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(54) Title: EXTRACELLULAR DNA AS A THERAPEUTIC TARGET IN NEURODEGENERATION

(57) Abstract: The invention relates to the use of deoxyribonuclease (DNase) enzyme for inhibiting progression and for prevention and treatment of neurodegeneration.

EXTRACELLULAR DNA AS A THERAPEUTIC TARGET IN NEURODEGENERATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/165,255, filed on May 22, 2015, the disclosure of which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates to the use of deoxyribonuclease (DNase) enzyme for inhibiting progression and for prevention and treatment of neurodegeneration.

BACKGROUND OF THE INVENTION

Neurodegeneration is a separate clinical pathological condition with progressive loss of structure and/or function of neurons, including death of neurons. Molecular pathways leading to neurodegeneration are highly disease-specific (e.g., accumulation of abnormally folded amyloid-beta and tau proteins in the brain in Alzheimer's disease patients; accumulation of alpha-synuclein in Parkinson's disease; accumulation of mutant Huntingtin in Huntington's disease; accumulation of TDP-43 and FUS protein aggregates in Amyelotropic Lateral Sclerosis (ALS); accumulation of mitochondrial DNA mutations and broken mitochondria division mechanics in aging) and result in neuronal cell death at advanced stages of disease progression. Programmed cell death including apoptosis seems to play a key role in the progression of neurodegeneration at late disease stage, as demonstrated by studies on animal models and cell lines (Radi E., et al., J Alzheimers Dis. 2014; 42).

Diseases involving clinical signs related to neurodegeneration affect almost 30 million individuals and lead to disability and death. Neurodegeneration usually leads to progressive nervous system dysfunction and is often associated with atrophy of the affected central or peripheral structures of the nervous system. Neurodegeneration is associated with a large group of neurological conditions with heterogeneous clinical and pathological expressions affecting specific subsets of neurons in specific functional anatomic systems. The non-limiting list of disorders where neurodegeneration has a significant impact on clinical and pathological picture is presented below:

- Alzheimer's disease
- Senile dementia of the Alzheimer type

- Pick's disease (lobar atrophy)
- Huntington's disease
- Multiple system atrophy combining dementia with ataxia and/or manifestations of Parkinson's disease
- Progressive supranuclear aplsy (Steel-Richardson-Olszewski)
- Diffuse Lewy body disease
- Corticodentatonigral degeneration
- Hallervorden-Spatz disease
- Progressive familial myoclonic epilepsy
- Paralysis agitans (Parkinson's disease)
- Striatonigral degeneration
- Progressive supranuclear palsy
- Torsion dystonia (torsion spasm; dystonia musculorum deformans)
- Spasmodic torticollis and other dyskinesis
- Familial tremor
- Gilles de la Tourette syndrome
- Cerebellar cortical degeneration
- Olivopontocerebellar atrophy (OPCA)
- Spinocerebellar degeneration (Friedreich's ataxia and related disorders)
- Syndrome of central autonomic nervous system failure (Shy-Drager syndrome)
- Amyotrophic lateral sclerosis
- Spinal muscular atrophy
- Primary lateral sclerosis
- Hereditary spastic paraplegia
- Peroneal muscular atrophy (Charcot-Marie-Tooth)
- Hypertrophic interstitial polyneuropathy (Dejerine-Sottas)
- Miscellaneous forms of chronic progressive neuropathy
- Pigmentary degeneration of the retina (retinitis pigmentosa)
- Hereditary optic atrophy (Leber's disease)
- Bipolar disorder
- Epilepsy
- Migraine

- Schizophrenia.

Traditionally, searches for new therapeutic modalities for neurodegeneration were focused on modulation of molecular pathways inside neuronal cells in order to prevent neuronal cell death and improve neuronal cells functionality. The histone acetyltransferase activators (US 20150119466), cyclin-dependent protein kinase 5 inhibitors (US 20150119348), neurotrophin mimetics (US 20150111903), semaphorin-4D blockers (US 20150110800), microsomal prostaglandin E synthase-1 inhibitors (US 20150087646), N-methyl-d-aspartate (NMDA) receptor inhibitors were proposed as agents useful in treatment of neurodegenerative disorders. NMDA receptor inhibitor memantine has been used clinically for treatment of many neurodegenerative disorders, including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's and Huntington's diseases. Memantine also shows promise as a treatment for other diseases associated with excessive NMDA receptor activation in the central nervous system (CNS), including glaucoma, multiple sclerosis, epilepsy and neuropathic pain (J. Johnson et al., Current Opinion in Pharmacology, 2006, V6, pp. 61-67).

Circulating extracellular (also called "cell free") nucleic acids were discovered more than 60 years ago (Anker P., Circulating DNA in plasma or serum, Clin Chim Acta. 2001; 313(1-2): 143-6). DNA levels in normal plasma samples are quite low with concentrations varying from 3.6 to 5.0 ng/ml. Normal plasma samples mainly contain DNA fragments of about 180 bp and to a much smaller extent larger fragments (e.g., fragments of 500 bp or larger). Circulating extracellular DNA has been described as therapeutic target in several diseases and conditions, including cancer, infection, diabetes, delayed-type hypersensitivity, and fertility (see, e.g., U.S. Patents Nos. 8,916,151; 8,871,200; 8,796,004; 8,710,012; 8,535,663; 8,431,123; 8,388,951; 7,612,032; PCT/IL2007/001250; PCT/IB2013/056321).

SUMMARY OF THE INVENTION

As specified in the Background Section, there is a great need in the art to develop new compositions and methods for treating neurodegeneration. The present invention addresses this and other needs by providing compositions and methods based on DNase enzyme.

Specifically, in one aspect, the invention provides a method for preventing, treating and/or inhibiting progression of neurodegeneration (e.g., primary neurodegeneration) in a patient in need thereof (e.g., a mammal such as human or an experimental animal model), comprising administering to said patient a therapeutically effective amount of a DNase enzyme.

In a related aspect, the invention provides a pharmaceutical composition comprising a DNase enzyme for use in preventing, treating and/or inhibiting progression of neurodegeneration (e.g., primary neurodegeneration).

In a further aspect, the invention provides a use of a DNase enzyme for preventing, treating and/or inhibiting progression of neurodegeneration in a patient in need thereof.

In one embodiment, the neurodegeneration is associated with an increased level of extracellular DNA (e.g., prokaryotic and/or human) in blood or cerebrospinal fluid or intestine of the patient, which level is higher than the control level (e.g., the level of extracellular DNA in blood or cerebrospinal fluid or intestine of a healthy age-matched individual or an average level of extracellular DNA in blood or cerebrospinal fluid or intestine of several healthy age-matched individuals). In one embodiment, the neurodegeneration is associated with a neurodegenerative disorder. Non-limiting examples of encompassed neurodegenerative disorders include, e.g., Alzheimer's disease (e.g., late-onset Alzheimer's disease), Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), and Huntington's disease. In one embodiment, the neurodegeneration is associated with a nervous system dysfunction such as, e.g., schizophrenia or bipolar disorder.

In one embodiment, the therapeutically effective amount of the DNase enzyme is sufficient to destroy said extracellular DNA (e.g., is sufficient to decrease the average molecular weight of said extracellular DNA [e.g., as measured by gel electrophoresis]) in blood or cerebrospinal fluid or intestine of the patient.

In one embodiment, said DNase is a recombinant DNase. In one embodiment, said DNase is DNase I. In one embodiment, said DNase has extended half-life (e.g., is conjugated with polysialic acid or is protected from binding to actin by modification of actin binding-site; see, e.g., Gibson et al., (1992) J. Immunol. Methods, 155, 249-256).

In one embodiment, said DNase is administered by intravenous, subcutaneous or intramuscular route. In one specific embodiment, said DNase is DNase I and is administered in the amount of at least 0.04 mg per kg per day or 0.05 - 10000 Kunitz units per kg per day during at least one day.

In another embodiment, said DNase is administered enterally (e.g., orally). In one specific embodiment, said DNase is DNase I and is administered in the amount of at least 0.04 mg per kg per day or 0.05 - 10000 Kunitz units per kg per day during at least one day.

In yet another embodiment, said DNase is administered into cerebrospinal fluid. In one specific embodiment, said DNase is DNase I and is administered in the amount of at least 0.1 mg per day.

These and other aspects of the present invention will be apparent to those of ordinary skill in the art in the following description, claims and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows accumulation of labeled extracellular DNA in rat brain parenchyma after itsd injection into carotid artery.

Figure 2 shows electrophoregrams of blood extracellular DNA of a patient before administration of DNase (left column) and one month after the start of DNase treatment (right column).

Figures 3A-B are T1 MRI images showing atrophy and extensive gliosis of the left frontoparietal region in the brain of a patient with severe Alzheimer's disease. Images (**A**) and (**B**) depict volume loss in end-stage Alzheimer's disease with mild changes to the periventricular white matter. (**A**) Coronal T1 MRI of showing marked progressive cortical atrophy of the parietal regions. (**B**) Transverse T1 MRI showing bilateral marked atrophy.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on an unexpected discovery that extracellular DNA (both eukaryotic and prokaryotic) is present in elevated levels in blood and cerebrospinal fluid (CSF) of patients suffering from neurodegeneration (e.g., neurodegeneration associated with Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Huntington's disease, schizophrenia, and bipolar disorder) and that said extracellular DNA penetrates blood brain barrier (BBB) and exerts neuronal toxicity. As further demonstrated herein, the administration of DNase enzymes results in treatment/amelioration of neurodegeneration and is accompanied by the reduction of the level of circulating extracellular DNA.

Definitions

The term "neurodegeneration" is used herein to refer to a separate clinical pathological condition with progressive loss of structure and/or function of neurons, including death of neurons. The neurodegeneration can be primary or secondary. Non-limiting examples of diseases involving primary neurodegeneration include, e.g., Alzheimer's disease (AD), Mild Cognitive Impairment (MCI), Parkinson's disease (PD), Huntington's disease (HD), prion-caused diseases, frontotemporal dementia (FTD), Lewy body dementia, vascular dementias, Amyotrophic Later Sclerosis (ALS), chronic traumatic encephalopathy (CTE), progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal

degeneration (CBGD), Pick's disease, olivopontocerebellar atrophy (OPCA), senile dementia of the Alzheimer type, progressive supranuclear palsy (Steel-Richardson-Olszewski), corticodentatonigral degeneration, Hallervorden-Spatz disease, progressive familial myoclonic epilepsy, striatonigral degeneration, torsion dystonia (e.g., torsion spasm; dystonia musculorum deformans), spasmodic torticollis and other dyskinesis, familial tremor, Gilles de la Tourette syndrome, cerebellar cortical degeneration, spinocerebellar degeneration (e.g., Friedreich's ataxia and related disorders), Shy-Drager syndrome, spinal muscular atrophy, primary lateral sclerosis, hereditary spastic paraplegia, peroneal muscular atrophy (Charcot-Marie-Tooth), hypertrophic interstitial polyneuropathy (Dejerine-Sottas), chronic progressive neuropathy, pigmentary degeneration of the retina (retinitis pigmentosa), and hereditary optic atrophy (Leber's disease). Secondary neurodegeneration is caused primarily by necrosis. Non-limiting examples of conditions, which may result in secondary neurodegeneration, include destruction of neurons by neoplasm, edema, hemorrhage, stroke, trauma, immune attack, hypoxia, poisoning, metabolic defects, and infections.

The terms "extracellular DNA" and "cell-free DNA" are used interchangeably to refer to extracellular DNA (of eukaryotic or prokaryotic origin) found in blood, cerebrospinal fluid (CSF) or intestine of a patient.

As used herein, the terms "deoxyribonuclease" and "DNase" are used to refer to any enzyme that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone. A wide variety of deoxyribonucleases is known and can be used in the methods of the present invention. Non-limiting examples of useful DNases include, e.g., DNase I (e.g., human recombinant DNase I or bovine pancreatic DNase I), analogues of DNase I (such as, e.g., DNase X, DNase gamma, and DNAS1L2), DNase II, phosphodiesterase I, lactoferrin, and acetylcholinesterase. DNase I cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, yielding 5'5'-phosphate-terminated polynucleotides with a free hydroxyl group on position 3', on average producing tetranucleotides. DNase I acts on single-stranded DNA, double-stranded DNA, and chromatin.

The term "about" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within an acceptable standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to ±20%, preferably up to ±10%, more preferably up to ±5%, and more preferably still up to ±1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an

order of magnitude, preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term "about" is implicit and in this context means within an acceptable error range for the particular value.

In the context of the present invention insofar as it relates to neurodegeneration or any of the specific disease conditions recited herein, the terms "treat", "treatment", and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition. Within the meaning of the present invention, the term "treat" also denotes to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease.

As used herein the term "therapeutically effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to a subject in need thereof. Within the context of the present invention, when the term "therapeutically effective" is used in connection with the use of deoxyribonuclease (DNase) to prevent, treat and/or inhibit progression of neurodegeneration or a specific disease associated with said neurodegeneration, it refers to an amount of DNase or a pharmaceutical composition containing DNase that is effective to relieve or alleviate at least one symptom associated with neurodegeneration or such disease, or to slow or reverse the progression of neurodegeneration or such disease. Note that when a combination of active ingredients is administered (e.g., a combination of DNase and another compound effective for preventing, treating and/or inhibiting progression of a disease associated with neurodegeneration) the effective amount of the combination may or may not include amounts of each ingredient that would have been effective if administered individually.

The phrase "pharmaceutically acceptable", as used in connection with compositions of the invention, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a subject (e.g., a mammal such as a human). Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

As used herein, the terms "subject" and "patient" refer to any mammal. In a preferred embodiment, the subject/patient is human.

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise.

In accordance with the present invention there may be employed conventional pharmacology and molecular biology techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.,* Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual,* Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook *et al.,* 1989"); *DNA Cloning: A Practical Approach,* Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (MJ. Gait ed. 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. (1985)); *Transcription and Translation* (B.D. Hames & S.J. Higgins, eds. (1984)); *Animal Cell Culture* (R.I. Freshney, ed. (1986»; *Immobilized Cells and Enzymes* (IRL Press, (1986)); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology,* John Wiley & Sons, Inc. (1994); among others.

Therapeutic Methods of the Invention

As demonstrated in the Examples section, below, both prokaryotic and eukaryotic extracellular DNA level in blood and cerebrospinal (CSF) fluid increases with worsening of neurological status in patients with senile dementia, Parkinson's and Alzheimer's` diseases. As further demonstrated herein, a large part of extracellular DNA in blood and CSF is intestinal bacterial DNA (e.g., derived from gastrointestinal microbiota). Hence, the monitoring of extracellular DNA from eukaryotic or prokaryotic origin can be useful for prognosis or monitoring progression of neurodegeneration. Monitoring may include, e.g., one or more of the following: determination of the amount of extracellular DNA, determination of the type of extracellular DNA (prokaryotic or eukaryotic, e.g., determined based on rRNA), determination of the presence and/or amount of specific DNA fragments (e.g., CpG motifs, CpG-like motifs, hyperconservative regions of 16S, rpoD, e.g., determined using RT-PCR or metagenomic analysis).

As further described herein, extracellular DNA from intestine, blood and CSF of patients suffering from neurodegenerative disorders can cross the blood brain barrier (BBB). This is unexpected, since BBB has been always considered as impermeable for large nucleic acid molecules (Evers MM. Antisense oligonucleotides in therapy for neurodegenerative disorders. Adv Drug Deliv Rev. 2015).

As further described herein, extracellular DNA from intestine, blood and CSF of patients suffering from neurodegeneration causes neuronal cell death and apoptosis. DNase treatment destroying such extracellular DNA significantly improves the nervous system function in these patients. The improvement assessment was performed according to

widely accepted clinical diagnostic criteria of cognitive decline such as MMSE, PANSS, physical function, and/or functional tasks (see, e.g., Holmes et al., (1999) The British Journal of Psychiatry, 174(1), 45-50; Os et al., (2006) Acta Psychiatrica Scandinavica, 113(2), 91-95; O'Shea et al., (2002) Physical therapy, 82(9), 888-897; Rochester et al., (2004) 85(10), 1578-1585).

In one aspect, the invention provides a method for preventing, treating and/or inhibiting progression of neurodegeneration in a subject in need thereof, which method comprises administering to the subject a therapeutically effective amount of a DNase enzyme (e.g., DNase I, analogues of DNase I [such as, e.g., DNase X, DNase gamma, DNAS1L2], DNase II, phosphodiesterase I, lactoferrin, or acetylcholinesterase), wherein said amount of the DNase enzyme is effective to prevent or ameliorate at least one symptom of neurodegeneration.

DNase doses useful in the methods of the invention depend on the severity and course of the neurodegeneration, previous or concurrent therapy, the patient's clinical history and response to DNase, as well as the discretion of the attending physician. Preferably, such doses range from 0.5 to 20 mg/kg/day or 500 to 20000 Kunitz units (KU)/kg/day.

The administration of a DNase enzyme according to the methods of the invention can be performed by any suitable route. Specific non-limiting examples of useful routes of administration include intravenous, subcutaneous, intramuscular, delivery to cerebrospinal fluid (CSF), enteral (e.g., oral), rectal (e.g., by enema), and intranasal.

According to the methods of the invention, DNase can be administered either alone or in combination with other treatments useful for inhibiting progression or treatment of neurodegenerative diseases or other encompassed nervous system dysfunctions (e.g., bipolar disorder, migraine, schizophrenia, epilepsy). Non-limiting examples of such additional treatments include, e.g., histone acetyltransferase activators, cyclin-dependent protein kinase 5 inhibitors, neurotrophin mimetics, semaphorin-4D blockers, microsomal prostaglandin E synthase-1 inhibitors, levodopa, and N-methyl-d-aspartate (NMDA) receptor inhibitors (e.g., memantine).

Pharmaceutical Compositions of the Invention

In certain embodiments, a DNase enzyme can be formulated in a pharmaceutical composition with a pharmaceutically acceptable carrier or excipient.

The formulations used in the methods of the invention may conveniently be presented in unit dosage form and may be prepared by methods known in the art. The amount of active

ingredients that can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration. The amount of active ingredients that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

In general, the formulations can be prepared with a liquid carrier, or a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Pharmaceutical compositions suitable for parenteral administration may comprise DNase in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions can also contain preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

Injectable depot forms can be made by forming microencapsule matrices of one or more active ingredients in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of active ingredient to polymer, and the nature of the particular polymer employed, the rate of active ingredient's release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the active ingredients in liposomes or microemulsions which are compatible with body tissue.

Formulations for oral administration can be in the form of capsules, cachets, pills, tablets, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid (e.g., as a mouthwash, as a composition to be swallowed, or as an enema), or as an oil-in-water or water-in-oil liquid emulsion, and the like, each containing a predetermined amount of one or more active ingredients.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more active ingredients (e.g., DNase and optionally another compound for treatment of a neurodegenerative disease) can be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

Suspensions, in addition to one or more active ingredients, can contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

The DNase compositions of the invention may further comprise agents, which facilitate DNase delivery across the blood brain barrier (BBB). Non-limiting examples of such useful agents include, e.g., an implantable reservoir (Omaya reservoir), polysialation of DNase, functionalized nanocarriers (e.g., nanoparticles coated with transferrin or transferrin receptor [TR] antibodies), exosomes, liposomes (e.g., liposomes coated with targeting molecules such as antibodies, Trojan Horses Liposomes [THL]), antibodies (e.g., antibodies against transferrin receptor [TR] or insulin receptor [HIR], BBB transmigrating Llama single

domain antibodies (sdAb)), chimeric peptides (e.g., Angiopeps derived from proteins expressing the Kunitz domain), low-density lipoprotein receptor related proteins 1 and 2 (LRP-1 and 2), diphtheria toxin receptor (DTR), mesenchyme stem cells, receptor-associated protein, apolipoprotein E, melanotransferrin/p97, etc.

EXAMPLES

The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described here. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this specification, and such variations can be made without departing from the invention in spirit or in scope. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which those claims are entitled.

Example 1. Extracellular circulating DNA from blood and CSF promotes neuronal cell death

For neuronal cultures, cerebral cortices were removed from embryonic day (E) 15–17 Sprague Dawley rat embryos. Cortical explants were dissected into pieces of about 200–400 μm^2 using fine needles and dissociated with the Papain Dissociation System (Worthington Biochemicals) according to the manufacturer's instructions and further kept on ice-cold minimum essential medium (Gibco). Neurons were plated on 13 mm diameter glass coverslips coated first with poly-D-lysine (10 μg/ml in PBS) followed by laminin (10 μg/ml in PBS) (Gibco) and cultured at 37°C in a humidified 8% CO_2 (v/v) atmosphere for 24–48 hrs in Neurobasal medium with 1% (v/v) Antibiotic-Antimycotic (Gibco).

Extracellular DNA was extracted from plasma and CSF of a patient with a severe stage of Alzheimer's disease using QIAamp Circulating Nucleic Acid Kit according to manufacturers' instructions. In order to assess biological effect of such extracellular DNA on neurons, samples of extracellular DNA were added to cortical neuronal cultures. Neuronal cell death was determined in dissociated cortical neurons cultured for 48 hours. After initial period, extracellular DNA samples (100 and 200 mkg/ml dose levels) were applied for a further 24 hours. Neuronal cell death was assessed by CytoTox-Glo™ Cytotoxicity Assay (Promega). Luminescence proportional to the number of dead cells was measured using a

Promega GloMax 96 luminometer and was expressed in relative luminescence units (RLU).

Induction of apoptosis marker caspase 3 was determined in dissociated cortical neurons cultured for 48 hours. After initial period, extracellular DNA samples (100 and 200 mkg/ml dose levels) were applied for a further 24 hours. Cells were than fixed in 4% (w/v) paraformaldehyde (PFA) and incubated for 1 hour with cleaved caspase 3 antibody (Abcam) diluted 1:500 in PBS. Cells were washed and incubated for 1 hour with goat anti-rabbit polyclonal Alexa Fluor 488 antibodies for caspase 3 (Invitrogen) in PBS prior to washing and counting.

Table 1

	Extracellular DNA 0		Extracellular DNA 100 mkg/ml		Extracellular DNA 200 mkg/ml	
	RLU	% Caspase positive	RLU	% Caspase positive	RLU	% Caspase positive
Extracellular DNA (blood)	1 100	3%	4 650	10%	12 700	32%
Extracellular DNA (CSF)	700	6%	6 250	15%	9 100	25%
Extracellular DNA (blood) + DNASE I* (5 mkg/ml)	900	2%	2 300	5%	3 200	9%
Extracellular DNA (CSF) + DNASE I* (5 mkg/ml)	1050	3%	1 700	8%	4 500	7%

*DNase I used was human recombinant DNASE 1 manufactured by Catalent (Madison, USA).

From the data in Table 1, it follows that extracellular DNA from both blood and CSF induces cytotoxicity and upregulation of apoptosis marker caspase 3 in cultured neurons in a dose dependent manner. DNase I protects neurons from toxicity induced by extracellular DNA.

Example 2. Evaluation of bacterial extracellular DNA in blood and CSF of a patient with Alzheimer's disease

Extracellular DNA was extracted from plasma and CSF of a patient with a severe stage of Alzheimer's disease and a healthy volunteer (age- and sex-matched) using QIAamp Circulating Nucleic Acid Kit according to manufacturers' instructions. Quantification of bacterial extracellular DNA was done using CFX96 Touch™ Real-Time PCR Detection System. We have used universal bacterial DNA PCR primers 1369F and 1492R (Bacterial 16S rRNA).

1369F (SEQ ID NO: 1)	CGGTGAATACGTTCYCGG
1492R (SEQ ID NO: 2)	GGWTACCTTGTTACGACTT

The threshold cycles are presented in the table below:

Table 2

Group	Sample	Cq
Alzheimer's disease	Serum	16.45
	CSF	18.10
Healthy volunteers	Serum	31.12
	CSF	29.09

As follows from the data in Table 2, serum and CSF of a patient with a severe stage of Alzheimer's disease contains significantly more bacterial DNA as compared to a healthy volunteer.

Example 3. Extracellular circulating DNA from blood penetrates the blood brain barrier

Extracellular DNA was extracted from plasma of a patient diagnosed with senile dementia using QIAamp Circulating Nucleic Acid Kit. Iodination of extracellular DNA was performed using Iodo-Gen reagent as described (Piatyszek A., et al., Analytical Biochemistry, 1988, V172, pp. 356-359). Specific activity of labeled extracellular DNA was approximately 30 mkCi/mkg. The preparation of labeled extracellular DNA in 1 ml of PBS solution (approx. 3.0 mkCi, 100 ng) was slowly infused (0.1 ml/mn) into carotid artery of an anesthetized female Sprague Dawley rat. In one hour, the rat was euthanized with

chloroform, the brain was removed and fixed in formalin solution. Paraffin sections were stained with Ilford L4 emulsion. Accumulation of the labeled extracellular DNA in brain was studied using standard visual histoautoradiographic technique (Figure 1). Accumulation of injected extracellular DNA in rat brain parenchyma indicates that it penetrates blood brain barrier (BBB).

Example 4. Use of DNase for treatment of senile dementia

Patient K., age 72, male has was diagnosed with senile dementia 4 years ago with gradual development of bradykinesia, deterioration of short-term memory and attention. Gradually the following symptoms progressed: aspontaneity, lack of initiative, emotional lability, drowsiness. For 3 years the patient has been taking levodopa. A moderate positive effect was observed. However, during the last 18 prior symptoms became more pronounced, also hypokinesia and postural disorders began to manifest.

The patient was administered bovine pancreatic DNase I (Samson Med, Russia) in the amount of 2500 Kunitz units/kg three times per day orally in capsules. The effectiveness of the DNase treatment was assessed using the mini-mental state examination scale (MMSE) after 14 and 30 days. The results of the study are shown in Table 3.

Table 3

MMSE score points		
D0	D14	D30
16	19	23

Human blood plasma DNA from the patient was quantified using real-time PCR (RT-PCR) assay for three different markers sequences: ALU (J1), c-MYC and b-GLOB (ALU primer was GTCAGGAGATCGAGACCATCCC (SEQ ID NO: 3), c-MYC primer was AAACACAAACTTGAACAGCTAC (SEQ ID NO: 4), b-GLOB primer was GGTTGGCCAATCTACTCCCAGG (SEQ ID NO: 5) two month prior to the DNase treatment and immediately before the start of the DNase treatment. The threshold cycles are presented in the table below.

Table 4

	Total cf DNA		
	Alu	**C-myc**	**β-Glob**
- 60 days	14.47±0.109	28.62±0.294	29.49±0.161
Prior treatment starts	14.6±0.012	29.42±0.021	31.86±0.817

Electrophoregrams (agarose gels) of blood extracellular DNA of the patient before the DNase treatment (left column) and one month after the start of the treatment (right column) are presented in Figure 2.

As demonstrated above, the progression of clinical symptoms in a patient with senile dementia is accompanied by elevation of the level of circulating extracellular DNA in blood. DNase treatment destroying blood extracellular DNA has a positive clinical effect on dementia symptoms.

Example 5. Relief of exacerbation of bipolar disorder using DNase

Patient: M., age 63, male. Diagnosis: primary illness — exacerbation of bipolar disorder, manic episode. At the beginning of the treatment, complained about being irritable, having a desire to scream away, nightmares. Examination revealed that the patient was highly active, would leave the room all of a sudden, manifested disinhibition (constantly asked questions, would often start to sing) and irritability (was easily irritated by repetitive questions).

Before the beginning of the DNase treatment, the total score of assessment using Excited Component of the Positive and Negative Syndrome Scale (PANSS-EC) amounted to 29. Patient received a one-time intravenous injection of human recombinant DNase I (Catalent, Madison, USA) in the amount of 50 Kunitz units/kg for 3 hours. After DNase treatment, a significant alleviation of the symptoms was observed, total score of PANSS-EC amounted to 8, no psychomotor excitement could be observed.

Therefore, the administration of DNase has a positive effect when treating exacerbation of bipolar disorder.

Example 6. Use of DNase for treatment of schizophrenia

Patient: L. age 38, female. Diagnosis: schizophrenia, paranoid type, paroxysmal course of the disease, paranoid-hallucinatory syndrome. Before the DNase treatment, the patient complained about hearing voices "inside her head", she also believed that her

colleagues were trying to poison her. The patient was sitting in the unnatural posture, also a pronounced tremor of the upper extremities was observed, the tremor would subside upon movement. Emotional disorders were discovered, as well as thought disorders (slow thinking, derailment, illogicality). The total score of assessment using the Positive and Negative Syndrome Scale (PANSS) amounted to 24 and 22, respectively.

The patient began taking bovine pancreatic DNase I (Samson Med, Russia) orally in capsules in the amount of 3000 Kunitz units (KU)/kg three times per day. The results of the study are shown in Table 5.

Table 5

Group	Scores according to the Positive and Negative Syndrome Scale (PANSS)	
	Positive Syndrome Scale, total score ± statistical deviation	Negative Syndrome Scale, total score ± statistical deviation
Before the treatment	24	22
On day 2 of the treatment	13	18
On day 5 of the treatment	11	13

Therefore, the administration of DNase has a positive effect when treating schizophrenia.

Example 7. Use of DNase for treatment of Alzheimer disease

Patient G., age 77, male. The patient was diagnosed with Alzheimer's disease based on clinical data and indicative MRI changes. Starting from month 14 after being diagnosed, the patient started taking Memantine. However, the clinical symptoms continued to deteriorate: memory impairments progressed, impairments of speech and coordination began to manifest, as well as disorientation. By month 20 after being diagnosed the patient had all of the above-mentioned symptoms progressing and stopped going around on his own. By the time the DNase administration began, the patient was in stupor, also urinary and fecal incontinence were observed.

Bovine pancreatic DNase I (Samson Med, Russia) was administered orally in capsules

in the amount of 4000 Kunitz units/kg three times per 24 hours. The effectiveness was assessed using the MMSE mini-scale, 5 and 30 days after the beginning of the DNase treatment. The results of the study are shown in Table 6.

Table 6.

Day of beginning of the treatment	**Treatment**	**MMSE score (points)**
Before the DNase treatment	Memantine	3
5	DNase	15
30	DNase	19

The human blood plasma DNA from the patient was quantified using conventional real time PCR (RT-PCR) assay for three different markers sequences: ALU (J1), c-MYC and b-GLOB (ALU primer was GTCAGGAGATCGAGACCATCCC (SEQ ID NO: 3), c-MYC primer was AAACACAAACTTGAACAGCTAC (SEQ ID NO: 4), b-GLOB primer was GGTTGGCCAATCTACTCCCAGG (SEQ ID NO: 5) in total fraction of patient's extracellular DNA one month prior to the DNase treatment, immideately before the treatment start, and 30 days after initiation of the DNase treatment. The results are presented at Table 7.

Table 7.

	Total cf DNA		
	Alu	**C-myc**	**β-glob**
30 Days before the DNase treatment	10.52±0.095	27.32±0.239	29.07 ±0.326
Immediately before the DNase treatment	11.72±0.056	27.86±0.161	30.2±0.345
30 days after the DNase treatment	10.48±0.027	26.78±0.129	29.2±0.379

As early as on day 5 after the beginning of the DNase treatment, a significant improvement of the cognitive functions was observed. Speech and thinking went back to normal, the patient was able to get up, get dressed and go around unaided.

Therefore, the administration of DNase has a positive effect on treatment of Alzheimer's disease. The positive clinical effect of DNase treatment develops alongside with

reduction of the level of circulating extracellular DNA in the blood.

Example 8. Use of DNase for treatment of Lewy Body Variant of Alzheimer's Disease

A 77-year-old Caucasian male was diagnosed with dementia secondary to late-onset Alzheimer's disease 30 months prior to his presentation at clinic, exhibiting behavioral disturbances, cognitive decline, and decreased ability to engage in activities of daily living. Approximately 14 months following the initial diagnosis, the patient began treatment with 10 mg of memantine per day (Reisberg et al., N Engl J Med. 2003; 348:1333-1341; Danysz et al., Br J Pharmacol. 2012; 167:324-352), though his cognitive condition continued to deteriorate, rapidly progressing to include such behavioral changes as aggressiveness and disinhibition, in addition to progressive amnesic syndrome, aphasia, bradykinesia, shuffling gait, loss of balance, and urinary incontinence. Further, the patient experienced a 20-pound weight loss, which is ordinarily indicative of a poor prognosis in patients suffering from Alzheimer's disease (Soto et al., Journal of Alzheimer's Disease (2012) 28:647-654; Soto et al., J Am Geriatr Soc. 2015; 63:651-658; White et al., J Am Geriatr Soc.1998; 46:1223-1227). Analysis of cranial MRIs revealed age-appropriate losses in volume and mild changes to the periventricular white matter (Figures 3A-B).

Thirteen months following initiation of memantine treatment, the patient's total scores on the Mini-Mental State Examination (MMSE) and Functional Assessment Staging Test (FAST) were 10 and 5 points, respectively. He lost points on orientation to time and place, attention, memory, and visuospatial construction, and the patient was noticeably slower in completing the tasks. The patient experienced additional difficulty in navigating turns and corners when walking, resulting in recurrent falls, and exhibited fluctuating levels of consciousness, alternating between periods of frank confusion and lucidity. However, he experienced no visual or auditory hallucinations.

A further three months later, and a total of 16 months following initiation of memantine treatment, the patient experienced further deterioration of cognitive function. The patient had fluctuating level of consciousness. His cognition fluctuated between periods of frank confusion and lucidity, however he had no visual or auditory hallucinations. He was unable to remember his name, the calendar date, day of the week, year, or place and could not recognize family members. Additional impairments included slurred speech, expressive aphasia, loss of bowel/bladder control, and lack of coordination marked by an inability to sit, stand, or walk unassisted. The patient became unresponsive to stimuli, with an MMSE score of 3 and a FAST score of 7.

One month later, the patient's relatives provided informed consent for treatment with 40 mg of human recombinant DNase I (1500 KU/mg; Samson Med, Russia) given orally 3 times a day in conjunction with his continued memantine therapy (10 mg daily). The DNase I was well tolerated, and no adverse or unanticipated events were registered.

The patient demonstrated considerable cognitive improvement beginning on the second day of DNase I treatment, becoming partially oriented to time and place, and once again recognizing and remember the names of family members. He further became able to dress himself, including tying shoelaces and buttons, as well as walk independently, feed himself, and use an exercise bike. Neurologic abnormalities affecting gait were significantly reduced. His MMSE score increased dramatically from 3 to 16, and his FAST score was reduced from 7 to 5. However, he continued to score low on the MMSE for measures of orientation to time and place, memory, and visuospatial construction.

Two months following the initiation of DNase I treatment (19 months following initiation of memantine treatment), the patient exhibited an MMSE score of 18 and a FAST score of 4. Moderate improvements in memory were observed, although visuospatial construction continued to decline. He was better able to speak and interact with others, recognize relatives, and actively attend to television programs. The patient further became able to perform calculations, play piano, chess, and walk independently.

The above data demonstrate that the administration of DNase has a positive effect on treatment of Alzheimer's disease. Treatment with DNase I in the present case allowed the patient to withdraw form a terminal state and resulted in significant improvements in cognitive and behavioral function, including the ability to walk and perform everyday tasks with near independence. Significant recovery was observed in all areas of cognitive and motor function, indicating the possibility of a DNase-sensitive target involved in generating the symptoms of Alzheimer's disease. Cell-free DNA, including bacteria-derived DNA, may be one such target (Holdenrieder et al., Clin Chem. 2005; 51:1544-1546).

Example 9. Use of DNase for treatment of Parkinson's Disease

Patient S., age 58, female, has been suffering from Parkinson's disease for 7 years. For the last 5 years she has been treated with levodopa. One month before the study, there was a significant deterioration in patient's condition, namely: significant increase of tremor of the right hand, appearance of involuntary movements of hands in the form of generalized choreoathetosis, rigidity of extremities, difficulties with getting up from the bed in the morning, frequent nocturnal urination (6-7 times).

The patient began taking bovine pancreatic DNase I (Samson Med, Russia) orally in

capsules in the amount of 1500 Kunitz units/kg three times per day. Assessment was done using unified Parkinson's disease rating scale (UPDRS), observation was performed during 6 months.

Human blood plasma DNA from the patient was quantified using conventional real time PCR (RT-PCR) assay for ALU sequence (TPALU1: GTAAGAGTTCCGTAACAGGACAGCT (SEQ ID NO: 6)) in total fraction of patient's extracellular DNA one month prior to the DNase treatment, immediately before the treatment, and 3 months after the initiation of the treatment. The threshold cycles are presented in Table 8.

Table 8.

Observation time, months	Cf DNA quantity (ALU)	UPDRS (points)
0	9.56±0.047	22
1	9.43±0.012	17
3	8.93±0.042	16
12	-	14
24	-	12

By month 6 of taking DNase I, a significant improvement of the patient's condition was observed: morning akinesia, rigidity of movements and tremor of hands decreased; the number of nocturnal urinations dropped to one time per night.

It follows, that DNase administration has a positive effect on treatment of Parkinson's disease. This positive effect develops alongside with reduction of the level of circulating extracellular DNA in the blood.

Example 10. Use of DNase for treatment of Amyotrophic Lateral Sclerosis

The studies were performed on mice with knockout of SOD1 enzyme, with hyperexpression of G93A-hSOD1 protein, which is a generally accepted model of amyotrophic lateral sclerosis (ALS) (Gurney, New England Journal of Medicine (1994) 331: 1721-1722). Two groups of animals were formed, each consisting of 10 animals (Jackson Laboratory, Bar Harbor, ME). The animals were housed in a room with 12 hour light cycle and provided with free access to water and food. Polysialated DNAse was prepared as described in PCT/GB2007/002839. Briefly: 20 molar excess of oxidized 26 kDa polysialic acid (PSA) was dissolved in buffer and the pH adjusted to 6.0. Human recombinant DNase I

DNAse (Catalent) and 50 mM (final concentration) sodium cyanoborohydride were then added, the pH re-adjusted, and reaction mixture brought to the required volume. Reactions were carried out at 37±1°C with gentle shaking for 18 hours. DNAse-PSA conjugates were purified using hydrophobic interaction chromatography (HIC) - Phenyl-sepharose matrix, starting buffer containing 2.0 M ammonium sulphate, elution in buffer without ammonium sulphate. Elution fractions are then applied to ion exchange matrix Q-sepharose Fast Flow, and eluted with buffer containing sodium chloride. Purification of conjugates was confirmed by size- exclusion-high performance liquid chromatography (SE-HPLC).

Mice from Group 1 received no treatment (the control group); mice from Group 2 received human polysialated recombinant DNase I dosed at 50 mg/kg subcutaneously once a day. Survival time of the animals was used as an assessment criterion. Results are shown in Table 9.

Table 9.

Day of the study	Percentage of surviving animals within the group (%)	
	Group 1	Group 2
60	70	100
90	50	100
120	20	100
150	0	70
180	0	60
210	0	50
240	0	20

It follows that administration of DNase I has a positive effect on survival of mice suffering from ALS.

Example 11. Use of DNase for treatment of systemic CNS atrophy

Huntington's disease was chosen as an example of systemic atrophies of the central nervous system. Studies were performed on R6/2 transgenic mice expressing exon 1 of the gene that encodes glutamine, which is a generally accepted model of Huntington's disease (Miller et al., Neuroscience (2008) 153: 329-337). Three groups of animals were formed, each consisting of 10 animals (Charles River Laboratories). T he animals were housed in a room with 12 hour light cycle and provided with free access to water and food. Mice from

Group 1 received no treatment; mice from Group 2 received human recombinant DNase I (Catalent , Madison, USA) in the amount of 25000 mg/kg (2 times per 24 hours, intramuscularly); mice from Group 3 received DNase in the amount of 25 mg/kg (once a week, into CSF, using ICV bolus injection technique). Survival time of the animals was used as an assessment criterion. Results are shown in Table 10.

Table 10.

Week	Survival in groups (%)		
	Group1	Group 2	Group3
6	100	100	100
9	70	100	100
12	10	100	100
15	0	100	100
18	0	100	90
21	0	70	60
24	0	50	40

Therefore, the administration of DNase I has a positive effect on treatment of Huntington's disease.

* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference in their entirety as if physically present in this specification.

CLAIMS

1. A method for preventing, treating and/or inhibiting progression of neurodegeneration in a patient in need thereof, comprising administering to said patient a therapeutically effective amount of a DNase enzyme.

2. The method of claim 1, wherein the neurodegeneration is primary neurodegeneration.

3. The method of claim 1 or 2, wherein the neurodegeneration is associated with an increased level of extracellular DNA in blood or cerebrospinal fluid or intestine of said patient, which level is higher than the control level of extracellular DNA in blood or cerebrospinal fluid or intestine.

4. The method of claim 3, wherein the control level is the level of extracellular DNA in blood or cerebrospinal fluid or intestine of a healthy age-matched individual or an average level of extracellular DNA in blood or cerebrospinal fluid of several healthy age-matched individuals.

5. The method of claim 3 or 4, wherein said extracellular DNA is of prokaryotic origin.

6. The method of claim 3 or 4, wherein said extracellular DNA is of human origin.

7. The method of any one of claims 1-6, wherein said therapeutically effective amount of the DNase enzyme is sufficient to destroy said extracellular DNA in blood or cerebrospinal fluid or intestine of the patient.

8. The method of any one of claims 1-6, wherein said therapeutically effective amount of the DNase enzyme is sufficient to decrease the average molecular weight of said extracellular DNA as measured by gel electrophoresis.

9. The method of any one of claims 1-8, wherein said DNase is a recombinant DNase.

10. The method of any one of claims 1-9, wherein said DNase is DNase I.

11. The method of any one of claims 1-10, wherein said DNase has extended half-life.

12. The method of claim 11, wherein said DNase is conjugated with polysialic acid.

13. The method of claim 11, wherein said DNase is protected from binding to actin by modification of actin binding site.

14. The method of any one of claims 1-13, wherein said DNase is administered by intravenous, subcutaneous or intramuscular route.

15. The method of claim 14, wherein said DNase is DNase I and is administered in the amount of at least 0.04 mg per kg per day during at least one day.

16. The method of claim 14, wherein said DNase is DNase I and is administered in the amount of 0.05 - 10000 Kunitz units per kg per day during at least one day.

17. The method of any one of claims 1-13, wherein said DNase is administered enterally.

18. The method of claim 17, wherein said DNase is administered orally.

19. The method of claim 17 or 18, wherein said DNase is DNase I and is administered in the amount of at least 0.04 mg per kg per day during at least one day.

20. The method of claim 17 or 18, wherein said DNase is DNase I and is administered in the amount of 0.05 - 10000 Kunitz units per kg per day during at least one day.

21. The method of any one of claims 1-13, wherein said DNase is administered into cerebrospinal fluid.

22. The method of claim 21, wherein said DNase is DNase I and is administered in the amount of at least 0.1 mg per day.

23. The method of any one of claims 1-22, wherein said patient has been diagnosed with Alzheimer's disease.

24. The method of claim 23, wherein said Alzheimer's disease is a late-onset Alzheimer's disease.

25. The method of any one of claims 1-22, wherein said patient has been diagnosed with Parkinson's disease.

26. The method of any one of claims 1-22, wherein said patient has been diagnosed with Amyotrophic Lateral Sclerosis.

27. The method of any one of claims 1-22, wherein said patient has been diagnosed with Huntington's disease.

28. The method of any one of claims 1-22, wherein said patient has been diagnosed with schizophrenia.

29. The method of any one of claims 1-22, wherein said patient has been diagnosed with bipolar disorder.

30. The method of any one of claims 1-29, wherein the patient is human.

31. The method of any one of claims 1-29, wherein the patient is an experimental animal model.

32. A pharmaceutical composition comprising a DNase enzyme for use in a method of preventing, treating and/or inhibiting progression of neurodegeneration, wherein said neurodegeneration is associated with an increased level of extracellular DNA in blood or cerebrospinal fluid or intestine.

33. The pharmaceutical composition of claim 32, wherein the neurodegeneration is primary neurodegeneration.

34. The pharmaceutical composition of claim 32 or 33, wherein said extracellular DNA is of prokaryotic origin.

35. The pharmaceutical composition of claim 32 or 33, wherein said extracellular DNA is of human origin.

36. The pharmaceutical composition of any one of claims 32-35, wherein the DNase is to be administered in an amount sufficient to destroy extracellular DNA in blood or cerebrospinal fluid or intestine of a patient.

37. The pharmaceutical composition of any one of claims 32-35, wherein the DNase is to be administered in an amount sufficient to decrease the average molecular weight of said extracellular DNA as measured by gel electrophoresis.

38. The pharmaceutical composition of any one of claims 32-37, wherein said DNase is a recombinant DNase.

39. The pharmaceutical composition of any one of claims 32-38, wherein said DNase is DNase I.

40. The pharmaceutical composition of any one of claims 32-39, wherein said DNase has extended half-life.

41. The pharmaceutical composition of claim 40, wherein said DNase is conjugated with polysialic acid.

42. The pharmaceutical composition of claim 40, wherein said DNase is protected from binding to actin by modification of actin binding site.

43. The pharmaceutical composition of any one of claims 32-42, wherein said DNase is to be administered by intravenous, subcutaneous or intramuscular route.

44. The pharmaceutical composition of claim 43, wherein said DNase is DNase I which is to be administered in the amount of at least 0.04 mg per kg per day during at least one day.

45. The pharmaceutical composition of claim 43, wherein said DNase is DNase I which is to be administered in the amount of 0.05 - 10000 Kunitz units per kg per day during at least one day.

46. The pharmaceutical composition of any one of claims 32-42, wherein said DNase is to be administered enterally.

47. The pharmaceutical composition of claim 46, wherein said DNase is to be administered orally.

48. The pharmaceutical composition of claim 46 or 47, wherein said DNase is DNase I which is to be administered in the amount of at least 0.04 mg per kg per day during at least one day.

49. The pharmaceutical composition of claim 46 or 47, wherein said DNase is DNase I which is to be administered in the amount of 0.05 - 10000 Kunitz units per kg per day during at least one day.

50. The pharmaceutical composition of any one of claims 32-42, wherein said DNase is to be administered into cerebrospinal fluid.

51. The pharmaceutical composition of claim 50, wherein said DNase is DNase I which is to be administered in the amount of at least 0.1 mg per day.

52. The pharmaceutical composition of any one of claims 32-51, wherein said neurodegeneration is associated with Alzheimer's disease.

53. The pharmaceutical composition of claim 52, wherein said Alzheimer's disease is a late-onset Alzheimer's disease.

54. The pharmaceutical composition of any one of claims 32-51, wherein said neurodegeneration is associated with Parkinson's disease.

55. The pharmaceutical composition of any one of claims 32-51, wherein said neurodegeneration is associated with Amyotrophic Lateral Sclerosis.

56. The pharmaceutical composition of any one of claims 32-51, wherein said neurodegeneration is associated with Huntington's disease.

57. The pharmaceutical composition of any one of claims 32-51, wherein said neurodegeneration is associated with schizophrenia.

58. The pharmaceutical composition of any one of claims 32-51, wherein said neurodegeneration is associated with bipolar disorder.

59. Use of a DNase enzyme for preventing, treating and/or inhibiting progression of neurodegeneration in a patient in need thereof.

60. The use of claim 59, wherein the neurodegeneration is primary neurodegeneration.

61. The use of claim 59 or 60, wherein the neurodegeneration is associated with an increased level of extracellular DNA in blood or cerebrospinal fluid or intestine of said patient, which level is higher than the control level of extracellular DNA in blood or cerebrospinal fluid or intestine.

62. The use of claim 61, wherein the control level is the level of extracellular DNA in blood or cerebrospinal fluid or intestine of a healthy age-matched individual or an average level of extracellular DNA in blood or cerebrospinal fluid of several healthy age-matched individuals.

63. The use of claim 61 or 62, wherein said extracellular DNA is of prokaryotic origin.

64. The use of claim 61 or 62, wherein said extracellular DNA is of human origin.

65. The use of any one of claims 59-64, wherein said therapeutically effective amount of the DNase enzyme is sufficient to destroy said extracellular DNA in blood or cerebrospinal fluid or intestine of the patient.

66. The use of any one of claims 59-64, wherein said therapeutically effective amount of the DNase enzyme is sufficient to decrease the average molecular weight of said extracellular DNA as measured by gel electrophoresis.

67. The use of any one of claims 59-66, wherein said DNase is a recombinant DNase.

68. The use of any one of claims 59-67, wherein said DNase is DNase I.

69. The use of any one of claims 59-68, wherein said DNase has extended half-life.

70. The use of claim 69, wherein said DNase is conjugated with polysialic acid.

71. The use of claim 69, wherein said DNase is protected from binding to actin by modification of actin binding site.

72. The use of any one of claims 59-71, wherein said DNase is administered by intravenous, subcutaneous or intramuscular route.

73. The use of claim 72, wherein said DNase is DNase I and is administered in the amount of at least 0.04 mg per kg per day during at least one day.

74. The use of claim 72, wherein said DNase is DNase I and is administered in the amount of 0.05 - 10000 Kunitz units per kg per day during at least one day.

75. The use of any one of claims 59-72, wherein said DNase is administered enterally.

76. The use of claim 75, wherein said DNase is administered orally.

77. The use of claim 74 or 75, wherein said DNase is DNase I and is administered in the amount of at least 0.04 mg per kg per day during at least one day.

78. The use of claim 74 or 75, wherein said DNase is DNase I and is administered in the amount of 0.05 - 10000 Kunitz units per kg per day during at least one day.

79. The use of any one of claims 59-71, wherein said DNase is administered into cerebrospinal fluid.

80. The use of claim 79, wherein said DNase is DNase I and is administered in the amount of at least 0.1 mg per day.

81. The use of any one of claims 59-80, wherein said patient has been diagnosed with Alzheimer's disease.

82. The use of claim 81, wherein said Alzheimer's disease is a late-onset Alzheimer's disease.

83. The use of any one of claims 59-80, wherein said patient has been diagnosed with Parkinson's disease.

84. The use of any one of claims 59-80, wherein said patient has been diagnosed with Amyotrophic Lateral Sclerosis.

85. The use of any one of claims 59-80, wherein said patient has been diagnosed with Huntington's disease.

86. The use of any one of claims 59-80, wherein said patient has been diagnosed with schizophrenia.

87. The use of any one of claims 59-80, wherein said patient has been diagnosed with bipolar disorder.

88. The use of any one of claims 59-80, wherein the patient is human.

89. The use of any one of claims 59-80, wherein the patient is an experimental animal model.

FIGURE 1

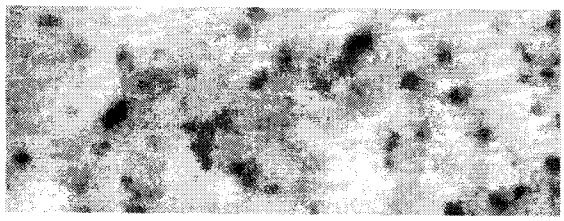


FIGURE 2

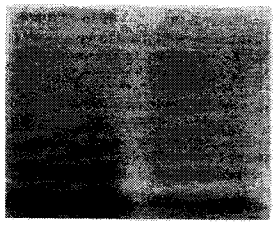


FIGURE 3A

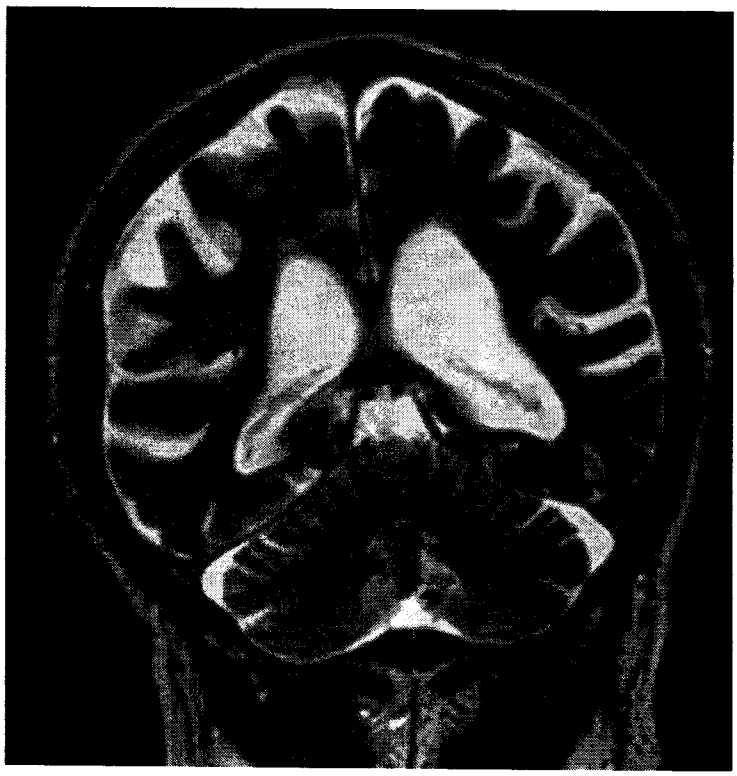
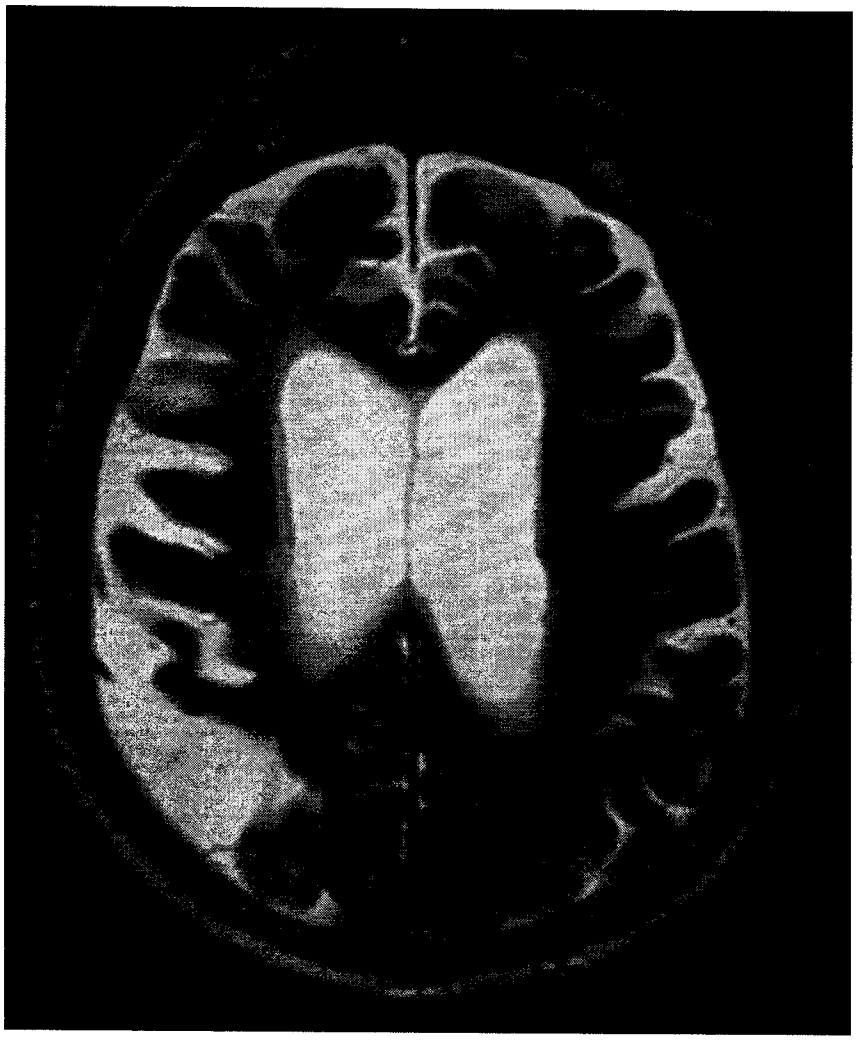


FIGURE 3B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/RU 2016/000284

A. CLASSIFICATION OF SUBJECT MATTER ***A61K 38/46*** *(2006.01)*
A61P 25/16 *(2006.01)*
A61P 25/28 *(2006.01)*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 38/46, A61P 25/16, 25/28

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, CIPO, ChemIDplus Advanced, DEPATISnet, DWPI, Depatisnet, EAPATIS, ESP@CE, ESP@CENET, Google, KIPRIS, MEDLINE, NCBI, PAJ, PCT Online, PatSearch, PubMed, RUPTO, STN, USPTO, WIPO, ВИНИТИ (VINITI), РМЖ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0252750 A1 (UNIVERSITY OF MASSACHUSETTS) 04.10.2012, abstract, claims 1, 3, 4, 6	1-4, 59-62
X Y	IL 199005 A (GENKIN DMITRY DMITRIEVICH) 30.04.2012, abstract, p.p.1-4	32-35 1-4, 59-62
Y	GLEBOVA KV. et al. Properties of extracellular DNA from the cerebrospinal fluid and blood plasma during Parkinson's disease. Bull Exp Biol Med., 2014 Apr; 156(6): 826-828, doi: 10.1007/s10517-014-2461-9. Epub 2014 May 3, abstract	1-4, 59-62
A	KADIOGLU E. et al. Detection of oxidative DNA damage in lymphocytes of patients with Alzheimer's disease. Biomarkers, 2004 Mar-Apr; 9(2):203-209. DOI: 10.1080/13547500410001728390, abstract	1-4, 32-35, 59-62
A	WO 2008/039989 (TRANSAVE INC et al.) 03.04.2008, abstract	1-4, 32-35, 59-62

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 October 2016 (07.10.2016)	10 November 2016 (10.11.2016)
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	Authorized officer D.Igumnov Telephone No. 495 531 65 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/RU 2016/000284

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

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

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

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

3. ☒ Claims Nos.: 5-31, 36-58, 63-89
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.