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(54) **METHODS FOR IMPROVING FRAILTY AND AGING**

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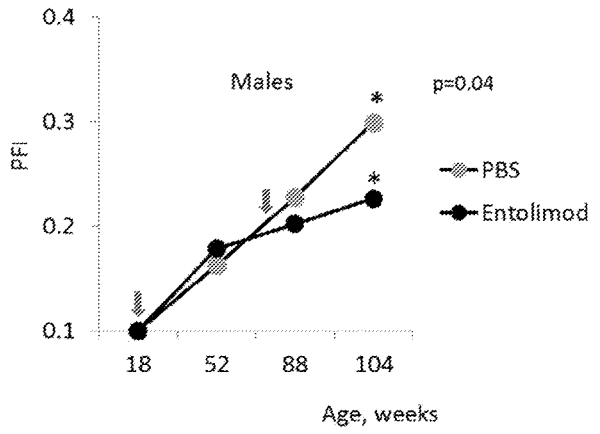
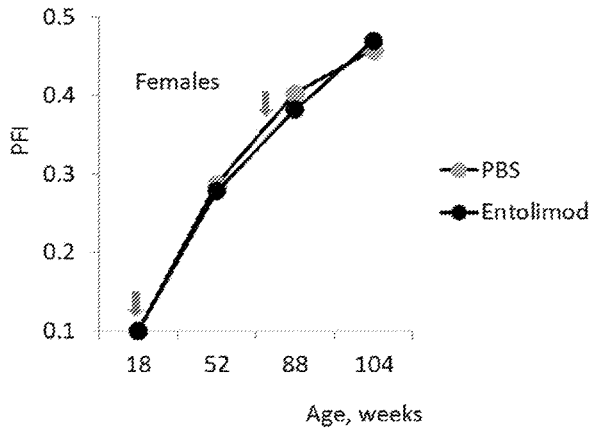
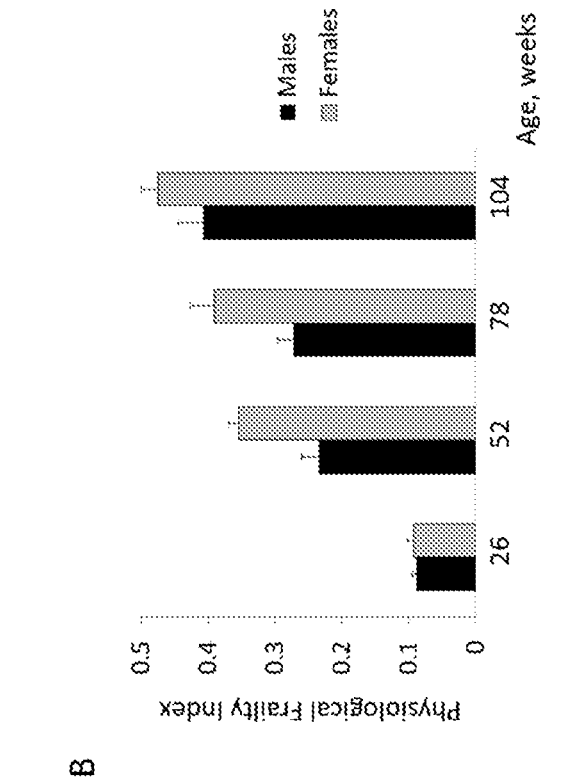
(57) **ABSTRACT**

In various aspects and embodiments provided are methods for treating age-related diseases or disorders and/or treating or preventing frailty in a patient. In certain embodiments the methods include administering a recombinant TLR5 agonist (e.g., a flagellin-based agent).

Specification includes a Sequence Listing.






B
Physiological Frailty Index
0.5
0.4
0.3
0.2
0.1
0
26
52
78
104
Age, weeks
Males
Females

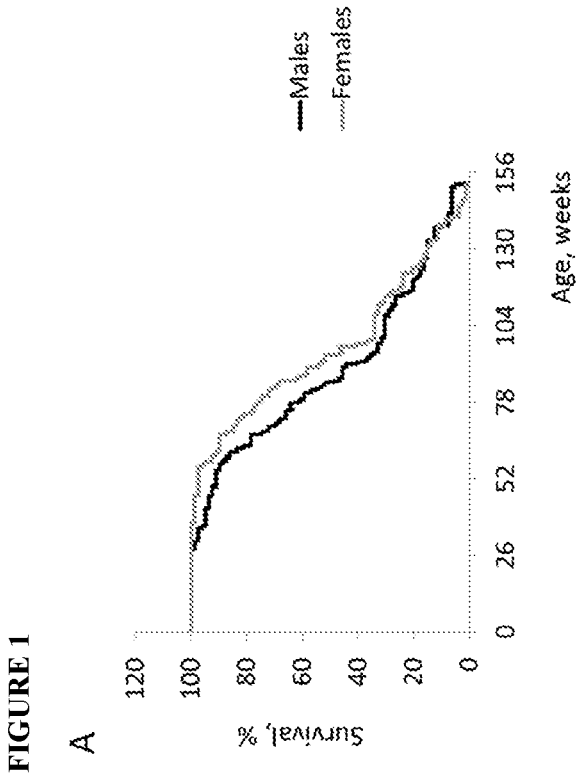

A
Survival, %
120
100
80
60
40
20
0
0
26
52
78
104
130
156
Age, weeks
Males
Females

FIGURE 1

FIGURE 2

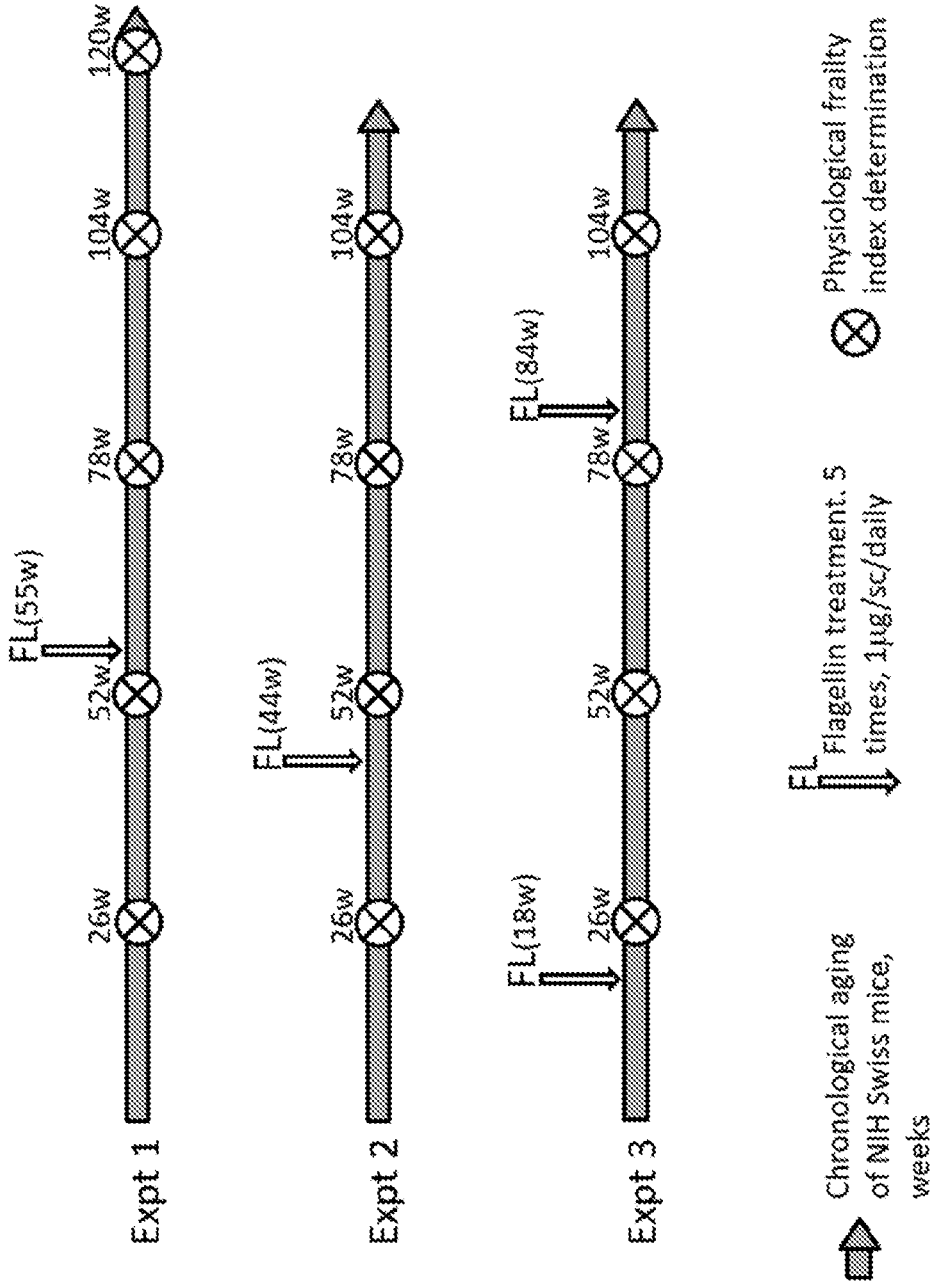
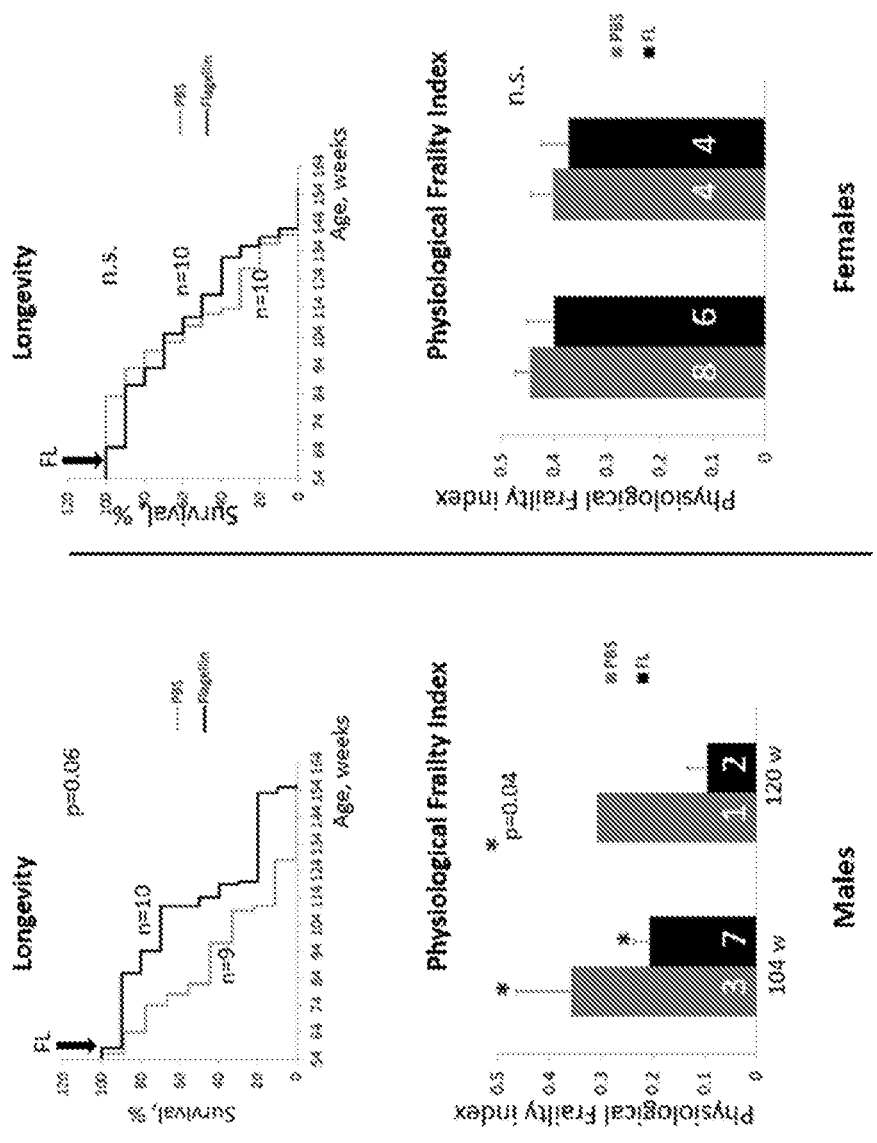

Expt 1
26w
52w
78w
104w
120w
FL(55w)
Expt 2
26w
52w
78w
104w
FL(44w)
Expt 3
26w
52w
78w
104w
FL(84w)
FL(18w)
Chronological aging of NIH Swiss mice, weeks
FL
Flagellin treatment. 5 times, 1μg/sc/daily
Physiological frailty index determination

FIGURE 3


Longevity
FL
p=0.06
n=10
n=9
Survival, %
Age, weeks
Physiological Frailty Index
p=0.04
104 w
120 w
Physiological Frailty Index
Males
Longevity
FL
n.s.
n=10
n=10
Survival, %
Age, weeks
Physiological Frailty Index
n.s.
Physiological Frailty Index
Females

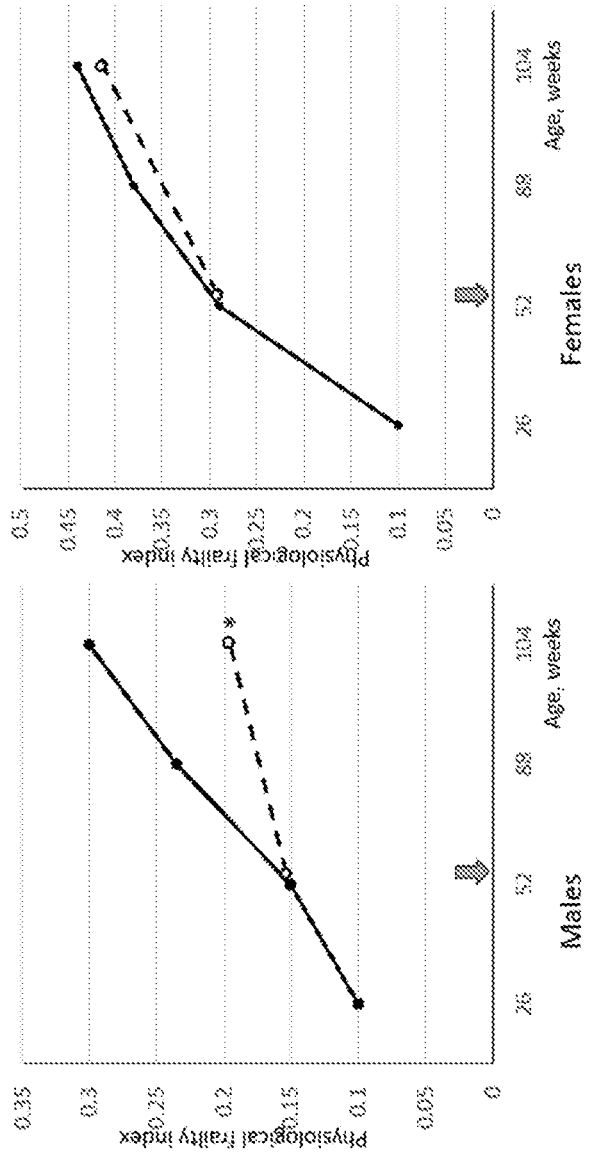

Physiological frailty index
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
26
52
88
104
Age, weeks
Females
Physiological frailty index
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
26
52
88
104
Age, weeks
Males

FIGURE 4

FIGURE 5

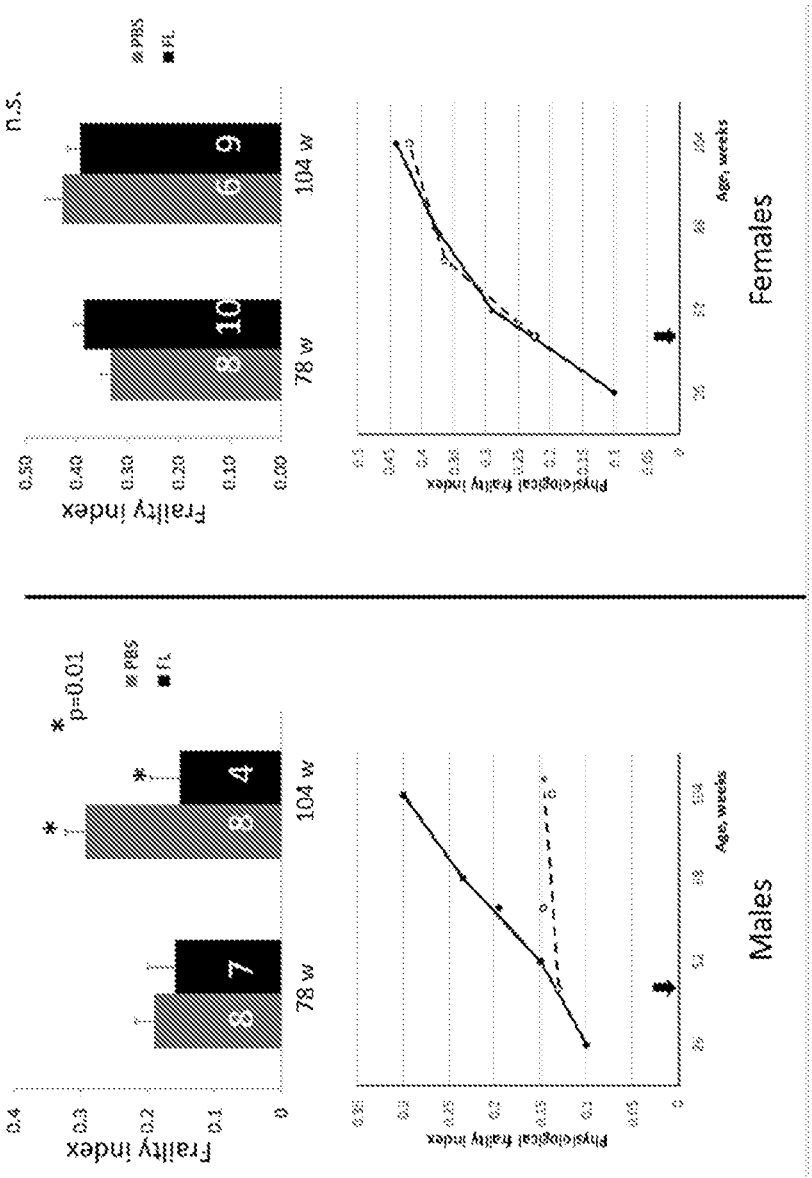
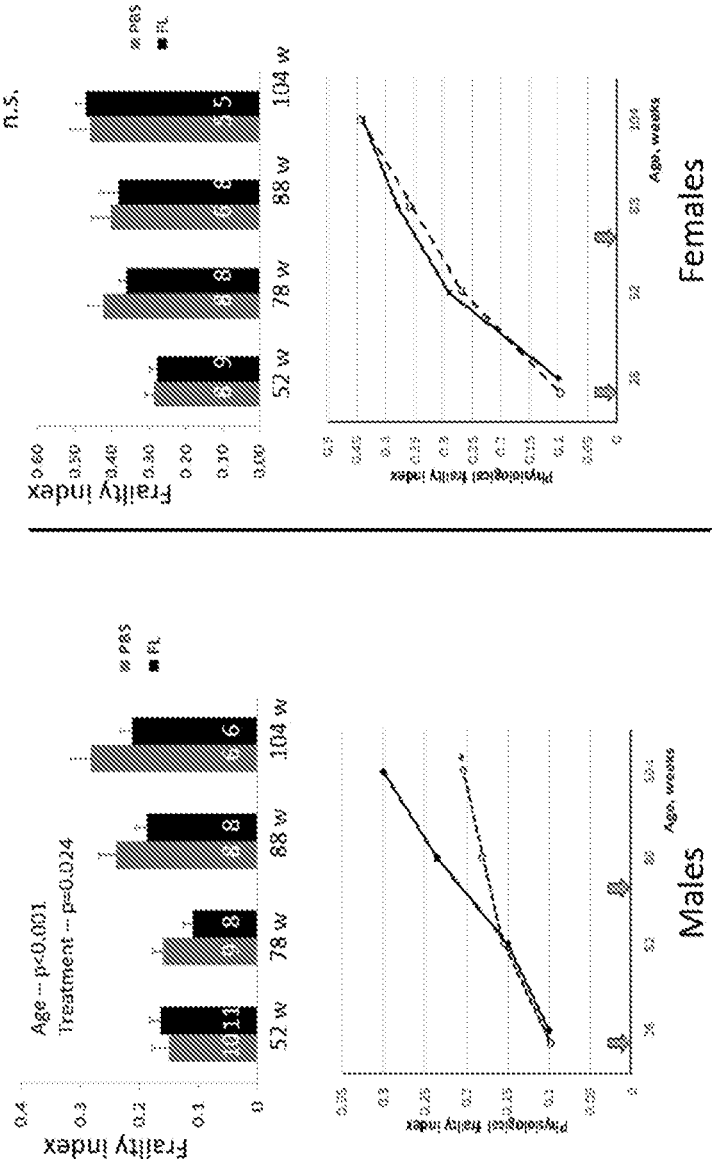

Frailty index
78 w
104 w
p=0.01
PBS
IL
n.s.
Females
Males
Age, weeks

FIGURE 6


n.s.
Frailty index
52 w
78 w
88 w
104 w
PBS
PL
Physiological frailty index
Age, weeks
Females
Age -- p<0.001
Treatment -- p=0.024
Males

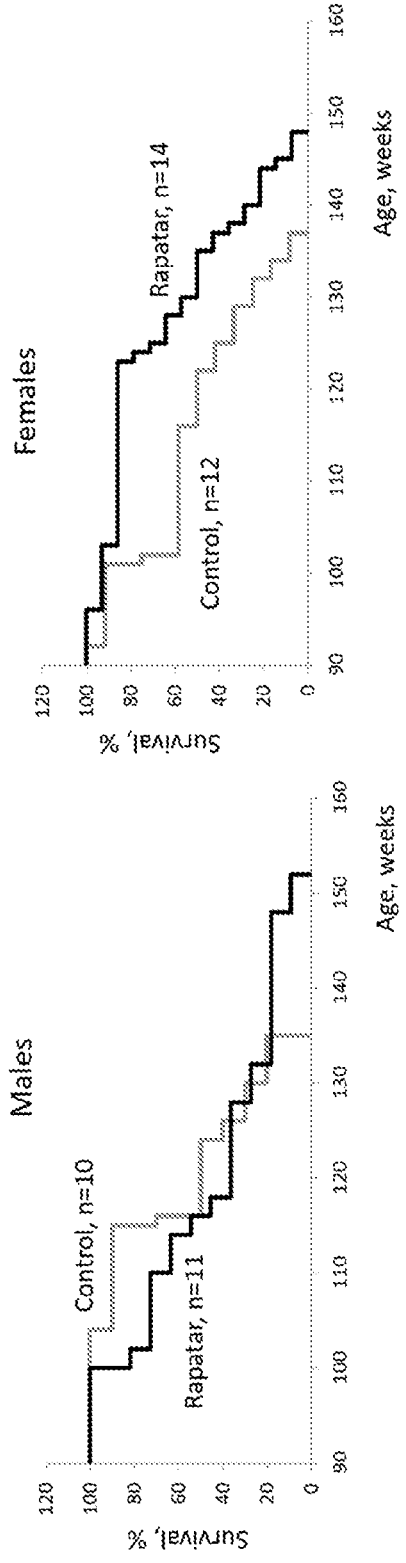

Females
Rapatar, n=14
Control, n=12
Survival, %
Age, weeks
Males
Control, n=10
Rapatar, n=11
Survival, %
Age, weeks

FIGURE 7

FIGURE 8

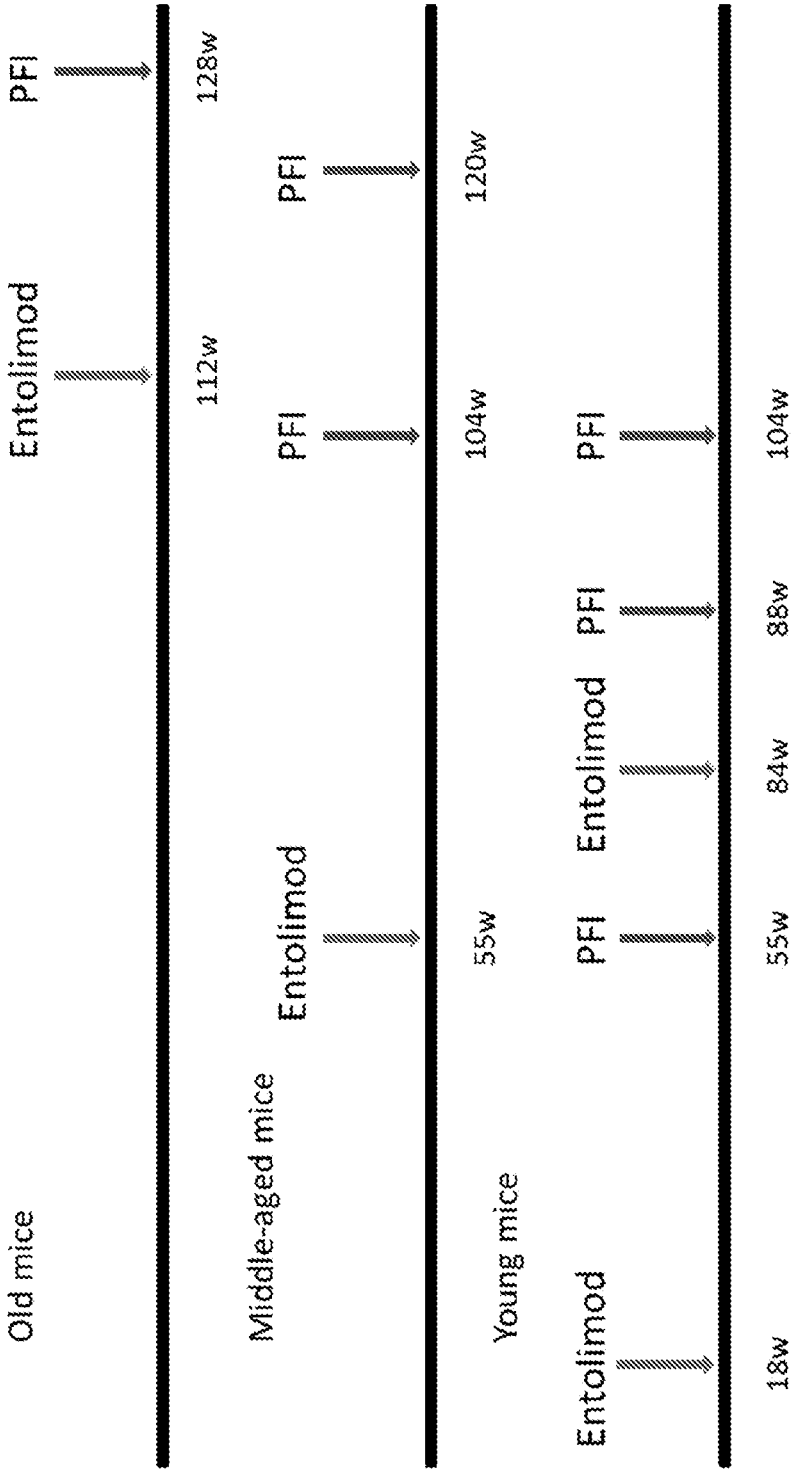

Old mice
Entolimod
112w
PFI
128w
Middle-aged mice
Entolimod
55w
PFI
104w
PFI
120w
Young mice
Entolimod
18w
PFI
55w
Entolimod
84w
PFI
88w
PFI
104w

FIGURE 9

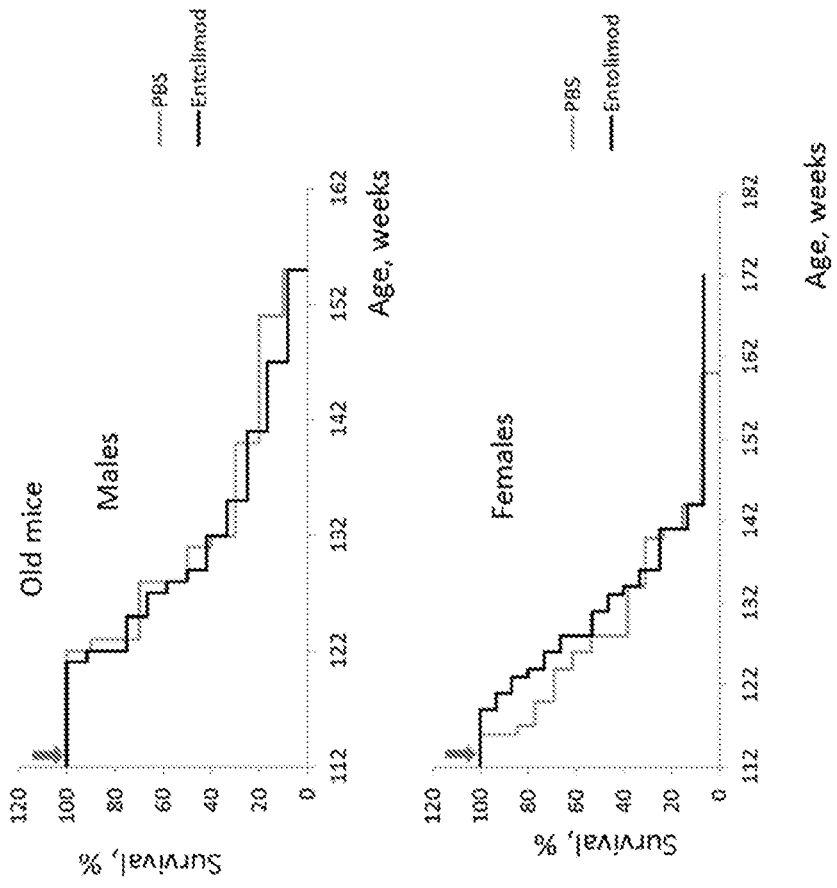

Old mice
Males
PBS
Entolimod
Survival, %
120
100
80
60
40
20
0
112
122
132
142
152
162
Age, weeks
Females
PBS
Entolimod
Survival, %
120
100
80
60
40
20
0
112
122
132
142
152
162
172
182
Age, weeks

FIGURE 10

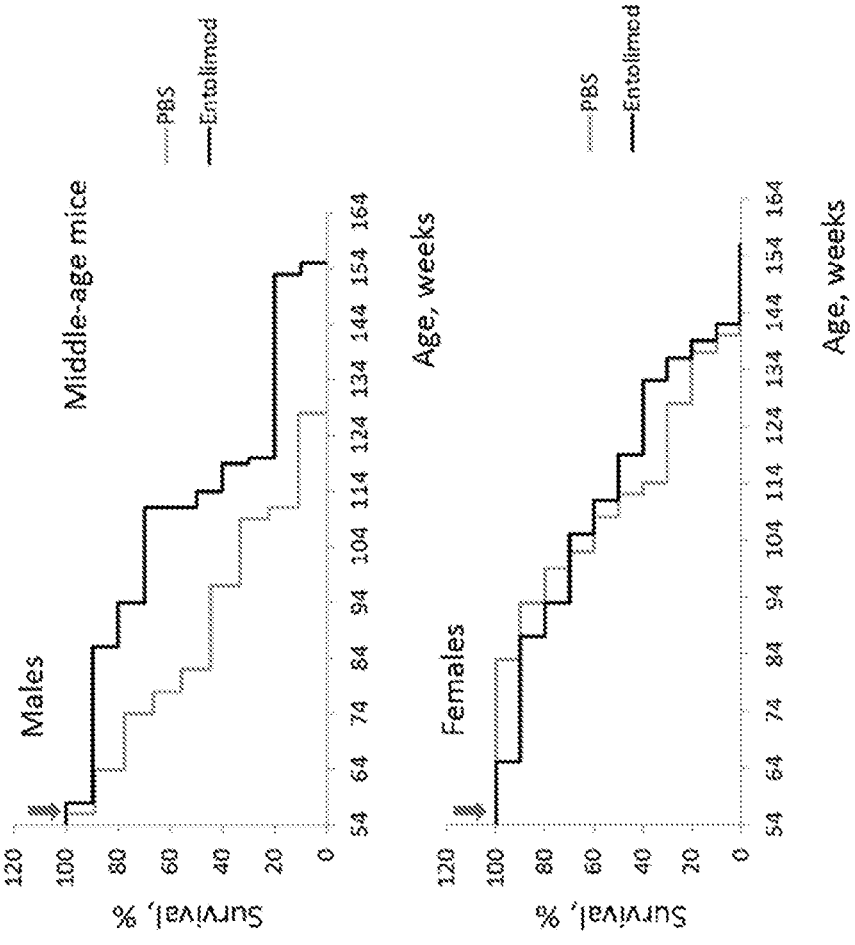
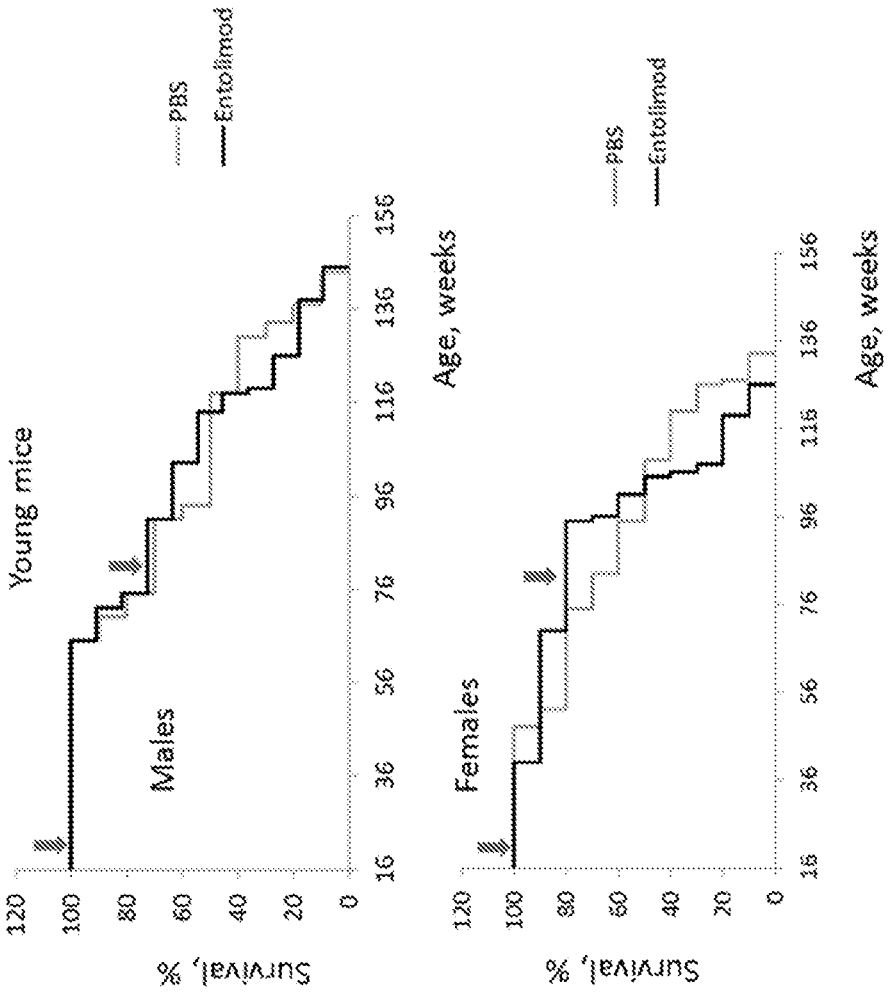

Middle-age mice
Males
Females
PBS
Entolimod
Survival, %
Age, weeks
120
100
80
60
40
20
0
54
64
74
84
94
104
114
124
134
144
154
164

FIGURE 11


Young mice
Males
Females
PBS
Entolimod
Survival, %
Age, weeks
120
100
80
60
40
20
0
16
36
56
76
96
116
136
156

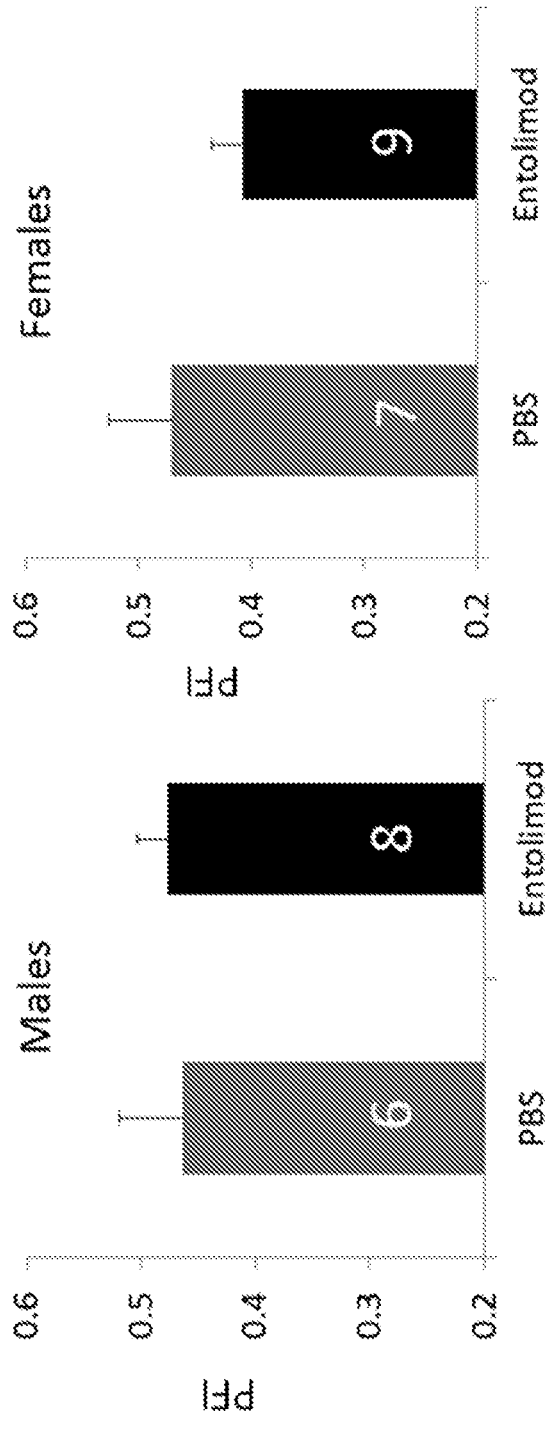


FIGURE 12

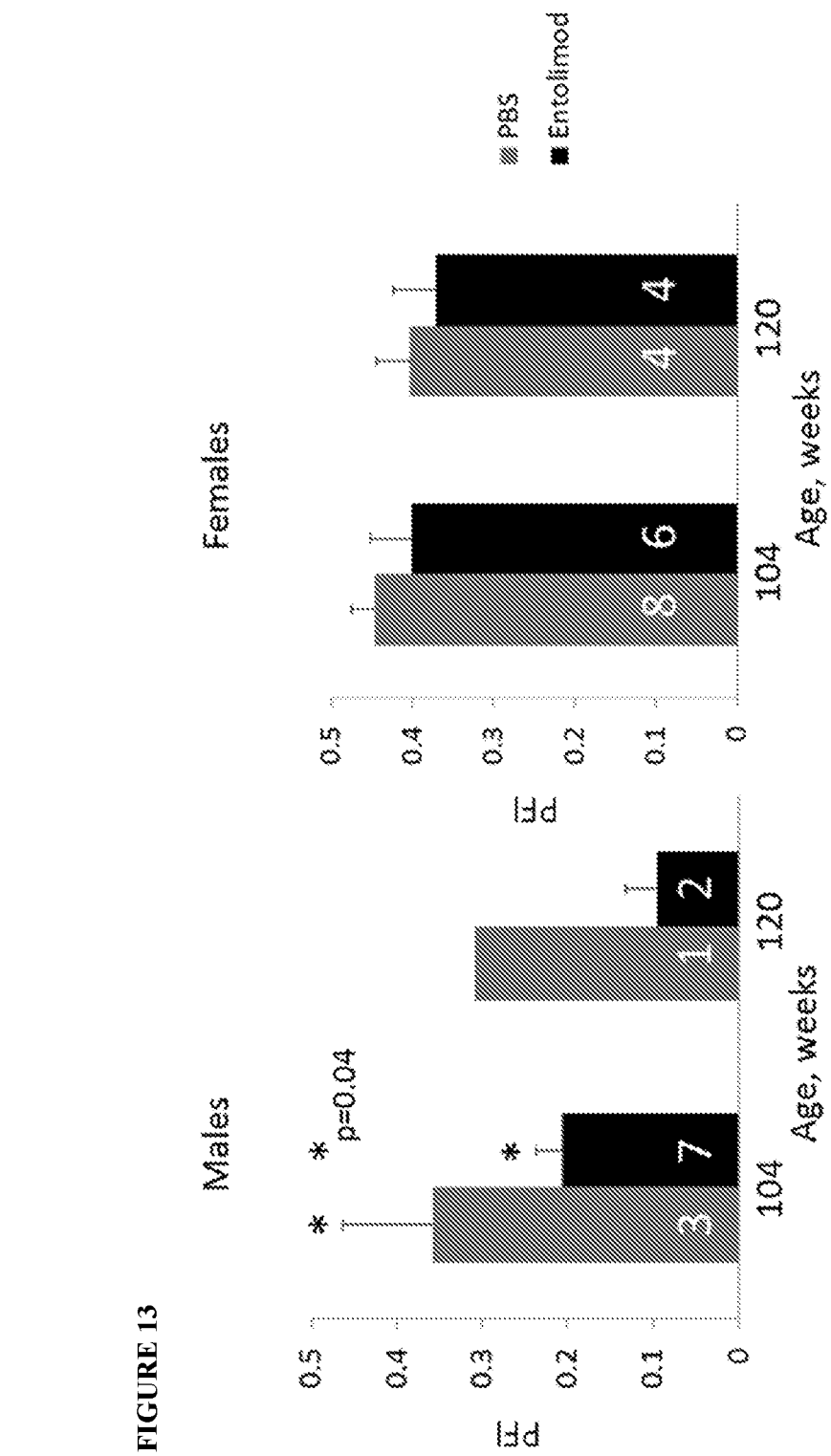

Females
Males
PBS
Entolimod
PFU
0.5
0.4
0.3
0.2
0.1
0
104
120
Age, weeks
8
6
4
4
3
7
1
2
*
*
*
p=0.04

FIGURE 13

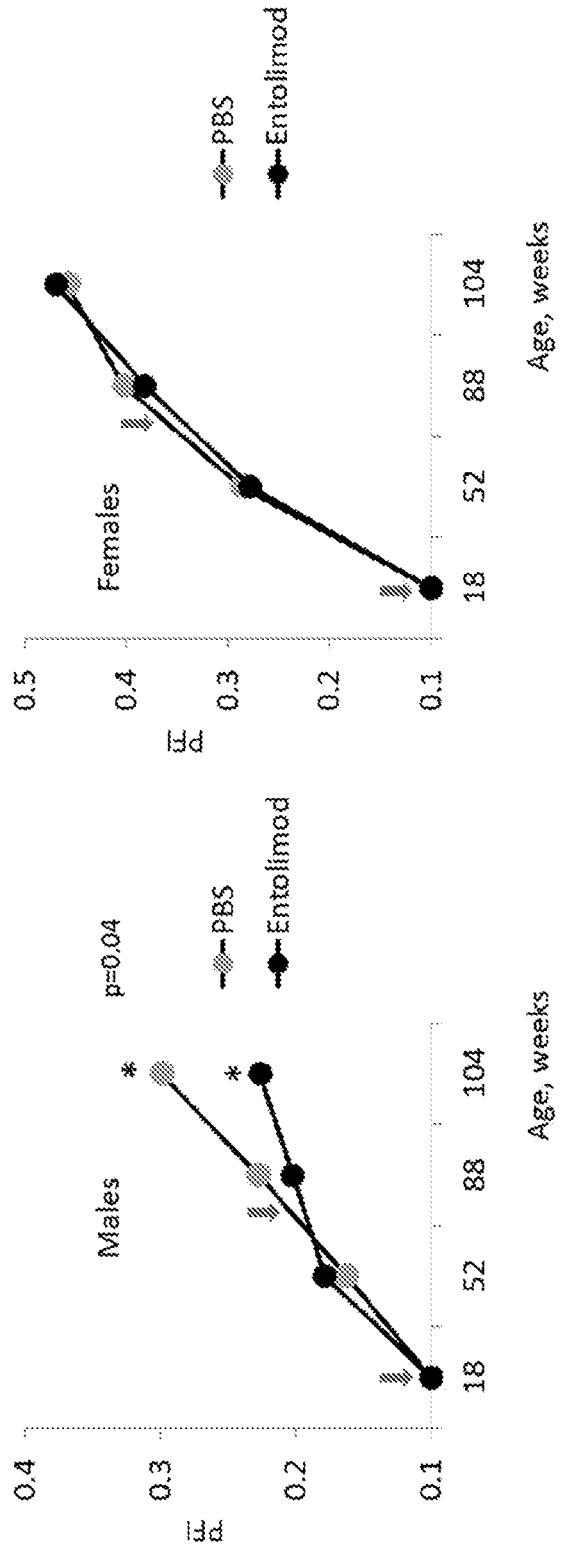

Females
Males
p=0.04
PBS
Entolimod
PD-1
0.5
0.4
0.3
0.2
0.1
18
52
88
104
Age, weeks
*

FIGURE 14

METHODS FOR IMPROVING FRAILTY AND AGING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/662,028, filed Apr. 24, 2018, the entire contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates to compositions and methods for treating age-related diseases and/or improving frailty.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (Filename: "GPI-002PC_ST25.txt"; Date created: Apr. 24, 2019; File size: 59.9 KB).

BACKGROUND

[0004] Aging of mammals is associated with accumulation of DNA damage in somatic cells, an increase in chronic systemic inflammation and reduced effectiveness of the immune system in clearing damaged cells.

[0005] TLR5 agonists derived from flagellin have been developed as therapies for various diseases. For example, entolimod is a pharmacologically-useful derivative of the natural TLR5 agonist flagellin currently being developed as a medical radiation countermeasure. In addition to its radioprotective activity, entolimod has demonstrated immunotherapeutic activity in preclinical cancer models.

[0006] There are currently no drugs or treatments that are conventionally used in medicine for prophylaxis and treatment of aging. Extension of healthy life and longevity has been documented by caloric restriction. A similar effect can be reached using mTOR inhibitors such as rapamycin. However, both require long-term applications. As such, pharmacological agents capable of slowing down the process of advancement of age-related frailty—both naturally occurring and accelerated by, e.g., cancer treatment—are needed.

SUMMARY OF THE INVENTION

[0007] Accordingly, the present invention provides, in certain aspects, methods of improving or reducing and/or treating or preventing frailty in a patient, where the method includes: identifying a patient desiring or in need of frailty treatment or prevention, and administering to said patient a recombinant TLR5 agonist, where the recombinant TLR5 agonist is not fused to a pathogenic protein antigen.

[0008] In some embodiments, the frailty is age-related. In some embodiments, frailty comprises an accumulation of deficiencies in major physiological functions, reduction of regeneration capabilities, impaired wound healing and increased risk of age-related diseases. For example, in some embodiments, frailty is associated with natural aging or accelerated aging. Frailty can be measured according to any number of indices or tests known to one of skill in the art. For example, one such index, the Physiological Frailty Index (PFI), includes measurement of one or more parameters selected from grip strength, systolic blood pressure, diastolic blood pressure, blood flow volume, number of blood neutrophils, percentage of blood neutrophils, number of blood monocytes, percentage of blood monocytes, number of lymphocytes, number of red blood cells, hemoglobin levels, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin levels, mean corpuscular hemoglobin concentration and keratinocyte-derived cytokine levels. Deviation from a reference standard in any one individual is known as a deficit, and the overall average PFI score of the individual is a ratio of deficits to the total number of parameters measured.

[0009] In some embodiments, the present invention provides methods of improving or reducing and/or treating or preventing frailty in a patient, as measured by a reduction in the PFI score of the patient. In some embodiments, methods and compositions of the present invention for improving or reducing and/or treating or preventing frailty in a patient include maintaining a PFI score over time so that the score increases at a rate slower than if the patient were not being administered the TLR5 agonist of the invention. In some embodiments of the present invention, the PFI score of the patient remains nearly the same over time. In further embodiments, methods of the present invention provide for a reduction in cellular senescence and immunosenescence associated with natural aging and/or accelerated aging (e.g., accelerated aging induced by, e.g., cancer or a cancer treatment).

[0010] In another aspect, the present invention provides for methods of treating or preventing an age-related disease or disorder in a patient, where the method includes: identifying a patient desiring or in need of treatment or prevention of an age-related disease or disorder, and administering to said patient a recombinant TLR5 agonist, where the recombinant TLR5 agonist is not fused to a pathogenic protein antigen. In some embodiments, the age-related disease or disorder is characterized by increased cellular senescence or immunosenescence.

[0011] In some embodiments, an age-related disease or disorder is selected from accelerated aging, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, cardiac diastolic dysfunction, benign prostatic hypertrophy, aortic aneurysm, emphysema, atherosclerosis, diabetes, pulmonary fibrosis, blindness, dementia, Alzheimer's disease, kidney dysfunction, osteoarthritis, low grade chronic sterile inflammation, herniated intervertebral disc, frailty, hair loss, hearing loss, vision loss, muscle fatigue, skin conditions, skin nevi, wrinkly skin, hyperpigmentation, scarring, keloid, rosacea, vitiligo, ichthyosis vulgaris, dermatomyositis, actinic keratosis, and sarcopenia.

[0012] In specific embodiments, methods of the present invention include treating or preventing accelerated aging. In some embodiments, accelerated aging is a Progeroid syndrome or symptom thereof, including, but not limited to, Hutchinson-Gilford progeria syndrome (HGPS), Werner syndrome (WS), Bloom syndrome (BS), Rothmund-Thomson syndrome (RTS), Cockayne syndrome (CS), xeroderma pigmentosum (XP), trichothiodystrophy (TTD), combined xeroderma pigmentosum-Cockayne syndrome (XP-CS), or restrictive dermopathy (RD). Subjects having one of these diseases or disorders typically have reduced longevity (i.e., lifespan).

[0013] In further embodiments, accelerated aging is induced by a cancer or a cancer treatment. For example, it is contemplated by the invention that a cancer treatment that induces an acceleration in the natural aging process is selected from one or more therapies consisting of radiotherapy, hormonal, tyrosine kinase inhibitor, anthracycline, alkylating agent, topoisomerase inhibitor, antimetabolites/cytotoxic drug, BRAF inhibitor, antitumor antibiotic, isoquinololine alkaloid, Bcl-2 inhibitor, hematopoietic cell transplantation (HCT), telomerase inhibitor, nucleoside analogue reverse-transcriptase inhibitor, DNA cross-linking agent, ribonucleotide reductase inhibitor, microtubule inhibitor, and miRNA.

[0014] In some embodiments, any cancer is contemplated for which the patient receives treatment that can induce accelerated aging. In an embodiment, the cancer for which a patient receives treatment is hematological cancer. Further, in some embodiments, the patient received the cancer treatment during childhood.

[0015] In further embodiments, the recombinant TLR5 agonist is administered to the patient for at least one week, or at least one month, or at least six months, or at least one year, or at least two years, or at least three years, or at least four years, or at least five years after the patient received the cancer treatment. In some embodiments, the patient no longer has cancer or the patient is in remission at the time the recombinant TLR5 agonist is administered.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The foregoing features of embodiments will be more readily understood by reference to the following detailed description, taken with reference to the accompanying drawings, in which:

[0017] FIG. **1**A-B. Longevity and chronological aging of NIH Swiss male and female mice. A. Kaplan-Meier survival curves for male and female NIH Swiss mice (n=79/group). No statistically significant difference between the two sexes was detected with mean lifespan 89.13+3.49 and 96.95+2.99 weeks for males and females respectively (p=0.368, log-rank test). B. Physiological Frailty Index (PFI) created for chronologically aged male and female NIH Swiss mice of different ages. Age-dependent increase in PFI reflects accumulation of health deficits observed in mice of both sexes.

[0018] FIG. **2**. Schematic diagram of experiment schedule for flagellin treatment. In each experimental group males and females received a short course of Flagellin injected s.c. daily (1 mg/injection) for five consecutive days: group 1 at the age of 55 weeks, group 2 at 44^{th} week, and group 3 received two courses at 18^{th} and 84^{th} weeks. PFI was evaluated when mice reach 26, 52, 78, 104, and 120 weeks.

[0019] FIG. **3**. Single five days course of flagellin administration at the age of 55 weeks (experimental group 1) increased mean survival of NIH Swiss male mice from 89±7.9 to 112.3±9.2 weeks. No effect on female mice survival rate was detected. Physiological Frailty Index was created for male and female mice at 104 and 120 weeks of age. Five days injection course of flagellin earlier in life significantly reduced PFI of male but not female mice.

[0020] FIG. **4**. Dynamic changes in Physiological Frailty Index in mice from experimental group 1. At 104 weeks, the average PFI in flagellin treated male group (dashed line) increased by 33% from the time of treatment at 55 weeks, while in control mice (solid line) it steadily increased by 100%. No effect was observed in the female group.

[0021] FIG. **5**. Dynamic changes in Physiological Frailty Index in mice from experimental group 2. Mice were injected with flagellin at the age of 44 weeks. The average PFI evaluated at 78 and 104 weeks demonstrated almost no changes in flagellin treated male group (dashed line) from the time of treatment, while in control mice it steadily increased more then 200% (solid line). No effect was observed in the female group.

[0022] FIG. **6**. Dynamic changes in Physiological Frailty Index in mice from experimental group 3. Mice were injected with flagellin twice at the age of 18 and 84 weeks. The average PFI evaluated four times at the age of 52, 78, 88, and 104 weeks demonstrated significantly smaller increase in flagellin treated male group (dashed line) as compared to control mice (solid line). No effect was observed in the female group.

[0023] FIG. **7**. Chronic administration of Rapatar (nano-formulated water soluble rapamycin, see Comas, et al., *Aging* (Albany N.Y.) 10:715-22 (2012)) extends lifespan of female, but not male NIH Swiss mice. Kaplan-Meier survival curves of mice that start receiving Rapatar in drinking water at 89 weeks of age. Chronic administration of rapamycin increases lifespan of female mice from 114.4±3.1 to 127.3±3.1 weeks (p=0.01, Kaplan-Meier log-rank test).

[0024] FIG. **8**. Schematic illustration of the timeline (not to scale) for treatment and evaluation of NIH Swiss mice. "Entolimod" arrows indicate timing of entolimod treatment (or PBS treatment in control groups) and "PFI" arrows indicate timing of PFI determination.

[0025] FIG. **9**. Effect of entolimod treatment on longevity of "old" mice. Kaplan-Meier survival curves are shown for groups of male and female NIH Swiss mice that received 5 daily SQ injections of entolimod (5 μg/mouse; dark line) or PBS (light line) at 113 weeks of age (arrow).

[0026] FIG. **10**. Effect of entolimod treatment on longevity of "middle-aged" mice. Kaplan-Meier survival curves are shown for groups of male and female NIH Swiss mice that received 5 daily SQ injections of entolimod (5 μg/mouse; dark line) or PBS (light line) at 55 weeks of age (arrow).

[0027] FIG. **11**. Effect of entolimod treatment on longevity of "young" mice. Kaplan-Meier survival curves are shown for groups of male and female NIH Swiss mice that received 5 daily SQ injections of entolimod (5 μg/mouse; dark line) or PBS (light line) at 18 weeks of age and at 84 weeks of age (arrow).

[0028] FIG. **12**. Treatment of NIH Swiss mice with entolimod at 112 weeks of age had no effect on mean PFI measured at 128 weeks of age. The number of animals evaluated for each group is shown in white within the bars of the graph

[0029] FIG. **13**. Treatment of NIH Swiss mice with entolimod at 55 weeks of age led to reduced PFI at 104 weeks and 120 weeks in males, but not females. The number of animals evaluated for each group is shown in white within the bars of the graph.

[0030] FIG. **14**. Dynamics of changes in PFI in male and female NIH Swiss mice after receiving entolimod treatment at 18 and 84 weeks of age. Mean PFI was determined at 18, 52, 84 and 104 weeks of age for entolimod-treated (black circles) and PBS-treated (grey circles) groups of mice. Timing of treatments is indicated by arrows.

DETAILED DESCRIPTION OF THE INVENTION

[0031] Some of the aspects and embodiments of this instant disclosure are based, at least in part, on the finding that TLR5 agonists (e.g., recombinant flagellin and/or flagellin-based agents, such as entolimod) that are not fused to a pathogenic protein antigen, can be effective, for example, in improving and/or treating or preventing frailty and/or treating or preventing age-related diseases or disorders, and/or preventing or slowing aging (including accelerated aging).

[0032] The aging process is manifested by a gradual accumulation of deficiencies in all major physiological functions, reduction of regeneration capabilities, impaired wound healing and increased risk of age-related diseases or disorders such as cancer, diabetes type 2, arthritis, Alzheimer and Parkinson diseases, atherosclerosis and others. Cumulatively, all these events can be described as a gradual increase in frailty and measured by a so-called "frailty index". Age-related increase in frailty can be expedited in people or animals that underwent cancer treatment by chemotherapy and radiation, which can be interpreted as accelerated aging.

[0033] Without wishing to be bound by theory, the present invention contemplates that the progression of natural aging, as well as aging accelerated by, e.g., cancer treatment, can be dramatically slowed down by activation of natural innate immunity mechanism of response to infection with bacteria that have flagella—an organelle for active moving that is built with the protein named flagellin; presence of such bacteria in the body is recognized by a cell surface receptor named Toll-like receptor 5 (TLR5). Binding of a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) to TLR5 triggers a physiological response leading to systemic mobilization of immune system accompanied with production of multiple bioactive factors (cytokines, chemokines, etc.) that have long-term effect on the organism manifested as a slowdown of frailty acquisition and improved health and quality of life of the treated organisms. Treatment with flagellin or its derivatives capable of activation of TLR5 can be projected as an approach to prevent and treat natural aging and premature accelerated aging caused by cancer treatment and other types of poisoning.

[0034] Aging is a gradual systemic pathological transformation of mammalian organism advancing with time. It is associated with accumulation of multiple deficiencies in functions of multiple organs and tissues and reduced regeneration capabilities leading to development of age-related chronic diseases or disorders including atherosclerosis, diabetes, pulmonary fibrosis, blindness, dementia, kidney dysfunction, osteoarthritis, and low grade chronic sterile inflammation as well as other age-related diseases and disorders contemplated herein. These conditions frequently coincide with a gradual development of geriatric syndromes including frailty, cognitive impairment and immobility. Aging is a natural and unavoidable process. Underlying causes of aging are still disputable; however, two features of aging are generally accepted as universal: an increase in DNA damage and development of systemic sterile chronic inflammation, both considered as major contributors of age-related pathologies.

[0035] In various embodiments, the present invention provides methods of reducing aging, or the multiple deficiencies causes thereof. In various embodiments, the present invention provides methods of reducing the amount or cellular impact of DNA damage. In various embodiments, the present invention provides methods of reducing the amount or cellular impact of systemic sterile chronic inflammation.

[0036] In various embodiments, the present invention provides methods of improving the cellular clearance of damaged cells, e.g. that may be functionally declined in aged subjects

[0037] In various embodiments, the present invention relates to treating or preventing cellular senescence, for example by reducing, halting, or delaying the senescence. Without intending to be bound by any particular theory, cellular aging (senescence) is considered to be caused by overstimulation and overactivation of signal transduction pathways such as the mTOR pathway, especially when the cell cycle is blocked, leading to cellular hyperactivation and hyperfunction. In turn, this causes secondary signal resistance and compensatory incompetence. Both cellular hyperfunction and signal-resistance cause organ damage (including in distant organs), manifested as aging (subclinical damage) and age-related diseases or disorders (clinical damage), eventually leading to organismal death. Non-limiting example of markers of cellular aging include cellular hypertrophy, permanent loss of proliferative potential, large-flat cell morphology and beta-Gal staining. In various embodiments, the present invention relates to modulating any of the markers of cellular aging.

Frailty and Frailty Indices

[0038] In various embodiments, the present invention provides methods of improving or reducing and/or treating or preventing frailty in a patient, wherein the method includes: identifying a patient desiring or in need of frailty treatment or prevention, and administering to said patient a recombinant TLR5 agonist, wherein the recombinant TLR5 agonist is not fused to a pathogenic protein antigen.

[0039] In some embodiments, frailty comprises an accumulation of deficiencies in major physiological functions, reduction of regeneration capabilities, impaired wound healing and increased risk of age-related diseases. For example, in some embodiments, frailty is associated with natural aging or accelerated aging. Frailty can be measured according to any number of indices or tests known to one of skill in the art. For example, one such index, the Physiological Frailty Index (PFI), includes measurement of one or more parameters selected from grip strength, systolic blood pressure, diastolic blood pressure, blood flow volume, number of blood neutrophils, percentage of blood neutrophils, number of blood monocytes, percentage of blood monocytes, number of lymphocytes, number of red blood cells, hemoglobin levels, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin levels, mean corpuscular hemoglobin concentration and keratinocyte-derived cytokine levels. Deviation from a reference standard in any one individual is known as a deficit, and the overall average PFI score of the individual is a ratio of deficits to the total number of parameters measured.

[0040] Frailty can manifest as vulnerability to stressors and a reduced capacity to withstand stress. For example, the disclosure of Buchner and Wagner 1992 *Clin Geriatr Med.* 1992 February; 8(1):1-17 is hereby incorporated by reference in its entirety. Frailty can manifest as loss of complex-

ity of homeostatic mechanisms (e.g., interconnectedness and/or feedback or feedforward). For example, the disclosure of Lipsitz 2002 *J Gerontol A Biol Sci Med Sci.* 2002 March; 57(3):B115-25. is hereby incorporated by reference in its entirety. Frailty can also manifest as disuse and/or a decrease in energy flow through an organism, as described in Bortz 2002, *J Gerontol A Biol Sci Med Sci.* 2002 May; 57(5):M283-8. which is hereby incorporated by reference in its entirety. Frailty can also manifest as homeostatic dysregulation, as described by Ferrucci 2005 *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 56, which is hereby incorporated by reference in its entirety.

[0041] There are several comprehensive approaches for quantitative assessment of aging-related accumulation of deficits and frailty in humans and animals. Individual organisms are heterogeneous in their health status and the rate of aging. To account for such heterogeneity, a Frailty Index (FI) has been introduced as a numerical score which is a ratio of the deficits present in a person to the total number of deficits considered in the study. Changes in the FI characterize the rate of individual aging. A similar approach has been applied to laboratory animals. Frailty index is considered as a reliable and broadly accepted measure of "biological age" and the degree of general health decline indicative of a reduction in the quality of life.

[0042] In certain aspects and embodiments, provided herein includes methods for improving and/or treating or preventing frailty and/or reducing frailty index in a patient. Frailty can be assessed in any of many methods known in the art. For example, frailty and methods to evaluate/index frailty are described in Hubbard, et al., *Ageing*, published electronically November, 2008 page 115-118; Cesari, et al., *Age and Ageing,* 43:10-12, 2014; and Mohler et al., *Experimental Gerontology,* 54:6-13, 2014, all of which are hereby incorporated by reference.

[0043] In various embodiments, a Frailty Index is calculated as described in U.S. Patent Application Publication No. 2015/0285823, which is incorporated herein by reference. For example, a description of the determination of the Frailty Index is provided. The Frailty Index was developed to assess a fit to frail range for the organisms of the same chronological age to address the notion that chronological age does not always reflect biologic age. Based on sixteen-item parameters (that include measurements of weight, grip strength, blood pressure, complete blood count, cytokine level analysis), FI is calculated as a ratio of the total number of deficits measured and are assigned a score of FI between 0 (no deficits=fit) and 1 (all deficits present=frail). Therefore, higher FI indicates poorer health of an organism. In this regard, a FI is provided as a useful tool for assessing a "fit" to "frail" range organisms of the same chronological age.

[0044] In certain embodiments, methods of the present invention reduce or prevent frailty in a subject as measured according to the Physiological Frailty Index (PFI), as described in Antoch et al. *Aging.* 2017; 9: 1-12 (hereby incorporated by reference in its entirety). For example, PFI can be determined for an individual subject with reference to a young reference subject. For each subject, various parameters are measured. These parameters include non-invasive measurements, including age, body weight, grip strength, and diastolic blood pressure. Additional blood chemistry measurements may also be determined, including white blood cell count, neutrophil count, neutrophil percentage, lymphocyte percentage, monocyte percentage, eosinophil percentage, red blood cell count, hemoglobin levels, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin levels, mean corpuscular hemoglobin concentration, platelet count, and mean platelet volume. For each parameter mean value and standard deviation are calculated. Subjects differing in more than one standard deviation (STDEV) from mean value in any single parameter are excluded from the reference group. The value for each parameter measured for subjects of older ages is compared with the corresponding value for the reference group and assigned a score. Values that differ less than 1 STDEV are assigned the score of 0 (no deficit, within the range of the reference group). Values that are different for one STDEV are scored as 0.25 (minimal deficit). Values that differ from the corresponding values in the reference group by 2 STDEV are scored as 0.5 and those that differ by 3 STDEV are scored as 0.75. If the value is above 3 STDEV, it is scored as 1 (extreme deficit). The number of deficits the individual subject expressed is calculated as a ratio of the total number of parameters measured and is referred to as Physiological Frailty Index (PFI).

[0045] In some embodiments, methods of the present invention reduce or improve and/or treat or prevent frailty in a subject, as measured by the PFI. For example, administering the recombinant TLR5 agonist to a subject in order to reduce or improve and/or treat or prevent frailty can result in a reduced PFI score. In some embodiments, a subject's PFI score is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%. In some embodiments, a subject's PFI score is reduced by about 25%-75%, about 25%-50%, or about 50% to 75%. In further embodiments, a subject's PFI score is reduced to no greater than 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1 or 0.5.

[0046] Further, frailty as an accumulation of deficits can be measured by the Rockwood frailty index, as described in Rockwood et al., *J Gerontol A Biol Sci Med Sci.* 2007 July; 62(7):722-727, which is incorporated by reference in its entirety. In embodiments, the present methods reduce or prevent frailty as assessed by the Rockwood frailty index.

[0047] Frailty as a biologic syndrome of decreased reserve resulting from cumulative declines across multiple physiologic systems can be measured by the Fried frailty score, as described in Fried et al., *J Gerontol A Biol Sci Med Sci.* 2001 March; 56(3):M146-56, which is incorporated by reference in its entirety. The Fried frailty score comprises a Physical Frailty Phenotype (PFP), which measures various parameters, such as weight loss of more than 10 pounds; weakness as related to grip strength; self-reported exhaustion; 15 feet walking speed; and amount of physical activity in Kcals per week. The Fried frailty score incorporates scoring of 0 (not frail), 1-2 (intermediate frailty), and greater than or equal to 3 (frail). In various embodiments, methods of the present invention reduce or improve and/or treat or prevent frailty in a subject, as measured by a Fried frailty score. For example, administering the recombinant TLR5 agonist to a subject in order to reduce or improve and/or treat or prevent frailty can result in a reduced Fried frailty score from 3 to 2, from 3 to 1, from 3 to 0, from 2 to 1, from 2 to 0 or from 1 to 0. Further, in some embodiments, administering the recombinant TLR5

agonist to a subject in order to reduce or improve and/or treat or prevent frailty results in a lack of increase of a subject's Fried frailty score.

[0048] Frailty can also be measured by the FRAIL Scale, as described in Abellean Van Kan et al., *J Am Med Dir Assoc.* 2008 February; 9(2):71-2. doi: 10.1016/j.jamda.2007.11.005, which is incorporated by reference in its entirety. The parameters measured in the FRAIL Scale include feelings of persistent fatigue; resistance (ability to climb a single flight of stairs); ambulation (ability to walk one block); more than five illnesses; and more than 5% loss of weight. The FRAIL Scale incorporates scoring of 0 (not frail), 1-2 (intermediate frailty), and greater than or equal to 3 (frail). In various embodiments, methods of the present invention reduce or improve frailty in a subject, as measured by a FRAIL Scale score. For example, administering the recombinant TLR5 agonist to a subject in order to reduce or improve frailty can result in a reduced FRAIL Scale score from 3 to 2, from 3 to 1, from 3 to 0, from 2 to 1, from 2 to 0 or from 1 to 0. Further, in some embodiments, administering the recombinant TLR5 agonist to a subject in order to reduce or improve and/or treat or prevent frailty results in a lack of increase of a subject's FRAIL Scale score.

[0049] In some embodiments the methods as provided herein improve (or reduce) frailty index, or delay or slow a decline in frailty using at least one accepted measure of frailty. In some embodiments the methods as provided herein improve (or reduce) frailty index, or delay or slow a decline in frailty using at least one accepted measure of frailty selected from the Frailty Index (FI), the Physiological Frailty Index (PFI), Fried frailty score, Rockwood frailty index, FRAIL Scale and the modified frailty index.

[0050] In some embodiments, the frailty comprises low lean mass, weakness, exhaustion, low energy expenditure and/or slow walking speed. In embodiments, the present methods reduce or prevent the onset or development of one or more of low lean mass, weakness, exhaustion, low energy expenditure and/or slow walking speed.

Age-Related Diseases and Disorders

[0051] The present invention contemplates methods involving administering a recombinant TLR5 agonist that is not fused to a pathogenic protein antigen. In various embodiments, the recombinant TLR5 agonist decreases cellular senescence in the patient having an age-related disease or disorder.

[0052] In some embodiments, the disease is cancer, age-related disease, tobacco-related disease, or skin wrinkles.

[0053] For example, in some embodiments, the methods provided herein are to prevent or treat age-related diseases or disorders such as Alzheimer's disease, type II diabetes, macular degeneration, chronic inflammation-based pathologies (e.g., arthritis), and/or to prevent development of cancer types known to be associated with aging (e.g., prostate cancer, melanoma, lung cancer, colon cancer, etc.), and/or with the purpose to restore function and morphology of aging tissues (e.g., skin or prostate), and/or with the purpose to improve morphology of tissue impaired by accumulated senescent cells (e.g., cosmetic treatment of pigmented skin lesions), and/or with the purpose to improve the outcome of cancer treatment by radiation or chemotherapy, and/or with the purpose to prevent recurrent and metastatic disease in cancer patients by elimination of dormant cancer cells. The disclosure is suitable for prophylaxis and/or therapy of human and non-human animal diseases and aging and age-related disorders.

[0054] In various examples, the disclosure relates to methods of treating an individual suspected of having or at risk for developing an age-related disease or disorder, including but not necessarily limited to Alzheimer's disease, Type II diabetes, macular degeneration, or a disease comprising chronic inflammation, including but not necessarily limited to arthritis.

[0055] In some embodiments, the methods described herein or for treatment of a patient identified as having or at risk of having a cardiovascular disease or disorder, inflammatory disease or disorder, pulmonary disease or disorder, neurological disease or disorder, metabolic disease or disorder, dermatological disease or disorder, age-related disease or disorder, a premature aging disease or disorder, and a sleep disorder.

[0056] The methods provided herein in certain aspects and embodiments are applicable to treating or preventing degenerative disorders that accompany aging. More particularly, the methods provided herein may provide improvements in 1) reducing the rate at which adipose tissue is lost, 2) reducing the rate at which muscle fibre diameter is reduced, and 3) reducing the rate at which skin tone deteriorates over time. These effects are likely to be seen more dramatically in aged recipients, i.e. those at an age greater than 50 years, especially those aged greater than 60 years or more, such as 65 years, 70 years and 75 years and greater. Also, candidate recipients include those whose lifestyle imposes age-accelerating effects, including tobacco smokers and users, alcohol and narcotic drug abusers, skin tanning enthusiasts, and the like.

[0057] Particular conditions and diseases or disorders that are treated by the present methods, in various embodiments, include sarcopenia. Sarcopenia is characterized first by a muscle atrophy (a decrease in the size of the muscle), along with a reduction in muscle tissue "quality," caused by such factors as replacement of muscle fibres with fat, an increase in fibrosis, changes in muscle metabolism, oxidative stress, and degeneration of the neuromuscular junction. Combined, these changes lead to progressive loss of muscle function and frailty.

[0058] Other conditions that are treated by the present method, in various embodiments, include cataracts, and so-called "signs of aging" such as wrinkling and discoloration of the skin, and overall dermal tone. Treatment by the present method is expected to reduce the rate at which fat and muscle that support skin tone are reduced, so that skin wrinkling also is reduced, delayed or eliminated. As well treatment is expected to have a benefit on the rate at which cataracts form in the eye.

Accelerated Aging (Cancer and Progeroid Syndrome)

[0059] In some embodiments, the present invention provides methods for reducing accelerated aging in a subject. For instance, in some embodiments, the present invention relates to the administration of a recombinant TLR5 agonist, e.g. flagellin or flagellin-based agent (such as entolimod) to a subject or patient to reduce accelerated aging associated with cancer and/or cancer treatments or Progeroid syndromes.

[0060] Exposure of younger individuals to genotoxic medical treatments or environment has been linked to a high

risk of premature development of multiple aging-associated conditions listed above and considered as accelerated aging.

[0061] One of the most common medical treatments of this type is cancer treatment. Cancer treatment frequently involves exposure of humans and animals to genotoxic stresses leaving numerous normal cells with damaged DNA, provoking accumulation of senescent cells and acquisition of chronic systemic inflammation. These conditions increase the risk of multiple diseases or disorders commonly associated with natural aging such as abnormal thyroid function, decreased bone mineral density and increased osteoporosis, infertility, compromised tissue regeneration, cardiotoxicity, pulmonary fibrosis and chronic sterile inflammation.

[0062] In various embodiments, the cancer being treated is selected from basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (e.g., small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (e.g. that associated with brain tumors), and Meigs' syndrome.

[0063] Acceleration of aging in cancer survivors is especially well documented in individuals that were successfully treated for cancer in their childhood. In fact, adults treated for childhood cancer are at increased risk of early development of chronic health conditions such as cardiovascular, pulmonary, hepatic, renal, and gonadal dysfunction, and secondary malignant neoplasms and increased rate of mortality. The rates of chronic diseases among survivors in their 20s are similar to rates among siblings in their 50s. Elevated rates of other aging-associated conditions, such as cognitive dysfunction, and reduced muscle strength, are also reported among childhood cancer survivors and appear decades earlier than expected. This and other studies suggest that some survivors of childhood cancer have a physiological frailty phenotype consistent with that found among older adults. Physiologic frailty among hematopoietic cell transplantation (HCT) survivors also suggests accelerated aging and is a predictor for premature mortality. Rates of frailty were eightfold higher among HCT survivors than among their siblings. Among survivors of HCT at least 10 years after transplant, the 15-year cumulative incidence of severe/life-threatening/fatal conditions was 41%.

[0064] In various embodiments, methods of the present invention include treating or preventing premature or accelerated aging. In some embodiments, accelerated aging is a symptom of any one of the Progeroid syndromes, including, but not limited to, Hutchinson-Gilford progeria syndrome (HGPS), Werner syndrome (WS), Bloom syndrome (BS), Rothmund-Thomson syndrome (RTS), Cockayne syndrome (CS), xeroderma pigmentosum (XP), trichothiodystrophy (TTD), combined xeroderma pigmentosum-Cockayne syndrome (XP-CS), or restrictive dermopathy (RD). Subjects having one of these diseases or disorders typically has reduced longevity (i.e., lifespan).

[0065] In various embodiments, the methods of the present invention modulate (e.g., increase or decrease) levels of inflammation in a subject. "Inflammation" is a normal response to a variety of acute stresses on the body, including infection, fever and injury. Other types of inflammation include increased levels of pro-inflammatory cytokines found within tissues and systemically in plasma. Inflammation may be associated with infections, but it occurs in response to virtually any type of injury or threat, including physical trauma, cold, burns from radiation, heat or corrosive materials, chemical irritants, bacterial or viral pathogens, localized oxygen deprivation (ischemia) or reperfusion (sudden reinfusion of oxygen to ischemic tissue), and others. It includes the classic symptoms of redness, heat, swelling, and pain, and may be accompanied by decreased function of the inflamed organ or tissue. It is a generalized reaction involving several effects that may tend to combat an injurious agent that may be present at the site where an injury or threat was detected, or it may tend to contain the injury or threat to its initial location, to keep it from spreading rapidly. Inflammation is a self-defensive reaction aimed at eliminating or neutralizing injurious stimuli, and restoring tissue integrity. Like peripheral inflammation, neuroinflammation can become a harmful process, and it is now widely accepted that it may contributes to the pathogenesis of many central nervous system disorders. CNS inflammation is commonly associated with some degree of tissue damage including, loss of myelin sheaths or loss of axons, and is a central theme in human patients with MS. The level of inflammation can be quantified by performing a simple blood test for a particular compound called C-reactive protein, or CRP.

[0066] In various embodiments, the methods of the present invention decrease levels of sterile chronic systemic inflammation in a subject. "Sterile chronic systemic inflammation", named "inflammaging" is a characteristic of aging. Chronic inflammation causes damage over time to organ systems like the heart, brain and kidneys, leading to disability or premature death. Blood vessels that supply these organs are vulnerable to inflammation, leading to vessel wall-thickening and narrowing of the blood passageway. Elevated CRP levels, measured over time, are an indicator of chronic inflammation in humans. Studies have shown that elevated levels of CRP correlate with an increased risk of heart attack and stroke. *Aging* is an intricate process that results from a combination of environmental, genetic, epigenetic, and stochastic factors. A chronic proinflammatory

status is a pervasive feature of aging. This chronic, low-grade, systemic inflammation occurring in the absence of overt infection (sterile inflammation) has been defined as "inflammaging" and represents a significant risk factor for morbidity and mortality in the elderly. Prattichizzo et al in (Inflammaging" as a Druggable Target: A Senescence-Associated Secretory Phenotype-Centered View of Type 2 Diabetes) *Oxid Med Cell Longev.* 2016 and Nasi et al in (*Aging* and inflammation in patients with HIV infection), *Clin Exp Immunol.* 2016 May 20, explore the connection between aging and inflammation.

[0067] In various embodiments, methods of the present invention treat or prevent age-related diseases or disorders in a subject. The term "age-related disease or disorder" includes but is not limited to a disease or disorder in an adult such as cancer, a metabolic disease, cardiovascular disease, tobacco-related disease, or skin wrinkles. Cancer includes but is not limited to prostate cancer, colon cancer, lung cancer, squamous cell cancer of the head and neck, esophageal cancer, hepatocellular carcinoma, gastric cancer, pancreatic cancer, ovarian cancer, or breast cancer. Age-related or tobacco-related disease or disorder includes cardiovascular disease, cerebrovascular disease, peripheral vascular disease, Alzheimer's disease, osteoarthritis, cardiac diastolic dysfunction, benign prostatic hypertrophy, aortic aneurysm, or emphysema.

[0068] In various embodiments, methods of the present invention mediate rejuvenation in a subject. The term "rejuvenation" refers to the results of reducing or preventing the progress of aging and/or reducing or preventing the progress of an age-related disease or disorder. The term "rejuvenating" refers to a process of improving parameters of frailty index and/or other markers of aging cell phenotypes or markers of age-related disease or disorder states, e.g., improved muscle endurance or strength, improved glucose tolerance, decreased presence of systemic or local inflammatory cytokines, improved mitochondrial function, and erasing epigenetic modifications participating in the cellular aging phenotype. In some embodiments, the loss or reduction of the expression at least one of the markers identified as having increased expression in adipose tissue macrophages (ATMs) from aged mice (Garg, S. K. et al. *Crit Rev Immunol.* 2014; 34(1). 1-14): CD11c, CD206, Mgl1, IL-6, TNF-alpha, Nos2, Ccr-7, IL-12, Arg1, Ccl-2, Ccr-1, Ccr-5, Ccr-9, Mcp-1, Cxcr-3, IL-1beta may also be considered a sign of rejuvenation.

Lifespan

[0069] In some embodiments, the present invention provides methods for increasing a subject's longevity or lifespan. For instance, in some embodiments, the present invention relates to the administration of a recombinant TLR5 agonist, e.g. flagellin or flagellin-based agent (such as entolimod) to a patient to increase longevity or lifespan.

[0070] For example, the present invention may increase a subject's longevity or lifespan by at least about 5, at least about 10, at least about 15, at least about 20, or at least about 25 years, as compared to a subject that is not administered the recombinant TLR5 agonist described herein and/or as compared to a life expectancy calculation, as described herein. Further, various embodiments of the present invention contemplate methods that reduce or decrease cellular senescence and/or immunosenescence in a subject.

[0071] In various embodiments, an increase in longevity or lifespan is assessed relative to a comparable population. For example, an increase in longevity or lifespan is assessed relative to a cohort—e.g. cohort LEB, the mean length of life of an actual birth cohort (all individuals born a given year) or a period—e.g. period LEB, the mean length of life of a hypothetical cohort assumed to be exposed, from birth through death, to the mortality rates observed at a given year. Such assessments can be made relative to various reports on lifespan and/or longevity in the art (e.g. World Health Organization (WHO)'s Health Status Statistics: Mortality). In some embodiments, the present methods provide for increased longevity or lifespan than what is expected relative to comparable populations. In some embodiments, the present methods provide for increased longevity or lifespan than what is expected relative to various reports on lifespan and/or longevity in the art (e.g. World Health Organization (WHO)'s Health Status Statistics: Mortality).

[0072] In further embodiments, an increase in longevity or lifespan is assessed with reference to one or more actuarial life tables, e.g. Life Tables For The United States Social Security Area 1900-2100 (Actuarial Study No. 120, Bell and Miller). In some embodiments, the present methods provide for increased longevity or lifespan than what is expected relative to one or more actuarial life tables.

Subjects

[0073] The methods provided herein can be used with a patient that is a mammal, including humans and non-human mammals. Non-human mammals treated using the present methods include domesticated animals (i.e., canine, feline, murine, rodentia, and lagomorpha) and agricultural animals (bovine, equine, ovine, porcine). In various examples, the individual to whom a compound or composition is administered is an individual who is at risk for, is suspected of having or has been diagnosed with an age-related disease or disorder.

[0074] In various embodiments of the present invention, the patient is a young human, a middle-aged human, or an elderly human. For example, in some embodiments, the patient is between about 18 and about 35 years, or between about 18 and about 30 years, or between about 18 and about 25 years, or between about 18 and about 20 years. In some embodiments, the patient is between about 36 and about 55 years, or between about 40 and about 55 years, or between about 45 and about 55 years, or between about 36 and about 50 years, or between about 36 and about 45 years, or between about 36 and about 40 years, or between about 40 and about 50 years old, or between about 45 and about 55 years old. In some embodiments, the patient is between about 56 and about 85 years, or between about 60 and about 85 years, or about 65 and about 85 years, or between about 70 and about 85 years, or between about 75 and about 85 years, or between 80 and about 85 years, or between 56 and about 80 years, or between 56 and about 75 years, or between 56 and about 70 years, or between 56 and about 65 years, or between 56 and about 60 years, or between about 60 years and about 80 years, or about 65 years and about 75 years.

[0075] In some embodiments, the patient is about 1, or about 2, or about 3, or about 4, or about 5, or about 6, or about 7, or about 8, or about 9, or about 10, or about 11, or about 12, or about 13, or about 14, or about 15, or about 16, or about 17, or about 18, or about 19, or about 20, or about

21, or about 22, or about 23, or about 24, or about 25, or about 26, or about 27, or about 28, or about 29, or about 30, or about 31, or about 32, or about 33, or about 34, or about 35, or about 36, or about 37, or about 38, or about 39, or about 40, or about 41, or about 42, or about 43, or about 44, or about 45, or about 46, or about 47, or about 48, or about 49, or about 50, or about 51, or about 52, or about 53, or about 54, or about 55, or about 56, or about 57, or about 58, or about 59, or about 60, or about 61, or about 62, or about 63, or about 64, or or about 65, or about 66, or about 67, or or about 68, or about 69, or about 70, or about 71, or about 72, or about 73, or about 74, or about 75, or about 76, or about 77, or about 78, or about 79, or about 80, or about 81, or about 82, or about 83, or about 84, or about 85 years old. In some embodiments, the patient is at least 55 years old.

[0076] A person of skill in the art will contemplate that age ranges with respect to "young," "middle-aged," and "elderly" definitions can vary based on geographic region, among other factors. Petry, *Gerontologist* 2002 February; 42(1):92-9 describes age-related definitions and is hereby incorporated by reference in its entirety.

[0077] In embodiments, the biological sex of the patient is male or female. In embodiments, the biological sex of the patient is male. In embodiments, the biological sex of the patient is female.

[0078] In embodiments, the biological sex of the patient is male and the patient is middle aged (e.g. between about 36 and about 55 years, or between about 40 and about 55 years, or between about 45 and about 55 years, or between about 36 and about 50 years, or between about 36 and about 45 years, or between about 36 and about 40 years, or between about 40 and about 50 years old, or between about 45 and about 55 years old). In some embodiments, the present methods, e.g. as applicable to a middle aged male patient, prevent or reduce the severity of one or more frailties and age-related diseases or disorders.

[0079] In various embodiments of the present invention, the subject is a patient. In some embodiments, the patient is a middle-aged human. For example, in some embodiments, the patient is between about 35 and 55 years old. In further embodiments, the biological sex of the patient is male.

[0080] In some embodiments of the methods provided herein, the patient is a mammal. In some embodiments of the methods provided herein, the patient is a human. In certain embodiments of the methods provided herein, the patient is a male.

TLR5 Agonists and Derivatives Thereof

[0081] Toll-like receptors (TLRs) play a central role in the initiation of cellular innate immune responses. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents and mediate the production of cytokines necessary for the development of effective immunity. There are 10 TLR genes in humans and 12 in mice. In particular, Toll-like receptor 5 (TLR5) is a transmembrane protein that recognizes bacterial flagellin and is highly expressed in the intestinal mucosa. Vertebrate organisms recognize the presence of potentially pathogenic flagella-carrying bacteria via signaling activated by a highly specific interaction of flagellin with TLR5 that triggers a cascade of signal transduction events aimed at activation and mobilization of natural defense mechanisms of innate immunity. Activation of TLR5 by entolimod (CBLB502), a pharmacologically-useful flagellin derivative, was capable of protecting animals from lethal total body irradiation.

[0082] As used herein, the term "TLR5 agonist" refers to a compound or peptide that selectively activates or increases normal signal transduction through TLR5. In some embodiments of the present invention, the TLR5 agonist is recombinant. In some embodiments, a TLR5 agonist has an EC50 of less than about 10^{-7}M; or less than 10^{-8} M; or less than 10^{-9}M; or less than 10^{-1} M; or less than $10^{-1\circ}$ M; or less than 10^{-11} M. In certain embodiments, a TLR5 agonist as provided herein has an EC50 of less than about 10^{-7}M; or less than 10^{-8} M; or less than 10^{-9}M; or less than 10^{-10} M; or less than 10^{-10} M; or less than 10^{-11}M in the flagellin bioactivity assay using HEK-Blue™-hTLR5 cells (Invivogen) as described in Lu Y., et al., *Biotechnol. Bioeng.* 110, 2073-2085 (2013) and in Lu and Swartz, *Sci Rep* 6:18379 (2016) or a similar TLR5 bioactivity assay.

[0083] In some embodiments, a TLR5 agonist that is not fused to a pathogenic protein as provided herein is a flagellin-based agent. As used herein, the term "flagellin" means flagellin polypeptide contained in a variety of Gram-positive or Gram-negative bacterial species. The nucleotide and amino acid sequences of flagellin from 22 bacterial species are provided in FIG. 7 of United States Patent Publication No. 2003/0044429, which is hereby incorporated by reference in its entirety. Therefore, the sequence differences between species is included within the meaning of the term. In certain embodiments a flagellin-based agent in accordance with the present disclosure includes an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity, or 100% identity with one or more of the flagellin from 22 bacterial species provided in FIG. 7 of United States Patent Publication No. 2003/0044429. The amino acid sequences of the conserved amino and carboxy terminus (important for TLR5 activity) from 21 species of bacteria are provided in FIG. 24A and 24B of U.S. Pat. No. 8,007,812, which is hereby incorporated by reference in its entirety.

[0084] In certain embodiments, a flagellin-based agent in accordance with the present disclosure includes a fragment of a flagellin protein or a flagellin-based agent. In some embodiments a flagellin based-agent or fragment thereof has activity as a TLR5 agonist. In various embodiments,

[0085] In some embodiments, the TLR5 agonist is a *Salmonella* flagellin protein, e.g. a recombinant *Salmonella* flagellin protein. In some embodiments, the TLR5 agonist is a *Salmonella dublin* flagellin protein, e.g. a recombinant *Salmonella dublin* flagellin protein. In various embodiments, the *Salmonella dublin* flagellin protein has the amino acid sequence of SEQ ID NO: 27, as shown below:

(SEQ ID NO: 27)

MAQVINTNSLSLLTQNNLNKSQSSLSSAIERLSSGLRINSAKDDAAGQA

IANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNNLQRVRELSVQ

ATNGINSDSDLKSIQDEIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQ

VGANDGETITIDLQKIDVKSLGLDGFNVNGPKEATVGDLKSSFKNVTGY

DTYAAGADKYRVDINSGAVVTDAAAPDKVYVNAANGQLTTDDAENNTAV

DLFKTTKSTAGTAEAKAIAGAIKGGKEGDTFDYKGVTFTIDTKTGDDGN

-continued

GKVSTTINGEKVTLTVADIATGAADVNAATLQSSKNVYTSVVNGQFTFD

DKTKNESAKLSDLEANNAVKGESKITVNGAEYTANATGDKITLAGKTMF

IDKTASGVSTLINEDAAAAKKSTANPLASIDSALSKVDAVRSSLGAIQN

RFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKAQILQQAGTSVL

AQANQVPQNVLSLLR.

[0086] In some embodiments, the present invention contemplates use of a TLR5 agonist comprising a polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 93% at least about 95%, or at least about 96%, or at least about 97% or at least about 98%, or at least about 99%, or 100% sequence identity to SEQ ID NO: 27. In various embodiments, the polypeptide having an amino acid sequence does not comprise a His tag.

[0087] In some embodiments of the methods provided herein, the TLR5 agonist that is not fused to a pathogenic protein antigen is entolimod (CBLB502). Entolimod (CBLB502) is a flagellin-related polypeptide (see, e.g., FIG. 7 of U.S. Patent Publication No. 2003/0044429, the contents of which are incorporated herein by reference in their entirety). As used herein"entolimod" (aka "CBLB502") refers to a polypeptide which has the sequence of SEQ ID NO: 1 of WIPO Patent Application WO/2016/109002 (hereby incorporated by reference in its entirety), as shown below:

(SEQ ID NO: 1)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKI

DVKSLGLDGFNVNSPGISGGGGGILDSMGTLINEDAAAAKKSTANPLAS

IDSALSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYA

TEVSNMSKAQILQQAGTSVLAQANQVPQNVLSLLR.

[0088] In some embodiments, the present invention contemplates use of a TLR5 agonist comprising a polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 93% at least about 95%, or at least about 96%, or at least about 97% or at least about 98%, or at least about 99%, or 100% sequence identity to SEQ ID NO: 1. In various embodiments, the polypeptide having an amino acid sequence does not comprise a His tag.

[0089] In some embodiments of the aspects and embodiments provided herein, the TLR5 agonist that is not fused to a pathogenic protein is a flagellin-based agent comprising a polypeptide having an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity, or 100% identity with one or more of CBLB502-S33ML (SEQ ID NO: 35 of WO/2016/019034), CBLB502-485CT (CBLB533, SEQ ID NO: 71 of WO/2016/019034), CBLB502-S33MX (CBLB543, SEQ ID NO: 150 of WO/2016/019034), CBLB502-S33 (SEQ ID NO: 17 of WO/2016/019034), Mutant 33ML (SEQ ID NO: 42 of WO 2016/019034) of International Patent Application WO 2016/019034 (hereby incorporated by reference in its entirety), as shown below, respectively:

CBLB502-S33ML (SEQ ID NO: 35 of WO/2016/019034)

(SEQ ID NO: 2)

MSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGA

LNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQRLEEIDRVSNQT

QFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSLGLDGFNVNSPG

ISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALSKVDAVRSSLG

AIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKAQILQQAG

TSVLAQANQVPQNVLSLLVPRGSHHHHHHG;

CBLB502-485CT (CBLB533, SEQ ID NO: 71 of WO/2016/019034)

(SEQ ID NO: 3)

MSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGA

LNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQRLEEIDRVSNQT

QFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSLGLDGFNVNSPG

STANPLASIDSALSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARS

RIEDADYATEVSNMSKAQILQQAGLVPRGSHHHHHHG;

CBLB502-S33MX (CBLB543, SEQ ID NO: 150 of WO/2016/019034)

(SEQ ID NO: 4)

MSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNAADGISIAQTTEGA

LNEINNNLQRVRELSVQATAGANADAALKAIQAEIQQRLEEIDRVSQQT

QAAAVKVLSQDNAMAIQVGANDGAAITIDLQKIDVKSLGLDGFNVNSPG

STANPLASIDSALSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARS

RIEDADYATEVSQMSKAQILQQAGTSVLAQANQVPQNVLSLLVPRGSHH

HHHHG;

CBLB502-S33 (SEQ ID NO: 17 of WO/2016/019034)

(SEQ ID NO: 5)

MRGSHHHHHHGMASMTGGQQMGRDLYDLVPRGSAKDPSGLRINSAKDDA

AGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNNLQRVRE

LSVQATNGTNSDSDLKSIQDEIQQRLEEIDRVSNQTQFNGVKVLSQDNQ

MKIQVGANDGETITIDLQKIDVKSLGLDGFNVNSPGISGGGGGILDSMG

TLINEDAAAAKKSTANPLASIDSALSKVDAVRSSLGAIQNRFDSAITNL

GNTVTNLNSARSRIEDADYATEVSNMSKAQILQQAGTSVLAQANQVPQN

VLSLLR;

and

Mutant 33ML (SEQ ID NO: 42 of WO/2016/019034)

(SEQ ID NO: 6)

MSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGA

LNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQRLEEIDRVSNQT

QFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSLGLDGFNVNSPG

STANPLASIDSALSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARS

RIEDADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSLLVPRGSHH

HHHHG.

[0090] In some embodiments, the present invention contemplates use of a TLR5 agonist comprising a polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 93% at least about 95%, or at least about 96%, or at least about 97% or at least about 98%, or at least about 99%, or 100% sequence identity to one or more of SEQ ID NOs: 2-6. In various embodiments, the polypeptide having an amino acid sequence does not comprise a His tag.

[0091] In some embodiments of the aspects and embodiments provided herein, the TLR5 agonist that is not fused to a pathogenic protein is a flagellin-based agent comprising a polypeptide having an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity or 100% identity with one or more of SEQ ID NOs: 243-252 of International Patent Application WO 2016/019134 (hereby incorporated by reference in its entirety), as shown below, respectively:

SEQ ID NO: 243 of WO 2016/019134 (SEQ ID NO: 7)

MGHHHHHHSGMEEFNMRINTNVAAMNTYSRLTAANTAKSNSLAKLSSGL

RINKAGDDAAGLAISEKMKSQIGGLTQAKRNAQDGISLVQTAEGALNET

HSILERMRDLAVQGSNGTLISSDRGSINKELKALHQELTRISNITEFNT

QKLFSQTKQKSVIFTFQIGANAGQTLSVAITAMSGEALLVSTDAKFSLN

AAGTNAGAMIKSIDAAIAKVSDQRADLGAVQNRLEHTINNLTATNENLS

DANSRIRDVDMAEEMMTFTKSNILSQAATSMLAQANAMPNSVLNLLQG;

SEQ ID NO: 244 of WO 2016/019134 (SEQ ID NO: 8)

MGHHHHHHSGMRINHNISALNAWRNIDQTQYSMSKTLERLSSGLRINRA

GDDAAGLAISEKMRGQIKGLNMAIKNAQDAISLIQTAEGALTEVHSILQ

RMRELAVQAASDTNTNVDREQIQKEIDQLREEIDRIARTTEFNIKKLLD

GKLEGFRSQVDAKVVTGGNINVQLGTVSSKAVEGTYVIEVGAAERAIMV

VDAAIHRVSTARAALGAIQNRLEHTISNLGVAAENLTAAESRIRDADMA

KEMMEFTKQQILLQSSMAMLAQSNTLPQNVLQLMR;

SEQ ID NO: 245 of WO 2016/019134 (SEQ ID NO: 9)

MGHHHHHHSGLNMAIKNAQDAISLIQTAEGALTEVHSILQRMRELAVQA

ASDTNTNVDREQIQKEIDQLREEIDRIARTTEFNIKKLLDGKLEGFRSQ

VDAKVVTGGNINVQLGTVSSKAVEGTYVIEVGAAERAIMVVDAAIHRVS

TARAALGAIQNRLEHTISNLG;

SEQ ID NO: 246 of WO 2016/019134 (SEQ ID NO: 10)

MGHHHHHHSGMSLRINNNIEALNAWRALNSTSNALQKSMEKLSSGLRIN

RAGDDAAGLAISEKLRAQIRGLNQAIRNAQDGISLIQTAEGGLSEIQNI

LQRMRELGVQAANGTLNNQDISAITTELNQLFNEIDRIAGATEFNIKNL

LAVSTGLVVTLQVGANAGQVIAFTIDNAGTASLGLSSADLAINDNASAS

AFISKVDSALQKVSTYRANLGSIQNRLEHTIANLGIASENLSASESRIR

DVDMAAEMMNFTKNQILQQAGVAILAQANQAPQAVLQLLR;

-continued

SEQ ID NO: 247 of WO 2016/019134 (SEQ ID NO: 11)

MGHHHHHHSGLNQAIRNAQDGISLIQTAEGGLSEIQNILQRMRELGVQA

ANGTLNNQDISAITTELNQLFNEIDRIAGATEFNIKNLLAVSTGLVVTL

QVGANAGQVIAFTIDNAGTASLGLSSADLAINDNASASAFISKVDSALQ

KVSTYRANLGSIQNRLEHTIANLG;

SEQ ID NO: 248 of WO 2016/019134 (SEQ ID NO: 12)

MGHHHHHHSGLNQAIRNAQDGISLIQTAEGGLSEIQNILQRMRELGVQA

ANGTLNNQDISAITTELNQLFNEIDRIAGATEFNTKNLLAAGTASLGLS

SADLAINDNASASAFISKVDSALQKVSTYRANLGSIQNRLEHTIANLG;

SEQ ID NO: 249 of WO 2016/019134 (SEQ ID NO: 13)

MGHHHHHHSASAFISKVDSALQKVSTYRANLGSIQNRLEHTIANLGPDG

LNQAIRNAQDGISLIQTAEGGLSEIQNILQRMRELGVQAANGTLNNQDI

SAITTELNQLFNEIDRIA;

SEQ ID NO: 250 of WO 2016/019134 (SEQ ID NO: 14)

MGHHHHHHSNNQDISAITTELNQLFNEIDRIAGATGSGGLSEIQNILQR

MRELGVQAANGTLNGGSASAFISKVDSALQKVSTYRANLGSIQNRLEHT

IANLG;

SEQ ID NO: 251 of WO 2016/019134 (SEQ ID NO: 15)

MGHHHHHHSGLAQASRNAQDAISIAQTAEGALDETQSILQRVRELGVQG

ANGTLTADDINALQAEVDQLIAEIDRIAGATEFNTQNLLDGSFTTKAFQ

VGANSGQNMTLTIGKMDTTTLGLSSADLAINDNAFANGAISTVDSALQK

VSAERAKLGAIQNRLEHTIANLG;
and

SEQ ID NO: 252 of WO 2016/019134 (SEQ ID NO: 16)

MGHHHHHHSGLAQASRQAQDAISIAQTAEGALDETQSILQRVRELGVQG

ADGTLTADDIDALQAEVDQLIAEIDRIAGATEFATQKLLDGSFTTKAFQ

VGAASGQDVTLTIGKVDTTTLGLSSADLAIDSAAFADGAISTVDSALQK

VSAERAKLGAIQNRLEHTIAQLG.

[0092] In some embodiments, the present invention contemplates use of a TLR5 agonist comprising a polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 93% at least about 95%, or at least about 96%, or at least about 97% or at least about 98%, or at least about 99%, or 100% sequence identity to one or more of SEQ ID NOs: 7-16. In various embodiments, the polypeptide having an amino acid sequence does not comprise a His tag.

[0093] In some embodiments of the aspects and embodiments provided herein, the TLR5 agonist that is not fused to a pathogenic protein is a flagellin-based agent comprising a polypeptide having an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity or 100% identity with one or more of SEQ ID NOs: 10, 12, 30, 32, 34, 36, 38, 40, 42, or 44 of International Patent Application

WO 2006/069198 (hereby incorporated by reference in its entirety), as shown below, respectively:

SEQ ID NO: 10 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKI

DVKSLGLDGFNVNSPGISGGGGGILDSMGTLINEDAAAAKKSTANPLAS

IDSALSKVDAVRSSLGAIQNRFDSAITNL;

SEQ ID NO: 12 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPFTSNIKGLTQASRNAN

DGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQ

RLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKS

LGLDGFNVNSPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSA

LSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVS

NMSKAQILQQAGTSVLAQANQVPQNVLSLLR;

SEQ ID NO: 30 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKI

DVKSLGLIPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALS

KVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNM

SKAQILQQAGTSVLAQANQVPQNVLSLLR;

SEQ ID NO: 32 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPFTSNIKGLTQASRNAN

DGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQ

RLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKS

LGLIPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALSKVDA

VRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKAQ

ILQQAGTSVLAQANQVPQNVLSLLR;

SEQ ID NO: 34 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKI

IPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALSKVDAVRS

SLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKAQILQ

QAGTSVLAQANQVPQNVLSLLR;

-continued

SEQ ID NO: 36 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPFTSNIKGLTQASRNAN

DGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQ

RLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKIIPGI

SGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALSKVDAVRSSLGA

IQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKAQILQQAGT

SVLAQANQVPQNVLSLLR;

SEQ ID NO: 38 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKI

DVKSLGLIPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALS

KVDAVRSSLGAIQNRFDSAITNL;

SEQ ID NO: 40 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKI

IPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALSKVDAVRS

SLGAIQNRFDSAITNL;

SEQ ID NO: 42 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQN

NLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQAS

RNANDGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQD

EIQQRLEEIDRVSNQIPGISGGGGGILDSMGTLINEDAAAAKKSTANPL

ASIDSALSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDAD

YATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSLLR;

and

SEQ ID NO: 44 of WO 2006/069198

(SEQ ID NO: 17)

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPFTSNIKGLTQASRNAN

DGISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQ

RLEEIDRVSNQIPGISGGGGGILDSMGTLINEDAAAAKKSTANPLASID

SALSKVDAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATE

VSNMSKAQILQQAGTSVLAQANQVPQNVLSLLR.

[0094] In some embodiments, the present invention contemplates use of a TLR5 agonist comprising a polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 93% at least about 95%, or at least about 96%, or at least about 97% or at least about 98%, or at least about 99%, or 100% sequence identity to one or more of SEQ ID

NOs: 17-26. In various embodiments, the polypeptide having an amino acid sequence does not comprise a His tag.

[0095] Examples of the pathogenic protein antigen that in some embodiments would not be fused to a TLR5 agonist and/or flagellin based agent as described herein include an α-helix domain of surface protein A (PspA) and pneumococcal surface protein A (PsaA) of *Streptococcus pneumonia*; subunit hemagglutinin (HA) and neuraminidase (NA) of influenza virus; and spike (S) protein of severe acute respiratory syndrome virus (SARS virus), and the like.

Dosage, Administration and Pharmaceutical Formulation

[0096] In embodiments, a pharmaceutical preparation of TLR5 agonist is used in the variousmethods and, in some embodiments, it may be in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The composition can, if desired, also contain other compatible therapeutic agents. Some pharmaceutical preparations can deliver the compounds of the disclosure in a sustained release formulation.

[0097] A of TLR5 agonist according to the invention, the dosage form may optionally be a liquid dosage form. Solutions can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose or an emulsifier such as polysorbate. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in *Remington's Pharmaceutical Sciences* (2003-20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999. Formulations optionally contain excipients including, but not limited to, a buffering agents, an anti-oxidant, a stabilizer, a carrier, a diluent, and an agent for pH adjustment. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins such as serum, albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN, PLURONICS or polyethylene glycol (PEG).

[0098] In treatment, the dose of of TLR5 agonist optionally ranges from about 0.0001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 5 mg/kg, about 0.15 mg/kg to about 3 mg/kg, 0.5 mg/kg to about 2 mg/kg and about 1 mg/kg to about 2 mg/kg of the subject's body weight. In other embodiments the dose ranges from about 100 mg/kg to about 5 g/kg, about 500 mg/kg to about 2 mg/kg and about 750 mg/kg to about 1.5 g/kg of the subject's body weight. For example, depending on the type and severity of the disease or disorder, about 1 .mu.g/kg to 15 mg/kg (e.g., 0.1-20 mg/kg) of agent is a candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage is in the range from about 1 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease or disorder symptoms occurs. However, other dosage regimens may be useful. Unit doses can be in the range, for instance of about 5 mg to 500 mg, such as 50 mg, 100 mg, 150 mg, 200 mg, 250 mg and 300 mg. The progress of therapy is monitored by conventional techniques and assays.

[0099] In some embodiments, a TLR5 agonist, e.g. flagellin or flagellin-based agent (such as entolimod) is administered to a human patient at an effective amount (or dose) of less than about 1 μg/kg, for instance, about 0.35 to about 0.75 μg/kg or about 0.40 to about 0.60 μg/kg. In some embodiments, the dose of a flagellin or flagellin-based agent (such as entolimod) is about 0.35 μg/kg, or about 0.40 μg/kg, or about 0.45 μg/kg, or about 0.50 μg/kg, or about 0.55 μg/kg, or about 0.60 μg/kg, or about 0.65 μg/kg, or about 0.70 μg/kg, or about 0.75 μg/kg, or about 0.80 μg/kg, or about 0.85 μg/kg, or about 0.90 μg/kg, or about 0.95 μg/kg or about 1 μg/kg. In various embodiments, the absolute dose of a flagellin or flagellin-based agent (such as entolimod) is about 2 μg/subject to about 45 μg/subject, or about 5 to about 40, or about 10 to about 30, or about 15 to about 25 μg/subject. In some embodiments, the absolute dose of a flagellin or flagellin-based agent (such as entolimod) is about 20 μg, or about 30 μg, or about 40 μg.

[0100] In various embodiments, the dose of TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) may be determined by the human patient's body weight. For example, an absolute dose of a flagellin or flagellin-based agent (such as entolimod) of about 2 μg for a pediatric human patient of about 0 to about 5 kg (e.g. about 0, or about 1, or about 2, or about 3, or about 4, or about 5 kg); or about 3 μg for a pediatric human patient of about 6 to about 8 kg (e.g. about 6, or about 7, or about 8 kg), or about 5 μg for a pediatric human patient of about 9 to about 13 kg (e.g. 9, or about 10, or about 11, or about 12, or about 13 kg); or about 8 μg for a pediatric human patient of about 14 to about 20 kg (e.g. about 14, or about 16, or about 18, or about 20 kg), or about 12 μg for a pediatric human patient of about 21 to about 30 kg (e.g. about 21, or about 23, or about 25, or about 27, or about 30 kg), or about 13 μg for a pediatric human patient of about 31 to about 33 kg (e.g. about 31, or about 32, or about 33 kg), or about 20 μg for an adult human patient of about 34 to about 50 kg (e.g. about 34, or about 36, or about 38, or about 40, or about 42, or about 44, or

about 46, or about 48, or about 50 kg), or about 30 μg for an adult human patient of about 51 to about 75 kg (e.g. about 51, or about 55, or about 60, or about 65, or about 70, or about 75 kg), or about 45 μg for an adult human patient of greater than about 114 kg (e.g. about 114, or about 120, or about 130, or about 140, or about 150 kg).

[0101] In certain embodiments, a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) in accordance with the methods provided herein is administered subcutaneously (s.c.), intraveneously (i.v.), intramuscularly (i.m.), intranasally or topically. Administration of a flagellin or flagellin-based agent (such as entolimod) described herein can, independently, be one to four times daily or one to four times per month or one to six times per year or once every two, three, four or five years. Administration can be for the duration of one day or one month, two months, three months, six months, one year, two years, three years, and may even be for the life of the human patient. The dosage may be administered as a single dose or divided into multiple doses. In some embodiments, a flagellin or flagellin-based agent (such as entolimod) is administered about 1 to about 3 times (e.g. 1, or 2 or 3 times). In some embodiments, a flagellin or flagellin-based agent (such as entolimod) is administered once.

[0102] In some embodiments of the methods provided herein, TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) is administered in one or more cycles. In certain embodiments of the methods as provided herein, a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) is administered in one or more cycles in which a cycle involves dosing a patient once per day for one day; or once a day for two days; or once a day for three days; or once a day for four days; or once a day for five days. In certain embodiments of the methods as provided herein, a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) is administered in one or more cycles as provided herein, and wherein no more than 5 cycles are administered per year; or no more than 3 cycles are administered per year; or no more than 2 cycles are administered per year.

[0103] Various modes of administration of a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) are contemplated herein. In one embodiment, a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) is administered parenterally. In some embodiments, a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) is administered by injection, e.g. intramuscular injection. In some embodiments, a TLR5 agonist, e.g. a flagellin or flagellin-based agent (such as entolimod) is by a single intramuscular injection. In some embodiments, administration is accomplished using a kit as described herein (e.g. via a unit dose form, e.g. a pre-loaded (a.k.a. pre-dosed or pre-filled) syringe or a pen needle injector (injection pen)).

Kits

[0104] The invention provides kits that can simplify the administration of any agent described herein. An illustrative kit of the invention comprises any composition described herein in unit dosage form. In one embodiment, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any agent described herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle. The kit can further comprise a label or printed instructions instructing the use of any agent described herein. The kit may also include a lid speculum, topical anesthetic, and a cleaning agent for the administration location. The kit can also further comprise one or more additional agent described herein. In one embodiment, the kit comprises a container containing an effective amount of a composition of the invention and an effective amount of another composition, such those described herein.

Definitions

[0105] With respect to the agents described herein, the terms "modulate" and "modulation" refers to the upregulation (i.e., activation or stimulation) or downregulation (i.e., inhibition or suppression) of a response. A "modulator" is an agent, compound, or molecule that modulates, and may be, for example, an agonist, antagonist, activator, stimulator, suppressor, or inhibitor. The terms "inhibit", "reduce", remove as used herein refer to any inhibition, reduction, decrease, suppression, downregulation, or prevention in expression, activity or symptom and include partial or complete inhibition of activity or symptom. Partial inhibition can imply a level of expression, activity or symptom that is, for example, less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% of the uninhibited expression, activity or symptom. The terms "eliminate" or "eradicate" indicate a complete reduction of activity or symptom.

[0106] As used herein, the term "a disorder" or "a disease" refers to any derangement or abnormality of function; a morbid physical or mental state. See Dorland's Illustrated Medical Dictionary, (W.B. Saunders Co. 27th ed. 1988).

[0107] As used herein, the term "treating" or "treatment" of any disease or disorder refers in one embodiment, to ameliorating the disease or disorder (i.e., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment "treating" or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, "treating" or "treatment" refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In yet another embodiment, "treating" or "treatment" refers to preventing or delaying the onset or development or progression of the disease or disorder.

[0108] As used herein, the term "abnormal" refers to an activity or feature which differs from a normal activity or feature. As used herein, the term "abnormal activity" refers to an activity which differs from the activity of the wild-type or native gene or protein, or which differs from the activity of the gene or protein in a healthy subject. The abnormal activity can be stronger or weaker than the normal activity. In one embodiment, the "abnormal activity" includes the abnormal (either over- or under-) production of mRNA transcribed from a gene. In another embodiment, the "abnormal activity" includes the abnormal (either over- or under-) production of polypeptide from a gene. In another embodiment, the abnormal activity refers to a level of a mRNA or polypeptide that is different from a normal level of the mRNA or polypeptide by about 15%, about 25%, about

35%, about 50%, about 65%, about 85%, about 100% or greater. In some embodiments, the abnormal level of the mRNA or polypeptide can be either higher or lower than the normal level of the mRNA or polypeptide. Yet in another embodiment, the abnormal activity refers to functional activity of a protein that is different from a normal activity of the wild-type protein. In some embodiments, the abnormal activity can be stronger or weaker than the normal activity. In some embodiments, the abnormal activity is due to the mutations in the corresponding gene, and the mutations can be in the coding region of the gene or non-coding regions such as transcriptional promoter regions. The mutations can be substitutions, deletions, insertions.

[0109] "Therapeutically effective amount" as used herein means the amount of a compound or composition (such as described herein) that causes at least one desirable change in a cell, population of cells, tissue, individual, patient or the like. In some embodiments a therapeutically effective amount as used herein means the amount of a compound or composition (such as described herein) that prevents or provides a clinically significant change in a disease or disorder or condition (e.g., reduce by at least about 30 percent, at least about 50 percent, or at least about 90 percent) or in one or more features of a disease or disorder or condition described herein.

EXAMPLES

[0110] The present disclosure will be further described in the following examples, which do not limit the scope of any invention or inventions described in the claims.

Example 1: Pharmacological Stimulation of TLR5 Improves Quality of Life and Reduces Frailty

[0111] This example describes a pharmacological flagellin-based agent and method of its use to prevent aging-related frailty and extend healthy life ("healthspan") and longevity ("lifespan").

[0112] Preclinical model of aging. General decline of the majority of physiological functions, regenerating capabilities, impaired wound healing, gradual elevation of the risk of a variety of diseases or disorders, increase in systemic sterile inflammation occur during lifetime of all mammals leads to acquisition of a combination of symptoms cumulatively defined as frailty. Increase in frailty observed in healthy animals and humans that is not provoked or accelerated by any particular pathology is named chronological aging. The severity of this condition grows with time and reaches critical degrees of severity when the organism approaches age that is close to its natural genetically determined lifespan. Chronological aging of laboratory mouse is a well-accepted model of human aging commonly used in the field of gerontology. Natural lifespan of most of strains of mice is about 2 years. Each strain is characterized by its prevalent spectrum of age-related diseases or disorders, which becomes a major cause of death, a phenomenon that may jeopardize other manifestations of aging and complicate studies of frailty. To minimize the influence of this factor, we used for our studies outbred NIH Swiss mice that are naturally less prone to genetic predisposition to certain specific pathologies. We have characterized the longevity of both genders of NIH Swiss mice that are maintained under conditions of minimized risk of the health risks (infections, poisoning, trauma, stress, etc.) other than age-related frailty. These conditions are provided in the Department of Laboratory Animals of Roswell Park Cancer Institute, where animals are maintained according to the Animal Welfare Guidelines of NIH. Under these conditions, animals have the highest chance to fully realize their natural lifespan and die from frailty that reflects endogenous processes of natural chronological aging (FIG. **1**). The adequacy of this model was confirmed by its capability to reveal the biological effect of factors that are known to modulate longevity as shown in FIG. **7**: treatment of mice with mTOR inhibitor rapamycin was shown to extend the lifespan of mice. Indeed, the results of treatment with mTOR inhibitor rapamycin appear to have the polar opposite effect of the results shown regarding administration of flagellin or entolimod.

[0113] Objective quantitative assessment of biological age was done by determination of a so-called "frailty index" (FI), a parameter which reflects the scale of accumulation of age-related deficits. Commonly used in gerontological clinics, FI was adapted to laboratory animals and was calculated for each animal as a function of the degrees of deviation of multiple measurable physiological and biochemical parameters from those of young and healthy animals. The resulting number, which we term "Physiological Frailty Index (PFI), gradually grows with life and reflects the biological age of animal. PFI is expressed as a score from "0" (no deficits, within the range of the reference group) to "1" (extreme deficits).

[0114] Flagellin of *Salmonella*. Flagellin, a bacterial protein, the major component of bacterial flagella, is the only known agonist of innate immunity receptor TLR5. *Salmonella* flagellin was synthesized as a recombinant protein in *E. coli* and affinity purified on Ni-containing column for its His tag followed by other purification steps (e.g., polymyxin column—to get rid of endotoxin) as previously described (Burdelya, L. G. et al. An Agonist of Toll-Like Receptor 5 Has Radioprotective Activity in Mouse and Primate Models. *Science* 320, 226-230 (2008)). The quality of the resulting product was controlled using a series of functional assays involving a panel of reporter cell lines expressing individual TLRs: it was capable of activating NF-kappaB signaling only in the cells expressing TLR5 but not other TLRs. Flagellin is stored in solution as deeply frozen aliquots.

[0115] Experimental design and rationale. Animals were maintained under strictly controlled conditions of temperature, day-night switch, healthy balanced diet, constant access to drinking water, sterile food and air. Their PFI was measured at the indicated time points throughout the animal life (FIG. **2**). Three groups containing equal proportions of males and females with individual PFIs falling into typical range were separated from the rest of the group at different times of their lives and received short courses of flagellin injected s.c. daily (1 μg/injection) for 5 consecutive days. Control animals received a vehicle (saline) injections. Specific times of injections are indicated in FIG. **2**: two groups received one course (44^{th} and 55^{th} week of life), while one group—two courses (18^{th} and 84th weeks of age) of flagellin. PFI was then determined later in life and animals were monitored daily until their death. Functionality of TLR5 signaling in NIH Swiss mice was established in our previous experiments, in which TLR5 agonist (flagellin and its pharmacologicalderivative entolimod) were shown capable of protecting animals from lethal total body irradiation. This experimental design was chosen to reveal the long-lasting effects of treatment with flagellin on mouse biological age

determined as PFI. It also allowed us to detect gender-related differences in organismal response to TLR5 agonist.

[0116] Effect of flagellin treatment on chronological aging in mice. The results of three independent experiments schematically described in FIG. **2** are provided in FIGS. **3-6**. All of them demonstrate a substantial slowdown in PFI growth in the groups that received flagellin treatment. This effect was limited to the male mice and was not seen in females. There are reports that estrogen receptors and estradiol modulate TLR5 expression and TLR5—dependent response to flagellin.

[0117] For example, FIG. **3** demonstrates that treatment of NIH Swiss males on the 56th week of age with five consequent daily s.c. injections of 1 μg/mouse of flagellin are translated into a substantially lower PFI nearly one year later—on the 104th week of age. The difference is statistically significant ($p=0.04$). Graphically, this effect is shown in FIG. **4**: at 104 weeks, the average PFI in flagellin treated male group (dashed line) increased by 33% from the time of treatment at 55 weeks, while in control mice it steadily increased by 100% (solid line). Consequently, the group of treated males demonstrated extended longevity vs. vehicle-treated control (average duration of life 22 weeks or 20% longer than control). Neither of the above was observed in female group.

[0118] Similar gender-specific effects of flagellin treatment on PFI was observed in Experimental groups 2 and 3 (FIGS. **5**, **6**). Importantly, the difference between the dynamics of PFI of control and flagellin-treated mice continued to grow within the whole duration of observation suggesting that a single week-long treatment with TLR5 agonist had a long-lasting physiological effect still visible in one-year post-treatment.

Example 2: Administration of Entolimod to Improve Frailty Index

[0119] To assess the effect of entolimod treatment on the longevity (lifespan) and overall health (healthspan) of animals, male and female NIH Swiss mice were given 5 daily injections of entolimod (5 μg/mouse, SQ) at different ages: "old" age (112 weeks of age), "middle-age" (55 weeks) and "young" age (18 weeks). Groups treated at "young" age received a second round of treatment at 84 weeks. Control groups consisted of gender- and age-matched mice given SQ injections of PBS instead of entolimod. The experimental design is illustrated in FIG. **8**. Mice were housed under standard conditions during the experiment and monitored for mortality and morbidity. Survival was recorded as an indicator of lifespan. In addition, at various times post-treatment (see FIG. **8**), tests were performed to determine PFI as a quantitative indicator of healthspan.

[0120] The treated mice were allowed to age naturally under standard housing conditions and their longevity was recorded based on IACUC-approved endpoint criteria for aging animals. FIGS. **9-11** show the impact of treatment with entolimod on lifespan in the age groups tested. Middle-aged male mice showed an increased life span.

[0121] Readouts of the aging process include not only absolute life span, or longevity, but also "health span", based upon an individual's overall health status. Potential effects of entolimod treatment on health span were evaluated for the treated NIH Swiss mice by determining their Physiological Frailty Index (PFI) at the times post-treatment indicated in FIG. **8**. PFI is a quantitative measure based on comparison of physiological parameters between test and reference groups. As shown in FIG. **12**, there was no difference in mean PFI values between control and entolimod-treated groups of mice that were treated at "old" age (112 weeks) and evaluated ~4 months later (at 128 weeks). Similar results were obtained for males and females. These data are consistent with the hypothesis (without wishing to be bound by theory) that treatment of old animals cannot slow down the aging process since the animals have already acquired a number of health deficits.

[0122] In contrast, treatment of middle-aged (55 week-old) mice with entolimod did have a beneficial effect on aging as measured by PFI at 104 weeks and 120 weeks, albeit only in males (FIG. **13**). Mean PFI was significantly lower in entolimod-treated males versus PBS-treated controls at the 104 weeks evaluation timepoint. The same trend was observed at 120 weeks. There was no difference in mean PFI detected in groups of females treated with entolimod versus PBS at 55 weeks of age when evaluated at either 104 or 120 weeks of age (FIG. **13**). A similar gender difference was observed in mice treated at a young age (18 weeks) followed by a second treatment at 84 weeks (see FIG. **8** for experimental design). When evaluated at middle-age (55 weeks), there was no difference in mean PFI between entolimod- and PBS-treated groups for either males or females (FIG. **14**). However, upon evaluation at 104 weeks, after having received a second treatment during middle-age (at 84 weeks), mean PFI was significantly lower in entolimod-treated males compared to the corresponding PBS-treated group. These mice also showed a noticeable decrease in PFI at 84 weeks. Similar to what was observed for mice treated at 55 weeks of age (FIG. **13**), the beneficial effect of entolimod treatment at 18 and 84 weeks was only detected in male mice.

[0123] In summary, these results show that a single 5-day course of treatment with entolimod significantly improved health status of mice at older ages, but only when treatment was administered during middle age (in our experiments, between 55 and 84 weeks of age) and only in males.

Example 3: Administration of Entolimod to a Patient to Improve Frailty Index

[0124] A 66-year old male patient is identified that has a recent history of declining frailty index as determined using the Frailty Index (FI), the Physiological Frailty Index (PFI), Fried frailty score, Rockwood frailty index, FRAIL Scale or the modified frailty index. A single cycle that includes one dose of entolimid per day for each of three consecutive days is administered to the patient. The frailty index of the patient is monitored following the entolimid administration using the Frailty Index (FI), the Physiological Frailty Index (PFI), Fried frailty score, Rockwood frailty index, FRAIL Scale or the modified frailty index. The decline in frailty index of the patient is reduced or frailty index is improved following the entolimid administration.

Example 4: Administration of Entolimod to a Pediatric Patient Who had Received Treatment for Leukemia

[0125] A 6 year-old male cancer survivor patient who had previously been treated with chemotherapy for leukemia is identified. One cycle that include one dose of entolimid per day for each of three consecutive days are administered to

the patient and a second identical entolimid cycle is administered six months later. The frailty index of the patient is monitored following the entolimid administration and no accelerated aging is observed in the patient.

[0126] While the disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those having skill in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the present disclosure as disclosed herein.

[0127] All references referred to herein are incorporated in their entirety. Various embodiments of the present invention may be characterized by the potential claims listed in the paragraphs following this paragraph (and before the actual claims provided at the end of this application). These potential claims form a part of the written description of this application. Accordingly, subject matter of the following potential claims may be presented as actual claims in later proceedings involving this application or any application claiming priority based on this application. Inclusion of such potential claims should not be construed to mean that the actual claims do not cover the subject matter of the potential claims. Thus, a decision to not present these potential claims in later proceedings should not be construed as a donation of the subject matter to the public.

[0128] The embodiments of the invention described above are intended to be merely illustrative; numerous variations and modifications will be apparent to those skilled in the art. All such variations and modifications are intended to be within the scope of the present invention as defined in any appended claims.

[0129] As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

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Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
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Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
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Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
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Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
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Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
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Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln
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Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp
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Leu Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val
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Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr
            260                 265                 270

Asn Leu Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile
        275                 280                 285

Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser Lys Ala Gln
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Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly
        35                  40                  45

Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser
    50                  55                  60

Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile
65                  70                  75                  80

Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn
                85                  90                  95

Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met
            100                 105                 110

Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu
        115                 120                 125

Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val Asn
    130                 135                 140

Ser Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu Asp Ser Met Gly
145                 150                 155                 160

Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala Asn
                165                 170                 175

Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val Asp Ala Val Arg
            180                 185                 190

Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr Asn
        195                 200                 205

Leu Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile Glu
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Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser Lys Ala Gln Ile
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Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly
        35                  40                  45

Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser
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Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile
65                  70                  75                  80

Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn
                85                  90                  95

Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met
            100                 105                 110

Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu
        115                 120                 125

Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val Asn
    130                 135                 140

Ser Pro Gly Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu
145                 150                 155                 160

Ser Lys Val Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg
                165                 170                 175

Phe Asp Ser Ala Ile Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn
            180                 185                 190

Ser Ala Arg Ser Arg Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser
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        35                  40                  45

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    50                  55                  60

Val Gln Ala Thr Ala Gly Ala Asn Ala Asp Ala Ala Leu Lys Ala Ile
65                  70                  75                  80

Gln Ala Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Gln
                85                  90                  95

Gln Thr Gln Ala Ala Ala Val Lys Val Leu Ser Gln Asp Asn Ala Met
            100                 105                 110

Ala Ile Gln Val Gly Ala Asn Asp Gly Ala Ala Ile Thr Ile Asp Leu
        115                 120                 125

Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val Asn
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Ser Pro Gly Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu
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Ser Lys Val Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg
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Phe Asp Ser Ala Ile Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn
            180                 185                 190

Ser Ala Arg Ser Arg Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser
        195                 200                 205

Gln Met Ser Lys Ala Gln Ile Leu Gln Gln Ala Gly Thr Ser Val Leu
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        35                  40                  45

Ala Ala Gly Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly
    50                  55                  60

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65                  70                  75                  80

Thr Thr Glu Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val
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Leu Lys Ser Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp
        115                 120                 125

Arg Val Ser Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln
    130                 135                 140

Asp Asn Gln Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile
145                 150                 155                 160

Thr Ile Asp Leu Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly
                165                 170                 175

Phe Asn Val Asn Ser Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu
            180                 185                 190

Asp Ser Met Gly Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys
        195                 200                 205

Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val
    210                 215                 220

Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser
225                 230                 235                 240

Ala Ile Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg
                245                 250                 255

Ser Arg Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser
            260                 265                 270

Lys Ala Gln Ile Leu Gln Gln Ala Gly Thr Ser Val Leu Ala Gln Ala
        275                 280                 285

Asn Gln Val Pro Gln Asn Val Leu Ser Leu Leu Arg
    290                 295                 300


<210> SEQ ID NO 6
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 6

Met Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly Gln
1               5                   10                  15

Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln Ala
            20                  25                  30

Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly
        35                  40                  45

Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser
    50                  55                  60

Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile
65                  70                  75                  80

Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn
                85                  90                  95

Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met
            100                 105                 110

Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu
        115                 120                 125

Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val Asn
    130                 135                 140

Ser Pro Gly Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu
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145                 150                 155                 160

Ser Lys Val Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg
                165                 170                 175

Phe Asp Ser Ala Ile Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn
            180                 185                 190

Ser Ala Arg Ser Arg Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser
        195                 200                 205

Asn Met Ser Lys Ala Gln Ile Leu Gln Gln Ala Gly Thr Ser Val Leu
    210                 215                 220

Ala Gln Ala Asn Gln Val Pro Gln Asn Val Leu Ser Leu Leu Val Pro
225                 230                 235                 240

Arg Gly Ser His His His His His His Gly
                245                 250


<210> SEQ ID NO 7
<211> LENGTH: 293
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 7

Met Gly His His His His His His Ser Gly Met Glu Glu Phe Asn Met
1               5                   10                  15

Arg Ile Asn Thr Asn Val Ala Ala Met Asn Thr Tyr Ser Arg Leu Thr
            20                  25                  30

Ala Ala Asn Thr Ala Lys Ser Asn Ser Leu Ala Lys Leu Ser Ser Gly
        35                  40                  45

Leu Arg Ile Asn Lys Ala Gly Asp Asp Ala Ala Gly Leu Ala Ile Ser
    50                  55                  60

Glu Lys Met Lys Ser Gln Ile Gly Gly Leu Thr Gln Ala Lys Arg Asn
65                  70                  75                  80

Ala Gln Asp Gly Ile Ser Leu Val Gln Thr Ala Glu Gly Ala Leu Asn
                85                  90                  95

Glu Thr His Ser Ile Leu Glu Arg Met Arg Asp Leu Ala Val Gln Gly
            100                 105                 110

Ser Asn Gly Thr Leu Thr Ser Ser Asp Arg Gly Ser Ile Asn Lys Glu
        115                 120                 125

Leu Lys Ala Leu His Gln Glu Leu Thr Arg Ile Ser Asn Thr Thr Glu
    130                 135                 140

Phe Asn Thr Gln Lys Leu Phe Ser Gln Thr Lys Gln Lys Ser Val Thr
145                 150                 155                 160

Phe Thr Phe Gln Ile Gly Ala Asn Ala Gly Gln Thr Leu Ser Val Ala
                165                 170                 175

Ile Thr Ala Met Ser Gly Glu Ala Leu Leu Val Ser Thr Asp Ala Lys
            180                 185                 190

Phe Ser Leu Asn Ala Ala Gly Thr Asn Ala Gly Ala Met Ile Lys Ser
        195                 200                 205

Ile Asp Ala Ala Ile Ala Lys Val Ser Asp Gln Arg Ala Asp Leu Gly
    210                 215                 220

Ala Val Gln Asn Arg Leu Glu His Thr Ile Asn Asn Leu Thr Ala Thr
225                 230                 235                 240

Asn Glu Asn Leu Ser Asp Ala Asn Ser Arg Ile Arg Asp Val Asp Met
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               245                 250                 255

Ala Glu Glu Met Met Thr Phe Thr Lys Ser Asn Ile Leu Ser Gln Ala
            260                 265                 270

Ala Thr Ser Met Leu Ala Gln Ala Asn Ala Met Pro Asn Ser Val Leu
        275                 280                 285

Asn Leu Leu Gln Gly
    290


<210> SEQ ID NO 8
<211> LENGTH: 280
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 8

Met Gly His His His His His His Ser Gly Met Arg Ile Asn His Asn
1               5                   10                  15

Ile Ser Ala Leu Asn Ala Trp Arg Asn Ile Asp Gln Thr Gln Tyr Ser
            20                  25                  30

Met Ser Lys Thr Leu Glu Arg Leu Ser Ser Gly Leu Arg Ile Asn Arg
        35                  40                  45

Ala Gly Asp Asp Ala Ala Gly Leu Ala Ile Ser Glu Lys Met Arg Gly
    50                  55                  60

Gln Ile Lys Gly Leu Asn Met Ala Ile Lys Asn Ala Gln Asp Ala Ile
65                  70                  75                  80

Ser Leu Ile Gln Thr Ala Glu Gly Ala Leu Thr Glu Val His Ser Ile
                85                  90                  95

Leu Gln Arg Met Arg Glu Leu Ala Val Gln Ala Ala Ser Asp Thr Asn
            100                 105                 110

Thr Asn Val Asp Arg Glu Gln Ile Gln Lys Glu Ile Asp Gln Leu Arg
        115                 120                 125

Glu Glu Ile Asp Arg Ile Ala Arg Thr Thr Glu Phe Asn Thr Lys Lys
    130                 135                 140

Leu Leu Asp Gly Lys Leu Glu Gly Phe Arg Ser Gln Val Asp Ala Lys
145                 150                 155                 160

Val Val Thr Gly Gly Asn Ile Asn Val Gln Leu Gly Thr Val Ser Ser
                165                 170                 175

Lys Ala Val Glu Gly Thr Tyr Val Ile Glu Val Gly Ala Ala Glu Arg
            180                 185                 190

Ala Ile Met Val Val Asp Ala Ala Ile His Arg Val Ser Thr Ala Arg
        195                 200                 205

Ala Ala Leu Gly Ala Ile Gln Asn Arg Leu Glu His Thr Ile Ser Asn
    210                 215                 220

Leu Gly Val Ala Ala Glu Asn Leu Thr Ala Ala Glu Ser Arg Ile Arg
225                 230                 235                 240

Asp Ala Asp Met Ala Lys Glu Met Met Glu Phe Thr Lys Gln Gln Ile
                245                 250                 255

Leu Leu Gln Ser Ser Met Ala Met Leu Ala Gln Ser Asn Thr Leu Pro
            260                 265                 270

Gln Asn Val Leu Gln Leu Met Arg
        275                 280
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<210> SEQ ID NO 9
<211> LENGTH: 168
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 9

Met Gly His His His His His His Ser Gly Leu Asn Met Ala Ile Lys
1 5 10 15

Asn Ala Gln Asp Ala Ile Ser Leu Ile Gln Thr Ala Glu Gly Ala Leu
20 25 30

Thr Glu Val His Ser Ile Leu Gln Arg Met Arg Glu Leu Ala Val Gln
35 40 45

Ala Ala Ser Asp Thr Asn Thr Asn Val Asp Arg Glu Gln Ile Gln Lys
50 55 60

Glu Ile Asp Gln Leu Arg Glu Glu Ile Asp Arg Ile Ala Arg Thr Thr
65 70 75 80

Glu Phe Asn Thr Lys Lys Leu Leu Asp Gly Lys Leu Glu Gly Phe Arg
85 90 95

Ser Gln Val Asp Ala Lys Val Val Thr Gly Gly Asn Ile Asn Val Gln
100 105 110

Leu Gly Thr Val Ser Ser Lys Ala Val Glu Gly Thr Tyr Val Ile Glu
115 120 125

Val Gly Ala Ala Glu Arg Ala Ile Met Val Val Asp Ala Ala Ile His
130 135 140

Arg Val Ser Thr Ala Arg Ala Ala Leu Gly Ala Ile Gln Asn Arg Leu
145 150 155 160

Glu His Thr Ile Ser Asn Leu Gly
165

<210> SEQ ID NO 10
<211> LENGTH: 285
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 10

Met Gly His His His His His His Ser Gly Met Ser Leu Arg Ile Asn
1 5 10 15

Asn Asn Ile Glu Ala Leu Asn Ala Trp Arg Ala Leu Asn Ser Thr Ser
20 25 30

Asn Ala Leu Gln Lys Ser Met Glu Lys Leu Ser Ser Gly Leu Arg Ile
35 40 45

Asn Arg Ala Gly Asp Asp Ala Ala Gly Leu Ala Ile Ser Glu Lys Leu
50 55 60

Arg Ala Gln Ile Arg Gly Leu Asn Gln Ala Ile Arg Asn Ala Gln Asp
65 70 75 80

Gly Ile Ser Leu Ile Gln Thr Ala Glu Gly Gly Leu Ser Glu Ile Gln
85 90 95

Asn Ile Leu Gln Arg Met Arg Glu Leu Gly Val Gln Ala Ala Asn Gly
100 105 110

Thr Leu Asn Asn Gln Asp Ile Ser Ala Ile Thr Thr Glu Leu Asn Gln
115 120 125

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Leu Phe Asn Glu Ile Asp Arg Ile Ala Gly Ala Thr Glu Phe Asn Thr
    130                 135                 140

Lys Asn Leu Leu Ala Val Ser Thr Gly Leu Val Val Thr Leu Gln Val
145                 150                 155                 160

Gly Ala Asn Ala Gly Gln Val Ile Ala Phe Thr Ile Asp Asn Ala Gly
                165                 170                 175

Thr Ala Ser Leu Gly Leu Ser Ser Ala Asp Leu Ala Ile Asn Asp Asn
            180                 185                 190

Ala Ser Ala Ser Ala Phe Ile Ser Lys Val Asp Ser Ala Leu Gln Lys
        195                 200                 205

Val Ser Thr Tyr Arg Ala Asn Leu Gly Ser Ile Gln Asn Arg Leu Glu
    210                 215                 220

His Thr Ile Ala Asn Leu Gly Ile Ala Ser Glu Asn Leu Ser Ala Ser
225                 230                 235                 240

Glu Ser Arg Ile Arg Asp Val Asp Met Ala Ala Glu Met Met Asn Phe
                245                 250                 255

Thr Lys Asn Gln Ile Leu Gln Gln Ala Gly Val Ala Ile Leu Ala Gln
            260                 265                 270

Ala Asn Gln Ala Pro Gln Ala Val Leu Gln Leu Leu Arg
        275                 280                 285


<210> SEQ ID NO 11
<211> LENGTH: 171
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 11

Met Gly His His His His His His Ser Gly Leu Asn Gln Ala Ile Arg
1               5                   10                  15

Asn Ala Gln Asp Gly Ile Ser Leu Ile Gln Thr Ala Glu Gly Gly Leu
            20                  25                  30

Ser Glu Ile Gln Asn Ile Leu Gln Arg Met Arg Glu Leu Gly Val Gln
        35                  40                  45

Ala Ala Asn Gly Thr Leu Asn Asn Gln Asp Ile Ser Ala Ile Thr Thr
    50                  55                  60

Glu Leu Asn Gln Leu Phe Asn Glu Ile Asp Arg Ile Ala Gly Ala Thr
65                  70                  75                  80

Glu Phe Asn Thr Lys Asn Leu Leu Ala Val Ser Thr Gly Leu Val Val
                85                  90                  95

Thr Leu Gln Val Gly Ala Asn Ala Gly Gln Val Ile Ala Phe Thr Ile
            100                 105                 110

Asp Asn Ala Gly Thr Ala Ser Leu Gly Leu Ser Ser Ala Asp Leu Ala
        115                 120                 125

Ile Asn Asp Asn Ala Ser Ala Ser Ala Phe Ile Ser Lys Val Asp Ser
    130                 135                 140

Ala Leu Gln Lys Val Ser Thr Tyr Arg Ala Asn Leu Gly Ser Ile Gln
145                 150                 155                 160

Asn Arg Leu Glu His Thr Ile Ala Asn Leu Gly
                165                 170


<210> SEQ ID NO 12
<211> LENGTH: 146
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 12

Met Gly His His His His His His Ser Gly Leu Asn Gln Ala Ile Arg
1 5 10 15

Asn Ala Gln Asp Gly Ile Ser Leu Ile Gln Thr Ala Glu Gly Gly Leu
20 25 30

Ser Glu Ile Gln Asn Ile Leu Gln Arg Met Arg Glu Leu Gly Val Gln
35 40 45

Ala Ala Asn Gly Thr Leu Asn Asn Gln Asp Ile Ser Ala Ile Thr Thr
50 55 60

Glu Leu Asn Gln Leu Phe Asn Glu Ile Asp Arg Ile Ala Gly Ala Thr
65 70 75 80

Glu Phe Asn Thr Lys Asn Leu Leu Ala Ala Gly Thr Ala Ser Leu Gly
85 90 95

Leu Ser Ser Ala Asp Leu Ala Ile Asn Asp Asn Ala Ser Ala Ser Ala
100 105 110

Phe Ile Ser Lys Val Asp Ser Ala Leu Gln Lys Val Ser Thr Tyr Arg
115 120 125

Ala Asn Leu Gly Ser Ile Gln Asn Arg Leu Glu His Thr Ile Ala Asn
130 135 140

Leu Gly
145

<210> SEQ ID NO 13
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 13

Met Gly His His His His His His Ser Ala Ser Ala Phe Ile Ser Lys
1 5 10 15

Val Asp Ser Ala Leu Gln Lys Val Ser Thr Tyr Arg Ala Asn Leu Gly
20 25 30

Ser Ile Gln Asn Arg Leu Glu His Thr Ile Ala Asn Leu Gly Pro Asp
35 40 45

Gly Leu Asn Gln Ala Ile Arg Asn Ala Gln Asp Gly Ile Ser Leu Ile
50 55 60

Gln Thr Ala Glu Gly Gly Leu Ser Glu Ile Gln Asn Ile Leu Gln Arg
65 70 75 80

Met Arg Glu Leu Gly Val Gln Ala Ala Asn Gly Thr Leu Asn Asn Gln
85 90 95

Asp Ile Ser Ala Ile Thr Thr Glu Leu Asn Gln Leu Phe Asn Glu Ile
100 105 110

Asp Arg Ile Ala
115

<210> SEQ ID NO 14
<211> LENGTH: 103
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 14

Met Gly His His His His His His Ser Asn Asn Gln Asp Ile Ser Ala
1 5 10 15

Ile Thr Thr Glu Leu Asn Gln Leu Phe Asn Glu Ile Asp Arg Ile Ala
20 25 30

Gly Ala Thr Gly Ser Gly Gly Leu Ser Glu Ile Gln Asn Ile Leu Gln
35 40 45

Arg Met Arg Glu Leu Gly Val Gln Ala Ala Asn Gly Thr Leu Asn Gly
50 55 60

Gly Ser Ala Ser Ala Phe Ile Ser Lys Val Asp Ser Ala Leu Gln Lys
65 70 75 80

Val Ser Thr Tyr Arg Ala Asn Leu Gly Ser Ile Gln Asn Arg Leu Glu
85 90 95

His Thr Ile Ala Asn Leu Gly
100

<210> SEQ ID NO 15
<211> LENGTH: 170
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 15

Met Gly His His His His His His Ser Gly Leu Ala Gln Ala Ser Arg
1 5 10 15

Asn Ala Gln Asp Ala Ile Ser Ile Ala Gln Thr Ala Glu Gly Ala Leu
20 25 30

Asp Glu Thr Gln Ser Ile Leu Gln Arg Val Arg Glu Leu Gly Val Gln
35 40 45

Gly Ala Asn Gly Thr Leu Thr Ala Asp Asp Ile Asn Ala Leu Gln Ala
50 55 60

Glu Val Asp Gln Leu Ile Ala Glu Ile Asp Arg Ile Ala Gly Ala Thr
65 70 75 80

Glu Phe Asn Thr Gln Asn Leu Leu Asp Gly Ser Phe Thr Thr Lys Ala
85 90 95

Phe Gln Val Gly Ala Asn Ser Gly Gln Asn Met Thr Leu Thr Ile Gly
100 105 110

Lys Met Asp Thr Thr Thr Leu Gly Leu Ser Ser Ala Asp Leu Ala Ile
115 120 125

Asn Asp Asn Ala Phe Ala Asn Gly Ala Ile Ser Thr Val Asp Ser Ala
130 135 140

Leu Gln Lys Val Ser Ala Glu Arg Ala Lys Leu Gly Ala Ile Gln Asn
145 150 155 160

Arg Leu Glu His Thr Ile Ala Asn Leu Gly
165 170

<210> SEQ ID NO 16
<211> LENGTH: 170
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

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<400> SEQUENCE: 16

Met Gly His His His His His His Ser Gly Leu Ala Gln Ala Ser Arg
1 5 10 15

Gln Ala Gln Asp Ala Ile Ser Ile Ala Gln Thr Ala Glu Gly Ala Leu
20 25 30

Asp Glu Thr Gln Ser Ile Leu Gln Arg Val Arg Glu Leu Gly Val Gln
35 40 45

Gly Ala Asp Gly Thr Leu Thr Ala Asp Asp Ile Asp Ala Leu Gln Ala
50 55 60

Glu Val Asp Gln Leu Ile Ala Glu Ile Asp Arg Ile Ala Gly Ala Thr
65 70 75 80

Glu Phe Ala Thr Gln Lys Leu Leu Asp Gly Ser Phe Thr Thr Lys Ala
85 90 95

Phe Gln Val Gly Ala Ala Ser Gly Gln Asp Val Thr Leu Thr Ile Gly
100 105 110

Lys Val Asp Thr Thr Thr Leu Gly Leu Ser Ser Ala Asp Leu Ala Ile
115 120 125

Asp Ser Ala Ala Phe Ala Asp Gly Ala Ile Ser Thr Val Asp Ser Ala
130 135 140

Leu Gln Lys Val Ser Ala Glu Arg Ala Lys Leu Gly Ala Ile Gln Asn
145 150 155 160

Arg Leu Glu His Thr Ile Ala Gln Leu Gly
165 170

<210> SEQ ID NO 17
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 17

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Pro Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln
35 40 45

Asn Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg
50 55 60

Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
65 70 75 80

Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
85 90 95

Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
100 105 110

Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
115 120 125

Ser Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser
130 135 140

Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
145 150 155 160

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Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln
                165                 170                 175

Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp
            180                 185                 190

Leu Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val
        195                 200                 205

Asn Ser Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu Asp Ser Met
    210                 215                 220

Gly Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala
225                 230                 235                 240

Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val Asp Ala Val
                245                 250                 255

Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr
            260                 265                 270

Asn Leu


<210> SEQ ID NO 18
<211> LENGTH: 276
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 18

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln Ala Ser Arg Asn Ala
        35                  40                  45

Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly Ala Leu Asn Glu
    50                  55                  60

Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser Val Gln Ala Thr
65                  70                  75                  80

Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile Gln Asp Glu Ile
                85                  90                  95

Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn Gln Thr Gln Phe
            100                 105                 110

Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met Lys Ile Gln Val
        115                 120                 125

Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu Gln Lys Ile Asp
    130                 135                 140

Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val Asn Ser Pro Gly Ile
145                 150                 155                 160

Ser Gly Gly Gly Gly Gly Gly Ile Leu Asp Ser Met Gly Thr Leu Ile Asn
                165                 170                 175

Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala Asn Pro Leu Ala Ser
            180                 185                 190

Ile Asp Ser Ala Leu Ser Lys Val Asp Ala Val Arg Ser Ser Leu Gly
        195                 200                 205

Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr Asn Leu Gly Asn Thr
    210                 215                 220

Val Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile Glu Asp Ala Asp Tyr
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225                 230                 235                 240

Ala Thr Glu Val Ser Asn Met Ser Lys Ala Gln Ile Leu Gln Gln Ala
                245                 250                 255

Gly Thr Ser Val Leu Ala Gln Ala Asn Gln Val Pro Gln Asn Val Leu
            260                 265                 270

Ser Leu Leu Arg
        275


<210> SEQ ID NO 19
<211> LENGTH: 323
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 19

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln
        35                  40                  45

Asn Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg
    50                  55                  60

Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
65                  70                  75                  80

Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
                85                  90                  95

Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
            100                 105                 110

Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
        115                 120                 125

Ser Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser
    130                 135                 140

Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
145                 150                 155                 160

Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln
                165                 170                 175

Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp
            180                 185                 190

Leu Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Ile Pro Gly Ile Ser
        195                 200                 205

Gly Gly Gly Gly Gly Ile Leu Asp Ser Met Gly Thr Leu Ile Asn Glu
    210                 215                 220

Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala Asn Pro Leu Ala Ser Ile
225                 230                 235                 240

Asp Ser Ala Leu Ser Lys Val Asp Ala Val Arg Ser Ser Leu Gly Ala
                245                 250                 255

Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr Asn Leu Gly Asn Thr Val
            260                 265                 270

Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile Glu Asp Ala Asp Tyr Ala
        275                 280                 285

Thr Glu Val Ser Asn Met Ser Lys Ala Gln Ile Leu Gln Gln Ala Gly
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    290                 295                 300

Thr Ser Val Leu Ala Gln Ala Asn Gln Val Pro Gln Asn Val Leu Ser
305                 310                 315                 320

Leu Leu Arg


<210> SEQ ID NO 20
<211> LENGTH: 270
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 20

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln Ala Ser Arg Asn Ala
        35                  40                  45

Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly Ala Leu Asn Glu
    50                  55                  60

Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser Val Gln Ala Thr
65                  70                  75                  80

Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile Gln Asp Glu Ile
                85                  90                  95

Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn Gln Thr Gln Phe
            100                 105                 110

Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met Lys Ile Gln Val
        115                 120                 125

Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu Gln Lys Ile Asp
    130                 135                 140

Val Lys Ser Leu Gly Leu Ile Pro Gly Ile Ser Gly Gly Gly Gly Gly
145                 150                 155                 160

Ile Leu Asp Ser Met Gly Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala
                165                 170                 175

Lys Lys Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser
            180                 185                 190

Lys Val Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe
        195                 200                 205

Asp Ser Ala Ile Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn Ser
    210                 215                 220

Ala Arg Ser Arg Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn
225                 230                 235                 240

Met Ser Lys Ala Gln Ile Leu Gln Gln Ala Gly Thr Ser Val Leu Ala
                245                 250                 255

Gln Ala Asn Gln Val Pro Gln Asn Val Leu Ser Leu Leu Arg
            260                 265                 270


<210> SEQ ID NO 21
<211> LENGTH: 316
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.
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<400> SEQUENCE: 21

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Pro Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln
35 40 45

Asn Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg
50 55 60

Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
65 70 75 80

Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
85 90 95

Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
100 105 110

Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
115 120 125

Ser Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser
130 135 140

Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
145 150 155 160

Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln
165 170 175

Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp
180 185 190

Leu Gln Lys Ile Ile Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu
195 200 205

Asp Ser Met Gly Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys
210 215 220

Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val
225 230 235 240

Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser
245 250 255

Ala Ile Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg
260 265 270

Ser Arg Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser
275 280 285

Lys Ala Gln Ile Leu Gln Gln Ala Gly Thr Ser Val Leu Ala Gln Ala
290 295 300

Asn Gln Val Pro Gln Asn Val Leu Ser Leu Leu Arg
305 310 315

<210> SEQ ID NO 22
<211> LENGTH: 263
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 22

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp

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            20                  25                  30

Pro Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln Ala Ser Arg Asn Ala
        35                  40                  45

Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly Ala Leu Asn Glu
    50                  55                  60

Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser Val Gln Ala Thr
65                  70                  75                  80

Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile Gln Asp Glu Ile
                85                  90                  95

Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn Gln Thr Gln Phe
            100                 105                 110

Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met Lys Ile Gln Val
        115                 120                 125

Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu Gln Lys Ile Ile
    130                 135                 140

Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu Asp Ser Met Gly Thr
145                 150                 155                 160

Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala Asn Pro
                165                 170                 175

Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val Asp Ala Val Arg Ser
            180                 185                 190

Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr Asn Leu
        195                 200                 205

Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile Glu Asp
    210                 215                 220

Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser Lys Ala Gln Ile Leu
225                 230                 235                 240

Gln Gln Ala Gly Thr Ser Val Leu Ala Gln Ala Asn Gln Val Pro Gln
                245                 250                 255

Asn Val Leu Ser Leu Leu Arg
            260


<210> SEQ ID NO 23
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 23

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln
        35                  40                  45

Asn Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg
    50                  55                  60

Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
65                  70                  75                  80

Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
                85                  90                  95

Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
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            100                 105                 110

Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
        115                 120                 125

Ser Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser
    130                 135                 140

Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
145                 150                 155                 160

Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln
                165                 170                 175

Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp
            180                 185                 190

Leu Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Ile Pro Gly Ile Ser
        195                 200                 205

Gly Gly Gly Gly Gly Ile Leu Asp Ser Met Gly Thr Leu Ile Asn Glu
    210                 215                 220

Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala Asn Pro Leu Ala Ser Ile
225                 230                 235                 240

Asp Ser Ala Leu Ser Lys Val Asp Ala Val Arg Ser Ser Leu Gly Ala
                245                 250                 255

Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr Asn Leu
            260                 265


<210> SEQ ID NO 24
<211> LENGTH: 261
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 24

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln
        35                  40                  45

Asn Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg
    50                  55                  60

Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
65                  70                  75                  80

Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
                85                  90                  95

Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
            100                 105                 110

Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
        115                 120                 125

Ser Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser
    130                 135                 140

Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
145                 150                 155                 160

Asn Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln
                165                 170                 175

Met Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp
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            180                 185                 190

Leu Gln Lys Ile Ile Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu
        195                 200                 205

Asp Ser Met Gly Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys
    210                 215                 220

Ser Thr Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val
225                 230                 235                 240

Asp Ala Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser
                245                 250                 255

Ala Ile Thr Asn Leu
            260


<210> SEQ ID NO 25
<211> LENGTH: 282
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 25

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln
        35                  40                  45

Asn Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg
    50                  55                  60

Leu Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly
65                  70                  75                  80

Gln Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln
                85                  90                  95

Ala Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu
            100                 105                 110

Gly Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu
        115                 120                 125

Ser Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser
    130                 135                 140

Ile Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser
145                 150                 155                 160

Asn Gln Ile Pro Gly Ile Ser Gly Gly Gly Gly Gly Ile Leu Asp Ser
                165                 170                 175

Met Gly Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr
            180                 185                 190

Ala Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val Asp Ala
        195                 200                 205

Val Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile
    210                 215                 220

Thr Asn Leu Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg Ser Arg
225                 230                 235                 240

Ile Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser Lys Ala
                245                 250                 255

Gln Ile Leu Gln Gln Ala Gly Thr Ser Val Leu Ala Gln Ala Asn Gln
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            260                 265                 270

Val Pro Gln Asn Val Leu Ser Leu Leu Arg
        275                 280


<210> SEQ ID NO 26
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 26

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Pro Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln Ala Ser Arg Asn Ala
        35                  40                  45

Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly Ala Leu Asn Glu
    50                  55                  60

Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser Val Gln Ala Thr
65                  70                  75                  80

Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile Gln Asp Glu Ile
                85                  90                  95

Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn Gln Ile Pro Gly
            100                 105                 110

Ile Ser Gly Gly Gly Gly Gly Ile Leu Asp Ser Met Gly Thr Leu Ile
        115                 120                 125

Asn Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala Asn Pro Leu Ala
    130                 135                 140

Ser Ile Asp Ser Ala Leu Ser Lys Val Asp Ala Val Arg Ser Ser Leu
145                 150                 155                 160

Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr Asn Leu Gly Asn
                165                 170                 175

Thr Val Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile Glu Asp Ala Asp
            180                 185                 190

Tyr Ala Thr Glu Val Ser Asn Met Ser Lys Ala Gln Ile Leu Gln Gln
        195                 200                 205

Ala Gly Thr Ser Val Leu Ala Gln Ala Asn Gln Val Pro Gln Asn Val
    210                 215                 220

Leu Ser Leu Leu Arg
225


<210> SEQ ID NO 27
<211> LENGTH: 505
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide.

<400> SEQUENCE: 27

Met Ala Gln Val Ile Asn Thr Asn Ser Leu Ser Leu Leu Thr Gln Asn
1               5                   10                  15

Asn Leu Asn Lys Ser Gln Ser Ser Leu Ser Ser Ala Ile Glu Arg Leu
            20                  25                  30
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Ser Ser Gly Leu Arg Ile Asn Ser Ala Lys Asp Asp Ala Ala Gly Gln
        35                  40                  45

Ala Ile Ala Asn Arg Phe Thr Ser Asn Ile Lys Gly Leu Thr Gln Ala
    50                  55                  60

Ser Arg Asn Ala Asn Asp Gly Ile Ser Ile Ala Gln Thr Thr Glu Gly
65                  70                  75                  80

Ala Leu Asn Glu Ile Asn Asn Asn Leu Gln Arg Val Arg Glu Leu Ser
                85                  90                  95

Val Gln Ala Thr Asn Gly Thr Asn Ser Asp Ser Asp Leu Lys Ser Ile
            100                 105                 110

Gln Asp Glu Ile Gln Gln Arg Leu Glu Glu Ile Asp Arg Val Ser Asn
        115                 120                 125

Gln Thr Gln Phe Asn Gly Val Lys Val Leu Ser Gln Asp Asn Gln Met
    130                 135                 140

Lys Ile Gln Val Gly Ala Asn Asp Gly Glu Thr Ile Thr Ile Asp Leu
145                 150                 155                 160

Gln Lys Ile Asp Val Lys Ser Leu Gly Leu Asp Gly Phe Asn Val Asn
                165                 170                 175

Gly Pro Lys Glu Ala Thr Val Gly Asp Leu Lys Ser Ser Phe Lys Asn
            180                 185                 190

Val Thr Gly Tyr Asp Thr Tyr Ala Ala Gly Ala Asp Lys Tyr Arg Val
        195                 200                 205

Asp Ile Asn Ser Gly Ala Val Val Thr Asp Ala Ala Ala Pro Asp Lys
    210                 215                 220

Val Tyr Val Asn Ala Ala Asn Gly Gln Leu Thr Thr Asp Asp Ala Glu
225                 230                 235                 240

Asn Asn Thr Ala Val Asp Leu Phe Lys Thr Thr Lys Ser Thr Ala Gly
                245                 250                 255

Thr Ala Glu Ala Lys Ala Ile Ala Gly Ala Ile Lys Gly Gly Lys Glu
            260                 265                 270

Gly Asp Thr Phe Asp Tyr Lys Gly Val Thr Phe Thr Ile Asp Thr Lys
        275                 280                 285

Thr Gly Asp Asp Gly Asn Gly Lys Val Ser Thr Thr Ile Asn Gly Glu
    290                 295                 300

Lys Val Thr Leu Thr Val Ala Asp Ile Ala Thr Gly Ala Ala Asp Val
305                 310                 315                 320

Asn Ala Ala Thr Leu Gln Ser Ser Lys Asn Val Tyr Thr Ser Val Val
                325                 330                 335

Asn Gly Gln Phe Thr Phe Asp Asp Lys Thr Lys Asn Glu Ser Ala Lys
            340                 345                 350

Leu Ser Asp Leu Glu Ala Asn Asn Ala Val Lys Gly Glu Ser Lys Ile
        355                 360                 365

Thr Val Asn Gly Ala Glu Tyr Thr Ala Asn Ala Thr Gly Asp Lys Ile
    370                 375                 380

Thr Leu Ala Gly Lys Thr Met Phe Ile Asp Lys Thr Ala Ser Gly Val
385                 390                 395                 400

Ser Thr Leu Ile Asn Glu Asp Ala Ala Ala Ala Lys Lys Ser Thr Ala
                405                 410                 415

Asn Pro Leu Ala Ser Ile Asp Ser Ala Leu Ser Lys Val Asp Ala Val
            420                 425                 430

Arg Ser Ser Leu Gly Ala Ile Gln Asn Arg Phe Asp Ser Ala Ile Thr
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        435                 440                 445

Asn Leu Gly Asn Thr Val Thr Asn Leu Asn Ser Ala Arg Ser Arg Ile
    450                 455                 460

Glu Asp Ala Asp Tyr Ala Thr Glu Val Ser Asn Met Ser Lys Ala Gln
465                 470                 475                 480

Ile Leu Gln Gln Ala Gly Thr Ser Val Leu Ala Gln Ala Asn Gln Val
                485                 490                 495

Pro Gln Asn Val Leu Ser Leu Leu Arg
            500                 505
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What is claimed is:

1. A method of treating or preventing frailty in a patient, said method comprising

(a) identifying a patient desiring or in need of frailty treatment or prevention, and

(b) administering to said patient a recombinant TLR5 agonist, wherein said TLR5 agonist is not fused to a pathogenic protein antigen.

2. The method of claim **1**, wherein frailty comprises an accumulation of deficiencies in major physiological functions, reduction of regeneration capabilities, impaired wound healing and/or increased risk of age-related diseases or disorders.

3. The method of either claim **1** or **2**, wherein frailty is measured according to the Physiological Frailty Index.

4. The method of claim **3**, wherein the Physiological Frailty Index comprises assessment of one or more parameters selected from grip strength, systolic blood pressure, diastolic blood pressure, blood flow volume, number of blood neutrophils, percentage of blood neutrophils, number of blood monocytes, percentage of blood monocytes, number of lymphocytes, number of red blood cells, hemoglobin levels, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin levels, mean corpuscular hemoglobin concentration and keratinocyte-derived cytokine levels.

5. The method of any one of the above claims, wherein the TLR5 agonist is a flagellin or a derivative thereof.

6. The method of claim **5**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 1.

7. The method of claim **6**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence that is SEQ ID NO: 1.

8. The method of claim **5**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence having at least 95% sequence identity to one of SEQ ID NOs: 2-27.

9. The method of claim **8**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence of one of SEQ ID NOs: 2-27.

10. The method of any one of the above claims, wherein the patient's Physiological Frailty Index is reduced by about 25% to about 75%.

11. The method of claim **10**, wherein the patient's Physiological Frailty Index is reduced by at least about 75%, or about 50%, or about 35%, or about 25%.

12. The method of claim **10**, wherein the frailty is associated with aging.

13. The method of any one of the above claims, wherein the patient is middle-aged.

14. The method of claim **13**, wherein the patient is between about 36 and about 55 years old.

15. The method of any one of the above claims, wherein the patient is elderly.

16. The method of claim **15**, wherein the patient is between about 56 and about 85 years old.

17. The method of any one of the above claims, wherein the biological sex of the patient is male.

18. The method of any one of claims **1-17**, wherein the biological sex of the patient is female.

19. A method of treating or preventing an age-related disease or disorder in a patient, said method comprising

(a) identifying a patient desiring or in need of treatment or prevention of an age-related disease or disorder, and

(b) administering to said patient a recombinant TLR5 agonist, wherein said TLR5 agonist is not fused to a pathogenic protein antigen.

20. The method of claim **19**, wherein the age-related disease or disorder is characterized by increased cellular senescence.

21. The method of either claim **19** or **20**, wherein the age-related disease or disorder is selected from accelerated aging, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, cardiac diastolic dysfunction, benign prostatic hypertrophy, aortic aneurysm, emphysema, atherosclerosis, diabetes, pulmonary fibrosis, blindness, dementia, Alzheimer's disease, kidney dysfunction, osteoarthritis, low grade chronic sterile inflammation, herniated intervertebral disc, frailty, hair loss, hearing loss, vision loss, muscle fatigue, skin conditions, skin nevi, wrinkly skin, hyperpigmentation, scarring, keloid, rosacea, vitiligo, ichthyosis vulgaris, dermatomyositis, actinic keratosis, and sarcopenia.

22. The method of claim **21**, wherein the age-related disease or disorder is accelerated aging.

23. The method of claim **22**, wherein the accelerate aging is a a Progeroid syndrome, or symptom thereof.

24. The method of claim **23**, wherein the Progeroid syndrome is selected from Hutchinson-Gilford progeria syndrome (HGPS), Werner syndrome (WS), Bloom syndrome (BS), Rothmund-Thomson syndrome (RTS), Cockayne syndrome (CS), xeroderma pigmentosum (XP), trichothiodystrophy (TTD), combined xeroderma pigmentosum-Cockayne syndrome (XP-CS), and restrictive dermopathy (RD).

25. The method of claim **22**, wherein the accelerated aging is induced by a cancer or a cancer treatment.

26. The method of claim **25**, wherein the cancer treatment is selected from one or more of radiotherapy, hormonal therapy, tyrosine kinase inhibitor, anthracycline, alkylating agent, topoisomerase inhibitor, antimetabolites/cytotoxic drug, BRAF inhibitor, antitumor antibiotic, isoquinololine alkaloid, Bcl-2 inhibitor, hematopoietic cell transplantation (HCT), telomerase inhibitor, nucleoside analogue reverse-transcriptase inhibitor, DNA cross-linking agent, ribonucleotide reductase inhibitor, microtubule inhibitor, and miRNA.

27. The method of any one of claims **25-26**, wherein the patient was previously afflicted with a cancer.

28. The method of claim **27**, wherein the patient is cancer survivor.

29. The method of claim **28**, wherein the cancer survivor has completed a cancer treatment and has no apparent evidence of active disease.

30. The method of any one of claims **25-29**, wherein the cancer is slectbasal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (e.g., small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (e.g. that associated with brain tumors), and Meigs' syndrome.

31. The method of any one of claims **19-30**, wherein the patient is middle-aged.

32. The method of claim **31**, wherein the patient is between about 36 and about 55 years old.

33. The method of any one of claims **19-30**, wherein the patient is elderly.

34. The method of claim **33**, wherein the patient is between about 56 and about 85 years old.

35. The method of any one of claims **25-34**, wherein the patient received the cancer treatment before the age of about 18, before the age of about 16, before the age of about 14, before the age of about 12, before the age of about 10, before the age of about 8, before the age of about 6, before the age of about 4, or before the age of about 2.

36. The method of any one of claims **25-35**, wherein the TLR5 agonist is administered to the patient for at least one week, or at least one month, or at least six months, or at least one year, or at least two years, or at least three years, or at least four years, or at least five years after the patient received the cancer treatment.

37. The method of any one of claims **25-36**, wherein the patient no longer has cancer or the patient is in remission at the time the TLR5 agonist is administered.

38. The method of any one of claims **19-37**, wherein administering the TLR5 agonist to the patient treats or prevents the age-related disease or disorder by decreasing cellular senescence.

39. The method of any one of claims **19-38**, wherein the TLR5 agonist is flagellin or a derivative thereof.

40. The method of claim **39**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 1.

41. The method of claim **40**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence that is SEQ ID NO: 1.

42. The method of claim **39**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence having at least 95% sequence identity to one of SEQ ID NOs: 2-27.

43. The method of claim **42**, wherein the TLR5 agonist comprises a polypeptide having an amino acid sequence of one of SEQ ID NOs: 2-27.

44. The method of any one of claims **19-43**, wherein the biological sex of the patient is male.

45. The method of any one of claims **19-43**, wherein the biological sex of the patient is female.

46. The method of any one of the preceding claims, wherein the TLR5 agonist is administered in one or more cycles.

47. The method of claim **46**, wherein the cycle involves dosing a patient once per day for one day, once a day for two days, once a day for three days, once a day for four days, or once a day for five days.

48. The method of claim **47**, wherein no more than 5 cycles, or no more than 3 cycles, or no more than 2 cycles are administered per year.

49. A TLR5 agonist for use in the treatment or prevention of frailty.

50. Use of a TLR5 agonist in the manufacture of a medicament for treatment or prevention of frailty.

51. A TLR5 agonist for use in the treatment or prevention of an age-related disease or disorder.

52. Use of a TLR5 agonist in the manufacture of a medicament for treatment or prevention of an age-related disease or disorder.

* * * * *