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(71) Applicant: **NEUERE, LLC** [US/US]; 155 Lambert Drive, Princeton, New Jersey 08540 (US).

(72) Inventor: **ANDERSON, Stephen**; 158 Springdale Road, Princeton, New Jersey 08540 (US).

(74) Agent: **WALES, Michele**; 11816 Centurion Way, Potomac, Maryland 20854 (US).

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(54) **Title:** AMBROXOL TO IMPROVE AND/OR EXTEND HEALTHSPAN, LIFESPAN AND/OR MENTAL ACUITY

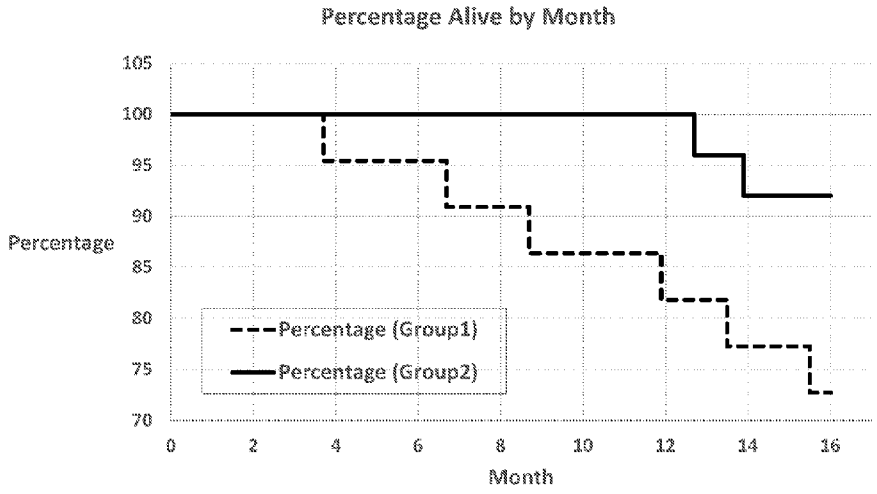

Figure 1

(57) **Abstract:** Compositions and methods for extending life expectancy are described herein. Specifically, ambroxol, ambroxol hydrochloride, and/or bromhexine can be used in a method for (a) treating, inhibiting, or reducing aging of a subject, (b) treating, inhibiting, or reducing an age-related symptom or an age-related disease in a subject, and/or (c) increasing the healthspan, lifespan, and/or mental acuity of a subject.

[Continued on next page]

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AMBROXOL TO IMPROVE AND/OR EXTEND HEALTHSPAN, LIFESPAN AND/OR MENTAL ACUITY

BACKGROUND OF THE INVENTION

Field of the Invention

[001] The invention relates to the use of ambroxol, ambroxol hydrochloride, and/or bromhexine to improve and/or extend healthspan, lifespan, and/or mental acuity in a subject. The invention further relates to inhibiting and/or reducing the aging process, including the reduction of symptoms associated with aging by using ambroxol, ambroxol hydrochloride, and/or bromhexine.

Discussion of the Related Art

[002] Growing evidence supports the linkage of metabolism and the aging process. Compared to *ad libitum* feeding, dietary restriction (DR) or calorie restriction (CR) consistently has been shown to extend lifespan and delay age-related diseases in evolutionarily diverse organisms. However, researchers still search for a "pill" that can mimic these results of dietary restriction to reduce the aging process without requiring diet changes.

[003] For example, the number of people aged 60 years and over has tripled since 1950, reaching 600 million in 2000 and surpassing 700 million in 2006. Furthermore, it is projected that the combined senior and geriatric population will reach 2.1 billion by 2050. With increased chronological age, progressive aging of organ systems also occurs. As a consequence, aged individuals have a dramatically increased risk of numerous debilitating diseases including bone fractures, cardiovascular disease, cognitive impairment, diabetes and cancer, leading to difficulty for the patient's well-being as well as stressing the health care system. Therefore, identifying ways to prevent or delay age-associated frailty and diseases is imperative for maintaining the health of our population as well as our nation's economy.

[004] Thus, need exists for a non-dietary based method of treatment for age-related symptoms and age-related diseases, such diseases including but not limited to cancer, diabetes, and cardiovascular disease thereby extending the healthspan, lifespan, and/or mental acuity of subjects.

SUMMARY OF THE INVENTION

[005] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

[006] The invention relates to compositions and methods for extending life expectancy. Specifically, ambroxol, ambroxol hydrochloride, and/or bromhexine can be used in a method for (a) treating, inhibiting, or reducing aging of a subject, (b) treating, inhibiting, or reducing an age-related symptom or an age-related disease in a subject, and/or (c) increasing the healthspan, lifespan, and/or mental acuity of a subject.

[007] Specifically, the invention relates to a method of prolonging healthspan, lifespan, and/or mental acuity of a subject, comprising administering to the subject an effective amount of ambroxol, ambroxol hydrochloride, and/or bromhexine. The invention also relates to ambroxol, ambroxol hydrochloride, and/or bromhexine, as a medicament for extending life expectancy and/or reducing aging or an age-related illness. The invention also relates to a composition for the treatment of extending life expectancy comprising a therapeutically effective amount of ambroxol, ambroxol hydrochloride, and/or bromhexine, together with a pharmaceutically acceptable excipient.

[008] In some embodiments, enantiomers, analogs, esters, amides, prodrugs, or metabolites of ambroxol and/or bromhexine, or a salt of ambroxol or bromhexine, particularly a pharmaceutically acceptable salt can be used. In preferred embodiments, bromhexine or a salt of bromhexine is used. In some embodiments, the salt of ambroxol or bromhexine is a hydrochloride.

[009] Thus, the invention relates to a method for increasing, promoting and/or improving lifespan, healthspan (e.g., healthy aging) and/or mental acuity in a subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol, ambroxol hydrochloride, and/or bromhexine. The invention further relates to treating, inhibiting, and/or reducing aging, an age-related symptom,

and/or an age-related disease in the subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol, ambroxol hydrochloride, and/or bromhexine.

[010] Examples of age-related symptoms and/or age-related diseases include, but are not limited to cardiovascular disease, a metabolic syndrome, a bone-loss disorder, a neurodegenerative disease, pre-diabetes, diabetes, obesity, osteoporosis, coronary artery disease, cerebrovascular disease, heart attack, stroke, peripheral arterial disease, aortic valve disease, stroke, mild cognitive impairment, pre-dementia, dementia, macular degeneration, and cataracts, hair thinning, hair graying, loss of mobility, loss of stamina, fatigue, increased susceptibility to infection, a metabolic change, a biochemical change, cardiac hypertrophy, heart failure, myocardial infarction, ischemia reperfusion injury, inflammatory disease, proinflammatory states, arthropathies, autoimmune diseases, and/or Alzheimer's Disease.

[011] For example, a daily dosage from about 20-500 mg/day, 50-150mg/day, 50-200mg/day, 50-250mg/day, 250-500mg/day, or 250mg-1000mg/day may be utilized. Higher or lower doses are also contemplated as it may be necessary to use dosages outside these ranges in some cases. The daily dosage may be divided, such as being divided equally into two to four times per day daily dosing.

[012] As described below, in a mouse model, it was unexpectedly found that the doses of ambroxol that improved age related symptoms were substantially lower than doses previously observed to effectively promote GCase chaperoning activity in the mouse. Thus, in preferred embodiments, long-term administration of ambroxol, bromhexine, or a salt thereof administered at a dose of approximately 50mg/day, 75mg/day, 100mg/day, 150mg/day, 200mg/day 250mg/day, 300mg/day, 350mg/day, 400mg/day, 450mg/day, 500mg/day, 550mg/day, 600mg/day, 650mg/day, 700mg/day, 750mg/day, 800mg/day, 850mg/day, 900mg/day, 950mg/day, 1000mg/day, 1050mg/day, 1100mg/day, 1150mg/day, 1200mg/day, or between 50-150mg/day, 50-200 mg/day, 50-250mg/day, 250-500 mg/day, or 250mg-1000mg/day, or less than 1000mg/day, or approximately 1mg/kg/day, 2mg/kg/day, 3mg/kg/day, 4mg/kg/day, 5mg/kg/day, 6mg/kg/day, 7mg/kg/day, 8mg/kg/day, 9mg/kg/day, 10mg/kg/day, 11mg/kg/day, 12mg/kg/day, and/or between 4-12mg/kg/day is expected to be effective in improving healthspan, lifespan, and/or mental acuity.

[013] In further embodiments, the invention includes a long-term method of inducing increasing and/or improving healthspan, lifespan, and/or mental acuity of a subject the method comprising administering a therapeutically effective amount of ambroxol, ambroxol hydrochloride, and/or bromhexine, wherein the administration of the compound is at least for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

[014] In further preferred embodiments, the lifespan, healthspan, mental acuity and/or healthy aging of the subject is extended, improved, or promoted by up to about 10%, 20%, 30%, 40%, 50%, 60%, or 70% as compared to untreated control subjects. In further preferred embodiments, the aging, the age-related symptoms, and/or the age-related diseases are treated, inhibited, and/or reduced in the subject by up to about 10%, 20%, 30%, 40%, 50%, 60%, or 70% as compared to untreated control subjects.

[015] In preferred embodiments, the subject is a mammal, and even in further preferred embodiments, the mammal is a human, a domesticated animal (e.g., a dog, a cat, a horse) or a farm animal (e.g., a cow or a pig).

BRIEF DESCRIPTION OF THE FIGURES

[016] The objects and features of the invention can be better understood with reference to the following detailed description and accompanying drawings.

[017] **Figure 1** provides animal data showing that ambroxol was able to increase lifespan in a mouse animal model. Group 1 represents control animals that were not administered ambroxol; Group 2 represents animals administered 50 mg/kg (body weight) of ambroxol daily as a chow supplement, starting at 2 months of age

[018] **Figure 2**. Mice were tested at age 7 months for novel place recognition. This is a cognitive test to measure short term working memory, involving recognition of a familiar object found in an unfamiliar place (see Magen et al (2012) *Eur. J. Neurosci.* **35**: 870-882; Magen and Chesselet (2011) *J. Parkinson's Disease* **1**: 217-227). A Discrimination Index (DI) = $(t_{novel} - t_{familiar})/(t_{novel} + t_{familiar})$ is used to assess the time spent exploring near the object in a novel place ("t_{novel}") vs. the total exploration time overall. Discrimination Index scores greater than zero are considered to indicate good place recognition memory.

[019] **Figure 3.** Effect of Ambroxol on basal macroautophagy in mouse cells. Mouse fibroblasts in culture (NIH3T3 cells) expressing the tandem reporter mCherry-

GFP-LC3 were exposed to the indicated concentrations of Ambroxol for 24 h in complete media. **Panel A**. Schematic of the autophagic compartments analyzed. **Panels B-D.** Number of autophagic vacuoles (AV) (**Panel B**); autophagosomes (APG) (**Panel C**); and autolysosomes (AUT) (**Panel D**). All values are mean +s.e.m. and quantifications were done in at least 2,500 cells per condition in three different experiments using high content microscopy. Differences with untreated (0 uM ambroxol) are significant for *p <0.05 **p <0.01 and ***p<0.001.

DETAILED DESCRIPTION

I. Definitions

[020] The following definitions are provided for specific terms which are used in the following written description.

[021] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. The term "a nucleic acid molecule" includes a plurality of nucleic acid molecules.

[022] The present invention can "comprise" (open ended) or "consist essentially of" the components of the present invention as well as other ingredients or elements described herein. As used herein, "comprising" means the elements recited, or their equivalent in structure or function, plus any other element or elements which are not recited. The terms "having" and "including" are also to be construed as open ended unless the context suggests otherwise. As used herein, "consisting essentially of' means that the invention may include ingredients in addition to those recited in the claim, but only if the additional ingredients do not materially alter the basic and novel characteristics of the claimed invention.

[023] As used herein, a "subject" is a vertebrate, preferably a mammal, more preferably a human, and even more preferably a domesticated animal, such as a pet, or a farm animal. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. In other preferred embodiments, the “subject” is a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), canine (e.g.,

a dog), feline (e.g., a cat), equine (e.g., a horse), a primate, simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), or an ape (e.g., gorilla, chimpanzee, orangutan, gibbon). In other embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g., murine, primate, porcine, canine, or rabbit animals) may be employed. In preferred embodiments, an “individual” or “patient” (as in the subject of the treatment) means mammals, particularly non-human primates, e.g. apes and monkeys, and most particularly humans. In preferred embodiments, subjects who are specifically excluded from the scope of the invention include humans suffering from a disease and/or humans (when the disease has a genetic basis) who are carriers of recessive alleles for the disease, wherein the disease is selected from a neurogenerative disease, such as, for example, Gaucher’s Disease, parkinsonism (including Parkinson’s Disease and Dementia with Lewy Bodies), Lysosomal Storage Disorders, Alzhemier’s Disease, and/or Pick’s disease.

[024] As understood herein, an "effective amount" of a pharmaceutical composition of the instant invention refers to an amount of the composition suitable to elicit a therapeutically beneficial response in the subject, e.g., extend and/or increase and/or improve healthspan, lifespan, and/or mental acuity, such as for example, increasing survival and/or healthy aging and/or to decrease morbidity or age-related illness in the subject.

[025] The term "dose" or "dosage" as used herein refers to physically discrete units suitable for administration to a subject, each dosage containing a predetermined quantity of the active pharmaceutical ingredient calculated to produce a desired response.

[026] The term "about" or "approximately" means within an acceptable range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2 fold, of a value. Unless otherwise stated, the term 'about' means within an acceptable error range for the particular value, such as ± 1-20%, preferably ± 1-10% and more

preferably ±1-5%. In even further embodiments, "about" should be understood to mean+/-5%.

[027] Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[028] All percentages and ratios used herein are by weight of the total composition unless otherwise indicated herein. All temperatures are in degrees Celsius unless specified otherwise. All measurements made are at 25°C and normal pressure unless otherwise designated.

[029] All ranges recited herein include the endpoints, including those that recite a range "between" two values. Terms such as "about," "generally," "substantially," "approximately" and the like are to be construed as modifying a term or value such that it is not an absolute, but does not read on the prior art. Such terms will be defined by the circumstances and the terms that they modify as those terms are understood by those of skill in the art. This includes, at very least, the degree of expected experimental error, technique error and instrument error for a given technique used to measure a value.

[030] Where used herein, the term "and/or" when used in a list of two or more items means that any one of the listed characteristics can be present, or any combination of two or more of the listed characteristics can be present. For example, if a composition of the instant invention is described as containing characteristics A, B, and/or C, the composition can contain A feature alone; B alone; C alone; A and B in combination; A and C in combination; B and C in combination; or A, B, and C in combination.

[031] The term “isolated compound” means a compound substantially free of contaminants or cell components with which the compound naturally occurs, or the reagents used in synthesis or the byproducts of synthesis. “Isolated” and “substantially free of contaminants” does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the compound in a form in which it can be used therapeutically.

[032] A "derivative" compound, as the term is used herein, refers to a second compound that is derived from a first compound, such as a brominated version of a non-brominated parent compound. For example, ambroxol is a derivative of bromhexine.

[033] As used herein, "lifespan" means the time until death.

[034] As used herein " healthspan" or "healthy aging" means the time of life living free (or optimally free) of serious disease.

[035] As used herein, "mental acuity" is a measure of a subject's cognitive abilities, such as ability to focus, attention span, and sharpness.

[036] As used herein, "nutrient sensing" is a cell's ability to sense and respond to fluctuations in nutrient levels as is described in Efeyan et al., "*Nutrient Sensing Mechanisms and Pathways*," Nature 517: 302–310 (2015) (hereby incorporated by reference in its entirety).

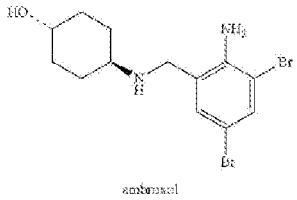
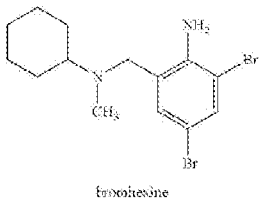
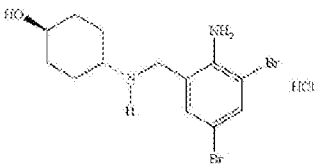
[037] As used herein "treating, inhibiting, and/or reducing aging, an age-related symptom, and/or an age-related disease" or the like means reducing the risk of occurrence, delaying the onset, slowing the progression, and/or reducing the severity and/or manifestation, of a sign of aging and/or degenerative disorder, and includes, but is not limited to, preventing the occurrence, development or progression of a sign of aging and/or degenerative disorder.

Compounds of the Invention

[038] Ambroxol, ambroxol hydrochloride, and/or bromhexine can be used as described herein. Table 1 shows the structures of these compounds. These compounds may be used to provide a long-term treatment of a subject to increase healthspan, lifespan, and/or mental acuity, such as by prolonging survival and/or reducing morbidity and/or age-related illnesses.

[039] Ambroxol, also known by its chemical name trans-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol, has the structure as shown in Table 1. An aspect of the invention also includes a medicament to increase and/or improve healthspan, lifespan, and/or mental acuity, comprising a pharmaceutically acceptable salt of ambroxol. In one embodiment, the salt is a hydrochloride salt. Bromhexine, also known by its chemical name 2-amino-3,5-dibromo-N-cyclohexyl-N-methylbenzenemethanamine, has the structure as shown in Table 1. Thus, another aspect

of the invention also includes a medicament to increase and/or improve healthspan, lifespan, and/or mental acuity, comprising a pharmaceutically acceptable salt of bromhexine. In one embodiment, the salt is a hydrochloride salt. Another aspect of the invention includes a pharmaceutically acceptable salt of a ambroxol and/or bromhexine.

TABLE 1	
Ambroxol	
Bromhexine	
Ambroxol Hydrochloride	

Preparation of Compounds of the Invention

[040] The compounds of the invention are known and may be prepared by methods known to the person skilled in the art of organic synthesis. For example, U.S. Patent Application publication number US2004/0242700, incorporated herein by reference in its entirety, provides a synthetic protocol for the preparation of ambroxol.

Salts of Compounds of the Invention

[041] For compounds that typically contain acidic or basic groups (such as carboxyl or amino groups) such groups will not necessarily be in the free base form. When referring to compounds of the invention, the reference is intended to include salt forms

of the compound. Within the scope of the invention, therefore, are salts of the active agent, especially salts of ambroxol and bromhexine. The preferred salts are pharmaceutically-acceptable salts. Also within the scope of the invention are salts of derivatives of ambroxol.

[042] The term "salts" embraces addition salts of free acids or free bases. The term "pharmaceutically-acceptable salt" refers to salts which possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for example utility in process of synthesis, purification or formulation of therapeutic compounds.

[043] Suitable pharmaceutically-acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and salfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β-hydroxybutyric, salicylic, galactaric, oxalic, malonic and galacturonic acid. Examples of pharmaceutically unacceptable acid addition salts include, for example, perchlorates and tetrafluoroborates. All of these acid addition salts may be prepared from ambroxol or bromhexine by reacting, for example, the appropriate acid with the compound.

[044] Suitable pharmaceutically acceptable base addition salts of ambroxol or bromhexine include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N′-

dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, m eglumine(N-methyl glucamine) and procaine. Examples of pharmaceutically unacceptable base addition salts include lithium salts and cyanate salts. All of these base addition salts may be prepared from ambroxol or bromhexine by reacting, for example, the appropriate base with the compound.

Pharmaceutical Compositions

[045] In an aspect, the invention includes a composition comprising a therapeutically effective amount of ambroxol or bromhexine or a pharmaceutically acceptable salt thereof, in conjunction with a pharmaceutically acceptable excipient for treatment of an individual to increase and/or improve healthspan, lifespan, and/or mental acuity, and/or to treat, inhibit, and/or reduce aging, age-related symptoms, and/or the age-related diseases. In another aspect, the invention includes a composition comprising a therapeutically effective amount of ambroxol or bromhexine or pharmaceutically acceptable salts thereof in conjunction with a pharmaceutically acceptable excipient to increase and/or improve healthspan, lifespan, and/or mental acuity, and/or to treat, inhibit, and/or reduce aging, age-related symptoms, and/or the age-related diseases in an individual. In another aspect, the invention includes a composition comprising a therapeutically effective amount of a derivative of ambroxol, including, but not limited to an enantiomer, analog, ester, amide, prodrug, or metabolite of ambroxol, or a pharmaceutically acceptable salt thereof, in conjunction with a pharmaceutically acceptable excipient for treatment of an individual to increase and/or improve healthspan, lifespan, and/or mental acuity, and/or to treat, inhibit, and/or reduce aging, age-related symptoms, and/or the age-related diseases.

[046] The active agent (e.g., ambroxol or bromhexine or a pharmaceutically acceptable salt thereof) may be administered in the form of a pharmaceutical composition, in combination with a pharmaceutically acceptable carrier. The active ingredient in such formulations may comprise from 0.1 to 99.99 weight percent. “Pharmaceutically acceptable carrier” means any carrier, diluent or excipient which is compatible with the other ingredients of the formulation and not deleterious to the recipient.

[047] The active agent is preferably administered with a pharmaceutically acceptable carrier selected on the basis of the selected route of administration and standard pharmaceutical practice. The active agent may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See Alphonso Gennaro, ed., Remington's Pharmaceutical Sciences, 18th Edition (1990), Mack Publishing Co., Easton, Pa. Suitable dosage forms may comprise, for example, tablets, capsules, solutions, parenteral solutions, troches, suppositories, or suspensions. For examples of the preparation of oral, topical, suppository and parenteral formulations of ambroxol, bromhexine, or other ambroxol derivatives, is disclosed in, for example, Examples 1-8 of WO2005/007146, or its equivalent US2005/00148747, incorporated herein by reference.

[048] In another embodiment, ambroxol or bromhexine or a salt thereof is used in the preparation of a medicament to increase and/or improve healthspan, lifespan, and/or mental acuity, and/or to treat, inhibit, and/or reduce aging, age-related symptoms, and/or the age-related diseases.

[049] For parenteral administration, the active agent may be mixed with a suitable carrier or diluent such as water, an oil (particularly a vegetable oil), ethanol, saline solution, aqueous dextrose (glucose) and related sugar solutions, glycerol, or a glycol such as propylene glycol or polyethylene glycol, or with a plant extract as a supplement. Solutions for parenteral administration preferably contain a water-soluble salt of the active agent. Stabilizing agents, antioxidant agents and preservatives may also be added. Suitable antioxidant agents include sulfite, ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol. The composition for parenteral administration may take the form of an aqueous or non-aqueous solution, dispersion, suspension or emulsion.

[050] For oral administration, the active agent may be combined with one or more solid inactive ingredients for the preparation of tablets, capsules, pills, powders, granules or other suitable oral dosage forms. For example, the active agent may be combined with at least one excipient such as fillers, binders, humectants, disintegrating agents, solution retarders, absorption accelerators, wetting agents, absorbents or lubricating agents. According to one tablet embodiment, the active agent may be combined

with carboxymethylcellulose calcium, magnesium stearate, mannitol and starch, and then formed into tablets by conventional tableting methods.

[051] The compositions are preferably formulated in a unit dosage form, each dosage containing from about 50 to about 1000 mg, more typically, about 250 to about 500 mg of active agent per unit dosage. The term "unit dosage form" refers to physically discrete units suitable as a unitary dosage for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

[052] In further preferred embodiments, ambroxol or bromhexine or its salt thereof is administered as several doses over a given period of time, e.g., a daily dose for a week or more. For example, a daily dosage from about 20-500 mg/day, 50-150mg/day, 50-200mg/day, 50-250mg/day, 250-500mg/day, or 250mg-1000mg/day may be utilized. Higher or lower doses are also contemplated as it may be necessary to use dosages outside these ranges in some cases. The daily dosage may be divided, such as being divided equally into two to four times per day daily dosing.

[053] In mouse models, it was unexpectedly found that the doses of ambroxol that improved age related symptoms were substantially lower than doses previously observed to effectively promote GCase chaperoning activity. Thus, in preferred embodiments, long-term administration of ambroxol or bromhexine or a salt thereof administered at a dose of approximately 50mg/day, 75mg/day, 100mg/day, 150mg/day, 200mg/day 250mg/day, 300mg/day, 350mg/day, 400mg/day, 450mg/day, 500mg/day, 550mg/day, 600mg/day, 650mg/day, 700mg/day, 750mg/day, 800mg/day, 850mg/day, 900mg/day, 950mg/day, 1000mg/day, 1050mg/day, 1100mg/day, 1150mg/day, 1200mg/day, or between 50-150mg/day, 50-200 mg/day, 50-250mg/day, 250-500 mg/day, or 250mg-1000mg/day, or less than 1000mg/day, or approximately 1mg/kg/day, 2mg/kg/day, 3mg/kg/day, 4mg/kg/day, 5mg/kg/day, 6mg/kg/day, 7mg/kg/day, 8mg/kg/day, 9mg/kg/day, 10mg/kg/day, 11mg/kg/day, 12mg/kg/day, and/or between 4-12mg/kg/day is expected to be effective in improving healthspan, lifespan, and/or mental acuity.

[054] In further embodiments, the invention includes a long-term method of inducing increasing and/or improving healthspan, lifespan, and/or mental acuity of a subject, and/or to treat, inhibit, and/or reduce aging, age-related symptoms, and/or the

age-related diseases, preferably in a human, the method comprising administering a therapeutically effective amount of ambroxol, bromhexine, or a salt thereof, wherein the administration of the compound is at least for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

[055] In further preferred embodiments, the lifespan, healthspan, mental acuity and/or healthy aging of the subject is extended, improved, or promoted by up to about 10%, 20%, 30%, 40%, 50%, 60%, or 70% as compared to untreated control subjects. In further preferred embodiments, the aging, the age-related symptoms, and/or the age-related diseases are treated, inhibited, and/or reduced in the subject by up to about 10%, 20%, 30%, 40%, 50%, 60%, or 70% as compared to untreated control subjects.

[056] The pharmaceutical compositions of the present invention may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydropropylmethylcellulose in varying proportions to provide the desired release profile, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes and/or microspheres.

[057] In general, a controlled-release preparation is a pharmaceutical composition capable of releasing the active ingredient at the required rate to maintain constant pharmacological activity for a desirable period of time. Such dosage forms provide a supply of a drug to the body during a predetermined period of time and thus maintain drug levels in the therapeutic range for longer periods of time than conventional non-controlled formulations.

[058] U.S. Pat. No. 5,674,533 discloses controlled-release pharmaceutical compositions in liquid dosage forms for the administration of moguisteine, a potent peripheral antitussive. U.S. Pat. No. 5,059,595 describes the controlled-release of active agents by the use of a gastro-resistant tablet for the therapy of organic mental disturbances. U.S. Pat. No. 5,591,767 describes a liquid reservoir transdermal patch for the controlled administration of ketorolac, a non-steroidal anti-inflammatory agent with potent analgesic properties. U.S. Pat. No. 5,120,548 discloses a controlled-release drug delivery device comprised of swellable polymers. U.S. Pat. No. 5,073,543 describes controlled-release formulations containing a trophic factor entrapped by a ganglioside-liposome vehicle. U.S. Pat. No. 5,639,476 discloses a stable solid controlled-release formulation having a coating derived from an aqueous dispersion of a hydrophobic acrylic polymer. Biodegradable microparticles are known for use in controlled-release

formulations. U.S. Pat. No. 5,354,566 discloses a controlled-release powder that contains the active ingredient. U.S. Pat. No. 5,733,566, describes the use of polymeric microparticles that release antiparasitic compositions.

[059] The controlled-release of the active ingredient may be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds. Various mechanisms of drug release exist. For example, in one embodiment, the controlled-release component may swell and form porous openings large enough to release the active ingredient after administration to a patient. The term "controlled-release component" in the context of the present invention is defined herein as a compound or compounds, such as polymers, polymer matrices, gels, permeable membranes, liposomes and/or microspheres, that facilitate the controlled-release of the active ingredient in the pharmaceutical composition. In another embodiment, the controlled-release component is biodegradable, induced by exposure to the aqueous environment, pH, temperature, or enzymes in the body. In another embodiment, sol-gels may be used, wherein the active ingredient is incorporated into a sol-gel matrix that is a solid at room temperature. This matrix is implanted into a patient, preferably a mammal, having a body temperature high enough to induce gel formation of the sol-gel matrix, thereby releasing the active ingredient into the patient.

[060] Compositions of the compounds of the invention that are suitable for administration intranasally or by inhalation are of particular interest.

[061] The compounds of the invention can be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, for example, in a dry blend with lactose in anhydrous or monohydrate form, preferably monohydrate, mannitol, dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose or trehalose, or as a mixed component particle, for example, mixed with phospholipids) from a dry powder inhaler or as an aerosol spray from a pressurized container, pump, spray, atomizer (preferably an atomizer using electrohydrodynamics to produce a fine mist), or nebulae, with or without the use of a suitable propellant, such as dichlorofluoromethane.

[062] The pressurized container, pump, spray, atomizer, or nebulae contains a solution or suspension of the active compound comprising, for example, ethanol (optionally, aqueous ethanol) or a suitable alternative agent for

dispersing, solubilizing, or extending release of the active, the propellant(s) as solvent and an optional surfactant, such as sorbitan trioleate or an oligolactic acid.

[063] Prior to use in a dry powder or suspension formulation, the drug product is micronized to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying.

[064] A suitable solution formulation for use in an atomizer using electrohydrodynamics to produce a fine mist may contain from 1 μg to 20 mg of the compound of the invention per actuation and the actuation volume may vary from 1 μL to 100 μL. A typical formulation may comprise the compound of the invention, propylene glycol, sterile water, ethanol and sodium chloride. Alternative solvents which may be used instead of propylene glycol include glycerol and polyethylene glycol. Capsules, blisters and cartridges (made, for example, from gelatin or HPMC) for use in an inhaler or insufflator may be formulated to contain a powder mix of ambroxol or bromhexine or a pharmaceutically acceptable salt thereof, a suitable powder base such as lactose or starch and a performance modifier such as L-leucine, mannitol, or magnesium stearate.

[065] Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled dual-, targeted- and programmed-release formulations. Sustained or controlled release can be obtained by using, for example, poly(D,L-lactic-co-glycolic acid).

Administration of Compounds of the Invention

[066] In a preferred embodiment, the compounds of the invention are administered orally to a patient. However, the compounds may be administered by any route, including by rectal, pulmonary, sublingual, and parenteral administration. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, intravaginal, intravesical (e.g., to the bladder), intradermal, transdermal, topical or subcutaneous administration.

[067] The dosing interval may be once a week, twice a week, every-other-day, once per day, typically once, twice, three times or four times per day with the doses given at

equal intervals throughout the day and night in order to maintain a constant presence of the drug. However, the skilled artisan will be aware that a treatment schedule can be optimized for any given patient, and that administration of compound may occur less frequently than once per day. The treatment may be carried out for as long a period as necessary.

[068] The specific dose of a compound according to the invention to obtain therapeutic benefit to increase and/or improve healthspan, lifespan, and/or mental acuity, and/or to treat, inhibit, and/or reduce aging, age-related symptoms, and/or the age-related diseases will, of course, be determined by the particular circumstances of the individual patient including the size, weight, age and sex of the patient, and the route of administration of the compound.

[069] For example, a daily dosage from about 20-500 mg/day, 50-150mg/day, 50-200mg/day, 50-250mg/day, 250-500mg/day, or 250mg-1000mg/day may be utilized. Higher or lower doses are also contemplated as it may be necessary to use dosages outside these ranges in some cases. The daily dosage may be divided, such as being divided equally into two to four times per day daily dosing.

[070] In mouse models, it was unexpectedly found that the predicted doses of ambroxol to improve age related symptoms as described herein occurred at doses substantially lower than doses previously observed to effectively promote GCase chaperoning activity. Thus, in preferred embodiments, long-term administration of ambroxol or bromhexine or a salt thereof administered at a dose of approximately 50mg/day, 75mg/day, 100mg/day, 150mg/day, 200mg/day 250mg/day, 300mg/day, 350mg/day, 400mg/day, 450mg/day, 500mg/day, 550mg/day, 600mg/day, 650mg/day, 700mg/day, 750mg/day, 800mg/day, 850mg/day, 900mg/day, 950mg/day, 1000mg/day, 1050mg/day, 1100mg/day, 1150mg/day, 1200mg/day, or between 50-150mg/day, 50-200 mg/day, 50-250mg/day, 250-500 mg/day, or 250mg-1000mg/day, or less than 1000mg/day, or approximately 1mg/kg/day, 2mg/kg/day, 3mg/kg/day, 4mg/kg/day, 5mg/kg/day, 6mg/kg/day, 7mg/kg/day, 8mg/kg/day, 9mg/kg/day, 10mg/kg/day, 11mg/kg/day, 12mg/kg/day, and/or between 4-12mg/kg/day is expected to be effective in improving healthspan, lifespan, and/or mental acuity.

[071] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[072] In accordance with the present invention, as described above or as discussed in the Examples below, there can be employed conventional clinical, chemical, cellular, histochemical, biochemical, molecular biology, microbiology and recombinant DNA techniques which are known to those of skill in the art. Such techniques are explained fully in the literature.

[073] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative example, make and utilize the compounds of the present invention and practice the claimed methods. The following working example therefore, specifically points out the preferred embodiments of the present invention, and is not to be construed as limiting in any way the remainder of the disclosure. Although the invention herein has been described with reference to embodiments, it is to be understood that these embodiments, and examples provided herein, are merely illustrative of the principles and applications of the present invention. It is therefore to be understood that numerous modifications can be made to the illustrative embodiments and examples, and that other arrangements can be devised without departing from the spirit and scope of the present invention as defined by the appended claims. All patent applications, patents, literature and references cited herein are hereby incorporated by reference in their entirety.

EXAMPLE

[074] The invention will now be further illustrated with reference to the following examples. It will be appreciated that what follows is by way of example only and that modifications to detail may be made while still falling within the scope of the invention.

Example 1: Animal Model Showing Ambroxol's Activity in Improving Lifespan

[075] Weaned male BDF1 mice were raised from pups, housed 1-4 animals per cage and fed Teklad 7013 NIH-31 rodent chow and water ad libitum. At 2 months of age, the mice were divided into two roughly equal groups of 22-25 animals. One group continued to be fed the Teklad chow as before (control); the other group was switched to Teklad chow formulated with ambroxol at 300 mg per kg of chow. This formulation was designed, based on the average *ad libitum* chow consumption of an adult male BDF1 mouse, to deliver 50 mg/kg/day (based on body mass) to each mouse in the treated group. Mice were maintained on this diet until they died naturally or until they reached 16 months of age, at which time the experiment ended. During the course of the experiment all mice were periodically removed from their cages and handled in the course of subjecting them to various sensorimotor, cognitive, and/or behavioral tests.

[076] **Figure 1** provides animal data showing that ambroxol was able to increase lifespan in a mouse animal model. Group 1 represents control animals that were not administered ambroxol; Group 2 represents animals administered 50 mg/kg of ambroxol daily as a chow supplement, starting at 2 months of age.

[077] Preliminary data also suggests that not only did ambroxol increase lifespan, but that when sacrificed, surviving animals of Group 2 appeared at least as healthy, on average, as did the Group 1 animals, indicating that healthspan was extended in parallel with lifespan.

Example 2: Animal Model Showing Ambroxol's Activity in Improving Cognitive Function

[078] In this experiment, three groups of mice, with at least 13 mice in each group, were given a cognitive acuity test at 7 months. A dose-related improvement in cognitive acuity was observed, with the "High Dose" Group (150 mg/kg/day) showing the best cognitive acuity results, the "Low Dose" (50 mg/kg/day) Group showing results in between the Control Group and the "High Dose" Group of animals, and the Control Group (no ambroxol) showing the base line cognitive acuity results.

[079] Moreover, these dosage results are unexpected and surprising as they are substantially lower than doses previously observed to effectively promote GCase

chaperoning activity in mice. See, e.g., Migdalska-Richards et al (2016) *Ann. Neurol.* **80**: 766-775.

Example 3: Ambroxol Induces Macroautophagy in Mouse Cells in Culture

[080]

Figure 3 shows the effect of ambroxol on basal macroautophagy in mouse cells. Mouse fibroblasts (NIH3T3) were obtained from the American Type Culture Collection (ATCC). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St.Louis, MO) in the presence of 10% newborn calf serum (NCS), 50μg/ml penicillin, and 50μg/ml streptomycin at 37 °C with 5% CO2. Cells plated in glass-bottom 96-well plates were treated for the indicated time and after fixation images were acquired using a high-content microscope (Operetta,PerkinElmer). Images of 9 different fields per well were captured, resulting in an average of 2,500-3,000 cells. Nuclei and puncta were identified using the manufacturer's software. The number of particles/puncta per cell was quantified using the `particle identifier' function in the cytosolic region after thresholding in non-saturated images. In all cases, focal plane thickness was set at 0.17μm and sections with maximal nucleus diameter were selected for quantification. Values are presented as number of puncta per cell section that in acquisition conditions represents 10-20% of the total puncta per cell. Macroautophagy activity in intact cells was measured upon transduction with lentivirus carrying the mCherry-GFP-LC3 tandem construct. Kimura S., Noda. T, & Yoshimori T "*Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3,*" Autophagy 3(5):452-460 (2007). Cells were plated on glass-bottom 96 well plates and fluorescence was read in both channels. Puncta positive for both fluorophores correspond to autophagosomes, whereas those positive for only the red fluorophore correspond to autolysosomes. Autophagic flux was determined as the conversion of autophagosomes (yellow) to autolysosomes (red only puncta).

[081] Without being bound by theory, ambroxol may, apart from its pharmacological activity as a GCase chaperone, be inhibiting nutrient sensing. By interfering with nutrient sensing, ambroxol may be triggering a response by the cells of the animal appropriate to the organism entering a nutrient-limited or fasting state, thus

sending the organism into a "catabolic signaling" mode characterized by transcription factor EB (TFEB) dephosphorylation, CLEAR network transcription, lysosome biogenesis, and autophagy induction, leading to improved lifespan, healthspan and/or cognitive acuity. *See, e.g.,* Efeyan et al., "*Nutrient Sensing Mechanisms and Pathways*," Nature 517: 302–310 (2015) (hereby incorporated by reference in its entirety). Thus, ambroxol, ambroxol hydrochloride, and/or bromhexine (including the low doses as described below), may systemically inhibit nutrient sensing resulting in the whole organism entering catabolic signaling mode.

[082] The human equivalent doses (HEDs), calculated from the "low" and "high" mouse doses of this study, are approximately 4mg/kg/day and 12mg/kg/day, which is approximately 250 mg/day or 750 mg/day for an average 62.5 kg human. Thus, in preferred embodiments, long-term administration of ambroxol or bromhexine or a salt thereof administered at a dose of approximately 50mg/day, 75mg/day, 100mg/day, 150mg/day, 200mg/day 250mg/day, 300mg/day, 350mg/day, 400mg/day, 450mg/day, 500mg/day, 550mg/day, 600mg/day, 650mg/day, 700mg/day, 750mg/day, 800mg/day, 850mg/day, 900mg/day, 950mg/day, 1000mg/day, 1050mg/day, 1100mg/day, 1150mg/day, 1200mg/day, or between 50-150mg/day, 50-200 mg/day, 50-250mg/day, 250-500 mg/day, or 250mg-1000mg/day, or less than 1000mg/day, or approximately 1mg/kg/day, 2mg/kg/day, 3mg/kg/day, 4mg/kg/day, 5mg/kg/day, 6mg/kg/day, 7mg/kg/day, 8mg/kg/day, 9mg/kg/day, 10mg/kg/day, 11mg/kg/day, 12mg/kg/day, and/or between 4-12mg/kg/day is expected to be effective in improving healthspan, lifespan, and/or mental acuity.

[083] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

[084] While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is Claimed

1. A method for increasing and/or improving lifespan, healthspan, and/or cognitive acuity in a subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof.

2. A method for treating, inhibiting, and/or reducing aging, an age-related symptom, and/or an age-related disease in the subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof.

3. The method of claim 2, wherein the age-related symptom and/or the age-related disease is selected from a cardiovascular disease, a metabolic syndrome, a bone-loss disorder, a neurodegenerative disease, pre-diabetes, diabetes, obesity, osteoporosis, coronary artery disease, cerebrovascular disease, heart attack, stroke, peripheral arterial disease, aortic valve disease, stroke, mild cognitive impairment, pre-dementia, dementia, macular degeneration, and cataracts, hair thinning, hair graying, loss of mobility, loss of stamina, fatigue, increased susceptibility to infection, a metabolic change, a biochemical change, cardiac hypertrophy, heart failure, myocardial infarction, ischemia reperfusion injury, inflammatory disease, proinflammatory states, arthropathies, autoimmune diseases, and/or Alzheimer's Disease.

4. The method of any one of claims 1-3, wherein the method promotes healthy aging in a subject.

5. The method of any one of claims 1-4, wherein the ambroxol salt is ambroxol hydrocholoride.

6. The method of any one of claims 1-5, wherein ambroxol or bromhexine or salt thereof is administered as several doses over a given period of time, e.g., a daily dose for a week or more.

7. The method of claim 6, wherein ambroxol or bromhexine or salt thereof is administered as for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

8. The method of any one of claims 1-7, wherein ambroxol or bromhexine or salt thereof is administered as a daily dose of about 20-500 mg/day, 50-150mg/day, 50-200mg/day, 50-250mg/day, 250-500mg/day, or 250mg-1000mg/day.

9. The method of any one of claims 1-8, wherein ambroxol or bromhexine or salt thereof is administered as a daily dose selected from:

(a) approximately 50mg/day, 75mg/day, 100mg/day, 150mg/day, 200mg/day 250mg/day, 300mg/day, 350mg/day, 400mg/day, 450mg/day, 500mg/day, 550mg/day, 600mg/day, 650mg/day, 700mg/day, 750mg/day, 800mg/day, 850mg/day, 900mg/day, 950mg/day, 1000mg/day, 1050mg/day, 1100mg/day, 1150mg/day, or 1200mg/day;

(b) between 50-150mg/day, 50-200 mg/day, 50-250mg/day, 250-500 mg/day or 250mg-1000mg/day;

(c) less than 1000mg/day;

(d) approximately 1mg/kg/day, 2mg/kg/day, 3mg/kg/day, 4mg/kg/day, 5mg/kg/day, 6mg/kg/day, 7mg/kg/day, 8mg/kg/day, 9mg/kg/day, 10mg/kg/day, 11mg/kg/day, or 12mg/kg/day; or

(e) between 4-12mg/kg/day.

10. The method according to any one of claims 1-9, wherein the lifespan of the subject is extended by up to about 10%, 20%, 30%, 40%, 50%, 60%, or 70% as compared to untreated control subjects.

11. The method of any one of claims 1-10, wherein the subject is mammalian, preferably human or a pet.

12. The method of any one of claims 1-11, wherein ambroxol or bromhexine or salt thereof inhibits nutrient sensing.

13. The method of any one of claims 1-12, wherein ambroxol or bromhexine or salt thereof induces autophagy.

14. The method of any one of claims 1-13, wherein the subject is not a human suffering from a disease and/or a human, when the disease has a genetic basis, who is a carrier of a recessive alleles for the disease, wherein the disease is selected from a neurogenerative disease.

15. The method of claim 14, wherein the neurogenerative disease is selected from Gaucher's Disease, parkinsonism (including Parkinson's Disease and Dementia with Lewy Bodies), Lysosomal Storage Disorders, Alzhemier's Disease, and/or Pick's disease.

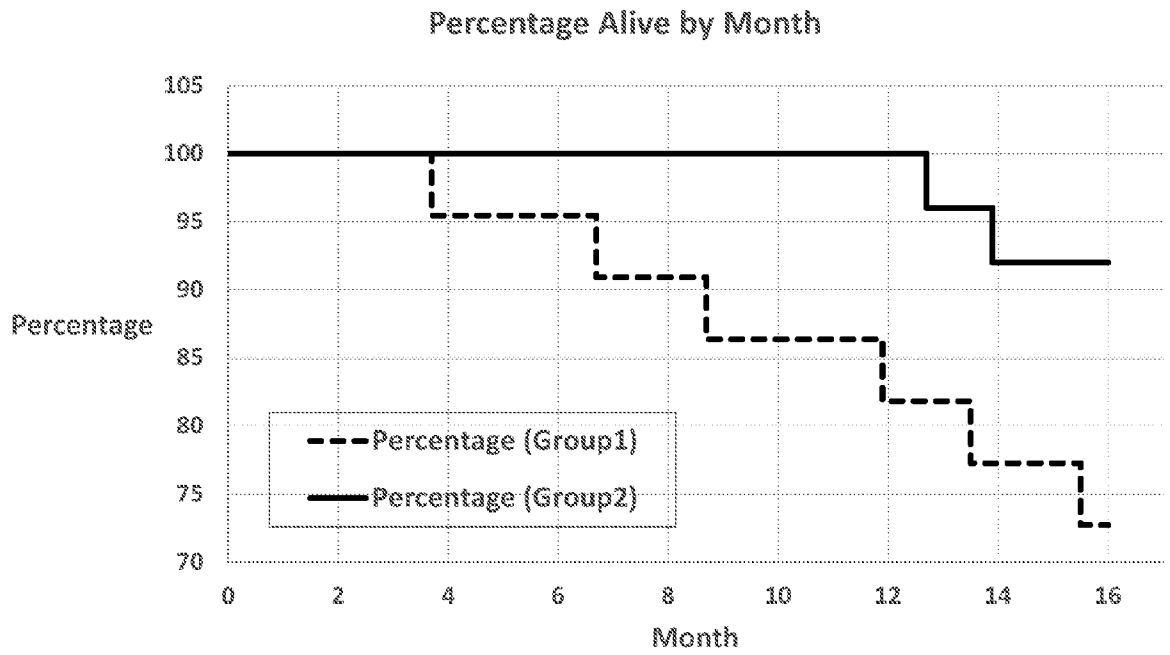
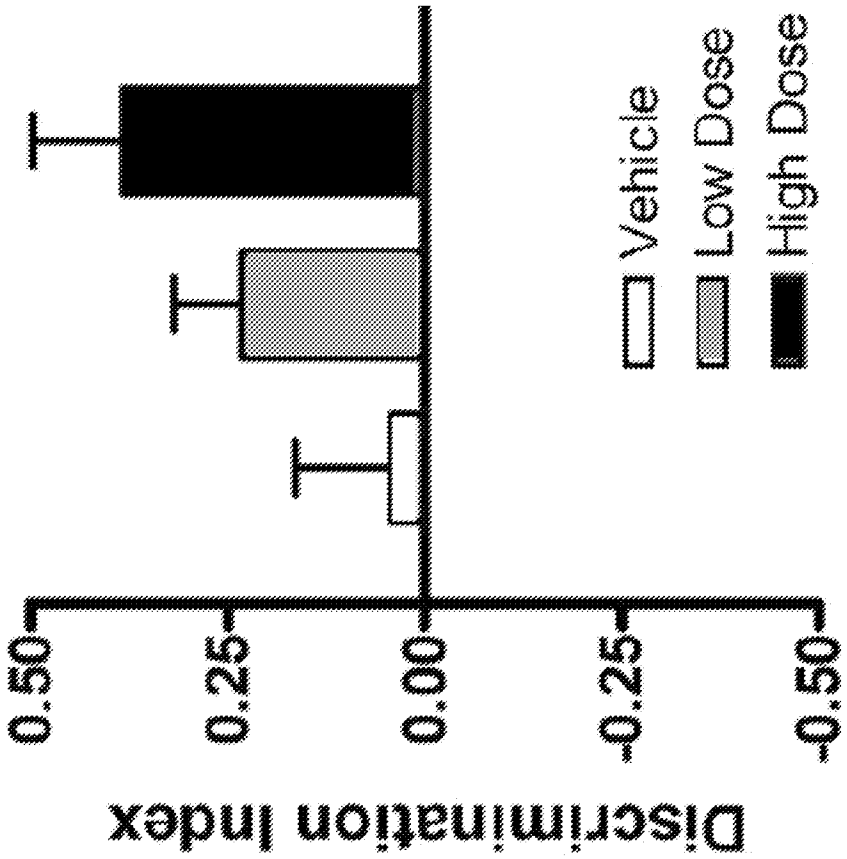

Percentage Alive by Month
105
100
95
90
85
80
75
70
Percentage
0
2
4
6
8
10
12
14
16
Month
Percentage (Group1)
Percentage (Group2)

Figure 1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0.25
0.00
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-0.50
Vehicle
Low Dose
High Dose

Figure 2

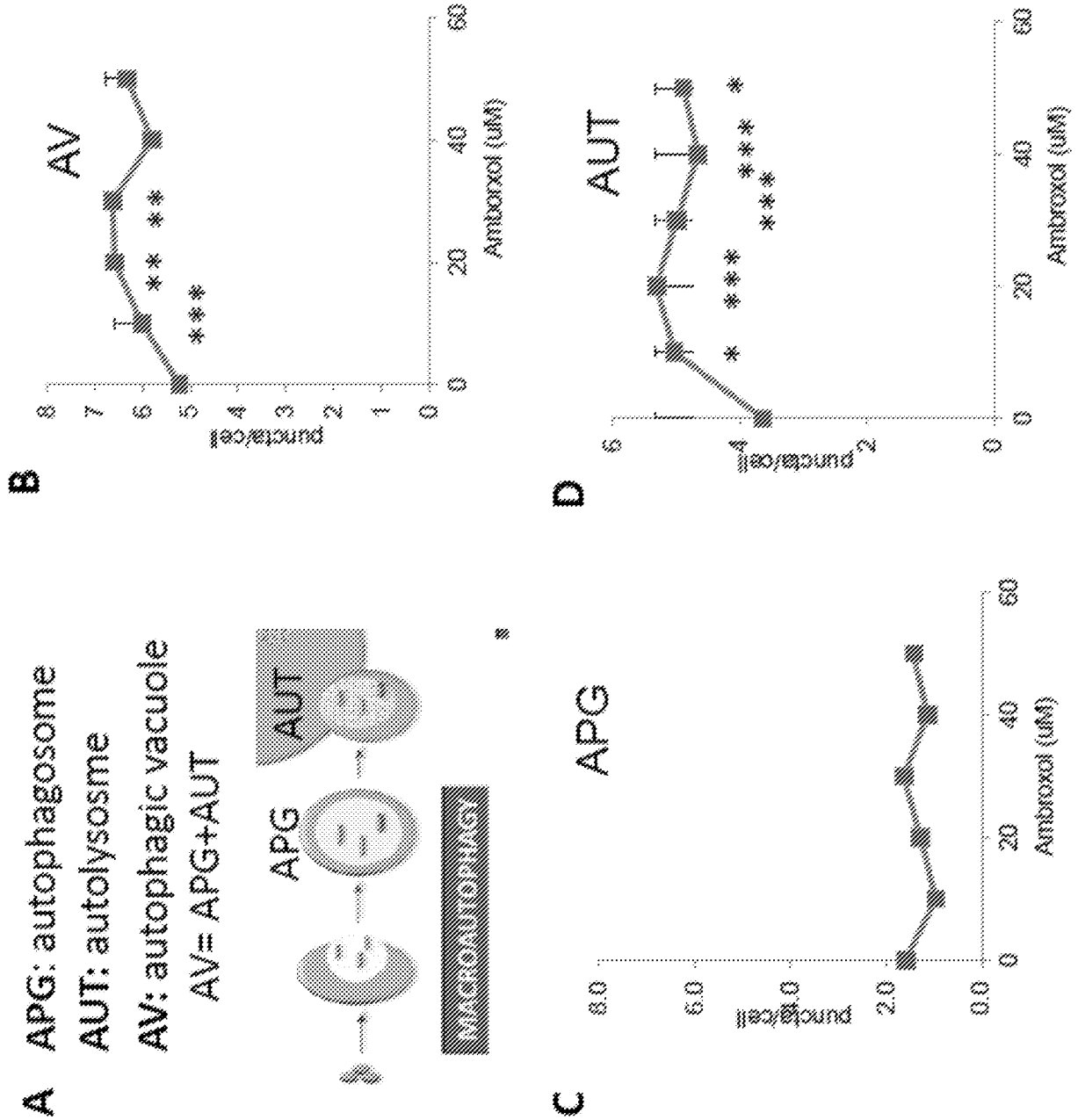
A
APG: autophagosome
AUT: autolysosme
AV: autophagic vacuole
AV= APG+AUT
APG
AUT
MACROAUTOPHAGY
B
AV
punctae/cell
Amiorixol (uM)
C
APG
punctae/cell
Amiorixol (uM)
D
AUT
punctae/cell
Amiorixol (uM)

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/042193

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/137 A61P17/14 A61P9/10 A61P39/06 A61P37/04
A61P9/14 A61P27/00 A61P3/00 A61P25/16 A61P25/28
A61P29/00 A61P27/12 A61P19/10 A61P19/02 A61P43/00

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)
A61K A61P

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, CHEM ABS Data, 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	HAORAN TAI ET AL: "Autophagy impairment with lysosomal and mitochondrial dysfunction is an important characteristic of oxidative stress-induced senescence", AUTOPHAGY, vol. 13, no. 1, 28 October 2016 (2016-10-28), pages 99-113, XP055509900, US ISSN: 1554-8627, DOI: 10.1080/15548627.2016.1247143 abstract page 105, column 1 - column 2, paragraph 1 page 109, column 1, paragraph 3 ----- -/--	1,2,4-15

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 September 2018	03/12/2018

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

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/096142 A1 (TOPOL ERIC [US] ET AL) 18 April 2013 (2013-04-18) claims 1,16 paragraphs [0004], [0183] - [0185]	1,2,4-15
X,P	J. Magalhaes ET AL: "Effects of ambroxol on the autophagy-lysosome pathway and mitochondria in primary cortical neurons", Scientific Reports, 1 January 2018 (2018-01-01), pages 1-12, XP055509940, DOI: 10.1038/s41598-018-19479-8 Retrieved from the Internet: URL:https://www.nature.com/articles/s41598-018-19479-8 [retrieved on 2018-09-26] abstract	1,2,4-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/042193

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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

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

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

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

1, 2, 4-15(all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 4-15(all partially)

   A method for increasing and/or improving lifespan in a subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof; a method for treating, inhibiting, and/or reducing ageing wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof

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2. claims: 1, 4-15(all partially)

   A method for increasing and/or improving healthspan in a subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof.

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3. claims: 1, 4-15(all partially)

   A method for increasing and/or improving cognitive acuity in a subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof.

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4. claims: 3(completely); 2, 4-15(partially)

   A method for treating, inhibiting, and/or reducing an age-related symptom, and/or an age-related disease in the subject, wherein said method comprises administering to the subject a therapeutically effective amount of ambroxol or bromhexine or salt thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/042193

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013096142 A1	18-04-2013	US 2011294833 A1	01-12-2011
		US 2013096142 A1	18-04-2013