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(54) **USE OF AKT PHOSPHORYLATION AS A BIOMARKER FOR PROGNOSING NEURODEGENERATIVE DISEASES AND TREATING SAME**

VERWENDUNG EINER AKT-PHOSPHORYLIERUNG ALS BIOMARKER ZUR PROGNOSE NEURODEGENERATIVER ERKRANKUNGEN UND ZU IHRER BEHANDLUNG

UTILISATION DE LA PHOSPHORYLATION D'AKT EN TANT QUE BIOMARQUEUR POUR LE PRONOSTIC ET LE TRAITEMENT DE MALADIES NEURODÉGÉNÉRATIVES

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**Description****FIELD OF THE INVENTION**

5 [0001] The present invention provides a method for assessing responsiveness to treatment of amyotrophic lateral sclerosis (ALS) with a peptide comprising SEQ ID NO:1, using Akt and phosphorylated Akt as biomarkers.

**BACKGROUND OF THE INVENTION**

10 [0002] The serine/threonine protein kinase Akt, also known as protein kinase B (PKB) or RAC-PK, was initially identified as one of the downstream targets of phosphatidylinositol-3 kinase (PI3K). Activated Akt plays a key role in mediating signals for cell growth, cell survival (anti-apoptotic), cell-cycle progression, differentiation, transcription, translation and glucose metabolism.

15 [0003] The Akt pathway is damaged in skeletal muscles of amyotrophic lateral sclerosis (ALS) patients, as in SOD1 mice (a mice model for ALS). Although no difference in Akt mRNA levels is found in ALS patients when compared to control subjects, at the protein level, ALS patients, have a significantly lower content of the active phosphorylated Akt protein in comparison to healthy control subjects (Leger et al., FASEB J. 2006; 20(3):583-585). Akt pathway dysfunction has also been shown in motoneurons of both sporadic and familial ALS patients (Dewil et al., Neuropathol Appl Neurobiol., 2007; 33(5):499-509).

20 [0004] The peptides LPPLPYP (SEQ ID NO: 1; also known as Stressin-1) and PYLPPL (SEQ ID NO: 2, where all residues are in the "D" isomeric form) were first disclosed in WO 2006/021954, where their efficacy in ameliorating stress-induced cell death and p53- mediated response was demonstrated. According to the disclosure, these peptides are also useful in treating inflammatory and autoimmune diseases. Nowhere in the background art was it taught or suggested that the activity of peptides comprising SEQ ID NO: 1 or SEQ ID NO: 2 or derivatives thereof is affected by the level of Akt and Akt phosphorylation, such that, the therapeutic effect of these peptides would be significantly higher in subjects having low levels of pAkt and, optionally, low levels of pAkt:tAkt ratio. In addition, the background art does not teach or even suggests the use of pAkt and pAkt:tAkt ratio as effective markers for ALS and moreover for staging the progression of ALS in ALS patients.

25 [0005] ALS is a devastating and rapidly fatal disease with currently only one available, FDA-approved, modestly effective treatment. The approved therapy, Rilutek® (riluzole), has a modest benefit estimated to be a three month extension in patient survival.

30 [0006] There is therefore an urgent need for new therapies. Recent attempts to find molecules that could provide a beneficial therapy for ALS include the finding of 1,4-Diaza-bicyclo[3.2.2]non-6-en-4-yl)-heterocyclyl-methanone ligands disclosed in US Patent Application, Publication No. 2010/0298306. The ligands are directed to treatment of any Nicotinic Acetylcholine Receptors, *inter alia*, ALS. In addition, US Patent Application, Publication No. 2010/0099700, discloses use of hydrogenated pyrido(4,3-b)indole for treating ALS. US Patent Application, Publication No. 2009/0324549 discloses methods for treating ALS comprising administration to a patient in need thereof, proteins and/or peptides characterized in that they originate from the gene which results from the retention of the intron 3 of the gene SMN (or survival motor neuron) identified in the gene bank with the access number AY876898.

35 [0007] WO 2011/017030 discloses a method of treating a disease associated with excess activation of monocytes to activated macrophages, including, *inter alia*, ALS said method comprising administering a therapeutically-effective amount of an oxidative agent to a subject in need thereof, wherein said oxidative agent is selected from the group consisting of non-halogen activated-oxygen compounds, non-oxygen activated-halogen compounds, and N-halo compounds. According to WO 2011/017030 the non-halogen activated- oxygen compounds are selected from potassium nitrate (KNO<sub>3</sub>), permanganate salts, ammonium cerium(IV) nitrate, hexavalent chromium compounds, chromate/dichromate compounds, ammonium silver nitrate, sulfoxides, persulfuric acid, osmium tetroxide (OsO<sub>4</sub>), nitric acid, nitrous oxide (N<sub>2</sub>O), hydrogen peroxide, organic peroxides, superoxides, and ozone; the non-oxygen activated-halogen compounds are selected from fluorine, chlorine, bromine, and iodine; the N-halo compounds are selected from the group consisting of N-halophthalimide, N-halosuccinimide, N-halosaccharin, N,N-dihalourethane, N-haloacetanilide, 1,3-dihalo-5,5- dimethylhydantoin, trihaloisocyanuric acid and sodium dihaloisocyanurate; and the oxidative agent is selected from 1,3-dichloro-5,5- dimethylhydantoin and chloramine-T.

40 [0008] The development of the first genetically based mouse model of ALS in 1994, energized the field of preclinical testing despite numerous unforeseen complexities along the way. Transgenic mutant SOD1 mice, the only ALS mouse models currently available, have mutations in the Cu/Zn Superoxide Dismutase 1 gene (SOD1) which account for ~20% of Familial ALS (FALS) cases, corresponding to 2-3% of all ALS cases. Transgenic mutant SOD1 mice exhibit all of the histopathological hallmarks observed clinically in sporadic and familial ALS.

45 [0009] Because there is no obvious mutational hotspot and no clear correlation between the level of enzymatic activity of the mutant SOD1 protein and the observed disease phenotype or clinical progression, SOD1 is thought to act primarily

via a toxic gain of function in ALS, although loss of function may also contribute to disease pathophysiology. It is generally thought that the different mutant SOD1 proteins are likely to cause ALS by a similar mechanism.

**[0010]** Several transgenic mouse models have been generated to model mutations found in FALS patients. In all of these mouse models, massive death of motor neurons in the ventral horn of the spinal cord and loss of myelinated axons in ventral motor roots ultimately leads to paralysis and muscle atrophy. All of these mouse models have been reported to exhibit the same histopathological hallmarks associated with ALS in humans: progressive accumulation of detergent-resistant aggregates containing SOD1 and ubiquitin and aberrant neurofilament accumulations in degenerating motor neurons. In addition to neuronal degeneration, reactive astroglia and microglia have also been detected in diseased tissue in the mice, similar to that observed in humans.

**[0011]** Despite these histopathological similarities, the timing of onset and rate of disease progression differ (often dramatically) among the various SOD1 transgenic mouse models.

**[0012]** ALS is commonly assessed by neurological score and weight loss. As used herein, the term "neurological score" and "neurologic score" are interchangeably used herein to describe the common standards for assessing the presence or stage of a neurologic disease, such as ALS. Some example of commonly used neurological scoring systems include: measurements of splay (or other measures of paralysis) and beam walk.

**[0013]** In SOD 1 mice, regardless of which neurological scoring system is used, scores are typically assessed for both hind legs of the SOD1 mice. The example neurological scoring system below employs a scale of zero to four ([www.researchals.org/uploaded\\_files/p4I\\_jax\\_sod1manual\\_20091202\\_29aPcx.pdf](http://www.researchals.org/uploaded_files/p4I_jax_sod1manual_20091202_29aPcx.pdf)). Example of score criteria used to assign each score under this system are as follows:

Score of 0: full extension of hind legs away from lateral midline when mouse is suspended by its tail, and mouse can hold this for two seconds, suspended two to three times.

Score of 1: collapse or partial collapse of leg extension towards lateral midline (weakness) or trembling of hind legs during tail suspension.

Score of 2: toes curl under at least twice during walking of 12 inches, or any part of foot is dragging along cage bottom/table.

Score of 3: rigid paralysis or minimal joint movement, foot not being used for generating forward motion.

Score of 4: mouse cannot right itself within 30 seconds after being placed on either side.

**[0014]** US Patent No. 7,659,243 discloses the use of angiogenin, or a fragment or variant thereof, to treat diseases characterized by neuronal injury or death, or axonal degeneration, especially neurodegenerative diseases such as ALS. According to the disclosure, the neuroprotective effect of angiogenin involves the activation of the PI3K/Akt pathway.

**[0015]** US Patent Nos. 7,030,090 and 7,517,857 disclose a peptide that stimulates Akt phosphorylation via activation of formyl peptide receptor or formyl peptide receptor-like 1, the peptide comprises an amino acid sequence of WX1X2MX3X4, where X1=K, R, E, H or D, X2=G, Y, H, E or W, X3=V or G and X4= D-Me or G.

**[0016]** US Patent No. 7,622,455 discloses a method of treating ALS comprising administering to the cerebrospinal fluid of a subject in need thereof antisense oligonucleotides complementary to SOD1 nucleic acids.

**[0017]** There is an unmet need for novel methods for slowing down the rate of progression of neurodegenerative diseases, such as ALS, assessing responsiveness to treatment of the disease, and staging the disease in a manner that is specific, safe and effective.

## SUMMARY OF THE INVENTION

**[0018]** The present invention provides a method for assessing responsiveness to treatment of ALS with a peptide comprising an amino acid sequence of SEQ ID NO: 1, or a pharmaceutical composition comprising same, the method comprising: assessing the level of pAkt or pAkt:tAkt ratio in bodily sample derived from a subject, wherein responsiveness to treatment is indicated by a pAkt level or pAkt:tAkt ratio significantly above a value of a pAkt level or pAkt:tAkt ratio in said subject prior to said treatment, and

wherein the sample is derived from the peripheral blood, the lymph system or a muscle of said subject.

**[0019]** Also disclosed are methods for treating neurodegenerative diseases, such as ALS and variants of this disease, the method comprises assessing pAkt level and the level of ratio pAkt to total Akt (tAkt) in a subject in need thereof, followed by administering to a subject having pAkt and/or pAkt:tAkt ratio significantly below a predetermined threshold level, a composition comprising a peptide having an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

**[0020]** While use of such peptides for ameliorating stress-induced cell death and p53-mediated response has been described, the present invention demonstrates that a peptide comprising SEQ ID NO:1 is useful in increasing the levels of Akt phosphorylation, thereby better defining the population that would benefit from treatment with the peptide.

**[0021]** It is now disclosed for the first time that a peptide having an amino acid sequence consisting of SEQ ID NO: 1

or SEQ ID NO:2 activates the Akt pathway. The peptide-induced activation of the Akt pathway in a specific manner was detected shortly after introducing said peptide to macrophages culture. The peptide's induced activation of the Akt pathway was also observed in vivo. Thus, these peptides are useful for treating diseases associated with low Akt phosphorylation (pAkt) or low pAkt:tAkt ratio.

5 [0022] Surprisingly, administering a peptide having an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2 to SOD1 mice enhanced survival and prolonged the life span of said mice in a significant manner (10 days longer; P=0.008) in comparison to non-treated mice. In addition to extended survival, this peptide significantly delays disease progression by delaying the appearance of the disease symptoms, primarily, the challenged mobility.

10 [0023] Without wishing to be bound by theory or mechanism, the advantages of this peptide over other treatments of ALS known to date may be attributed to the fact that the peptide was tested, and shown to be effective not only before disease onset but also after disease onset including at very late stage of disease.

[0024] Advantageously, use of this peptide is relatively safe, as administration of the peptide in a dose that is four times higher than the effective dose, daily, for 28 days, did not initiate any detectable side effect in healthy mice. Accordingly, this peptide is suitable for chronic use. In addition, this peptide was shown effective when administered intravenously (IV), intraperitoneally (IP) or in combination of these two modes of delivery.

15 [0025] The present invention also highlights the use of Akt phosphorylation and pAkt:tAkt ratio as biomarkers that are significantly useful for establishing responsiveness to treatment with a peptide comprising an amino acid sequence of SEQ ID NO:1. It is shown that high levels of pAkt and high levels of pAkt:tAkt ratio (e.g. above 1) correlate with responsiveness to treatment of ALS with a peptide comprising an amino acid sequence of SEQ ID NO: 1.

20 [0026] There is disclosed a method for prognosticating the progression of a neurodegenerative disease in a subject, comprising (a) assessing the value of at least one marker selected from: pAkt and pAkt:tAkt ratio, in a bodily sample derived from the subject; and (b) obtaining the ratio between the value of the marker and the value of the marker in a control sample, wherein a level of pAkt or pAkt:tAkt ratio significantly below a control value indicates a rapid disease.

25 [0027] There is disclosed a method for treating a neurodegenerative disease in a subject in need thereof, comprising administering to a subject having a rapidly progressing neurodegenerative disease a therapeutically effective amount of an agent capable of activating a Akt pathway, thereby treating the neurodegenerative disease.

[0028] There is disclosed a method for treating a neurodegenerative disease in a subject in need thereof, comprising (a) assessing the level of pAkt and pAkt:tAkt ratio in a bodily sample derived from the subject; and (b) administering a therapeutically effective amount of a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and an analog or a derivative thereof, to a subject having pAkt level or pAkt:tAkt ratio significantly below a control value.

30 [0029] There is disclosed a method for treating amyotrophic lateral sclerosis (ALS) in a subject in need thereof, comprising (a) assessing the level of pAkt and pAkt:tAkt ratio in bodily sample derived from the subject; and (b) administering a therapeutically effective amount of a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and an analog or a derivative thereof, to a subject having pAkt level or pAkt:tAkt ratio significantly below a control value.

35 [0030] According to an aspect of some embodiments of the present invention there is provided a method for assessing responsiveness to treatment of ALS with a peptide comprising an amino acid sequence of SEQ ID NO: 1, or a pharmaceutical composition comprising same, the method comprising: assessing the level of pAkt or pAkt:tAkt ratio in bodily sample derived from a subject, wherein responsiveness to treatment is indicated by a pAkt level or pAkt:tAkt ratio significantly above a value of a pAkt level or pAkt:tAkt ratio in the subject prior to the treatment, and wherein the sample is derived from the peripheral blood, the lymph system or a muscle of said subject.

40 [0031] There is disclosed a kit for diagnosing a neurodegenerative disease or prognosticating its progression in a subject comprising i) means for collecting a bodily sample from a subject and ii) means for determining the level of pAkt and tAkt in the sample.

[0032] According to the invention, the disease is amyotrophic lateral sclerosis (ALS).

45 [0033] An agent capable of activating the Akt pathway may comprise a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and an analog or a derivative thereof, a insulin-like growth factor 1 (IGF-I), vascular endothelial growth factor (VEGF), angiogenin, naphtho[1,2-b]furan-4,5-dione (NFD), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), growth factor-beta (TGF-beta), glial-cell-line-derived neurotrophic factor (GDNF), Hepatic growth factor (HGF), fibroblast growth factor (FGF) or epidermal growth factor (EGF).

50 [0034] An agent capable of activating the Akt pathway may be administered by a route of administration selected from the group consisting of: oral, transdermal, parenteral, transmucosal, intrathecal, intracerebroventricular (ICV), intranasal, sublingual, intravenous and intraperitoneal.

55 [0035] According to the invention, the bodily sample is derived from the peripheral blood, the lymph system or a muscle of the subject.

[0036] According to some embodiments of the invention, the peripheral blood or the lymph system comprises lymphocytes.

[0037] According to some embodiments of the invention, the peptide is comprised in a pharmaceutical composition in combination with at least one more therapeutic drug.

5 [0038] According to some embodiments of the invention, the at least one more therapeutic drug is selected from the group consisting of: an oxidative agent, non-halogen activated-oxygen compounds, non-oxygen activated-halogen compounds, N-halo compounds and riluzole.

[0039] According to some embodiments of the invention, the pharmaceutical composition further comprises a pharmaceutical acceptable excipient, carrier or diluent.

10 [0040] Treating may comprise attenuating the progression of the disease, alleviating symptoms of the disease, delaying the appearance of disease symptoms or improving management of the disease.

[0041] According to some embodiments of the invention, the administering the peptide by a route of administration selected from the group consisting of: oral, transdermal, parenteral, transmucosal, intrathecal, intracerebroventricular (ICV), intranasal, sublingual, intravenous and intraperitoneal.

15 [0042] According to some embodiments of the invention, the peptide consists of an amino acid sequence of SEQ ID NO: 1.

[0043] According to some embodiments of the invention, the means for determining the levels of pAkt comprise at least one antibody directed to pAkt.

20 [0044] According to some embodiments of the invention, the means for determining the levels of tAkt comprise at least one antibody directed to tAkt.

[0045] These and other embodiments of the present invention will become apparent in conjunction with the figures, description and claims that follow.

25 [0046] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

30 **BRIEF DESCRIPTION OF THE FIGURES**

[0047] Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

35 [0048] In the drawings:

40 FIG. 1A presents Akt phosphorylation in macrophages treated with a peptide having the amino acid sequence of SEQ ID NO: 1.

FIG. 1B shows Akt phosphorylation in mice treated with the peptide of SEQ ID NO: 1 as compared to non-treated mice.

45 FIG. 2 shows survival curve after disease onset (week 15 onwards) of treated (light squares) and untreated (control; dark squares) SOD1 mice together with the extent of significance, as evaluated by Chi test (\* for  $P < 0.05$ ; \*\* for  $P < 0.01$  and \*\*\* for  $P < 0.001$ ). Mice were treated with the peptide of the invention starting at disease onset as indicated by weigh loss (average - 90 days, 400  $\mu\text{g}$  200 IP + 200 IV) and were compared to control (n = 21+21) mice (untreated or treated with PBS).

50 FIG. 3 exhibits the average neurologic disability score in treated (light squares) and untreated (control; dark squares) SOD1 mice together with the extent of significance, as evaluated by Student's T test (\* for  $P < 0.05$ ; \*\* for  $P < 0.01$  and \*\*\* for  $P < 0.001$ ). Mice were treated with the peptide of the invention starting at disease onset (average 90 days, 400  $\mu\text{g}$  200 IP + 200 IV) and were compared to control (n = 21+21) mice (untreated or treated with PBS).

**DETAILED DESCRIPTION OF THE INVENTION**

55 [0049] The present invention provides a method for assessing responsiveness to treatment of ALS with a peptide comprising an amino acid sequence of SEQ ID NO: 1, or a pharmaceutical composition comprising same, the method comprising: assessing the level of pAkt or pAkt:tAkt ratio in bodily sample derived from a subject, wherein responsiveness to treatment is indicated by a pAkt level or pAkt:tAkt ratio significantly above a value of a pAkt level or pAkt:tAkt ratio in said subject prior to said treatment, and wherein the sample is derived from the peripheral blood, the lymph system or

a muscle of said subject.

**[0050]** The term "treating" as used herein includes prophylactic and therapeutic uses, and refers to the alleviation of symptoms of a particular disease in a patient, and/or improvement of an ascertainable measurement associated with a particular disorder. More specifically, in the context of this invention the disease is ALS and "treating a disease associated with low levels of Akt phosphorylation", means attenuating the progression of said ALS and/or alleviating symptoms of said ALS and/or improving management of said ALS. This modulation can be measured by assessing the level of Akt phosphorylation upon treatment and prior to treatment, where assessment of Akt phosphorylation may be carried out in ways which are routine in the art, for example, mass spectroscopy.

**[0051]** As used herein the term "subject" refers to a mammalian subject, e.g. human subject. The subject may be of any age and gender.

**[0052]** Examples of neurodegenerative diseases include, but are not limited to, Amyotrophic Lateral Sclerosis (ALS), primary lateral sclerosis (PLS), spinal muscular atrophy (SMA), Alzheimer's disease, Parkinson's disease, glaucoma, macular degeneration, hypoxia, fulminant toxic liver, kidney failure, infertility, type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, autoimmune uveitis, graft versus host disease, graft rejection, arthritis, systemic inflammatory response syndrome (SIRS), inflammatory bowel disease (IBD), adult respiratory distress syndrome (ARDS), psoriasis, atherosclerosis.

**[0053]** According to the invention, the neurodegenerative disease is ALS.

**[0054]** As used herein "amyotrophic lateral sclerosis (ALS)" also referred to as "Lou Gehrig's disease" refers to a progressive, fatal, neurodegenerative disease caused by the degeneration of motor neurons, the nerve cells in the central nervous system that control voluntary muscle movement. The term ALS includes sporadic and familial ALS, ALS at any rate of progression (i.e. rapid or slow progression) and ALS at any stage (e.g. prior to onset, at onset and late stages of ALS).

**[0055]** ALS typically causes muscle weakness and atrophy throughout the body as both the upper and lower motor neurons degenerate, ceasing to send messages to muscles. Unable to function, the muscles gradually weaken, develop fasciculations (twitches) because of denervation, and eventually atrophy because of that denervation. Affected subjects may ultimately lose the ability to initiate and control all voluntary movement; bladder and bowel sphincters and the muscles responsible for eye movement are usually, but not always, spared.

**[0056]** Cognitive function is generally spared except in certain situations such as when ALS is associated with frontotemporal dementia. However, there are reports of more subtle cognitive changes of the frontotemporal type in many patients when detailed neuropsychological testing is employed. Sensory nerves and the autonomic nervous system, which controls functions like sweating, generally remain functional. ALS as used herein refers to all the above exemplary manifestations. ALS, as used herein refers to hereditary and sporadic ALS.

**[0057]** Both hereditary causes and environmental risks may contribute to onset of disease. For instance, an inherited genetic defect on chromosome 21 (coding for superoxide dismutase) is associated with approximately 20 % of familial cases of ALS. This mutation is believed to be autosomal dominant. The most common ALS causing SOD1 mutation in North America is A4V, characterized by an exceptionally rapid progression from onset to death. The children of those diagnosed with familial ALS have a higher risk factor for developing the disease; however, those who have close family members diagnosed with sporadic ALS have no greater a risk factor than the general population, suggesting an environmental or other non-genetic cause.

**[0058]** Furthermore, environmental causative factors have been suggested for the increased incidence of ALS. These include, prolonged exposure to a dietary neurotoxin called BMAA produced by cyanobacteria which is one of several possible neurotoxic compounds found in the seed of the cycad *Cycas circinalis*, a tropical plant found in Guam; Exposure to pesticides; toxic exposure such as nerve gas.

**[0059]** The treatment of ALS may be initiated at any stage of the disease, including following detection of ALS symptoms.

**[0060]** Detection of ALS may be determined by the appearance of different symptoms depending on which motor neurons in the body are damaged first (and consequently which muscles in the body are damaged first). In general, ALS symptoms include the earliest symptoms which are typically obvious weakness and/or muscle atrophy. Other symptoms include muscle fasciculation (twitching), cramping, or stiffness of affected muscles, muscle weakness affecting an arm or a leg and/or slurred and nasal speech. Most ALS patients experience first symptoms in the arms or legs. Others first notice difficulty in speaking clearly or swallowing. Other symptoms include difficulty swallowing, and loss of tongue mobility. A small proportion of patients experience respiratory difficulties.

**[0061]** The symptoms may be also classified by the part of neuronal system that is degenerated, namely, upper motor neurons and lower motor neurons. Symptoms of upper motor neuron degeneration include tight and stiff muscles (spasticity) and exaggerated reflexes (hyperreflexia) including an overactive gag reflex. Symptoms of lower motor neuron degeneration include muscle weakness and atrophy, muscle cramps, and fleeting twitches of muscles that can be seen under the skin (fasciculations). To be diagnosed with ALS, patients must have signs and symptoms of both upper and lower motor neuron damage that cannot be attributed to other causes.

**[0062]** Alternatively, treatment may be initiated at progressive stages of the disease, e.g. when muscle weakness and

atrophy spread to different parts of the body and the subject has increasing problems with moving [e.g. the subject may suffer from tight and stiff muscles (spasticity), from exaggerated reflexes (hyperreflexia), from muscle weakness and atrophy, from muscle cramps, and/or from fleeting twitches of muscles that can be seen under the skin (fasciculations)], swallowing (dysphagia), speaking or forming words (dysarthria).

5 **[0063]** As mentioned above, the method of the invention involves the use of a peptide comprising SEQ ID NO:1, which maybe referred to as a "peptide of the invention".

**[0064]** Addition of amino acid residues may be performed at either terminus of the peptides of the invention for the purpose of providing a "linker" by which the peptides of this invention can be conveniently bound to a carrier. Such linkers are usually of at least one amino acid residue and can be of 40 or more residues, more often of 1 to 10 residues. Typical

10 **[0065]** A peptide of the invention may also be conjugated to itself or aggregated in such a way as to produce a large complex containing the peptide. Such large complex may be advantageous because it has new biological properties such as longer half-life in circulation or greater activity.

15 **[0066]** Peptidomimetics are small molecules that can bind to proteins by mimicking certain structural aspects of peptides and proteins. They are used extensively in science and medicine as agonists and antagonists of protein and peptide ligands of cellular and other receptors, and as substrates and substrate analogs for enzymes.

20 **[0067]** A primary goal in the design of peptide mimetics has been to reduce the susceptibility of mimics to cleavage and inactivation by peptidases. In one approach, one or more amide bonds have been replaced in an essentially isosteric manner by a variety of chemical functional groups, including, but not limited to urea bond, carbamate bond, sulfonamide bond, hydrazine bond, or any other covalent bond. In another approach, a variety of uncoded or modified amino acids such as D-amino acids and N-methyl amino acids have been used to modify mammalian peptides.

**[0068]** As mentioned, treating the neurodegenerative disease may be affected by administration of a therapeutically effective amount of an agent capable of activating a Akt pathway.

25 **[0069]** As used herein, the phrase "agent capable of activating a Akt pathway" refers to a molecule that upregulates phosphorylation of Akt per se or a down-stream signaling effector thereof (i.e., indirect activation).

**[0070]** The peptide comprising an amino acid sequence of SEQ ID NO: 1 can be administered to the subject per se or as part of a pharmaceutical composition.

30 **[0071]** As used herein a "pharmaceutical composition" refers to a preparation comprising the peptide of the invention, with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to a patient in need thereof.

**[0072]** The composition of the invention may be administered by any conventional and appropriate route of administration, including, but not limited to, oral, intravenous, intramuscular, subcutaneous, intrathecal, topical, rectal, buccal, inhalational, intranasal transdermal, parenteral, transmucosal, sublingual, intravenous and intraperitoneal.

35 **[0073]** Hereinafter, the term "oral administration" includes, but is not limited to, administration by mouth for absorption through the gastrointestinal tract (peroral) wherein the drug is swallowed, or for trans-mucosal absorption in the oral cavity by buccal, gingival, lingual, sublingual and oro-pharyngeal administration. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. The oral composition can optionally contain inert pharmaceutical excipients such as thickeners, diluents, flavorings, dispersing aids, emulsifiers, binders, preservatives and the like.

40 **[0074]** The term "parenteral administration" as used herein indicates any route of administration other than via oral administration and includes, but is not limited to, administration by intravenous drip (IV) or bolus injection, intraperitoneal (IP), intrathecal, subcutaneous, or intra muscular injection, topical, transdermal, rectal, intranasal (IN) administration or by inhalation.

45 **[0075]** According to one embodiment, the peptide as set forth in SEQ ID NO: 1 is administered via a combination of two routes (e.g. intraperitoneal and intravenous routes).

**[0076]** According to one embodiment, the peptide of the invention is administered orally.

**[0077]** Formulations for parenteral administration include but are not limited to sterile aqueous solutions which can also contain buffers, diluents and other suitable additives.

50 **[0078]** For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants for example DMSO, or polyethylene glycol are generally known in the art.

55 **[0079]** Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers.

**[0080]** In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration

should be in dosages suitable for the chosen route of administration.

**[0081]** Alternatively, the compounds of the present invention can be incorporated into oral liquid preparations such as aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, for example. Moreover, formulations containing these compounds can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can contain conventional additives, like suspending agents, such as sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats; emulsifying agents, such as lecithin, sorbitan monooleate, or acacia; nonaqueous vehicles (which can include edible oils), such as almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol; and preservatives, such as methyl or propyl p-hydroxybenzoate and sorbic acid.

**[0082]** For administration by inhalation, the peptides for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e. g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the peptide and a suitable powder base such as lactose or starch.

**[0083]** The pharmaceutical compositions of the invention are also useful for topical application. As used herein, the term "topical" means "pertaining to a particular surface area", e.g. skin and mucosa, and the topical agent applied to a certain area of said surface will affect only the area to which it is applied. The formulations of the peptides/peptide analogs may be administered topically as a gel, ointment, cream, emulsion, sustained release formulation including a transdermal patch, and may comprise liposomes and any other pharmaceutically acceptable carrier suitable for administration of the drug topically. The pharmaceutical compositions herein described may also comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers such as polyethylene glycols.

**[0084]** The preparation of pharmaceutical compositions which contain peptides or polypeptides as active ingredients is well known in the art. Typically, such compositions are prepared as injectable, either as liquid solutions or suspensions, however, solid forms, which can be suspended or solubilized prior to injection, can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is mixed with inorganic and/or organic carriers, which are pharmaceutically acceptable and compatible with the active ingredient. Carriers are pharmaceutically acceptable excipients (vehicles) comprising more or less inert substances when added to a pharmaceutical composition to confer suitable consistency or form to the composition. Suitable carriers are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents, which enhance the effectiveness of the active ingredient.

**[0085]** Toxicity and therapeutic efficacy of the peptides described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e. g., by determining the  $IC_{50}$  (the concentration which provides 50 % inhibition) and the  $LD_{50}$  (lethal dose causing death in 50 % of the tested animals) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

**[0086]** The amount of active agent used in an administration composition of the present invention is an amount effective to accomplish the purpose of the particular active agent for the target indication. The amount of active agent in the compositions typically is a pharmacologically, biologically, therapeutically, or chemically effective amount. However, the amount can be less than that amount when the composition is used in a dosage unit form because the dosage unit form may contain a plurality of compounds or active agents in a single composition or may contain a divided pharmacologically, biologically, therapeutically, or chemically effective amount. The total effective amount can then be administered in cumulative units containing, in total, an effective amount of the active agent.

**[0087]** A therapeutically effective amount of a peptide of the invention is an amount that when administered to a patient for treating a neurodegenerative disease, is capable of attenuating the progression of said disease, alleviating symptoms of said disease and improving management of said disease.

**[0088]** Although an appropriate dosage of a peptide of the invention varies depending on the administration route, age, body weight, sex or conditions of the patient, and should be determined by the physician in the end, the dose suitable for adult humans can generally be between about 0.2-2000 mg/kg body weight, about 0.2-1500 mg/kg body weight, about 0.2-1000 mg/kg body weight, about 0.2-500 mg/kg body weight, about 0.2-200 mg/kg body weight, about 0.2-100 mg/kg body weight, about 1-2000 mg/kg body weight, about 1-1500 mg/kg body weight, about 1-1000 mg/kg body weight, about 1-500 mg/kg body weight, about 1-100 mg/kg body weight, or preferably between about 1-200 mg/kg.

**[0089]** The pharmaceutical compositions of the present invention comprise one or more compounds of the present invention, and one or more excipients, carriers or diluents.

**[0090]** Carriers are pharmaceutically acceptable vehicles comprising more or less inert substances when added to a pharmaceutical composition to confer suitable consistency or form to the composition. Suitable carriers are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents, which enhance the effectiveness of the active ingredient.

**[0091]** Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

**[0092]** The compositions comprising the compounds and active agents have utility in the delivery of active agents to selected biological systems and in an increased or improved bioavailability of the active agent compared to administration of the active agent without the delivery agent. Delivery can be improved by delivering more active agent over a period of time, or in delivering active agent in a particular time period (such as to effect quicker or delayed delivery) or over a period of time (such as sustained delivery).

**[0093]** Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

**[0094]** The present invention further contemplates administration of other therapeutic drugs to the subject. Exemplary drugs which may be administered include, but are not limited to, oxidative agents, non-halogen activated-oxygen compounds, non-oxygen activated-halogen compounds, N-halo compounds and riluzole.

**[0095]** As mentioned above, in accordance with the present invention, the level of Akt phosphorylation (pAkt) and pAkt:tAkt ratio is assessed.

**[0096]** As used herein, the term "Akt" refers to the serine/threonine-specific protein kinase also known as Protein Kinase B (PKB). Three genes in the Akt family, in humans: Akt1 (also called Akt), Akt2, and Akt3, encode for enzymes that are members of this serine/threonine-specific protein kinase family. Akt1 is involved in cellular survival pathways, by inhibiting apoptotic processes and is able to induce protein synthesis pathways, which renders it a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. Since it can block apoptosis, and thereby promote cell survival, Akt1 has been implicated as a major factor in many types of cancer. Akt2 is an important signaling molecule in the Insulin signaling pathway and is required to induce glucose transport. The role of Akt3 is less clear, though it appears to be predominantly expressed in brain.

**[0097]** As used herein, the term "pAkt" refers to the phosphorylated form of Akt. According to exemplary embodiments, the Akt is phosphorylated on serine and/or threonine site(s), such as serine 308 and threonine 473.

**[0098]** As used herein, the term "pAkt:tAkt ratio" refers to the level of phosphorylated Akt divided by the level of total Akt in the cell or biological sample.

**[0099]** Phosphorylation is probably the most common of protein post translational modifications (PTMs), with 30 % of eukaryotic proteins estimated to be modified this way. Phosphorylation is essential to the cell by playing a central role in signal transduction cascades, regulation of protein activity and protein-protein interactions. Protein phosphorylation can be detected as a mass shift (+79.99 Da) in mass spectra, which corresponds to the addition of  $HPO_3$  to a peptide, generally at serine, threonine or tyrosine residues.

**[0100]** Methods for measuring protein and peptide phosphorylation (i.e. pAkt) are known in the art and include Kinase Activity Assays, Western blots (i.e. with an anti-pAkt antibody) or Enzyme-Linked Immunosorbent Assays (ELISA, i.e. with an anti-pAkt antibody) and Mass Spectrometry (e.g. Donahue et al., Methods in Enzymology, Volume 434, p. 131-150). Similarly, total Akt (tAkt) levels may be assessed using e.g. Western blot (i.e. with an anti-Akt antibody) or Enzyme-Linked Immunosorbent Assay (ELISA i.e. with an anti-Akt antibody).

**[0101]** Without being bound by any theory or mechanism, Akt pathway dysfunction in ALS patients may result from multiple effects of the underlying ALS disease process. Akt is the major signaling pathway activated by at least four major factors and hormones: GH, IGF1, HGF and VEGF. Akt pathway induction by all four is impaired in ALS:

- i. GH levels are drastically reduced as a direct result of oxidative stress and other primary disease processes of ALS
- ii. The IGF1 signaling pathway, a major fallback option of GH, is disturbed by up-regulation of IGFBP1 and direct carbonylation damage to the IGF1 Receptor.
- iii. Later in the disease, IGF1, HGF and VEGF levels are also decreased, further reducing the natural stress response of the neuro-musculature system.

**[0102]** As shown in Examples 4 and 5 of the Examples section which follows, the present inventors have shown that pAkt correlates with the state of the disease, i.e. in rapidly progressing ALS disease the ratio of muscle/lymph pAkt is significantly lower compared to slow progressing ALS disease. Moreover, the present inventors have shown that pAkt can be used to assess responsiveness to treatment, i.e. when the subject responds to treatment, there is a significant

elevation in pAkt levels.

**[0103]** Assessing the level of pAkt or pAkt:tAkt ratio may thus be carried out prior to treatment, upon treatment and following treatment and may be used for various applications as specified in further detail below.

**[0104]** According to one embodiment, there is provided a method of assessing responsiveness to treatment of a disease (e.g. ALS) with the peptide of the present invention, the method comprising: assessing the level of pAkt and pAkt:tAkt ratio in bodily sample derived from the muscle, the blood or lymphatic system of the subject (e.g. comprising lymphocytes), wherein responsiveness to treatment is indicated by a pAkt level or pAkt:tAkt ratio which is significantly above a value of pAkt level and pAkt:tAkt ratio in the subject prior to the treatment.

**[0105]** As used herein, the terms "above" or "increase" as used herein, refer to at least about 10 %, at least about 20 %, at least about 30 %, at least about 40 %, at least about 50 %, at least about 60 %, at least about 70 %, at least about 80 %, at least about 90 % or at least about 100 % higher level of pAkt level or pAkt:tAkt ratio in the subject following treatment as compared to a level of same in a subject prior to treatment or to commonly used control samples taken from sick individual (e.g. an individual with a disease, e.g. ALS, corresponding to the subject being treated).

**[0106]** Statistical significance may be evaluated by any method known in the art, such as, Student's T-test, Analysis of variance (ANOVA) and Chi-square test, among others.

**[0107]** The present invention also highlights the use of Akt phosphorylation as a biomarker. Disclosed is a method for assessing whether the progression of ALS is slow or rapid.

**[0108]** By "rapid progression" or "rapid disease" it is meant that the symptoms of ALS progress continuously and significant degradation of motor neurons can be observed within less than a year. Rapid disease in human patients corresponds to survival of up to 4 years from diagnosis. In contrast, "slow progression" or "slow disease" refers to a condition where the ALS symptoms appear slowly with long periods, i.e. many months or years. Slow disease in human patients also typically corresponds to survival of more than 4 years from diagnosis.

**[0109]** A kit may comprise i) means for collecting a bodily sample from a subject ii) means for determining the level of the markers pAkt and tAkt (total Akt) in the sample and iii) a standard sample for comparison of the tested sample.

**[0110]** The means for determining the levels of said markers may comprise at least one antibody directed to pAkt and at least one antibody directed to total Akt. The level of pAkt and tAkt may be determined by Western blot analysis, or applying Akt phosphorylation assays as been demonstrated by Armentero et al. (Neurobiol Aging, Jan. 25, 2010). The level of pAkt and tAkt may be determined by FACS (fluorescence-activated cell sorting) assays.

**[0111]** A kit may further comprise a standard, a calibration curve or an index indicating a control value of the pAkt and the tAkt.

**[0112]** A control value may correspond to pAkt level or pAkt:tAkt ratio in a sample comprising a bodily sample of a healthy individual, a bodily sample of an individual not afflicted with any neurodegenerative disease, such as ALS, a bodily sample of an individual afflicted with a slowly progressing neurodegenerative disease, such as ALS.

**[0113]** In certain embodiments, the detection of pAkt and tAkt may be performed using an immunoassay such as an enzyme-linked immunosorbent assay (ELISA) testing kit. In such assays samples are typically incubated in the presence of an immobilized first specific binding agent (e.g. an antibody) capable of specifically binding pAkt or an immobilized first specific binding agent capable of binding tAkt. Binding of pAkt or tAkt to said first specific binding agent may be measured using any one of a variety of known methods, such as using a labeled second specific binding agent capable of specifically binding pAkt or tAkt, respectively, (at a different epitope) or capable of specifically binding the first specific binding agent.

**[0114]** Exemplary specific binding agents include e.g. monoclonal antibodies, polyclonal antibodies, and antibody fragments such as recombinant antibody fragments, single-chain antibodies (scFv) and the like.

**[0115]** Exemplary anti-pAkt antibodies which may be used in accordance with the present teachings may be commercially purchased from e.g. Cell Signaling Technology (e.g. # 9275), Millipore (e.g. STAR phospho-Akt1 (Thr308) ELISA kit), BioLegend and Eptomics Inc..

**[0116]** Exemplary anti-tAkt antibodies (i.e. anti-Akt antibodies) which may be used in accordance with the present teachings may be commercially purchased from e.g. Cell Signaling Technology (e.g. # 9272), Millipore (e.g. STAR Akt ELISA kit), Enzo Life Sciences Inc., BioLegend and Proteintech Group Inc.

**[0117]** In some embodiments, various conventional tags or labels may be used, such as a radioisotope, an enzyme, a chromophore or a fluorophore. A typical radioisotope is iodine-125 or sulfur-35. Typical enzymes for this purpose include horseradish peroxidase, horseradish galactosidase and alkaline phosphatase.

**[0118]** Alternately, other immunoassays may be used; such techniques are well known to the ordinarily skilled artisan and have been described in many standard immunology manuals and texts.

**[0119]** In some embodiments, the methods of the invention are suitable for automated or semi-automated analysis, and may enable clinical, medium or high-throughput screening of multiple samples. For example, automated ELISA systems such as Biotest's Quickstep® ELISA Processor, Maxmat Automated microwell ELISA analyzer (Maxmat S.A., France), or DSX™ Four-Plate System (Dynex Technologies) may conveniently be used.

**[0120]** Other suitable assays include for example flow cytometry assays (such as singleplex and multiplex bead-based

Luminex® assays (Invitrogen).

**[0121]** Alternately, pAkt or tAkt may be captured on an antibody microarray. The antibody microarray comprises an anti-pAkt antibody or an anti-tAkt antibody, or, for example, a combination of anti-pAkt antibodies and anti-tAkt antibodies. In general, the sample (e.g., peripheral blood) obtained from the subject is placed on the active surface of a chip for a sufficient time to allow binding. Then, unbound molecules are washed from the surface using a suitable eluant, such as phosphate buffered saline. In general, the more stringent the eluant, the more tightly pAkt or tAkt must be bound to be retained after the wash. As a result, the retained pAkt or tAkt can be detected by appropriate means.

**[0122]** Additional exemplary assays may be based on dipstick technology, as demonstrated, for example, in U.S. Pat. Nos. 4,632,901; 4,313,734; 4,786,589 5,656,448 and EP 0125118. For example, U.S. Pat. No. 4,632,901, discloses a flow-through type immunoassay device comprising antibody (specific to a target antigen analyte) bound to a porous membrane or filter to which is added a liquid sample. As the liquid flows through the membrane, target analyte binds to the antibody. The addition of sample is followed by addition of labeled antibody. The visual detection of labeled antibody provides an indication of the presence of target antigen analyte in the sample. EP 0125118 discloses a sandwich type dipstick immunoassay in which immunochemical components such as antibodies are bound to a solid phase. The assay device is "dipped" for incubation into a sample suspected of containing unknown antigen analyte. Enzyme-labeled antibody is then added, either simultaneously or after an incubation period. The device next is washed and then inserted into a second solution containing a substrate for the enzyme. The enzyme-label, if present, interacts with the substrate, causing the formation of colored products which either deposit as a precipitate onto the solid phase or produce a visible color change in the substrate solution.

**[0123]** For example, the method may be performed by the steps comprising:

- a) collecting a bodily sample from the subject;
- b) contacting the sample, under conditions such that a specific antigen-antibody complex may be formed, with at least one antibody, said antibody being directed to either pAkt or tAkt;
- c) quantifying the amount of antigen-antibody complex formed, wherein said amount is indicative of the amount of pAkt or tAkt in said sample.

**[0124]** An antibody "directed to" an antigen, as used herein is an antibody which is capable of specifically binding the antigen. The term "specifically bind" as used herein means that the binding of an antibody to an antigen is not competitively inhibited by the presence of non-related molecules. Antibodies directed to pAkt and antibodies directed to tAkt may be prepared using well known methods, for example as detailed hereinabove. Alternatively, antibodies, or ELISA kits for determining the presence of these antigens, may be purchased from a variety of sources.

**[0125]** The following examples are to be considered merely as illustrative and nonlimiting in nature. It will be apparent to one skilled in the art to which the present invention pertains that many modifications, permutations, and variations may be made without departing from the scope of the invention.

**[0126]** As used herein the term "about" refers to  $\pm 10\%$ .

**[0127]** The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

**[0128]** The term "consisting of means "including and limited to".

**[0129]** The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

**[0130]** As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

**[0131]** Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

**[0132]** Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

**[0133]** As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily

developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0134] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[0135] Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

## EXAMPLES

[0136] Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

[0137] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996). Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.

### Example 1

#### The effect of the peptide of SEQ ID NO: 1 on the Akt pathway in cells

[0138] Raw264.7 macrophages were treated with 50 mM of the peptide having the amino acid sequence of SEQ ID NO: 1 ( $10 \times 10^5$  per well) in quadruplicates. Akt phosphorylation was found to be induced shortly after introduction of the peptide (Figure 1A). Akt and pAkt were detected using western blot analysis with Cell Signaling Technology #9272 and #9275 antibodies. This observation demonstrates that the peptide triggers activation of the Akt pathway in a specific manner.

[0139] Next, mice were injected with the peptide of SEQ ID NO: 1 (400  $\mu$ l) and after 30 minutes lymph nodes were assessed for pAkt, compared to lymph samples obtained from non-treated mice. Akt phosphorylation was measured using ELISA compared to a standard, using Millipore's STAR Akt and STAR phospho-Akt1 (Thr308) ELISA kits.

[0140] As shown in Figure 1B, Akt phosphorylation was higher in treated mice compared to non-treated mice.

### Example 2

#### The effect of the peptide of SEQ ID NO: 1 on survival of a mice model for ALS

[0141] ALS is characterized by apoptosis of motor neurons. The SOD1G93A mouse model of familial ALS was used in the following examples. Initially, doses less than optimal were applied, and model irregularities due to gene counts were detected. These deficiencies were repaired in later studies.

## EP 2 715 350 B1

**[0142]** Mice were purchased from Jackson and were administered with a dose of at least 200 microgram a day of the peptide of SEQ ID NO: 1. Overall, the studies included 142 mice. Eventually all mice died of neurologic disease. Mice (a few) that died before the detection of a symptomatic disease were not included, as recommended by Scott et al. (Amyotroph Lateral Scler. 2008; 9(1):4-15). Of the 142 mice, 99 mice were treated intraperitoneally (IP), intranasally (IN), intravenously (IV) or with a combined delivery, and 43 mice were used as control (untreated or treated with PBS).

**[0143]** Allocation of the mice to the experimental groups was done by matching weight and, when applicable (particularly in study III) also by gender match. In studies II and III, where treatment started after disease onset, the primary grouping criteria was date of onset. In fact, age at onset and weight at onset reflect to a certain degree the disease severity, thereby allowing to perform an even distribution of animals according to the phenotypic manifestation of the disease. Although genotypic distribution and separation of siblings is recommended, siblings in the same groups were used, preferring the phenotypic criteria as an indicator of the severity of the disease itself, and as in many cases too many of the mice were from the same pedigree.

**[0144]** Exclusion (censoring) criteria were employed to exclude data that do not include any measurements taken from animals that died before detection of the neurological damage or animals that died due to non-disease associated reasons (such as physical wounds).

**[0145]** In this example, 40 female mice (purchased from Jackson) were included. 30 mice were treated (IN or IV; peptide of SEQ ID NO: 1) and 10 mice were controls. Disease onset was measured by Rotarod exertion value (measuring mobility on a Rotarod vs. time), defined as Rotarod values below 180 (i.e. 3 min.). The animals were divided into groups according to weight match.

Table 1: Study design

Group	Treatment	Route	Schedule	Age at treatment initiation	n
1	PBS (control)	IV	Daily	75 days	10
2	200 µg peptide	IV	Daily	75 days	10
3	400 µg peptide	IV	Daily	75 days	10
4	400 µg peptide	IN	Daily	75 days	10

Table 2: Total survival of mice treated with the peptide

Group	Survival of 50 % (days)	Survival Mean (days)	P (Logrank)	Added survival compared to control (days)
Control	125	128.1		-
400 IN	139	132.4		4-14
200 IV	145	144.3	0.02	16-20
400 IV	139	136.2		8-14
All treatment groups	139	139.2	0.017*	11-14

\* Significance equivalent to p=0.05 for 3 combined groups

**[0146]** The results summarized in Table 2 above clearly indicate that the peptide of the invention induces a significant (p=0.05) increase in survival, additional 11 to 14 days as compared to control, irrespective of the disease stage. The significantly prolonged survival induced by the peptide of the invention is also shown in Figure 2.

**[0147]** Similar results are shown in the 'all stages' columns of Table 3, below. As detailed in Table 3, the most pronounced effect of the protein of the invention is exerted during late stages of the disease, which is characterized by low Rotarod index (less than 120).

Table 3: Disease duration at different stages of the disease (late and early) upon treatment with the peptide of the invention at 200 µg or 400 µg for 7 successive days<sup>a</sup>

Group	Late disease (Y<120) <sup>a</sup>	Early disease (180>Y>120) <sup>b</sup>	All stages of the disease
	Duration (days)	Duration (days)	Duration (days)
400 µg IN (n=10)	26.9	17.7	44.6
200 µg IV (n=10)	34.8	16.2	51.0
400 µg IV (n=10)	25.7	15.8	41.5
All treatments (n=30)	29.2	16.6	45.7
Control (10)	15.5	15.7	31.2

<sup>a</sup>Mice stayed on the Rotarod for less than 120 seconds, i.e. Rotarod index (Y) < 120.  
<sup>b</sup>Mice that stayed on the Rotarod for more than 120 seconds but less than 180 seconds.

[0148] The results indicate that daily treatments with the peptide of the invention via any way of administration (IN or IV) leads to increased survival at advanced (late) stages of the disease (measured by Rotarod exertion values below 120 seconds). Treatment with the peptide of the invention prolonged the duration at the late stage by 88 % while not affecting disease onset or disease progression at the earlier stages. In this study, onset in all groups was at an average age of 93-97 days, and there were no differences between the groups. Late-stage disease started at age of about 110 days.

[0149] In another study (Figure 2) the differences between 21 mice treated with 400 microgram (200 IP + 200 IV) of the SEQ ID NO: 1 peptide and 21 non-treated mice are observed in weeks 18-20. At week 18, 21/21 treated vs. 16/21 non-treated manifested at 20 % difference in survival (\*, P=0.02); at week 19, 19/21 treated vs. 9/21 non-treated manifested 50 % difference in survival (\*\*\*, P=0.001); and at week 20, 14/21 treated vs. 6/21 non-treated manifested a 40 % difference in survival (\*\*, P=0.005).

[0150] The following analysis (Table 4, below) shows the effect of the peptide of the invention on all treatments, irrespective of the stage of disease, but subject to the method of administration.

Table 4: Summary of 50 % survival in all groups (treatments and control)

Study	Treatment	Age at treatment initiation (days)	No. of Treatment animals (number of groups)	No. of Control animals (number of groups)	Average ±SD of added survival (days)	Significance (Logrank)	Average added 50 % survival
Total	200-600 µg IP/IV/IN	89.75	99(9)	43 (3)	*7.2±12.6	0.008	10

§Disease onset was observed  
 \*Adjusted according to control values

[0151] The data shows that the 50 % survival of the 99 mice treated with the peptide of SEQ ID NO: 1 was about 10 days longer, significantly (P=0.008) as compared to control. The most effective route of administration seems to be a combined IV/IP administration.

**Example 3**

**The effect of the peptide of the invention on disease progression**

[0152] The protective effect of the peptide of the invention is not only limited to prolonged survival, but also involves significant delay in the progression of disease symptoms, as monitored by the neurologic disability score, thereby postponing development of end-stage disease.

[0153] Mice were administered with the peptide of SEQ ID NO: 1 or placebo (PBS) starting at disease onset as measured by weight loss. It was at average age of about 90 days. The mice were treated daily. During the period of weeks 16-19, the neurologic score was assessed, and was found lower by a factor of about 2 in treated mice compared

to controls (Figure 3).

**Example 4**

5 **The effect of the peptide of SEQ ID NO: 1 on the Akt pathway in vivo**

[0154] Twenty SOD1 mice at the age of 75 days (i.e. prior to disease onset) were treated daily with 200 µg IV + 200 µg IP of the peptide of SEQ ID NO: 1 or with PBS, and monitored for body weight and neurological score. At the age of 120 days, the mice were sacrificed, and phosphorylated Akt (pAkt) and total Akt (tAkt) in lymphocytes (lymph node) and muscle cells (hind legs) were measured (see Tables 5-6, below). Two treated mice died prior to age 120 days without any preceding signs of neurologic damage. Two non-treated mice died prior to age 120 days after detection of severe signs of neurologic disease.

15 Table 5: The effect of the peptide of SEQ ID NO: 1 on Akt phosphorylation

	Muscles		Lymph node	
	pAkt	pAkt/tAkt	pAkt	pAkt/tAkt
Control (n=8)	150	1.61	95	0.96
SEQ ID NO: 1 (n=8)	149	1.83	129	1.35
Difference	-1 %	+14 %	+36%	+42%

25 Table 6: Treatment with the peptide of SEQ ID NO: 1 significantly increases the combined pAkt/Akt ratio of lymph nodes and muscles

Treatment	Lymph node pAKT ratio	Muscle pAKT ratio	Average pAKT	Average pAKT ratio	Percentage of mice with high pAkt content
PBS	0.96	1.61	121	1.27	12.5 %
SEQ ID NO: 1	1.35	1.83	140	1.61	62.5 %
Percent change	42%	14%	16%	26%	
P value	0.11	0.45	<b>0.037</b>	0.07	<b>0.04</b> chi square

[0155] The peptide of SEQ ID NO: 1 increased the phosphorylation level of lymphocytes by 36 %, and even more in terms of the pAkt/tAkt ratio (42 %). There was a minimal effect on muscle cells, which were predominantly highly phosphorylated (70 % higher pAkt/tAkt ratio in muscles compared to lymphocytes, before treatment with the peptide of the invention). The average increase of pAkt in both muscles and lymphocytes was statistically significant - P=0.04.

[0156] Preliminary measurements of weight loss and neurologic score among non-treated mice with naturally elevated peripheral (muscle and lymph) pAkt showed differences in clinical parameters, as detailed in Table 7, below. This table demonstrates that low lymphocyte and muscle pAkt corresponds to rapid disease (characterized by high neurologic score and weight loss at 120 days) wherein relatively high lymphocyte pAkt and muscle pAkt corresponds to slow disease (i.e. a disease characterized by low neurologic score and weight loss at 120 days).

[0157] It is to be understood that a combination of weight loss and neurological score is considered one of the most reliable and gentle (i.e. not introducing additional stressors) ways to assess the presence, progression and staging of ALS.

50 Table 7: The effect of Akt phosphorylation on disease parameters in non-treated SOD1 mice aged 120 days.

	Muscle pAkt	Lymphocyte pAkt	Muscle-lymph pAkt	Neurologic score	Previous week weight loss
Lower peripheral pAkt (3)	138	94	116	0.67	4.7 %

EP 2 715 350 B1

(continued)

	Muscle pAkt	Lymphocyte pAkt	Muscle-lymph pAkt	Neurologic score	Previous week weight loss
Higher peripheral pAkt (3)	151	111	131	0.33	1.4 %
Change	+9 %	+18 %	+13 %	-50 %	-71 %

[0158] The correlation of Akt phosphorylation with disease progression is even more striking when peripheral (muscle and lymph) pAkt of mice with rapid disease, both treated and non-treated, is compared to peripheral pAkt of mice with slow disease, in both treated and non-treated SOD1 mice, as described in Table 8 below.

Table 8: The effect of Akt phosphorylation on rapid and slow disease

	Neurologic score	Muscle+lymph pAkt
Score 0.5-1.25 (n=7, SOD1 mice with Rapid Disease)	0.8	120
Score 0-0.25 (n=9, SOD1 mice with Slow Disease)	0.05	139
Significance		P=0.01

[0159] In this study, animals at day 120 were divided into groups by the disease state. The results of this analysis indicate that the pAKT correlates with the state of the disease.

[0160] Specifically, in mice where the disease progressed rapidly (neurological score of 0.8 at 120 days), the ratio of muscle:lymph pAkt is significantly (P=0.01) lower than this ratio in mice where the disease progressed slowly (neurological score of 0.05 at 120 days). Thus, the results indicate that pAkt phosphorylation is a reliable indication for distinguishing the stage of the ALS disease (slow/fast).

[0161] When these mice were divided according to pAkt ratio of lymph node and muscle, 10 mice with low pAkt had a significantly (P=0.005) 10 times higher clinical score (0.6) as compared to 6 mice with high pAkt (0.06).

[0162] Thus, the peptide of the invention might employ increased pAkt in both muscles and lymphocytes for reduction of ALS progression rate in SOD1 mice, and might contribute to the treatment of sporadic ALS.

Example 5

Responsiveness to treatment

[0163] The following analysis reflects the significant therapeutic effect of the peptide of the invention on ALS and emphasizes the importance in assessing responsiveness to treatment in terms of increased biomarker during the treatment.

[0164] Mice, age 75 days, were treated for 45 days with 400 µg of the peptide of SEQ ID NO: 1, administered as follows: 200 µg IV and 200 µg IP. At age of 120 days, 6 of the peptide treated mice responded to treatment, having a slow disease e.g. low weight loss (only 3.3 %) and low neurological score (0.08) compared to placebo (PBS) treated mice which kept losing weight, reaching a weight loss of 5.4 % and neurological score of 0.8. The six responding mice had 49 % higher pAkt (P=0.01) compared to the placebo treated mice.

[0165] Two other mice treated with the peptide of the invention did not respond to treatment, having a significantly higher neurological score of 1.1 (93 %; P=0.05) and higher weight loss (6.3 %). Those mice resulted with a significantly lower pAkt phosphorylation (-37 %; P=0.004 in comparison with the responding group).

[0166] The data is summarized in Table 9, below.

Table 9: pAkt in responding and non-responding SOD1 mice at 120 days

	Lymphocyte pAkt	Effect on pAkt*	Average score (age 110-120 days)	Effect on score**	Weight loss§
Placebo (PBS; n=10)	95 (8 survivors, 2 died earlier)	100%	0.8	100%	5.4 %

(continued)

	Lymphocyte pAkt	Effect on pAkt*	Average score (age 110-120 days)	Effect on score**	Weight loss§	
5	<b>Responding (SEQ ID NO: 1; n=6)</b>	142	+49 % pAkt* P=0.01	0.08	-90 % score*  P=0.02	3.3 %
10	<b>Non responding (SEQ ID NO: 1; n=2)</b>	90	-37 % pAkt** P=0.004	1.1	+1400 %** score P=0.05	6.3 %
15	*The effect of treatment on pAkt and neurologic score relative to placebo (100 %). ** The effect of treatment on pAkt and neurologic score in non-responders relative to responders §Average weight loss (compared to the weight on the week prior to treatment)					

**Example 6****Measuring the effect of the peptide of SEQ ID NO: 1 on slow and rapid disease**

20 **[0167]** SOD1 mice were treated IP and IV using the peptide of SEQ ID NO: 1 starting at disease onset, detected by weight loss, at an average age of 90 days.

25 **[0168]** In order to check if mice with rapid disease respond to treatment differently from mice with slow disease, a comparison was made separately of the 50 % control and treated mice that survived less than or equal to the median survival and 50 % control and treated mice that survived longer than the median survival.

30 **[0169]** According to this comparison, demonstrated in Table 10 below, SD values were 40 % lower for treated mice, demonstrating that treatment reduced the diversity and range between rapid and slow disease, making the rapid disease look more like slow disease, and the extension of survival was much larger (11 days comparing to 3 days), and much more significant (P=0.0001 compared to P=0.8), for mice with fast disease.

Table 10: Systemic treatment with the peptide of SEQ ID NO: 1 starting at the onset of weight loss (age 90 days)

	N control / treatment	SD controls / treatment	50 % fast control / treatment	50 % slow control / treatment	
35	<b>PBS</b>	21	13.8	125.2	146.7
	SEQ ID NO: 1	21	8.7	136.0	149.7
	Significance			P=0.0001	P=0.8

40 **[0170]** The absence of an effect on long-term survivors was also reflected by the survival and disease progression curves: they showed the convergence of control and treatment curves for long-term surviving mice and at late study stages, which demonstrate the lack of effect on the fraction of 25 % of mice with the longest survival (Figures 2 and 3)

**[0171]** Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art.

45 **[0172]** Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

## SEQUENCE LISTING

**[0173]**

<110> Yeda Research and Development Co. Ltd.

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Pevsner-Fischer, Meirav

EP 2 715 350 B1

<120> USE OF AKT PHOSPHORYLATION AS A BIOMARKER FOR PROGNOSING NEURODEGENERATIVE DISEASES AND TREATING SAME

<130> 53388

<150> US 61/488,806

<151> 2011-05-23

<160> 2

<170> PatentIn version 3.5

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<211> 7

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<223> Stressin-1 synthetic peptide

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<221> misc\_feature

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<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> Stressin-1 sequence reversed synthetic peptide

<400> 2

Pro Tyr Pro Leu Pro Pro Leu  
1 5

Claims

1. A method for assessing responsiveness to treatment of ALS with a peptide comprising an amino acid sequence of SEQ ID NO: 1, or a pharmaceutical composition comprising same, the method comprising: assessing the level of pAkt or pAkt:tAkt ratio in bodily sample derived from a subject, wherein responsiveness to treatment is indicated by a pAkt level or pAkt:tAkt ratio significantly above a value of a pAkt level or pAkt:tAkt ratio in said subject prior to said treatment, and wherein the sample is derived from the peripheral blood, the lymph system or a muscle of said subject.
2. The method according to claim 1, wherein said peripheral blood or said lymph system comprises lymphocytes.

**Patentansprüche**

5 1. Verfahren zum Bestimmen des Antwortverhaltens auf die Behandlung von ALS mit einem Peptid, aufweisend eine Aminosäuresequenz der SEQ ID NO: 1, oder eine pharmazeutische Zusammensetzung, die selbige aufweist, wobei das Verfahren aufweist: Bestimmen des Spiegels von pAkt oder pAkt:tAkt-Verhältnisses in einer von einem Probanden abgeleiteten Körperprobe, wobei die Ansprechbarkeit auf die Behandlung durch einen pAkt-Spiegel oder pAkt:tAkt-Verhältnis wesentlich über einem Wert eines pAkt-Spiegels oder pAkt:tAkt-Verhältnisses in dem Probanden vor der Behandlung angezeigt wird, und

10 wobei die Probe aus dem peripheralen Blut, dem Lymphsystem oder einem Muskel des Probanden abgeleitet ist.

2. Verfahren nach Anspruch 1, wobei das periphere Blut oder das Lymphsystem Lymphozyten aufweist.

15 **Revendications**

20 1. Procédé d'évaluation de la réponse au traitement de la SLA avec un peptide comprenant une séquence d'acides aminés de SEQ ID NO : 1, ou une composition pharmaceutique le comprenant, le procédé comprenant : l'évaluation du niveau de pAkt ou du rapport pAkt:tAkt dans un échantillon corporel provenant d'un sujet, dans lequel la réponse au traitement est indiquée par un niveau de pAkt ou un rapport pAkt:tAkt considérablement supérieur à une valeur d'un niveau de pAkt ou d'un rapport pAkt:tAkt chez ledit sujet avant ledit traitement, et dans lequel l'échantillon provient du sang périphérique, du système lymphatique ou d'un muscle dudit sujet.

25 2. Procédé selon la revendication 1, dans lequel ledit sang périphérique ou ledit système lymphatique comprend des lymphocytes.

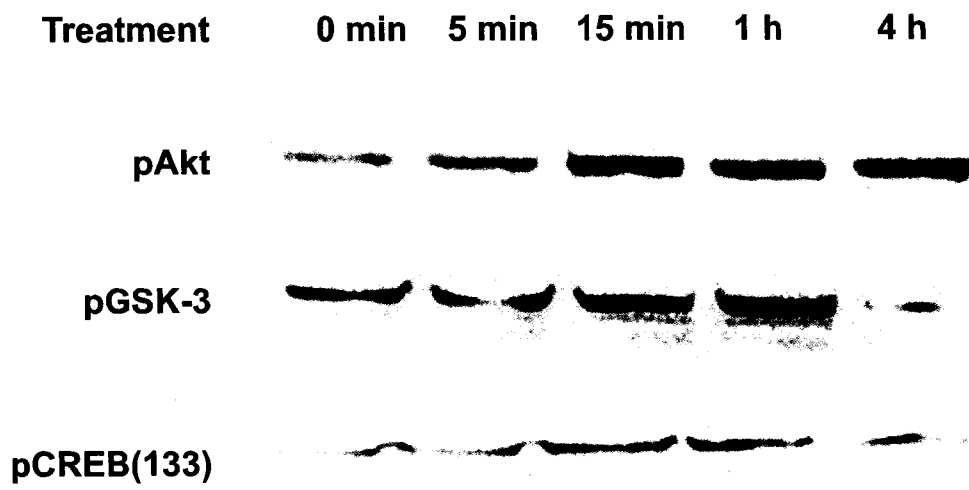
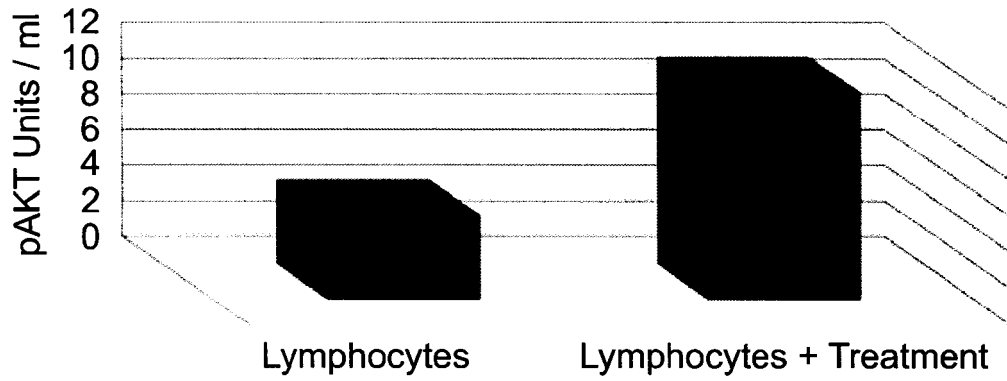
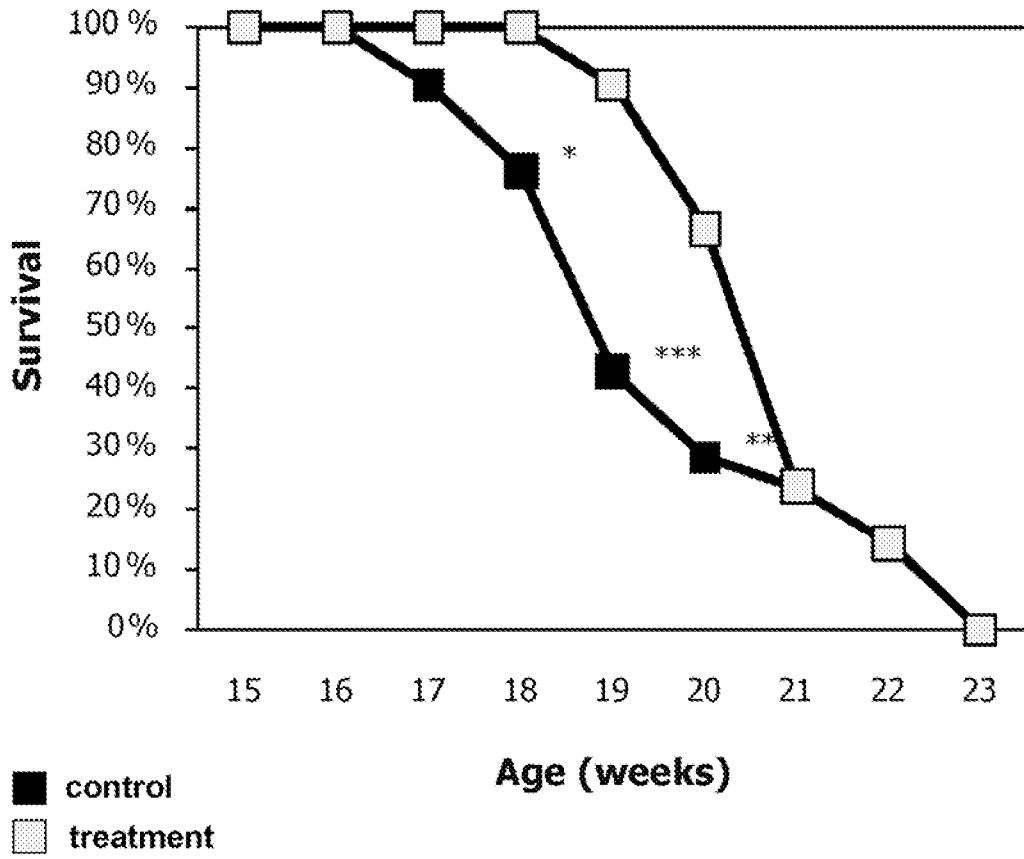


FIG. 1A

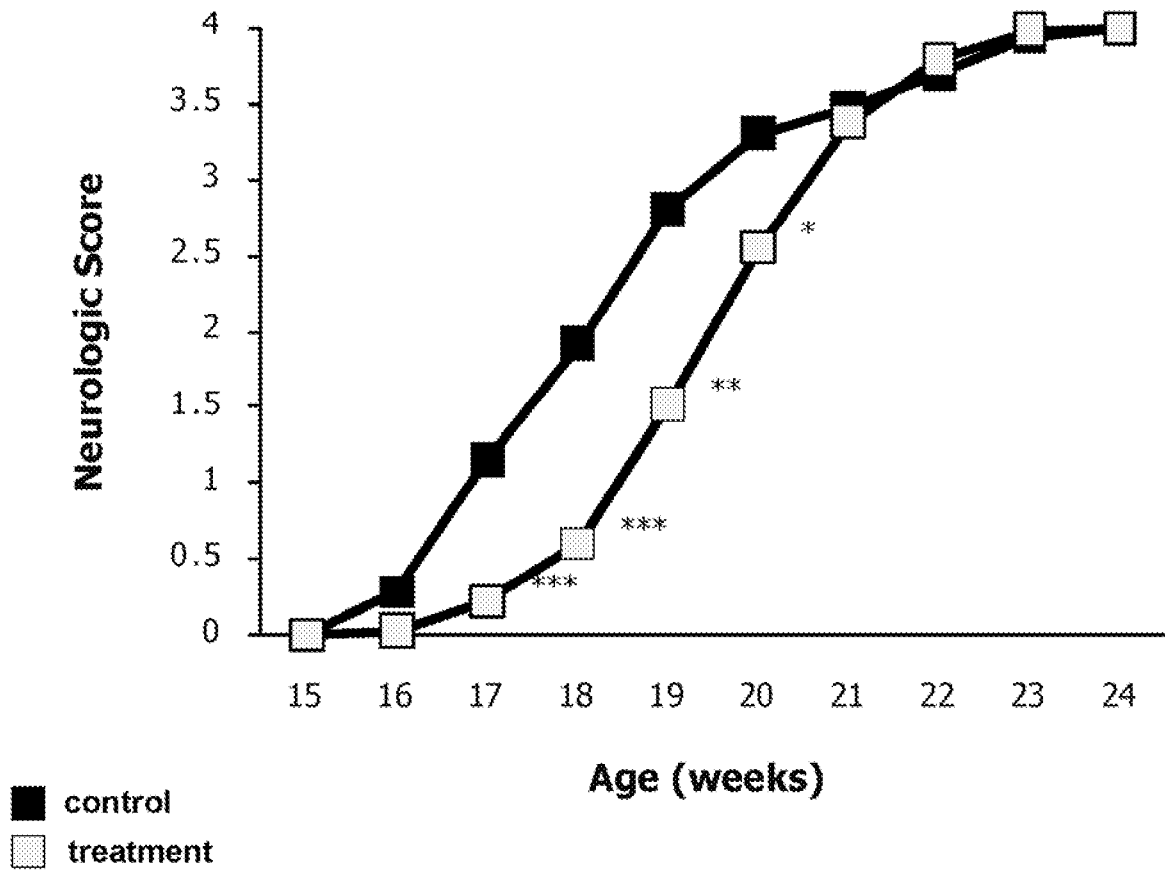
**Quantified ELISA of pAkt in lymphocytes in response to treatment of mice with the peptide of seq id no. 1**



**FIG. 1B**



**FIG. 2**



**FIG. 3**

## REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	使用akt磷酸化作为预测神经退行性疾病和治疗神经退行性疾病的生物标志物		
公开(公告)号	<a href="#">EP2715350A4</a>	公开(公告)日	2015-08-05
申请号	EP2012788741	申请日	2012-05-23
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IPC分类号	G01N33/53 A61K38/17 A61K39/00		
CPC分类号	A61K31/343 A61K38/08 A61K38/1808 A61K38/1825 A61K38/1833 A61K38/1841 A61K38/185 A61K38/1866 A61K38/1891 A61K38/30 A61K45/06 A61P21/00 A61P25/28 G01N33/6896 G01N2800/52 A61K2300/00 C07K7/06 G01N33/54306 G01N33/6854 G01N2800/28		
优先权	61/488806 2011-05-23 US		
其他公开文献	EP2715350A2 EP2715350B1		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

本发明涉及使用pAkt或pAkt : tAkt比率作为生物标志物的神经退行性疾病的预后。