and Type 2), or the presence of a pathological condition associated with AGEs such as, for example, atherosclerosis, inflammation, retinopathy, nephropathy, stroke, endothelial cell dysfunction, neurodegenerative disorders or cancer. In addition, subjects may be identified for treatment based on their age. For example, a human over 75 years of age may be treated for sarcopenia, while a human under 30 years of age might not be identified as in need of treatment for sarcopenia. Alternatively, any of the mammals or subjects identified above may be excluded from the patient population in need of treatment for sarcopenia.

[64] A human subject may be identified as having sarcopenia or in need of treatment if (1) the subject is at least 25 years old and (2) his or her measured muscle mass and measured muscle function are two standard deviations or more below the mean value for healthy 25 year olds of the same gender and no alternative pathology has been

identified to account for the reduced muscle mass and reduced muscle function. Preferably, a subject being treated for sarcopenia is at least 40 years old. More preferably, a subject being treated for sarcopenia is at least 50 years old. Most preferably, a subject being treated for sarcopenia is at least 60 years old. Alternatively, a subject may be identified as having sarcopenia or in need of treatment if (1) his or her gait speed is less than 1.0 m/s across a 4 m course and (2) he or she has an objectively measured low muscle mass, such as, for example, an appendicular mass relative to the square of height less than or equal to 7.23 kg/m^2 for male subjects or less than or equal to 5.67 kg/m^2 for female subjects.

[65] Any mammal that could develop neurodegenerative disorders 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 neurodegenerative disorder.

[66] In the case of cancer, a 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. 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. Administration of antibody and subsequent testing may be repeated until the desired therapeutic result is achieved.

[67] **EXAMPLES**

[68] Example 1 (Prophetic): An AGE-RNAse containing vaccine in a human subject.

[69] AGE-RNAse is prepared by incubating RNAse in a phosphate buffer solution containing 0.1-3 M glucose, glucose-6-phosphate, fructose or ribose for 10-100 days. The AGE-RNAse solution is dialyzed and the protein content is measured. Aluminum hydroxide or aluminum phosphate, as an adjuvant, is added to 100 μg of the AGE-RNAse. Formaldehyde or formalin is added as a preservative to the preparation. Ascorbic acid is added as an antioxidant. The vaccine also includes phosphate buffer to adjust the pH and glycine as a protein stabilizer.

[70] The composition is injected into a human subject subcutaneously. The subject's muscle mass is measured at the time of injection to establish a baseline muscle mass value. The patient's muscle mass is measured again after one month. The one-month muscle mass value is compared to the baseline value. Additional injections are performed and additional muscle mass measurements are taken every month until the muscle mass measurement indicates no change, or an increase, from the baseline value.

[71] Example 2 (Prophetic): Injection regimen for an AGE-RNAse containing vaccine in a human subject.

[72] The same vaccine as described in Example 1 is injected into a human subject. The titer of antibodies to AGE-RNAse is determined by ELISA after two weeks. Additional injections are performed after three weeks and six weeks, respectively. Further titer determination is performed two weeks after each injection.

[73] Example 3 (Prophetic): An AGE-hemoglobin containing vaccine in a human subject.

[74] AGE-hemoglobin is prepared by incubating human hemoglobin in a phosphate buffer solution containing 0.1-3 M glucose, glucose-6-phosphate, fructose or ribose for 10-100 days. The AGE-hemoglobin solution is dialyzed and the protein content is measured. All vaccine components are the same as in Example 1, except AGE-hemoglobin is substituted for AGE-RNAse.

[75] Administration is carried out as in Example 1, or as in Example 2. The number of senescent cells in the subject's adipose tissue is measured at the time of injection to establish a baseline number of senescent cells. The number of senescent cells in the subject's adipose tissue is measured again two months after injection and is compared to the baseline number of senescent cells. Additional injections are performed and additional senescent cell measurements are taken every two months to determine if the number of senescent cells in adipose tissue is increasing or decreasing, or if there is no change in the number of senescent cells in adipose tissue.

[76] Example 4 (Prophetic): An AGE-human serum albumin containing vaccine in a human subject.

[77] AGE-human serum albumin is prepared by incubating human serum albumin in a phosphate buffer solution containing 0.1-3 M glucose, glucose-6-phosphate, fructose or ribose for 10-100 days. The AGE-human serum albumin solution is dialyzed and the protein content is measured. All vaccine components are the same as in Example 1, except AGE-human serum albumin is substituted for AGE-RNAse. Administration is carried out as in Example 1, or as in Example 2.

[78] Example 5: *In vivo* study of the administration of anti-AGE antibody.

[79] To examine the effects of an anti-AGE 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-AGE antibody raised against carboxymethyl lysine conjugated with keyhole limpet hemocyanin. A control reference of physiological saline was used in the control animals.

[80] 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.

[81] 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.

[82] $p16^{INK4a}$ 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 $\Delta\Delta Ct$ = ΔCt mean control Group (2) – ΔCt mean experimental Group (1 or 3 or 5); Fold Expression= $2^{-\Delta\Delta Ct}$.

[83] 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**	

[84] 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.

[85] 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 (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.

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

[87] The mass of the gastrocnemius muscle was also measured, to determine the effect of antibody administration on a classic sign of aging, sarcopenia. The results are shown 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.

[88] 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

[89] 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.

REFERENCES

[90] 1. International Application Pub. No. WO 2009/143411 to Gruber (26 Nov. 2009).

[91] 2. U.S. Patent No. 5,702,704 to Bucala (issued December 30, 1997).

[92] 3. U.S. Patent No. 6,380,165 to Al-Abed *et al.* (issued April 30, 2002).

[93] 4. U.S. Patent No. 6,387,373 to Wright *et al.* (issued May 14, 2002).

[94] 5. U.S. Patent No. 4,217,344 to Vanlerberghe *et al.* (issued August 12, 1980).

[95] 6. U.S. Patent No. 4,917,951 to Wallach (issued April 17, 1990).

[96] 7. U.S. Patent No. 4,911,928 to Wallach (issued March 27, 1990).

[97] 8. U.S. Patent Application Publication Pub. No. US 2010/226932 to Smith *et al.* (September 9, 2010).

[98] 9. Baker, D.J. *et al.*, "Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-associated disorders", Nature, Vol. 479, pp. 232-236, (2011).

[99] 10. 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).

[100] 11. Lindsey, J. B. *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).

[101] 12. 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).

[102] 13. Ahmed, E. K. *et al.*, "Protein Modification and Replicative Senescence of WI-38 Human Embryonic Fibroblasts", *Aging Cells*, Vol. 9, 252, 260 (2010).

[103] 14. 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).

[104] 15. Vlassara, H. *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. USA*, Vol. 82, 5588, 5591 (1985).

[105] 16. Roll, P. *et al.*, "Anti-CD20 Therapy in Patients with Rheumatoid Arthritis", *Arthritis & Rheumatism*, Vol. 58, No. 6, 1566-1575 (2008).

[106] 17. Kajstura, J. *et al.*, "Myocite Turnover in the Aging Human Heart", *Circ. Res.*, Vol. 107(11), 1374-86, (2010).

[107] 18. de Groot, K. *et al.*, "Vascular Endothelial Damage and Repair in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis", *Arthritis and Rheumatism*, Vol. 56(11), 3847, 3847 (2007).

[108] 19. Manesso, E. *et al.*, "Dynamics of β-Cell Turnover: Evidence for β-Cell Turnover and Regeneration from Sources of β-Cells other than β-cell Replication in the HIP Rat", *Am. J. Physiol. Endocrinol. Metab.*, Vol. 297, E323, E324 (2009).

[109] 20. Kirstein, M. *et al.*, "Receptor-specific Induction of Insulin-like Growth Factor I in Human Monocytes by Advanced Glycosylation End Product-modified Proteins", *J. Clin. Invest.*, Vol. 90, 439, 439-440 (1992).

[110] 21. Murphy, J. F., "Trends in cancer immunotherapy", *Clinical Medical Insights: Oncology,* Vol. 14(4), 67-80 (2010).

[111] 22. Flint, S. J. *et al.*, "Principles of Virology", ASM Press (2000).

[112] 23. Buskas, T. *et al.*, "Immunotherapy for Cancer: Synthetic Carbohydrate-based Vaccines", *Chem. Commun.*, Vol. 28(36), 5335-349 (2009).

[113] 24. Beier, K. C. *et al.*, "Master Switches of T-cell Differentiation", *Eur. Respir. J.*, Vol. 29, 804-12 (2007).

[114] 25. Schmidlin H. *et al.*, "New Insights in the Regulation of Human B Cell Differentiation", *Trends Immunol.*, Vol. 30(6), 277-85 (2009).

[115] 26. Vogel, F. R. *et al.*, "A compendium of vaccine adjuvants and excipients", *Pharmaceutical Biotechnology*, Vol. 6, pp. 141-228 (1995).

[116] 27. Coler, R. N. *et al.*, "Development and Characterization of Synthetic Glucopyranosyl Lipid Adjuvant System as a Vaccine Adjuvant", *PLoS ONE*, Vol. 6(1): e16333 (2011).

[117] 28. Cheadle, E. J. *et al.*, "Bugs as Drugs for Cancer", *Immunology*, Vol. 107, 10-19 (2002).

[118] 29. Jiang, Z-H. *et al.* "Synthetic vaccines: the role of adjuvants in immune targeting", *Current Medicinal Chemistry*, Vol. 10(15), pp. 1423-39 (2003).

[119] 30. Virella, G. *et al.*, "Autoimmune Response to Advanced Glycosylation End-Products of Human LDL", *Journal of Lipid Research*, Vol. 44, 487-493 (2003).

[120] 31. Ameli, S. *et al.*, "Effect of Immunization With Homologous LDL and Oxidized LDL on Early Atherosclerosis in Hypercholesterolemic Rabbits", *Arteriosclerosis, Thrombosis, and Vascular Biology*, Vol. 16, 1074 (1996).

[121] 32. "Vaccine Excipient & Media Summary", available online at www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/B/excipient-table-2.pdf (The Pink Book, Epidemiology and Prevention of Vaccine-Preventable Diseases, 12th Ed. Second Printing, September 2013).

[122] 33. "Sarcopenia", available online at en.wikipedia.org/wiki/Sarcopenia (November 14, 2014).

[123] 34. "What is sarcopenia?", available online at www.iofbonehealth.org/what-sarcopenia (2014).

[124] 35. Blahd, W., "Sarcopenia with aging", available online at www.webmd.com/healthy-aging/sarcopenia-with-aging (August 3, 2014).

[125] 36. "Keyhole limpet hemocyanin", available online at en.wikipedia.org/wiki/Keyhole_limpet_hemocyanin (April 18, 2014).

[126] 37. "CML-BSA Product Data Sheet", available online at www.cellbiolabs.com/sites/default/files/STA-314-cml-bsa.pdf (2010).

[127] 38. "CML (N-epsilon-(Carboxymethyl)Lysine) Assays and Reagents", available online at www.cellbiolabs.com/cml-assays (Accessed on December 15, 2014).

[128] 39. Cruz-Jentoft, A. J. *et al.*, "Sarcopenia: European consensus on definition and diagnosis", *Age and Ageing*, Vol. 39, pp. 412-423 (April 13, 2010).

[129] 40. Rolland, Y. *et al.*, "Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives", *J. Nutr. Health Aging*, Vol. 12(7), pp. 433-450 (2008).

[130] 41. Mera, K. *et al.*, "An autoantibody against N^{ε}-(carboxyethyl)lysine (CEL): Possible involvement in the removal of CEL-modified proteins by macrophages",

Biochemical and Biophysical Research Communications, Vol. 407, pp. 420-425 (March 12, 2011).

[131] 42. Reddy, S. *et al.*, "N^{ε}-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins", *Biochemistry*, Vol. 34, pp. 10872-10878 (August 1, 1995).

[132] 43. Naylor, R. M. *et al.*, "Senescent cells: a novel therapeutic target for aging and age-related diseases", *Clinical Pharmacology & Therapeutics*, Vol. 93(1), pp.105-116 (December 5, 2012).

[133] 44. Katcher, H. L., "Studies that shed new light on aging", *Biochemistry (Moscow)*, Vol. 78(9), pp. 1061-1070 (2013).

[134] 45. Fielding, R. A., *et al.*, "Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences", *Journal of the American Medical Directors Association*, Vol. 12(4), pp. 249-256 (May 2011).

[135] 46. Fu, M-X., et al., "The advanced glycation end product, N^{ε}-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions", The Journal of Biological Chemistry, Vol. 271, No. 17, pp. 9982-9986 (April 26, 1996).

WHAT IS CLAIMED IS:

1. A method of treating or preventing a disease or disorder associated with cellular senescence, comprising immunizing a subject in need thereof against AGE-modified proteins or peptides of a cell.

2. A method of treating a subject with a disease or disorder associated with cellular senescence, comprising:

administering a first vaccine comprising a first AGE antigen; and

administering a second vaccine comprising a second AGE antigen;

wherein the second AGE antigen is different from the first AGE antigen.

3. The method of any of the preceding claims, wherein the disease or disorder is selected from the group consisting of sarcopenia, inflammation, the onset of cataracts, the onset of loss of adipose tissue, the onset of lordokyphosis, auto-immune disorders, atherosclerosis, neurodegenerative disorders, cancer, and cancer metastases.

4. The method of any of the preceding claims, wherein the immunizing comprises administering a vaccine comprising an AGE antigen.

5. The method of any of the preceding claims, wherein the vaccine comprises

(a) the AGE antigen,

(b) an adjuvant,

(c) optionally, a preservative, and

(d) optionally, an excipient.

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

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

8. The method of any of the preceding claims, wherein the vaccine is administered in an amount effective to cause the immune system to produce antibodies to cells having AGE-modified proteins or peptides.

9. The method of any of the preceding claims, wherein the subject does not have diabetes.

10. The method of any of the preceding claims, wherein the AGE antigen is an AGE-modified protein or peptide selected from the group consisting of AGE-RNAse, AGE-human hemoglobin, AGE-human serum albumin, AGE-low density lipoprotein, AGE-collagen IV, AGE-antithrombin III, AGE-calmodulin, AGE-insulin, AGE-ceruloplasmin, AGE-collagen, AGE-cathepsin B, AGE-albumin, 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, and mixtures thereof.

11. The method of any of the preceding claims, wherein the AGE antigen comprises at least one protein or peptide that exhibits AGE modifications selected from the group consisting of carboxymethyllysine, carboxyethyllysine, pentosidine, pyrraline, FFI, AFGP, and ALI.

12. The method of any of the preceding claims, wherein the AGE antigen comprises a carboxymethyllysine-modified protein or peptide.

13. The method of any of the preceding claims, wherein
the vaccine is sterile, and
the vaccine is in unit dosage form.

14. The method of any of the preceding claims, wherein
the vaccine is sterile, and
the vaccine is in multidosage form.

15. Use of an AGE antigen for the manufacture of a medicament for treating or preventing a disease or disorder associated with cellular senescence.

16. The use of any of the preceding claims, wherein the disease or disorder is selected from the group consisting of sarcopenia, inflammation, the onset of cataracts, the onset of loss of adipose tissue, the onset of lordokyphosis, auto-immune disorders, atherosclerosis, neurodegenerative disorders, cancer, and cancer metastases.

17. The use of any of the preceding claims, wherein the medicament comprises
(a) the AGE antigen,
(b) an adjuvant,
(c) optionally, a preservative, and
(d) optionally, an excipient.

18. The use of any of the preceding claims, wherein the AGE antigen comprises a carboxymethyllysine-modified protein or peptide.

19. A composition comprising an AGE antigen for use in treating or preventing a disease or disorder associated with cellular senescence.

20. A composition comprising an AGE antigen for use in treating sarcopenia.

21. A composition comprising an AGE antigen for use in promoting tissue or organ regeneration.

22. A composition comprising an AGE antigen for use in promoting regenerative processes or overcoming aging effects.

23. A composition comprising an AGE antigen for use in treating atherosclerosis.

24. A composition comprising an AGE antigen for use in preventing or delaying the onset of cataracts.

25. A composition comprising an AGE antigen for use in preventing or delaying the onset of loss of adipose tissue.

26. A composition comprising an AGE antigen for use in preventing or delaying the onset of lordokyphosis.

27. A composition comprising an AGE antigen for use in treating inflammation or auto-immune disorders.

28. A composition comprising an AGE antigen for use in treating neurodegenerative disorders.

29. A composition comprising an AGE antigen for use in treating cancer or cancer metastases.

30. A composition comprising an AGE antigen for use in increasing health span.

31. The composition of any of the preceding claims, wherein the composition comprises a vaccine.

32. The composition of any of the preceding claims, wherein the vaccine comprises

(a) the AGE antigen,

(b) an adjuvant,

(c) optionally, a preservative, and

(d) optionally, an excipient.

33. The composition of any of the preceding claims, wherein the AGE antigen comprises a carboxymethyllysine-modified protein or peptide.

34. The composition of any of the preceding claims, wherein the composition is in unit dosage form.

35. The composition of any of the preceding claims, wherein the treating comprises administering a vaccine to a subject in an amount effective to cause the immune system to produce antibodies to cells expressing AGE-modified proteins or peptides.

36. The method of any of the preceding claims, further comprising testing the patient to determine if the disease or disorder associated with cellular senescence has been ameliorated, and

repeating the immunizing, if necessary.

37. A method of reducing the number of AGE-modified cells in a patient, comprising administering a vaccine comprising an AGE antigen.

38. The method of any of the preceding claims, further comprising testing the patient to determine if senescent cells have been killed, and

repeating the administering, if necessary.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/039076

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/44
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/135616 A1 (SIWA CORP [US]; GRUBER LEWIS [US]) 4 October 2012 (2012-10-04) paragraphs [0014] - [0024]; claims 1-21	1-38
X	MUSTSHIKA WIDA MASHITAH ET AL: "Immunization of AGE-modified albumin inhibits diabetic nephropathy progression in diabetic mice", DIABETES, METABOLIC SYNDROME AND OBESITY: TARGETS AND THERAPY, 1 August 2015 (2015-08-01), page 347, XP055319784, DOI: 10.2147/DMSO.S86332 title, abstract, Result sections	1-38

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
18 November 2016	02/12/2016

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Hongying Liu ET AL: "Abstract 154: Vaccination Using Advanced Glycation End Product of Low-Density Lipoprotein Pulsed Dendritic Cells Reduces Atherosclerosis in Diabetic Apoe-/- Mice ¦ Arteriosclerosis, Thrombosis, and Vascular Biology", Arteriosclerosis, Thrombosis, and Vascular Biology, 1 January 2012 (2012-01-01), pages 1-4, XP055319781, Retrieved from the Internet: URL:http://atvb.ahajournals.org/content/32/Suppl_1/A154 [retrieved on 2016-11-15] abstract	1-38
X	LIN ZHU ET AL: "Immunization with advanced glycation end products modified low density lipoprotein inhibits atherosclerosis progression in diabetic apoE and LDLR null mice", CARDIOVASCULAR DIABETOLOGY, BIOMED CENTRAL, LONDON, GB, vol. 13, no. 1, 13 November 2014 (2014-11-13), page 151, XP021204122, ISSN: 1475-2840, DOI: 10.1186/S12933-014-0151-6 title, abstract, result sections	1-38
X	WO 2016/044252 A2 (SIWA CORP [US]) 24 March 2016 (2016-03-24) paragraphs [0014], [0015]; claims 1-26	1-38
X	US 5 766 590 A (FOUNDS HENRY W [US] ET AL) 16 June 1998 (1998-06-16) claims 1-12	1-38
A	WAEL N. SAYEJ ET AL: "Advanced Glycation End Products Induce Obesity and Hepatosteatosis in CD-1 Wild-Type Mice", BIOMED RESEARCH INTERNATIONAL, vol. 6, no. 39, 1 January 2016 (2016-01-01), pages 41434-12, XP055318512, US ISSN: 2314-6133, DOI: 10.1155/2012/847246	1-38

-/--

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/039076

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VELANDAI SRIKANTH ET AL: "Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease", NEUROBIOLOGY OF AGING., vol. 32, no. 5, 1 May 2011 (2011-05-01), pages 763-777, XP055318514, US ISSN: 0197-4580, DOI: 10.1016/j.neurobiolaging.2009.04.016	1-38
A	DARREN J. BAKER ET AL: "Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders", NATURE, vol. 479, no. 7372, 1 November 2011 (2011-11-01), pages 232-236, XP055074325, ISSN: 0028-0836, DOI: 10.1038/nature10600	1-38
A	S DRINDA ET AL: "Identification of the advanced glycation end products Nvarepsilon-carboxymethyllysine in the synovial tissue of patients with rheumatoid arthritis", ANNALS OF THE RHEUMATIC DISEASES, vol. 61, no. 6, 1 June 2002 (2002-06-01), pages 488-492, XP055237629, GB ISSN: 0003-4967, DOI: 10.1136/ard.61.6.488	1-38
A	WO 99/14587 A1 (PICOWER INST MED RES [US]) 25 March 1999 (1999-03-25) cited in the application	1-38
T	US 2016/215043 A1 (GRUBER LEWIS S [US]) 28 July 2016 (2016-07-28)	
T	US 2016/175413 A1 (GRUBER LEWIS S [US]) 23 June 2016 (2016-06-23)	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/039076

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012135616 A1	04-10-2012	NONE	
WO 2016044252 A2	24-03-2016	NONE	
US 5766590 A	16-06-1998	US 5698197 A	16-12-1997
		US 5744318 A	28-04-1998
		US 5766590 A	16-06-1998
		ZA 9600004 B	02-10-1996
WO 9914587 A1	25-03-1999	AU 758645 B2	27-03-2003
		AU 9494098 A	05-04-1999
		CA 2303824 A1	25-03-1999
		EP 1018005 A1	12-07-2000
		JP 2001516877 A	02-10-2001
		US 2002022234 A1	21-02-2002
		WO 9914587 A1	25-03-1999
US 2016215043 A1	28-07-2016	NONE	
US 2016175413 A1	23-06-2016	NONE	