Title: MIF FOR USE IN METHODS OF TREATING SUBJECTS WITH A NEURODEGENERATIVE DISORDER

Abstract: Described herein are materials and methods of prophylaxis and therapy for subjects with (or at risk for) a neurodegenerative disorder, such as amyotrophic lateral sclerosis, Alzheimer's Disease, Parkinson's Disease and Huntington's Disease.
MIF FOR USE IN METHODS OF TREATING SUBJECTS WITH A NEURODEGENERATIVE DISORDER

FIELD OF THE INVENTION

[0001] The present disclosure is directed to materials and methods of prophylaxis and therapy for subjects with (or at risk for) a neurodegenerative disorder, such as amyotrophic lateral sclerosis, Parkinson’s Disease, Alzheimer’s Disease and Huntington’s Disease.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] This application contains, as a separate part of disclosure, a Sequence Listing in computer-readable form (filename: 46889A_SeqListing.txt; created: March 14, 2013; 15,870 bytes), which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease or motor neuron disease (MND), is one of several neurodegenerative diseases of the central nervous system. ALS is the most common adult onset motor neuron disease, affecting one in every 20,000 individuals, with an average age of onset of 50-55 years. ALS is characterized by rapidly progressive degeneration of motor neurons in the brain, brainstem, and spinal cord (Cleveland et al., Nat. Rev. Neurosci., 2, 806-19, 2001). The median survival of patients from time of diagnosis is five years.

[0004] ALS exists in both sporadic and familial forms. Familial ALS (FALS) comprises only 5-10% of all ALS cases. Over the last decade, a number of basic and clinical research studies have focused on understanding the familial form of the disease, which has led to the identification of eight genetic mutations related to FALS. Transgenic mice expressing point mutants of the Cu/Zn superoxide dismutase-1 (SOD1) gene develop an age-dependent progressive motor weakness similar to human ALS due to a toxic gain of function (Rosen et al., Nature, 362, 59-62, 1993), Rosen et al. Hum Mol Genet, 3, 981-987, 1994), and Borchelt et al., Proc. Natl. Acad. Sci. USA, 91, 8292-8296, 1994).

[0005] A number of different model systems, including SOD1 transgenic mice, in vitro primary motor neuron cultures or spinal cord slice cultures, in vivo imaging studies, and postmortem examination of tissue samples, have been utilized to understand the pathogenesis of ALS (Subramaniam et al., Nat. Neurosci., 5, 301-307, 2002), Nagai et al., J. Neurosci., 21,
9246-9254, 2001), Menzies et al. Brain, 125, 1522-1533, 2002), Kim et al. J. Neuropathol. Exp. Neurol., 62:88-103, 2003), and Ranganathan et al., Am. J. Pathol., 162, 823-835, 2003). Although these studies have yielded therapeutic targets and several clinical trials, there are no drugs that delay disease onset or prolong long-term survival of ALS patients. Riluzole (Rilutek®, Aventis), a glutamate antagonist, currently is the only FDA-approved medication available to treat ALS. Riluzole, however, extends life expectancy by only a few months (Miller et al., Amyotrophic Lateral Sclerosis & Other Motor Neuron Disorders, 4, 191-206, 2003). Creatine and α-tocopherol have shown some efficacy in relieving the symptoms of ALS in SOD1 transgenic mice, but exhibit minimal efficacy in human ALS patients (Groeneveld et al., Annals of Neurology, 53, 437-45, 2003 and Desnuelle et al., Amyotrophic Lateral Sclerosis & Other Motor Neuron Disorders, 2, 9-18, 2001).

[0006] Some forms of protein therapy have been beneficial. For example, improvement in survival was produced by administering insulin-like growth factor 1 either transgenically (Dobrowolny et al., J Cell Biol, 168(2):193-9, 2005) or through AAV2-delivery via intramuscular (IM) injection and subsequent retrograde axonal transport to motor nerves (Kaspar et al., Science, 301(5634):839-42, 2003). Two other proteins that have shown therapeutic promise as neuroprotective agents are erythropoietin (Iwasaki et al., Neurol Res, 24(7):643-6, 2002) and vascular endothelial growth factor (VEGF) (Brockington, et al., Neuropathol Appl Neurobiol, 30(5):427-46, 2004; Storkebaum et al., Bioessays, 26(9):943-54, 2004). The latter is of interest because genetic analysis has implicated hypomorphic variants in the VEGF gene as a risk factor for ALS (Lambrechts et al., Nat Genet, 34(4):383-94, 2003). Moreover, mice that lack hypoxia-responsive promoter elements develop a slowly progressive motor neuron disease (Oosthuyse et al., Nat Genet, 28(2):131-8, 2001). Subsequently, it was documented that lentiviral delivery of VEGF to the spinal cord of ALS mice delays death (Azzouz et al., Nature, 429(6990):413-7, 2004. Two independent investigators have reported that infusion of VEGF into the cerebrospinal fluid in ALS mice (Zheng et al., Ann Neurol, 56(4):564-7, 2004) and rats (Storkebaum et al., Nat Neurosci, 8(1): p. 85-92, 2005) also slow the disease course.

SUMMARY OF THE INVENTION

[0007] The present invention has numerous aspects and is based in part on discoveries described herein involving macrophage migratory inhibitory factor (MIF) molecular and cell biology, including discoveries including the chaperone-like activity of MIF, and more particularly chaperone-like activity towards SOD1 protein, and more particularly towards
mutant SOD1; and the effect of MIF on the association and/or accumulation of SOD1 (especially mutant SOD1) with mitochondria or other organelles in cells from the central nervous system (CNS) and other insights.

[0008] The invention includes materials and methods for prophylaxis and for treatment. Prophylaxis is especially contemplated for subjects identified as being at elevated risk for neurodegenerative disorders, e.g., due to a predisposing genetic risk factor.

[0009] In one aspect, described herein is a method of treatment comprising administering a composition to a mammalian subject at risk for, or having, a neurodegenerative disorder (including, but not limited to, Amyotrophic lateral sclerosis (ALS), Alzheimer’s Disease, Parkinson’s Disease and Huntington’s Disease), wherein the composition comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity.

[0010] In another aspect, described herein is a method of palliating a deleterious effect of mutant SOD1 in a cell comprising contacting the cell with a composition that comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity in the cell.

[0011] In any of the methods described herein the composition is optionally administered in an amount effective to reduce accumulation of misfolded SOD1 in cells obtainable from CNS of the subject. In some embodiments, the composition is administered to a mammalian subject identified as having a familial or genetic increased risk for the neurodegenerative disorder, in a prophylactically effective amount. In some embodiments, the composition is administered to a mammalian subject with the neurodegenerative disorder, in a therapeutically effective amount.

[0012] Numerous exemplary agents are described in the application, and incorporated into this summary of invention by reference. In some variations, the agent for use in the methods described herein comprises at least one substance selected from the group consisting of:

(a) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;
(b) a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(c) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 11;

(d) a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 11, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(e) a polypeptide comprising a MIF mutant, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(f) a fragment of any one of (a)-(e), wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(g) N-terminal deletion fragments of any one of (a)-(e) wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(h) C-terminal deletion fragments of any one of (a)-(e) and (g), wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(i) a variant of any one of (a)-(h) wherein the cysteine at position 60 of SEQ ID NO: 2 is deleted or replaced with another amino acid, and wherein the polypeptide (variant) exhibits chaperone activity towards a mutant SOD1 protein;

(j) a variant of any one of (a)-(i) lacking an N-terminal methionine, and wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(k) a variant of any one of (a)-(j), wherein the proline corresponding to position 2 of SEQ ID NO: 2 is deleted or replaced with another amino acid, and wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(l) a polynucleotide comprising, or consisting essentially of, or consisting of, a nucleotide sequence that encodes the polypeptide of any one of (a)-(k), optionally attached to one or more heterologous coding sequences or non-coding sequences (such as expression control sequences);

(m) a vector, especially an expression vector, comprising the polynucleotide of (l);
(n) a cell transformed or transfected with (l) or (m), that expresses the encoded polypeptide; and

(o) a compound that increases MIF chaperone activity in CNS cells.

[0013] In some embodiments, the agent comprises a polynucleotide encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the agent comprises a polynucleotide encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 9, a variant of SEQ ID NO: 2 in which Cys60 has been replaced with a serine. It should be understood that replacement with other amino acids, or deletion of the cysteine, also is contemplated.

[0014] In some embodiments, the agent comprises an oligonucleotide that increases MIF expression of activity in cells from CNS. The oligonucleotide, in some embodiments, is an antisense oligonucleotide that binds to a nucleotide sequence that inhibits MIF expression in cells from CNS, thereby upregulating expression of the MIF protein in the CNS. In some embodiments, the antisense oligonucleotide is at least partly complementary to the microRNA-451 sequence of SEQ ID NO: 4 or SEQ ID NO: 5.

[0015] In some embodiments, the agent comprises a CNS precursor cell transformed or transfected with a polynucleotide that encodes a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein. The cell is optionally isolated from CNS of the mammalian subject, wherein the transforming or transfecting is performed ex vivo, and wherein the cell or its progeny are re-administered to the same subject after the transforming or transfecting. In another variation, the cell is isolated from one mammalian subject and transplanted into the CNS of a different mammalian subject of the same species. In some embodiments, the cell is expanded after the transforming or transfecting, and wherein progeny cells are re-administered to the subject after the expanding.

[0016] In some embodiments, the agent comprises a compound, such as a small molecule, that increases MIF chaperone activity in CNS cells, thereby upregulating expression of the MIF protein in CNS cells.

[0017] The methods described herein optionally further comprises screening a subject for a SOD1 mutation prior to the administering step. In some embodiments, the screening step optionally comprises assaying a biological sample (e.g., spinal fluid or cells obtainable from CNS of a subject) from the subject for evidence that the SOD1 mutation is present in the
subject. Exemplary cells obtainable or present in the CNS of a subject include, but are not limited to, glial cells, glial cell precursors, astrocytes, oligodendrocytes, neural cells and neuronal progenitor cells. It will also be appreciated that an inherited mutation in SOD1 would be detectable in nucleic acid from essentially any type of cell. In some embodiments, the screening step optionally comprises analyzing a medical record for evidence that the SOD1 mutation is present in the subject. In some embodiments, the medical record comprises genomic nucleotide sequence information.

[0018] The assaying step optionally comprises analyzing nucleic acid from the subject for a mutation that codes for SOD1^{G93A} or SOD1^{G85R}, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

[0019] In some embodiments, the mammalian subject has a mutation (e.g., a missense mutation) in a superoxide dismutase 1 (SOD1) gene. In some embodiments, the mutation is associated with SOD1 misfolding, SOD1 self-aggregation, or SOD1 association with one or more cellular structures such as mitochondria, endoplasmic reticulum or endosomes in cells from CNS of the subject.

[0020] Screening methods to identify small molecule modulators of MIF expression or activity (e.g., increase MIF expression and/or MIF chaperone activity in cells of the CNS) are also contemplated as aspects of the invention. An exemplary screening method comprises contacting a CNS cell with a test small molecule and determining the quantity of the MIF mRNA or protein as described herein. In some embodiments, the method comprises contacting a CNS cell with a test compound and determining the quantity of MIF mRNA or protein, MIF chaperone activity, and/or the quantity of decreased SOD1 misfolding, SOD1 self-aggregation, or SOD1 association with one or more cellular structures such as mitochondria, endoplasmic reticulum or endosomes in cells from CNS cells in the presence and absence of the test small molecule, as described herein. An increased MIF chaperone activity (and/or decreased SOD1 misfolding, SOD1 self-aggregation, or SOD1 association with one or more cellular structures) in the presence of the test small molecule identifies the test small molecule as an agonist of MIF activity, and decreased MIF chaperone activity identifies the candidate small molecule as an antagonist of MIF activity.

[0021] In some embodiments, the small molecule mitigates SOD1 misfolding, SOD1 self-aggregation or SOD1 association with one or more cellular structures such as mitochondria, endoplasmic reticulum or endosomes in cells from CNS.
In some variations, small molecules are screened in a cell free assay. For example, a MIF protein and a SOD1 protein (which may be a mutant SOD1) are contacted together in the presence and absence of a test molecule, and measureable decreases in SOD1 misfolding, aggregation, or association with cellular structures (if mitochondria, endoplasmic reticulum, endosomes, or other structures are included in the assay) identifies the test molecule as a molecule that beneficially modulate MIF activity.

In some embodiments, the missense mutation codes for an amino acid alteration selected from the group consisting of SOD1$^{G93A}$ and SOD1$^{G85R}$, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

The composition described herein can be administered by any route. In some embodiments, the composition is administered by intrathecal or intravascular administration. In some embodiments, the composition is administered to the cerebrospinal fluid of the subject.

Aspects of the invention that are described herein as methods (especially methods that involve treatment) can alternatively be described as (medical) uses of reagents or therapeutics. For example, in one variation, the invention is a use of a composition that comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity. In another variation, the invention is a use of a composition for palliating a deleterious effect of a mutant SOD1 in a cell, wherein the composition comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity in the cell.

The agents and compositions described herein for use in treatment are themselves aspects of the invention also, e.g., as compositions of matter.

In the treatment methods (or uses) described herein, the methods optionally comprises administering a standard of care therapeutic to the subject in combination with the agent described herein. With respect to any combination treatment described herein, the agent composition can be administered simultaneously with the other active agents, which may be in admixture with the agent or may be in a separate composition. Each composition
preferably includes a pharmaceutically acceptable diluent, adjuvant, or carrier. When the agents are separately administered, they may be administered in any order. In some embodiments, the standard of care therapeutic is selected from the group consisting of standard of care therapeutic selected from the group consisting of gabapentin (Neurontin®), Myotrophin® (Insulin-like Growth Factor 1, IGF-1), brain-derived neurotrophic factor (BDNF), BFGF, Rilutek® (riluzole), SR57746A, metal chelators (e.g., D-penicillamine), erythropoietin, VEGF, creatine, cyclosporin, CoQ10, inhibitors of tubulin/filament assembly, diazepam, and various vitamins (e.g., C, E and B).

[0028] Additional aspects of the invention are defined or summarized in the following numbered paragraphs:

[0029] 1. A method of treatment comprising administering a composition to a mammalian subject at risk for, or having, a neurodegenerative disorder, wherein the composition comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity.

[0030] 2. The use of a composition for neurodegenerative disorder prophylaxis or therapy, wherein the composition comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity.

[0031] 3. The method or use according to paragraphs 1 or 2, wherein the composition further comprises a pharmaceutically acceptable excipient, adjuvant, diluents, or carrier in admixture with the agent.

[0032] 4. The method or use according to any of paragraphs 1-3, wherein the mammalian subject is human.

[0033] 5. The method or use according to any one of paragraphs 1-4, comprising administering the composition to a mammalian subject with the neurodegenerative disorder, in a therapeutically effective amount.

[0034] 6. The method or use according to any one of paragraphs 1-4, comprising administering the composition to a mammalian subject identified as having a familial or
genetic increased risk for the neurodegenerative disorder, in a prophylactically effective amount.

[0035] 7. The method or use of any one of paragraphs 1-6, wherein the mammalian subject has a mutation in an superoxide dismutase 1 (SOD1) gene.

[0036] 8. The method or use of paragraph 7, wherein the mutation is associated with SOD1 misfolding, SOD1 self-aggregation or SOD1 association with mitochondria, endoplasmic reticulum or endosomes in cells from CNS of the subject.

[0037] 9. The method or use of any one of paragraphs 7-8, wherein the SOD1 mutation is a missense mutation.

[0038] 10. The method or use of paragraph 9, wherein the missense mutation codes for an amino acid alteration selected from the group consisting of SOD1\textsuperscript{G93A} and SOD1\textsuperscript{G85R}, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

[0039] 11. The method of any one of paragraphs 3-10, further comprising screening a subject for a SOD1 mutation prior to the administering step.

[0040] 12. The method of paragraph 11, wherein the screening step comprising assaying a biological sample from the subject for evidence that the SOD1 mutation is present in the subject.

[0041] 13. The method of paragraph 12, wherein the biological sample comprises spinal fluid or cells obtainable from CNS of a subject.

[0042] 14. The method of paragraph 13, wherein the cells obtainable from CNS of a subject are selected from the group consisting of glial cells, glial cell-precursors, astrocytes, oligodendrocytes, neural cells and neuronal progenitor cells.

[0043] 15. The method of paragraph 12, wherein the assaying comprises analyzing nucleic acid from the subject for a mutation that codes for SOD1\textsuperscript{G93A} or SOD1\textsuperscript{G85R}, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

[0044] 16. The method of paragraph 12, wherein the assaying comprises analyzing SOD1 protein or cell from CNS of the subject for evidence of SOD1 misfolding, SOD1 self-aggregation or SOD1 association with mitochondria, endoplasmic reticulum or endosomes in the cell.
[0045] 17. The method of paragraph 12, wherein the screening comprises analyzing a medical record for evidence that the SOD1 mutation is present in the subject.

[0046] 18. The method of paragraph 17, wherein the medical record comprises genomic nucleotide sequence information.

[0047] 19. The method or use according to any one of paragraphs 1-18, wherein the composition is administered in an amount effective to reduce accumulation of misfolded SOD1 in cells obtainable from CNS of the subject.

[0048] 20. The method or use of any one of paragraphs 1-19, wherein the agent comprises at least one substance selected from the group consisting of:

(a) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;

(b) a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(c) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 11;

(d) a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 11, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(e) a polypeptide comprising a MIF mutant, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(f) a fragment of any one of (a)-(e), wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(g) N-terminal deletion fragments of any one of (a)-(e) wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(h) C-terminal deletion fragments of any one of (a)-(e) and (g), wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(i) a variant of any one of (a)-(h) wherein the cysteine at position 60 of SEQ ID NO: 2 is deleted or replaced with another amino acid, and wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;
(j) a variant of any one of (a)-(i) lacking an N-terminal methionine, and wherein
the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(k) a variant of any one of (a)-(i), wherein the proline corresponding to position 2
of SEQ ID NO: 2 is deleted or replaced with another amino acid, and wherein the polypeptide
exhibits chaperone activity towards a mutant SOD1 protein;

(l) a polynucleotide comprising a nucleotide sequence that encodes the
polypeptide of any one of (a)-(k);

(m) a vector comprising the polynucleotide of (l); and

(n) a cell transformed or transfected with (l) or (m), that expresses the encoded
polypeptide.

[0049] 21. The method or use of paragraph 20, wherein the agent comprises an
expression vector that contains a promoter sequence operatively connected to a
polynucleotide that comprises nucleotide sequence that encodes a polypeptide comprising an
amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide
exhibits chaperone activity towards a mutant SOD1 protein.

[0050] 22. The method or use of paragraph 21, wherein the expression vector contains a
promoter sequence operatively connected to polynucleotide that comprises a nucleotide
sequence that encodes a polypeptide comprising an amino acid sequence at least 95%
identical to SEQ ID NO: 2.

[0051] 23. The method or use of paragraph 21, wherein the expression vector contains a
promoter sequence operatively connected to a polynucleotide that comprises a nucleotide
sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2,
or a fragment thereof that exhibits chaperone activity towards a mutant SOD1 protein.

[0052] 24. The method or use of paragraph 21, wherein the encoded polypeptide
comprises the amino acid sequence set forth in SEQ ID NO: 2.

[0053] 25. The method or use of paragraph 21, wherein the polynucleotide comprises a
cDNA or fragment thereof that encodes a polypeptide or fragment thereof that exhibits
chaperone activity towards a mutant SOD1 protein.

[0054] 26. The method or use of paragraph 21, wherein the encoded polypeptide exhibits
chaperone activity towards a mutant SOD1 protein characteristic of MIF, and exhibits
reduced oxidoreductase activity compared to wild type MIF (SEQ ID NO: 2).
27. The method or use of paragraph 21, wherein the polynucleotide encodes a MIF polypeptide containing a MIF$^{C66S}$ mutation (SEQ ID NO: 9).

28. The method or use according to any one of paragraphs 20-26, wherein the vector is a replication-deficient viral vector.

29. The method or use of any one of paragraphs 20-26, wherein the vector is an adeno-associated viral (AAV) vector.

30. The method or use of paragraph 27, wherein the AAV vector is an AAV9 vector.

31. The method or use of any one of paragraphs 21-30, wherein the promoter is selected from the group consisting of CMV promoter, Prp promoter, VACHT promoter, GFAP promoter, CD11b promoter and Plp promoter.

32. The method or use of paragraph 20, wherein the agent comprises a CNS precursor cell transformed or transfected with a polynucleotide that encodes a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein.

33. The method or use of paragraph 32, wherein cell is isolated from CNS of the mammalian subject, wherein the transforming or transfecting is performed ex vivo, and wherein the cell or its progeny are re-administered to the subject after the transforming or transfecting.

34. The method or use of paragraph 33, wherein the cell is expanded after the transforming or transfecting, and wherein progeny cells are re-administered to the subject after the expanding.

35. The method of use of paragraph 20, wherein the polynucleotide encodes MIF-2 or P1G-MIF.

36. The method or use of any one of paragraphs 1-19, wherein the agent comprises an oligonucleotide that increases MIF expression or activity in cells from CNS.

37. The method or use of paragraph 36, wherein the oligonucleotide is an antisense oligonucleotide that binds to a nucleotide sequence that inhibits MIF expression in cells from CNS, thereby upregulating expression of the MIF protein in the CNS.
38. The method or use of paragraph 37, wherein the antisense oligonucleotide is at least partly complementary to the microRNA sequence of SEQ ID NO: 4 or SEQ ID NO: 5.

39. The method or use of paragraph 38, wherein the antisense oligonucleotide is 15 to 30 nucleotides in length.

40. The method or use of any one of paragraphs 36-39, wherein the oligonucleotide comprises a nucleotide sequence that is complementary to bases 1 to 9 of SEQ ID NO: 4.

41. The method or use of any one of paragraphs 1-40, wherein the neurodegenerative disorder is selected from amyotrophic lateral sclerosis (ALS), Alzheimer’s Disease, Parkinson’s Disease, and Huntington’s Disease.

42. The method or use of paragraph 41, wherein the neurodegenerative disorder is ALS.

43. The method or use of paragraph 42, wherein the ALS is sporadic ALS.

44. The method or use of paragraph 42, wherein the ALS is familial ALS.

45. The method or use of one of paragraphs 1-44, wherein the composition is administered by intrathecal administration.

46. The method or use of any one of paragraphs 1-44, wherein the composition is administered to the spinal cord of the subject.

47. The method or use of paragraph 46, wherein the composition is administered to the lumbar of the subject.

48. The method or use of any one of paragraphs 1-44, wherein the composition is administered to the cerebrospinal fluid of the subject.

49. The method or use of any one of paragraphs 1-44, wherein the composition is administered intravascularly.

50. The method or use of any one of paragraphs 1-49, further comprising administering to the subject a standard of care therapeutic selected from the group consisting of gabapentin (Neurontin®), Myotrophin® (Insulin-like Growth Factor 1, IGF-1), brain-derived neurotrophic factor (BDNF), BFGF, Rilutek® (riluzole), SR57746A, metal chelators (e.g., D-penicillamine), erythropoietin, VEGF, creatine, cyclosporin, CoQ10, inhibitors of tubulin/filament assembly, diazepam, and various vitamins (e.g., C, E and B).
51. A method of palliating a deleterious effect of mutant SOD1 in a cell comprising contacting the cell with a composition that comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity in the cell.

52. Use of a composition for palliating a deleterious effect of a mutant SOD1 in a cell, wherein the composition comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity in the cell.

53. The method of paragraph 51 that comprises:

54. The method or use of paragraph 53, wherein the deleterious effect comprises the formation or accumulation misfolded SOD1 in the cell, or inhibition of mitochondrial activity by the SOD1.

55. The method or use of any one of paragraphs 51-54, wherein the mutant SOD1 is selected from the group consisting of SOD1<sup>G93A</sup> and SOD1<sup>G85R</sup>, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

56. A method of treating a mammalian subject having a disorder associated with accumulation of misfolded SOD1 in CNS cells of the subject, the method comprising administering to the subject an effective amount of a composition that comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity.

57. The method of paragraph 56, wherein the disorder is a neurodegenerative disorder.
[0088] The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document.

[0089] In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations defined by specific paragraphs above. For example, certain aspects of the invention that are described as a genus, and it should be understood that every member of a genus is, individually, an aspect of the invention. Also, aspects described as a genus or selecting a member of a genus, should be understood to embrace combinations of two or more members of the genus. Although the applicant(s) invented the full scope of the invention described herein, the applicants do not intend to paragraph subject matter described in the prior art work of others. Therefore, in the event that statutory prior art within the scope of a paragraph is brought to the attention of the applicant(s) by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a paragraph to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a paragraph. Variations of the invention defined by such amended paragraphs also are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0090] Figure 1. The cytosol determines mutant SOD1 association with mitochondria. (A) Schematic outlining the different purification steps. (B) Spinal cord but not liver cytosol of two different mutant SOD1 rats induces mutant SOD1 association with mitochondria, as demonstrated by immunoblotting. VDAC1 immunoblot shows comparable numbers of mitochondria in each assay. The input showing comparable amounts of cytosolic fractions is also displayed. (C) Recombinant mutant SOD1G85R was measured for its association with mitochondria in the absence or presence of liver cytosol.

[0091] Figure 2. MIF inhibits the association of mutant SOD1 with mitochondria. (A) Schematic outlining the different steps toward MIF identification. (B) The gel filtration fractions were measured for their activity to inhibit association of recombinant mutant SOD1 with mitochondria. (C) MIF distribution correlates perfectly with inhibitory activity of the
fractions as determined by western blot (D) The activity of recombinant MIF was measured by immunoblot for the ability to inhibit recombinant mutant SOD1 association with mitochondria. VDAC immunoblots show comparable amounts of mitochondria.

[0092] Figure 3. A factor present in unaffected tissues inhibits the association of mutant SOD1 with mitochondria. Spinal cord but not liver cytosol of mutant SOD1G93A rat (symptomatic) induces mutant SOD1 association with mitochondria, as demonstrated by immunoblotting. The ability of liver cytosol to inhibit this association is proteinase K resistant and heat sensitive. Moreover, this activity is Ca2+-, hsp70- and hsp90- independent. Cytochrome c immunoblot shows comparable numbers of mitochondria in each assay. The input showing comparable amounts of cytosolic fractions is also displayed.

[0093] Figure 4. Coomassie staining shows the fraction with the simplest protein composition that was subjected to mass spectrometry analysis. The gel filtration fractions were measured for their activity to inhibit association of recombinant mutant SOD1 with mitochondria. Cytochrome c immunoblots show comparable amounts of mitochondria.

[0094] Figure 5. Immunoblotting revealed that only MIF presence fractionates perfectly with inhibitory activity as determined by western blot.

[0095] Figure 6. Distribution of MIF expression through different mouse tissues. (A) The level of MIF expression in different mouse tissues is shown by western blot. Ponceau staining is shown as a loading control. (B) Immunoprecipitation of misfolded SOD1 using B8H10 antibody was performed from different tissues expressing varying levels of MIF. Tissue sample numbers are as indicated in Figure 6A.

[0096] Figure 7. MIF inhibits the association of mutant SOD1 with mitochondria in a concentration dependent manner. The activity of increased concentrations of recombinant MIF was measured by immunoblot for the ability to inhibit recombinant mutant SOD1 association with mitochondria. VDAC immunoblots show comparable amounts of mitochondria.

[0097] Figure 8. MIF inhibition of mutant SOD1 association with mitochondria is specific. The activity of recombinant MIF, hsp27, cyclophilin-A or glutathione peroxidase was measured by immunoblot for the ability to inhibit recombinant mutant SOD1 association with mitochondria.

[0098] Figure 9. MIF inhibits the accumulation of misfolded SOD1. (A) Recombinant hSOD1 wild type, hSOD1G93A or hSOD1G85R were subjected to immunoprecipitation
using DSE2 in the absence or presence of recombinant MIF. The immunoprecipitates were immunoblotted using an SOD1 antibody. MIF levels in the unbound fraction are shown. (B) MIF and (C) MIFC60S expressed in NSC-34 motor neuron-like cells suppress misfolded SOD1 accumulation as determined by immunoprecipitation using B8H10 antibody. MIF levels are shown in the unbound fraction.

[0099] Figure 10. MIF inhibits the association of mutant SOD1 with endosomal membranes in NSC-34 cells. MIFC60S expressed in NSC-34 motor neuron-like cells suppress mutant SOD1 association with endosomal membranes in a concentration dependent manner as determined by immunoblot using anti SOD1 antibody. Calnexin levels are shown as loading control and MIF levels are shown in the cytosolic fraction.

[00100] Figure 11 is a map of an AAV9 expression vector comprising MIF-IRES-GFP.

[00101] Figure 12 is an alignment of the amino acid sequences of MIF proteins identified in various species.

DETAILED DESCRIPTION

[00102] The present application is based in part on the discoveries that macrophage migratory inhibitory factor (MIF) demonstrates chaperone-like activity towards mutant SOD1, and that this activity may be deficient in cells from the central nervous system (CNS) with a mutant SOD1 protein. The accumulation of misfolded SOD1 in cells from the CNS is associated with ALS and other neurodegenerative disorders (Boillée et al., Neuron 52:39-59, 2006 and Boillée et al., Science, 312:1389-1392, 2006). As described in the Examples, the chaperone-like activity of MIF inhibits or decreases accumulation of misfolded SOD1 and inhibits SOD1 aggregation with mitochondria, and other organelles including endoplasmic reticulum and endosomes (e.g., represented in the light endosomal preparation of subcellular fractionation) in CNS cells, and this beneficial effect can be achieved in CNS cells by increasing MIF in such cells.

[00103] In one aspect, described herein is a method of treatment comprising administering a composition to a mammalian subject at risk for, or having a neurodegenerative disorder, wherein the composition comprises at least one agent selected from the group consisting of: (a) polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; (b) a polynucleotide comprising a nucleotide sequence that encodes the polypeptide of (a); and (c) an agent that increases endogenous MIF expression or MIF chaperone activity. In
some embodiments, the composition is administered in an amount effective to reduce accumulation of misfolded SOD1 in cells from the CNS of the subject. The “administering” shall include physically administering. In jurisdictions where compatible with laws or rules governing patent-eligible subject matter, “administering” shall further include the act of prescribing a controlled substance that a human subject self-administers or that a needed professional other than the prescribing authority physically administers.

[00104] Subjects “at risk for” having a neurodegenerative disorder are subjects having a SOD1 mutation that results in SOD1 misfolding, SOD1 aggregation and SOD1 mitochondrial membrane association in a cell from the CNS of the subject. Subjects with a family member diagnosed with a neurodegenerative disorder or having a SOD1 mutation that results in SOD1 misfolding, SOD1 aggregation and SOD1 mitochondrial membrane association in a cell from the CNS are also considered to be at risk for the neurodegenerative disorder. In addition, subjects are “at risk for” having a neurodegenerative disorder if they have a mutation in TDP-43, FUS/TLS, alpha synuclein, amyloid beta and huntintin, which is associated with misfolding or aggregation of these proteins and with development of one or more neurodegenerative disorders selected from ALS, Alzheimer’s disease and Huntington’s disease. A subject is considered to “at risk” before the subject begins to exhibit symptoms or other signs of onset of the disorder.

[00105] The term “MIF protein chaperone activity” as used herein refers to the ability of the MIF protein to reduce SOD1 misfolding, SOD1 aggregation and SOD1 mitochondrial membrane association as demonstrated according to any of the in vitro assays described in Example 1.

[00106] Exemplary neurodegenerative disorders for prophylaxis or therapy according to the invention include amyotrophic lateral sclerosis (ALS) (familial or sporadic), Alzheimer’s Disease, Parkinson’s Disease, and Huntington’s Disease.

[00107] Standard medical criteria are used to identify ALS symptoms in a subject, or diagnose ALS in a subject. The natural history of ALS is well documented (Munset T. L., 1992, The natural history of amyotrophic lateral sclerosis. In: Handbook of Amyotrophic Lateral Sclerosis, Smith R A (eds.), Chapter 2, pp. 39-63, Marcel Dekker, Inc.: New York, the entire disclosure of which is herein incorporated by reference). The presenting symptoms of ALS include muscle wasting or weakness of the hands or legs. Occasionally, cramps and fasciculations precede the common presenting symptoms. Bulbar symptoms consisting of
dysarthria or dysphagia typically appear as the disease progresses, but can also be the presenting complaints in some subjects.

[00108] Standard criteria for diagnosis of ALS have been established by the World Federation of Neurology, and is described in Brooks et al., "El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis," Amyotroph. Lateral. Scler. Other Motor Neuron Disord. 2000 December; 1(5):293-9, the entire disclosure of which is herein incorporated by reference. For example, the "El Escorial" criteria for the diagnosis of ALS require: (1) the presence of (a) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination; (b) evidence of upper motor neuron (UMN) degeneration by clinical examination; and (c) a progressive spread of symptoms or signs within a region or to other regions as determined by history or examination; and (2) the absence of (a) electrophysiological or pathological evidence of other disease processes that might explain the signs of LMN or UMN degeneration; and (b) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs. Typical LMN and UMN signs indicative of ALS are given in Table 1.

[00109] Table 1.

<table>
<thead>
<tr>
<th>LMN signs, weakness, atrophy, fasciculations</th>
<th>Brainstem</th>
<th>Cervical</th>
<th>Thoracic</th>
<th>Lumbosacral</th>
</tr>
</thead>
<tbody>
<tr>
<td>jaw, face, palate, tongue, larynx</td>
<td>neck, arm, hand, diaphragm</td>
<td>back, abdomen</td>
<td>back, foot, abdomen, leg, foot</td>
<td></td>
</tr>
<tr>
<td>UMN signs, pathologic spread of reflexus, clonus, etc.</td>
<td>clonic jaw, jerk, gag reflex, exaggerated snout reflex, pseudobulbar features, forced yawning, deep tendon reflex and spastic tone</td>
<td>clonic deep tendon reflex, Hoffman reflex, pathologic deep tendon reflex, spastic tone, preserved reflex in weak wasted limb</td>
<td>loss of superficial abdominal reflexes, pathologic deep tendon reflex, spastic tone</td>
<td>clonic deep tendon reflex, extensor plantar response, pathological deep tendon reflex, spastic tone, preserved reflex in weak wasted limb</td>
</tr>
</tbody>
</table>

[00110] In addition to the "El Escorial" criteria for diagnosing ALS (Brooks et al., (2000), supra), one or more of the following standard clinical evaluations can be used to identify ALS symptoms or assess progress/prevention of ALS in a subject:

1. Quantitative strength and functional markers. The TUFTS Quantitative Neuromuscular Examination (TQNE) is a well standardized, reliable, validated test to measure strength and function in ALS. The test involves measurement of maximum
voluntary isometric contraction (MVIC) of eight muscle groups in the arms using a strain
gauge tensiometer.

(2) Functional measures. The ALS Functional Rating Scale (ALSFRS) is an easily
administered ordinal rating scale used to determine patient’ assessment of their ability and
independence for ten functional activities. Validity has been established by correlating
ALSFRS scores with change in strength over time.

(3) Neuropathologic examination of muscle, peripheral nerve and other tissue
biopsies. The presence of neuropathologic features such as chronic denervation/reinervation
in affected muscle tissue, scattered hypertrophied muscle fibers, necrotic muscle fibers,
inflammatory cell infiltration and giant axonal swellings in intramuscular nerves are all
indicative of ALS. An overview of neuropathologic findings in patients with ALS is
presented in, for example, A. Hirano (1996), "Neuropathology of ALS: an overview,"
Neurology, 47 (Suppl 2): S63-S66, the entire disclosure of which is herein incorporated by
reference.

[00111] Standard medical criteria are use to identify or diagnose other neurodegenerative
disorders in a subject. For example, and with respect to Alzheimer’s Disease, such criteria
include, but are not limited to Diagnostic and Statistical Manual of Mental Disorders, third
edition (DSM-III) Alzheimer’s Disease Diagnostic and Treatment Center (ADDT),
International Statistical Classification of Diseases, 10th Revision (ICD-10), National Institute
of Neurological Disorders and Stroke-Association Internationale pour la Recherche et
PEnseignement en Neurosciences (NINDS-AIREN) and Diagnostic and Statistical Manual of
Mental Disorders, Fourth Edition (DSM-IV). See Pohjasvaara et al, Stroke 2000, 31, 2952-
2957. Clinical characterization of a patient as mild cognitive impairment is well within the
skill of the practitioner. Such testing of a patient to elucidate such a condition involves
performing a series of mental tests. The methods for clinical diagnosis are widely reviewed
and are discussed in, e.g., Petersen et al., Arch. Neurol. 1999, 56, 303-308, the disclosure of
which is incorporated herein by reference in its entirety.

[00112] Medical diagnosis of the onset of Huntington’s Disease can be made following the
appearance of physical symptoms specific to the disease (Walker, Lancet, 369:218-228,
2007. Genetic testing can be used to confirm a physical diagnosis if there is no family history
of HD. A physical examination, sometimes combined with a psychological examination, can
determine whether the onset of the disease has begun (Walker, Lancet, 369:218-228, 2007).
Excessive unintentional movements of any part of the body are often the reason for seeking
medical consultation. If these are abrupt and have random timing and distribution, they suggest a diagnosis of HD. Cognitive or psychiatric symptoms are rarely the first diagnosed; they are usually only recognized in hindsight or when they develop further. How far the disease has progressed can be measured using the unified Huntington’s disease rating scale which provides an overall rating system based on motor, behavioral, cognitive, and functional assessments (Rao et al., Gait Posture, 29:433-436, 2009 and UHDRS and Database, Huntington Study Group, last update February 2009). Medical imaging, such as computerized tomography (CT) and magnetic resonance imaging (MRI), only shows visible cerebral atrophy in the advanced stages of the disease.

[00113] In another aspect, described herein is a method of palliating a deleterious effect of mutant SOD1 in a cell from CNS comprising contacting the cell with a composition that comprises at least one agent selected from the group consisting of: (a) polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; (b) a polynucleotide comprising a nucleotide sequence that encodes the polypeptide of (a); and (c) an agent that increases endogenous MIF expression or MIF chaperone activity. In some embodiments, the method comprises screening a mammalian subject for a SOD1 mutation, and administering to a subject identified from the screening as having the SOD1 mutation the composition, in an amount effective to palliate the deleterious effect of the SOD1 mutation in a cell from the CNS of the subject. Exemplary deleterious effects include, but are not limited to, the formation or accumulation misfolded SOD1 in the cell, or inhibition of mitochondrial activity by the SOD1 in the cell.

[00114] In yet another aspect, described herein is a method of treating a mammalian subject having a disorder associated with accumulation of misfolded SOD1 in cells from CNS of the subject, the method comprising administering to the subject an effective amount of a composition that comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity. Cells from CNS of a subject include, but are not limited to, glial cells, glial cell-precursors, astrocytes, oligodendrocytes, neural cells (i.e., neurons) and neuronal progenitor cells.

[00115] The methods described herein optionally comprise administering an inhibitor of SOD1 expression or activity to the mammalian subject. In some embodiments, the inhibitor of SOD1 expression or activity is an inhibitory nucleic acid, such as an antisense
oligonucleotide that binds to a nucleotide sequence that inhibits SOD1 expression in a cell from CNS of the subject, thereby inhibiting expression of the SOD1 protein in the cell.

[00116] In some embodiments, the methods described herein comprise administering the composition to a mammalian subject identified as having a familial or genetic increased risk for the neurodegenerative disorder, in a prophylactically effective amount. The term “prophylactically effective amount” as used herein refers to an amount of the agent effective to delay onset of the neurodegenerative disorder. The method is “prophylactic” when it contributes to a measurable delay in the onset of a disease or any one or more symptoms used to diagnose a disease, e.g., provides a measure of protection, prevention, or delay of onset of disease, or delay of onset of symptoms used to diagnose a disease in a subject. While it often will be apparent to a subject and/or the subject’s caregiver(s) that a measure of protection, prevention, or delay of onset of disease, or delay of onset of symptoms used to diagnose a disease has been achieved (e.g., based on experience with the disease in other subjects), prophylaxis also is demonstrated and quantifiable in the context of a controlled study, where a measure of protection, prevention, or delay of onset of disease, or delay of onset of disease symptoms is achieved in a group of treated subjects, compared to a group of untreated controls, for example. If a standard of care regimen for prophylaxis exists for a particular disease or condition, then the use of the agents for therapy described herein can be compared against the standard of care therapeutics in a controlled study.

[00117] In some embodiments, the methods described herein comprise administering a “therapeutically effective amount.” The term “therapeutically effective amount” refers to an amount that slows neurodegenerative progression or provides an improvement in an indicator of neurodegenerative disorder progression, as described below in greater detail.

[00118] In some embodiments, the methods disclosed herein will result in a slowing of the neurodegenerative disorder progression. As used herein, "slowing neurodegenerative disorder progression" means the delay of a clinically undesirable change in one or more disabilities in an individual suffering from a neurodegenerative disorder, such as ALS, and is assessed by methods routinely practiced in the art, for example, the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS), pulmonary function tests, and muscle strength measurements. Such methods are herein referred to as "indicators of ALS disease progression." Such delay can be shown in a controlled study comparing the rate of progression of disease in treated subjects versus untreated controls. Slowing progression
alternatively means the lessening of the severity of a clinically undesirable changes as assessed at a particular point in time, compared to untreated controls.

[00119] An "improvement in a indicator of neurodegenerative disorder progression" as used herein refers to slowing of the rate of change in one or more of the indicators of a neurodegenerative disorder progression, such as ALS disease progression. An improvement in an indicator of ALS disease progression also includes a lack of a measurable change in one or more of the indicators of ALS disease progression. An improvement in an indicator of ALS disease progression additionally includes a positive change in one of the indicators of ALS disease progression described herein, such as, for example, an increase in an ALSFRS-R score. It is within the abilities of a physician to identify a slowing of disease progression in an individual suffering from ALS, using one or more of the disease assessment tests described herein. A physician may administer to the individual diagnostic tests, such as additional pulmonary function tests or muscle strength measurement tests, to assess the rate of disease progression in an individual suffering from ALS.

[00120] A slowing of neurodegenerative disorder progression may further comprise an "increase in survival time" in an individual suffering from the neurodegenerative disorder, e.g., ALS. A physician can use one or more of the disease assessment tests described herein to predict an approximate survival time of an individual suffering from ALS. A physician may additionally use the known disease course of ALS accompanied by a particular ALS mutation to predict survival time.

[00121] The "revised ALS functional rating scale" or "ALSFRS-R" is used by physicians and is a validated rating instrument for monitoring the progression of disability in ALS patients. The ALSFRS-R includes 12 questions that ask a physician to rate his or her impression of an ALS patient's level of functional impairment in performing one of ten common tasks, for example, climbing stairs. Each task is rated on a five-point scale, where a score of zero indicates an inability to perform a task and a score of four indicates normal ability in performing a task. Individual item scores are summed to produce a reported score of between zero (worst) and 48 (best).

[00122] The identifying steps of the methods described herein optionally comprise screening the subject for the presence of a mutation in a superoxide dismutase (SOD1) gene prior to the administering step. In the context of assaying, the term “mutation” includes addition, deletion, and/or substitution of one or more nucleotides in a SOD1 gene sequence.
Mutation(s) in the SOD1 gene are, in some embodiments, associated with SOD1 misfolding, SOD1 self-aggregation or SOD1 association with mitochondria in cells from CNS. In some embodiments, the mutation in the SOD1 gene is a missense mutation. Exemplary missense mutations include, but are not limited to missense mutations that result in SOD1<sup>G93A</sup>, SOD1<sup>G37R</sup>, SOD1<sup>G85R</sup>, SOD1<sup>H46R</sup>, relative to the wild type SOD1 amino acid sequence of SEQ ID NO: 8. Other SOD1 mutations that are associated with ALS are known in the art and are described in, for example, Battistini et al., European Neurological Journal, March 2010, the disclosure of which is incorporated herein by reference in its entirety.

[00123] The screening step comprises, in some embodiments, assaying a biological sample from the subject for evidence that the SOD1 mutation is present in the subject. Suitable biological samples include any tissue or fluid that contains a nucleic acid or SOD1 protein from the subject including, but are not limited to, blood, cells from CNS (including but not limited to, glial cells, glial cell-precursors, astrocytes, oligodendrocytes, neural cells and neuronal progenitor cells) and spinal fluid.

[00124] In some embodiments, the assaying comprises analyzing nucleic acid from the subject for a mutation that codes for SOD1<sup>G93A</sup>, SOD1<sup>G37R</sup>, SOD1<sup>G85R</sup>, and/or SOD1<sup>H46R</sup>, relative to the wild type SOD1 amino acid sequence of SEQ ID NO: 8. In the context of assaying, the term “nucleic acid of a subject” is intended to include nucleic acid obtained directly from the mammalian subject (e.g., DNA, or RNA obtained from a biological sample such as spinal fluid or cells from CNS, such as neural cells isolated from spinal fluid); and also nucleic acid derived from nucleic acid obtained directly from the subject. By way of non-limiting examples, well known procedures exist for creating cDNA that is complementary to RNA derived from a biological sample from a subject, and for amplifying (e.g., via polymerase chain reaction (PCR)) DNA or RNA derived from a biological sample obtained from a subject. Any such derived polynucleotide which retains relevant nucleotide sequence information of the subject’s own DNA/RNA is intended to fall within the definition of “nucleic acid of a subject” for the purposes of the present invention. In some embodiments, the assaying comprises analyzing SOD1 protein expressed in the sample or a cell from the CNS of the subject for evidence of SOD1 misfolding, SOD1 self-aggregation or SOD1 association with mitochondria in the cell. Such evidence of aberrant protein behavior is scored as evidence that a mutation is present. Evidence of a protein mutation also can be detected by using an antibody that preferentially recognizes an epitope of a mutant SOD1 compared to wild type SOD1. Exemplary antibodies that preferentially recognize an epitope
of a mutant SOD1 compared to wild type SOD1, include, but are not limited to B8H10 and C4F6 (both of which are commercially available from Medimabs).

[00125] Genetic diagnosis of a mutation in a SOD1 gene can be performed using any technologies for assaying DNA for a mutation. The nucleic acid sequence data can be obtained by any means known in the art. The assaying step may involve any techniques available for analyzing nucleic acid to determine its characteristics, including but not limited to well-known techniques such as single-strand conformation polymorphism analysis (SSCP) (Orita et al., Proc Natl. Acad. Sci. USA, 86: 2766-2770, 1989); non-radioactive PCR-single strand conformation polymorphism analysis; DNA and/or RNA hybridization; heteroduplex analysis (White et al., Genomics, 12: 301-306, 1992); denaturing gradient gel electrophoresis analysis (Fischer et al., Proc. Natl. Acad. Sci. USA, 80: 1579-1583, 1983); and Riesner et al., Electrophoresis, 10: 377-389,1989); DNA sequencing (manual or automated) Sanger et al., Proc. Natl. Acad. Sci. USA, 74:5463-5467 (1977); RNase cleavage (Myers et al., Science, 230: 1242-1246, 1985); chemical cleavage of mismatch techniques (Rowley et al., Genomics, 30: 574-582, 1995; and Roberts et al., Nucl. Acids Res., 25: 3377-3378, 1997); restriction fragment length polymorphism (RFLP) analysis; single nucleotide primer extension analysis (Shumaker et al., Hum. Mutat., 7: 346-354, 1996); and Pastinen et al., Genome Res., 7: 606-614, 1997); 5’ nuclease assays (Pease et al., Proc. Natl. Acad. Sci. USA, 91:5022-5026, 1994); DNA Microchip analysis (Ramsay, G., Nature Biotechnology, 16: 40-48, 1999; and Chee et al., U.S. Patent No. 5,837,832); analysis using a single nucleotide polymorphism (SNP) chip containing SNP’s from throughout the genome (e.g., Infinium HD BeadChip) or from a portion of the genome, such as the mitochondrial genome; ligase chain reaction (Whiteley et al., U.S. Patent No. 5,521,065); cloning for polymorphisms; denaturing high pressure liquid chromatography (DHPLC); clamped denaturing gel electrophoresis (CDGE); denaturing gradient gel electrophoresis (DGGE); mobility shift analysis; restriction enzyme analysis; chemical mismatch cleavage (CMC); RNase protection assays; and use of polypeptides that recognize nucleotide mismatches, such as E. coli mutS protein; and allel-specific PCR. See generally, Schafer and Hawkins, Nature Biotechnology, 16: 33-39, 1998; Li et al., Nucleic Acids Research, 28(2): e1 (i-v) (2000); Liu et al., Biochem Cell Bio 80:17-22 (2000); and Burczak et al., Polymorphism Detection and Analysis, Eaton Publishing, 2000; Sheffield et al., Proc. Natl. Acad. Sci. USA, 86:232-236 (1989); Flavell et al., Cell, 15:25-41 (1978); Geever et al., Proc. Natl. Acad. Sci. USA, 78:5081-5085 (1981); Cotton et al., Proc. Natl. Acad. Sci. USA, 85:4397-4401 (1985); Myers et al., Science 230:1242-1246

[00126] In some embodiments, the screening comprises analyzing a database or other record (e.g., a medical record) for evidence that a SOD1 mutation is present in the genome of the subject. Exemplary medical records include partial or complete genome sequencing records or single nucleotide polymorphism (SNP) analysis.

[00127] The assaying optionally involves sequencing of nucleic acid to determine nucleotide sequence thereof, using any available sequencing technique. (See, e.g., Sanger et al., *Proc. Natl. Acad. Sci. (USA)*, 74: 5463-5467, 1977 (dideoxy chain termination method); Mirzabekov, TIBTECH, 12: 27-32, 1994 (sequencing by hybridization); Drmanac et al., *Nature Biotechnology*, 16: 54-58, 1998; U.S. Patent No. 5,202,231; and Science, 260: 1649-1652, 1993 (sequencing by hybridization); Kieleczawa et al., *Science*, 258:1787-1791, 1992 (sequencing by primer walking); (Douglas et al., *Biotechniques*, 14:824-828, 1993 (Direct sequencing of PCR products); and Akane et al., *Biotechniques* 16: 238-241, 1994; Maxam and Gilbert, *Meth. Enzymol.*, 65: 499-560, 1977 (chemical termination sequencing), all incorporated herein by reference in their entireties). The analysis may entail sequencing of the entire gene genomic DNA sequence, or portions thereof; or sequencing of the entire gene coding sequence or portions thereof. In some circumstances, the analysis may involve a determination of whether an individual possesses a particular gene allelic variant, in which case sequencing of only a small portion of nucleic acid -- enough to determine the sequence of a particular codon or codons characterizing the allelic variant -- is sufficient. This approach is appropriate, for example, when assaying to determine whether one family member inherited the same allelic variant that has been previously characterized for another family member, or, more generally, whether a person’s genome contains an allelic variant that has been previously characterized and correlated with a neurodegenerative disorder.

[00128] The assaying optionally comprises performing a hybridization assay to determine whether nucleic acid from the subject has a nucleotide sequence identical to or different from one or more reference sequences. The hybridization involves, in some embodiments, a determination of whether nucleic acid derived from the subject will hybridize with one or more oligonucleotides, wherein the oligonucleotides have nucleotide sequences that correspond identically to a portion of the gene sequence, or that correspond identically except for one mismatch, insertion, or deletion. The hybridization conditions are selected to
differentiate between perfect sequence complementarity and imperfect matches differing by one or more bases. Such hybridization experiments thereby can provide single nucleotide polymorphism sequence information about the nucleic acid from the human subject, by virtue of knowing the sequences of the oligonucleotides used in the experiments.

[00129] Several of the techniques outlined above involve an analysis wherein one performs a polynucleotide migration assay, e.g., on a polyacrylamide electrophoresis gel, under denaturing or non-denaturing conditions. Nucleic acid derived from the subject is subjected to gel electrophoresis, usually adjacent to one or more reference nucleic acids. The nucleic acid from the subject and the reference sequence(s) are subjected to similar chemical or enzymatic treatments and then electrophoresed under conditions whereby the polynucleotides will show a differential migration pattern, unless they contain identical sequences. (See generally Ausubel et al. (eds.), Current Protocols in Molecular Biology, New York: John Wiley & Sons, Inc. (1987-1999); and Sambrook et al., (eds.), Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press (1989), both incorporated herein by reference in their entirety.)

[00130] The polynucleotide sequences encoding the gene protein product (e.g., SOD1) may be used in hybridization or PCR assays of fluids (e.g., spinal fluid) to detect expression of the appropriate protein. Such methods may be qualitative or quantitative in nature and may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits.

[00131] The various techniques for identifying/selecting subjects as candidates for treatment or prophylaxis are not mutually exclusive. Thus, variations of the invention include analysis of two, three, four, or more of the aforementioned parameters, e.g., existence of an affected genetic relative; genetic testing for mutations in the SOD1 genes; biochemical testing of gene product activity (e.g., SOD1 misfolding, SOD1 self-aggregation or SOD1 association with mitochondria in the cell from CNS); and expression level of MIF mRNA or protein, biochemical testing of MIF chaperone activity.

[00132] In some embodiments, the methods described herein optionally comprise administering to the subject a standard of care therapeutic as a co-therapy for the disorder. The standard of care therapeutic can be any agent or agents used by medical professionals to treat the disorder or its symptoms. Exemplary agents include, but are not limited to,
gabapentin (Neurontin®), Myotrophin® (Insulin-like Growth Factor 1, IGF-1), brain-derived neurotrophic factor (BDNF), BFGF, Rilutek® (riluzole), SR57746A, metal chelators (e.g., D-penicillamine), erythropoietin, VEGF, creatine, cyclosporin, CoQ10, inhibitors of tubulin/filament assembly, diazepam, and various vitamins (e.g., C, E and B).

[00133] In some embodiments, the methods described herein optionally comprise administering to the subject an agent that inhibits the expression or activity of SOD1. For example, in some embodiments, the agent that inhibits the expression of activity of SOD1 is an antisense oligonucleotide, such as an antisense oligonucleotide described in Smith et al., J. Clin. Invest., 116:2290-2296, 2006. Suitable doses for administration of the SOD1 antisense oligonucleotide are also disclosed in Smith et al. (supra). The SOD1 antisense oligonucleotide is optionally administered by continuous intrathecal infusion for a period of time of about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days or longer.

[00134] MIF, Fragments and Variants Thereof

[00135] In some embodiments, the agent for use in accordance with the methods described herein is a polypeptide that exhibits MIF protein chaperone activity. In some embodiments, the polypeptide comprises the human wild type amino acid sequence of MIF set forth in SEQ ID NO: 2, which is encoded by the nucleotide sequence of SEQ ID NO: 1. MIF proteins have been identified in other species including mouse (Genbank Accession No. NP_034928.1), rat (Genbank Accession No. NP_112313.1), cow (Genbank Accession No. NP_001028780.1), zebrafish (NP_001036786), pig (Genbank Accession No. NP_001070681), African clawed frog (Genbank Accession No. NP_001083650) and sheep (Genbank Accession No. NP_001072123).

[00136] Fragments of MIF polypeptides are also contemplated for use in the methods described herein. For purposes of the therapeutic compositions, methods, and uses of the invention, the term “fragment of MIF” refers to a polypeptide that includes a sufficient portion of the wild type MIF such that the polypeptide retains the chaperone activity for MIF that is demonstrated in Example 1. The fragment optionally is attached to heterologous sequences that do not eliminate this chaperone activity. Deletion variants, described below, are examples of fragments. For example, in some embodiments, the agent comprises a MIF fragment that comprises at least 10, at least 15, at least 20, at least 25, at least 30, at least 40,
at least 50, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, at least 100, at least 105 or at least 110 amino acids of SEQ ID NO: 2 wherein the fragment exhibits chaperone activity towards a mutant SOD1 protein.

[00137] A polypeptide for use in a method of the invention can be modified by techniques in the art. Exemplary modified polypeptides include, polypeptide variants and polypeptide derivatives. The term “polypeptide variant” refers to a polypeptide sequence that contains at least one amino acid substitution, deletion, or insertion in the wild type amino acid sequence, wherein the variant retains the chaperone biological activity of wild type polypeptide. The term “polypeptide derivative” refers to a polypeptide that is covalently modified by conjugation to therapeutic or diagnostic agents, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) or insertion or substitution by chemical synthesis of non-natural amino acids into the wild type sequence.

[00138] Deletion variants are polypeptides wherein at least one amino acid residue of any amino acid sequence is removed. Deletions can be effected at one or both termini of the protein, or with removal of one or more residues within (i.e. internal to) the polypeptide. Methods for preparation of deletion variants are routine in the art. See, e.g., Sambrook et al. (1989) Molecular Cloning: A Laboratory Guide, Vols 1-3, Cold Spring Harbor Press, the disclosure of which is incorporated herein by reference in its entirety. In some embodiments, the deletion variant comprising a polypeptide in which the Met at position 1 of SEQ ID NO: 1 is deleted. For example, in some embodiments, the polypeptide having chaperone activity towards a mutant SOD1 protein comprises amino acids 2-115 of SEQ ID NO: 2, or a fragment thereof that exhibits the chaperone activity..

[00139] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing hundreds or more residues, as well as internal sequence insertions of one or more amino acids. As with any of the different variant types described herein, insertional variants can be designed such that the resulting polypeptide retains the same biological properties or exhibits a new physical, chemical and/or biological property not associated with the parental polypeptide from which it was derived. Methods for preparation of insertion variants are also routine and well known in the art (Sambrook et al., supra).
[00140] Fusion proteins comprising a MIF polypeptide, and a heterologous polypeptide, are another variant contemplated by the invention. Non-limiting examples of heterologous polypeptides which can be fused to polypeptides of interest include proteins with long circulating half-life, such as, but not limited to, immunoglobulin constant regions (e.g., Fc region); marker sequences that permit identification of the polypeptide of interest; sequences that facilitate purification of the polypeptide of interest; and sequences that promote formation of multimeric proteins. In some embodiments, a receptor fragment is fused to alkaline phosphatase (AP). Methods for making Fc or AP fusion constructs are found in WO 02/060950.

[00141] Substitution variants are those in which at least one residue in the polypeptide amino acid sequence is removed and a different residue is inserted in its place. Modifications in the biological properties of the polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. In certain embodiments of the invention, substitution variants are designed, i.e. one or more specific (as opposed to random) amino acid residues are substituted with a specific amino acid residue. Typical changes of these types include conservative substitutions and/or substitution of one residue for another based on similar properties of the native and substituting residues.

[00142] Conservative substitutions are shown in Table 2. The most conservative substitution is found under the heading of “preferred substitutions.” If such substitutions result in no change in biological activity, then more substantial changes may be introduced and the products screened.

<table>
<thead>
<tr>
<th>Original</th>
<th>Exemplary</th>
<th>Preferred Residue Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>val; leu; ile</td>
<td>val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>lys; gln; asn</td>
<td>lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>gln; his; asp, lys; gln</td>
<td>gln</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>glu; asn</td>
<td>glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>ser; ala</td>
<td>ser</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>asn; glu</td>
<td>asn</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Common Side-Chain Properties</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Glu (E)</td>
<td>asp; gln</td>
<td></td>
</tr>
<tr>
<td>Gly (G)</td>
<td>ala</td>
<td></td>
</tr>
<tr>
<td>His (H)</td>
<td>asn; gln; lys; arg</td>
<td></td>
</tr>
<tr>
<td>Ile (I)</td>
<td>leu; val; met; ala; phe; norleucine</td>
<td></td>
</tr>
<tr>
<td>Leu (L)</td>
<td>norleucine; ile; val; met; ala; phe</td>
<td></td>
</tr>
<tr>
<td>Lys (K)</td>
<td>arg; gln; asn</td>
<td></td>
</tr>
<tr>
<td>Met (M)</td>
<td>leu; phe; ile</td>
<td></td>
</tr>
<tr>
<td>Phe (F)</td>
<td>leu; val; ile; ala; tyr</td>
<td></td>
</tr>
<tr>
<td>Pro (P)</td>
<td>ala</td>
<td></td>
</tr>
<tr>
<td>Ser (S)</td>
<td>thr</td>
<td></td>
</tr>
<tr>
<td>Thr (T)</td>
<td>ser</td>
<td></td>
</tr>
<tr>
<td>Trp (W)</td>
<td>tyr; phe</td>
<td></td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>trp; phe; thr; ser</td>
<td></td>
</tr>
<tr>
<td>Val (V)</td>
<td>ile; leu; met; phe; ala; norleucine</td>
<td></td>
</tr>
</tbody>
</table>

[00143] Amino acid residues which share common side-chain properties are often grouped as follows.

1. Hydrophobic: norleucine, met, ala, val, leu, ile;
2. Neutral hydrophilic: cys, ser, thr;
3. Acidic: asp, glu;
4. Basic: asn, gln, his, lys, arg;
5. Residues that influence chain orientation: gly, pro; and
6. Aromatic: trp, tyr, phe.

[00144] In addition, an alignment of MIF amino acid sequences from multiple species would provide guidance with respect to residues that are conserved and preferably left unchanged or with conservative substitutions, versus residues that vary between species, are
less likely to contribute to critical structure, and are more readily accepting of substitution, deletion, or insertion. Modifications made outside of these conserved motifs is less likely to alter the ability of the polypeptide to exhibit chaperone activity towards a mutant SOD1 protein.

[00145] In some embodiments, the MIF variant polypeptide is MIF<sup>C60S</sup>, relative to the wild type MIF amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the MIF variant polypeptide is a polypeptide devoid of cytokine activity, but retains the chaperone-like activity described herein in Example 1.

[00146] In some embodiments, the polypeptide with MIF chaperone activity is a P1G-MIF mutant (SEQ ID NO: 10) that is devoid of tautomerase activity but retains partial cytokine activity based on partial interaction with CD74. The P1G-MIF mutant comprises an amino acid sequence in which the proline at position 2 of SEQ ID NO: 2 is replaced with a glycine. The amino acid sequence of the P1G-MIF mutant is set forth in SEQ ID NO: 13. In some embodiments, the polypeptide with MIF chaperone activity is D-dopachrome tautomerase (DDT or MIF-2) (SEQ ID NO: 11) that retains tautomerase activity and CD74 binding.

[00147] Variants can also be described with respect to the percent sequence identity shared with a wild type sequence. For example, the MIF variants that comprise an amino acid sequence at least 80% (or at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more) identical to the amino acid sequence of SEQ ID NO: 2 or fragment thereof, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein are also contemplated.

[00148] The foregoing descriptions of modifications of wild type polypeptides are not intended to as being mutually exclusive. Variants are contemplated that combine two or more types of these modifications, for example, insertions, deletions, substitutions, fusions, and/or derivatives. For example, fragments of P1G-MIF or MIF<sup>C60S</sup>, or variants thereof that share amino acid sequence similarity and retain activity, are contemplated.

[00149] Polynucleotides

[00150] The invention embraces polynucleotides that encode any of the polypeptides with MIF chaperone activity described herein, and constructs and compositions comprising the polynucleotides, and methods/uses of them. By virtue of the universal genetic code, the
amino acid sequence of a polypeptide defines a set of polynucleotides that encode it, and all are contemplated for practice of the invention.

[00151] In some embodiments, the polynucleotide encoding a polypeptide with MIF chaperone activity is a cDNA comprising the nucleotide sequence set forth in SEQ ID NO: 1. In some embodiments, the polynucleotide encoding a polypeptide with MIF chaperone activity is a genomic sequence comprising the nucleotide sequence set forth in SEQ ID NO: 3.

[00152] Also provided are polynucleotides that hybridize under moderately stringent or high stringency conditions to the complete non-coding strand, or complement, of such polynucleotides. Complementary molecules are useful as templates for synthesizing coding molecules, and for making stable double-stranded polynucleotides. Due to the well-known degeneracy of the universal genetic code, one can synthesize numerous polynucleotide sequences that encode the polypeptides described herein. All such polynucleotides are contemplated as part of the invention. Such polynucleotides are useful for recombinant expression of polypeptides of the invention in vivo or in vitro (e.g., for gene therapy).

[00153] A genus of similar polypeptides can alternatively be defined by the ability of encoding polynucleotides to hybridize to the complement of a nucleotide sequence that corresponds to the cDNA sequence encoding the polypeptide. For example, the invention provides a polynucleotide that comprises a nucleotide sequence that hybridizes under moderately stringent or high stringency hybridization conditions to the complement of any specific nucleotide sequence of the invention, and that encodes a polypeptide as described herein that exhibits MIF protein chaperone activity (e.g., towards a SOD1 mutant protein).

[00154] The term “highly stringent conditions” refers to hybridization/wash conditions selected to only permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Exemplary highly stringent hybridization conditions are as follows: hybridization at 65°C for at least 12 hours in a hybridization solution comprising 5X SSPE, 5X Denhardt’s, 0.5% SDS, and 2 mg sonicated non homologous DNA per 100 ml of hybridization solution; washing twice for 10 minutes at room temperature in a wash solution comprising 2X SSPE and 0.1% SDS; followed by washing once for 15 minutes at 65°C with 2X SSPE and 0.1% SDS; followed by a final wash
for 10 minutes at 65°C with 0.1X SSPE and 0.1% SDS. Moderate stringency washes can be achieved by washing with 0.5X SSPE instead of 0.1X SSPE in the final 10 minute wash at 65°C. Low stringency washes can be achieved by using 1X SSPE for the 15 minute wash at 65°C, and omitting the final 10 minute wash. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook et al., (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

[00155] In some embodiments, the polynucleotide comprises a nucleotide sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to any nucleotide sequence that encodes a wild-type MIF protein, fragments or variants as described herein that demonstrate MIF chaperone activity.

[00156] In some variations, the polynucleotide optionally includes 3’ untranslated sequence from a MIF gene. In such cases, the polynucleotides described herein optionally comprise one or more mutations in the 3’ UTR of the MIF mRNA. Such mutations have been shown to enhance stability of the MIF mRNA. See Bandres et al., Clin. Cancer Res., 15:2281, 2009, the disclosure of which is incorporated herein by reference in its entirety. In some embodiments, one or more bases selected from the group consisting of 921-928 if SEQ ID NO: 3 are deleted and replaced with another amino acid. In some embodiments, the nucleotide at position 921 and 922 of SEQ ID NO: 3 are replaced with guanines, the nucleotide at position 923 of SEQ ID NO: 3 is replaced with a thymine, the nucleotide at position 924 of SEQ ID NO: 3 is replaced with an adenine, the nucleotide at position 925 of SEQ ID NO: 3 is replaced with cytosine, the nucleotide at position 926 of SEQ ID NO: 3 is replaced with thymine, the nucleotide at position 927 of SEQ ID NO: 3 is replaced with guanine and the nucleotide at position 928 of SEQ ID NO: 3 is replaced with adenine.

[00157] Expression vectors are useful for recombinant production of polypeptides of the invention. Expression vectors of the invention may also include sequences encoding one or more selectable markers that permit identification of host cells bearing the construct. Expression constructs may also include sequences that facilitate, and preferably promote,
homologous recombination or other integration in a host cell. Preferred expression vectors of 
the invention also include sequences necessary for replication in a host cell. Expression 
vectors are discussed in more detail below under the heading “Gene Therapy.”

Exemplary expression control sequences include promoter/enhancer sequences, 
(e.g., cytomegalovirus promoter/enhancer (Lehner et al., J. Clin. Microbiol., 29:2494-2502, 
1991; Boshart et al., Cell, 41:521-530, 1985); Rous sarcoma virus promoter (Davis et al., 
Hum. Gene Ther., 4:151, 1993); Tie promoter (Korhonen et al., Blood, 86(5): 1828-1835, 
1995); or simian virus 40 promoter for expression in the target mammalian cells, the 
promoter being operatively linked upstream (i.e. 5’) of the polypeptide coding sequence. In 
another variation, the promoter is an neuronal-specific promoter, glial-cell-specific promoter 
or CNS cell-specific promoter. Suitable promoters for use in connection with the present 
invention include, but are not limited to, prion protein (Prp) promoter, vesicular acetylcholine 
transporter (VACHT) promoter, glial fibrillary acidic protein (GFAP) promoter, CD11b, 
proteolipid protein (Plp) promoter and cytomegalovirus (CMV) promoter.

The polynucleotides of the invention may also optionally include a suitable 
polyadenylation sequence (e.g., the SV40 or human growth hormone gene polyadenylation 
sequence) operably linked downstream (i.e. 3’) of the polypeptide coding sequence.

In another aspect, the invention provides vectors comprising a polynucleotide of 
the described herein. Such vectors are useful, e.g., for amplifying the polynucleotides in host 
cells to create useful quantities thereof, and for expressing polypeptides of the invention 
using recombinant techniques. In preferred embodiments, the vector is an expression vector 
wherein the polynucleotide of the invention is operatively linked to a polynucleotide 
comprising an expression control sequence. Autonomously replicating recombinant 
expression constructs such as plasmid and viral DNA vectors incorporating polynucleotides 
of the invention are specifically contemplated. Expression control DNA sequences include 
promoters, enhancers, and operators, and are generally selected based on the expression 
systems in which the expression construct is to be utilized. Preferred promoter and enhancer 
sequences are generally selected for the ability to increase gene expression, while operator 
sequences are generally selected for the ability to regulate gene expression.

The polynucleotide may further optionally comprise sequences whose only 
intended function is to facilitate large scale production of the vector, e.g., in bacteria, such as 
a bacterial origin of replication and a sequence encoding a selectable marker. However, in
one embodiment, such extraneous sequences are at least partially cleaved off prior to administration to humans according to methods of the invention. One can manufacture and administer such polynucleotides for gene therapy using procedures that have been described in the literature for other transgenes. See, e.g., Isner et al., Circulation, 91: 2687-2692 (1995); and Isner et al., Human Gene Therapy, 7: 989-1011 (1996); incorporated herein by reference in their entirety.

[00162] Inhibitory Nucleic Acids

[00163] In another aspect, the agent for use in the methods described herein comprises an inhibitory nucleic acid that increases MIF expression or activity in a cell from the CNS. Inhibitory nucleic acids useful in the present methods and compositions include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, molecules comprising modified bases, locked nucleic acid molecules (LNA molecules), antagonimirs, peptide nucleic acid molecules (PNA molecules), and other oligomeric compounds or oligonucleotide mimetics which hybridize to at least a portion of the target nucleic acid and modulate its function. In some embodiments, the inhibitory nucleic acids include antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof. See, e.g., WO 2010040112. For further disclosure regarding inhibitory nucleic acids, please see US2010/0317718 (antisense oligos); US2010/0249052 (double-stranded ribonucleic acid (dsRNA)); US2009/0181914 and US2010/0234451 (LNA molecules); US2007/0191294 (siRNA analogues); US2008/0249039 (modified siRNA); and WO2010/129746 and WO2010/040112 (inhibitory nucleic acids).

[00164] The inhibitory nucleic acid, in some embodiments, is an antisense oligonucleotide that binds to a nucleotide sequence that inhibits MIF expression in a cell from the CNS, thereby upregulating expression of the MIF protein. In some embodiments, the antisense oligonucleotide is at least partly complementary to the microRNA sequence of SEQ ID NO: 4 (microRNA-451). MicroRNA-451 has been shown to regulate MIF production with a perfectly inverse correlation (Bandres et al., Clin. Cancer Res., 15:2281, 2009). The design and delivery of antisense oligonucleotides targeting microRNA has been described in
Horwich et al., Nat. Protoc., 3:1537-1549, 2008, the disclosure of which is incorporated herein by reference in its entirety.

[00165] The oligonucleotide optionally comprises a nucleotide sequence that is at least 80% (or at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more) identical to SEQ ID NO: 6 (ACTCAGTAATGGTAACGGTT). Described differently, exemplary oligonucleotides differ by 0, 1, 2, 3 or 4 bases over their length relative to SEQ ID NO: 6. In some embodiments, the oligonucleotide comprises the nucleotide sequence set forth in SEQ ID NO: 6.

[00166] The antisense oligonucleotide described herein is complementary to and hybridizes to a target segment of microRNA-451. The term "target segment" as used herein means a sequence of microRNA-451 to which one or more antisense oligonucleotides are complementary. Multiple antisense oligonucleotides complementary to a given target segment may or may not have overlapping sequences. In some embodiments, the sequence of the antisense oligonucleotide is complementary to bases 17 to 25 of SEQ ID NO: 5 (human microRNA-451 transcript), these bases represent a target segment of microRNA-451. Other contemplated target segments of microRNA-451 within SEQ ID NO: 5 include, but are not limited to bases 1 to 5 of SEQ ID NO: 5, bases 1 to 10 of SEQ ID NO: 5, bases 5 to 10 of SEQ ID NO: 5, bases 10 to 15 of SEQ ID NO: 5, bases 10 to 20 of SEQ ID NO: 5, bases 15 to 20 of SEQ ID NO: 5, bases 20 to 30 of SEQ ID NO: 5, bases 25 to 30 of SEQ ID NO: 5, bases 30 to 40 of SEQ ID NO: 5, bases 35 to 40 of SEQ ID NO: 5, bases 40 to 50 of SEQ ID NO: 5, bases 45 to 50 of SEQ ID NO: 5, bases 50 to 60 of SEQ ID NO: 5, bases 55 to 60 of SEQ ID NO: 5, bases 60 to 70 of SEQ ID NO: 5, bases 65 to 70 of SEQ ID NO: 5, bases 70 to 82 of SEQ ID NO: 5 and bases 75 to 82 of SEQ ID NO: 5.

[00167] In some embodiments, the sequence of the antisense oligonucleotide is complementary to bases 1 to 9 of SEQ ID NO: 4 (mature microRNA-451 sequence), thus these bases also represent a target segment of microRNA-451. Other contemplated target segments of microRNA-451 within SEQ ID NO: 4 include, but are not limited to, bases 1 to 5 of SEQ ID NO: 4, bases 1 to 10 of SEQ ID NO: 4, bases 5 to 10 of SEQ ID NO: 4, bases 10 to 15 of SEQ ID NO: 4, bases 10 to 20 of SEQ ID NO: 4 and bases 15 to 20 of SEQ ID NO: 4.
In some embodiments, the antisense oligonucleotide described herein comprises from about 15 to about 30 (or from about 17 to about 25, or from about 19 to about 25, or from about 15 to about 20) bases in length. Thus, in some embodiments, the antisense oligonucleotide that comprises about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, or about 30 bases in length. It is understood that non-complementary bases may be included in such inhibitory nucleic acids; for example, an inhibitory nucleic acid 30 nucleotides in length may have a portion of 15 bases that is complementary to the targeted miRNA.

The inhibitory nucleic acids useful in the present methods are sufficiently complementary to the target microRNA-451, e.g., hybridize sufficiently well and with sufficient biological functional specificity, to give the desired effect. "Complementary" refers to the capacity for pairing, through base stacking and specific hydrogen bonding, between two sequences comprising naturally or non-naturally occurring (e.g., modified as described above) bases (nucleosides) or analogs thereof. 100% complementarity is not required. As noted above, inhibitory nucleic acids can comprise universal bases, or inert abasic spacers that provide no positive or negative contribution to hydrogen bonding. Base pairings may include both canonical Watson-Crick base pairing and non-Watson-Crick base pairing (e.g., Wobble base pairing and Hoogsteen base pairing). It is understood that for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), that cytosine-type bases (C) are complementary to guanosine-type bases (G), and that universal bases such as such as 3-nitropyrrrole or 5-nitroindole can hybridize to and are considered complementary to any A, C, U, or T. Nichols et al., Nature, 1994;369:492-493 and Loakes et al., Nucleic Acids Res., 1994:22:4039-4043. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U, or T. See Watkins and SantaLucia, Nucl. Acids Research, 2005; 33 (19): 6258-6267.

It is understood in the art that a complementary nucleic acid sequence need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. A complementary nucleic acid sequence for purposes of the present methods is specifically hybridizable when binding of the sequence to the target MIF microRNA molecule interferes with the normal function of the target MIF microRNA to cause a loss of activity (e.g., inhibiting MIF-associated expression with consequent up-regulation of MIF gene expression) and there is a sufficient degree of complementarity to avoid non-specific binding of the
sequence to non-target sequences under conditions in which avoidance of the non-specific binding is desired, e.g., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed under suitable conditions of stringency. Exemplary hybridization conditions are discussed elsewhere herein.

[00171] In some embodiments, the inhibitory nucleic acid comprises one or more modifications comprising: a modified sugar moiety, and/or a modified internucleoside linkage, and/or a modified nucleotide and/or combinations thereof. It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the modifications described herein may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide.

[00172] In some embodiments, the inhibitory nucleic acid comprises at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other preferred embodiments, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher Tm (i.e., higher target binding affinity) than; 2'-deoxyoligonucleotides against a given target.

[00173] A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide; these modified oligos survive intact for a longer time than unmodified oligonucleotides. Specific examples of modified oligonucleotides include those comprising modified backbones, for example, phosphorothioates, phosphorodiesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly CH2-OH-CH2, CH2-N(CH3)-O-CH2 (known as a methylene(methylimino) or MMI backbone), CH2--O--N (CH3)-CH2, CH2, N (CH3)-N (CH3)-CH2 and O-N (CH3)-CH2 backbone, wherein the native phosphodiester backbone is represented as O- P- O- CH2; amide backbones (see De Mesmaeker et al. Ace. Chem. Res. 1995, 28:366-374); morpholino backbone structures (see Summerton and Weller, U.S. Pat. No. 5,034,506); peptide nucleic acid (PNA) backbone (wherein the phosphodiester backbone of the oligonucleotide is
replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone, see Nielsen et al., Science 1991, 254, 1497). Phosphorus-containing linkages include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3′alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3′-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3′-5′ to 5′-3′ or 2′-5′ to 5′-2′; see U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, the disclosure of which are incorporated herein by reference in their entireties.


[00176] Modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S
and CH2 component parts; see US patent nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

[00177] Modified oligonucleotides are also known that include oligonucleotides that are based on or constructed from arabinonucleotide or modified arabinonucleotide residues. Arabinonucleosides are stereoisomers of ribonucleosides, differing only in the configuration at the 2'-position of the sugar ring. In some embodiments, a 2'-arabino modification is 2'-F arabino. In some embodiments, the modified oligonucleotide is 2'-fluoro-D-arabinonucleic acid (FANA) (as described in, for example, Lon et al., Biochem., 41:3457-3467, 2002 and Min et al., Bioorg. Med. Chem. Lett., 12:2651-2654, 2002; the disclosures of which are incorporated herein by reference in their entirety). Similar modifications can also be made at other positions on the sugar, particularly the 3' position of the sugar on a 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide.

PCT Publication No. WO 99/67378 discloses arabinonucleic acids (ANA) oligomers and their analogues for improved sequence specific inhibition of gene expression via association to complementary messenger RNA.


[00179] In some embodiments, the modification(s) include locked nucleic acids (LNAs) (e.g., as described in International Publication No. WO 2008/043753, U.S. Pat. Nos. 6,268,490; 6,734,291; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,060,809; 7,084,125; and 7,572,582; and U.S. Pre-Grant Pub. Nos. 20100267018; 20100261175; and 20100035968; Koshkin et al. Tetrahedron 54, 3607-3630 (1998); Obika et al. Tetrahedron Lett. 39, 5401-5404 (1998); Jepsen et al., Oligonucleotides 14:130-146 (2004); Kauppinen et al., Drug Disc. Today 2(3):287-290 (2005); and Ponting et al., Cell 136(4):629-641 (2009), and references cited therein. The disclosures of each of these documents is incorporated herein by reference in its entirety. LNAs include ribonucleic acid analogues wherein the
ribose ring is “locked” by a methylene bridge between the 2’-oxygen and the 4’-carbon – i.e., oligonucleotides containing at least one LNA monomer, that is, one 2’-O,4’-C-methylene-β-D-ribofuranosyl nucleotide.

[00180] One or more substituted sugar moieties can also be included, e.g., one of the following at the 2’ position: OH, SH, SCH₃, F, OCN, OCH₃, OCH₃, OCH₃ O(CH₂)ₙ CH₃, O(CH₂)ₙ NH₂ or O(CH₂)ₙ CH₃ where n is from 1 to about 10; Ci to C10 lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF₃; OCF₃; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH₃; SO₂ CH₃; ONO₂; NO₂; N₃; NH₂; heterocycloalkyl; heterocycloalkenyl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. In some embodiments, the modification(s) include 2’-methoxyethoxy [2’-O-CH₂CH₂OCH₃, also known as 2’-O-(2-methoxyethyl)] (Martin et al, Helv. Chim. Acta, 1995, 78, 486). In some embodiments, the modification(s) include 2’-methoxy (2’-O-CH₃), 2’-propoxy (2’-OCH₂ CH₂CH₃) and 2’-fluoro (2’-F).

[00181] In some embodiments, the inhibitory nucleic acids are chimeric oligonucleotides that contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. Chimeric inhibitory nucleic acids may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Patent Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

[00182] Inhibitory nucleic acids can also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleobases include nucleobases found only
infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2′ deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, isocytosine, pseudoisocytosine, as well as synthetic nucleobases, e.g., 2-aminoadenine, 2- (methylamino)adenine, 2- (imidazolylalkyl)adenine, 2- (aminoalkylamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 5-propynyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine, 6-aminopurine, 2-aminopurine, 2-chloro-6-aminopurine and 2,6-diaminopurine or other diaminopurines. See, e.g., Kornberg, “DNA Replication,” W. H. Freeman & Co., San Francisco, 1980, pp75-77; and Gebeyehu, G., et al. Nucl. Acids Res., 15:4513 (1987)). In some embodiments, the modified nucleobases comprise other synthetic and natural nucleobases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudo-uracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5- bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7 and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

A "universal" base known in the art, e.g., inosine, can also be included. 5-Me-C substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2<0>C. (Sanghvi, in Crooke, and Lebleu, eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions.

methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, et al., eds., “Antisense Research and Applications,” CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications. Modified nucleobases are described in US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

[00185] Inhibitory oligonucleotides can be administered directly or delivered to cells by transformation or transfection via a vector, including viral vectors or plasmids, into which has been placed DNA encoding the inhibitory oligonucleotide with the appropriate regulatory sequences, including a promoter, to result in expression of the inhibitory oligonucleotide in the desired cell, as described elsewhere herein.

[00186] In some embodiments, the inhibitory nucleic acids are chemically linked to one or more moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide. For example, one or more inhibitory nucleic acids, of the same or different types, can be conjugated to each other; or inhibitory nucleic acids can be conjugated to targeting moieties with enhanced specificity for a cell type or tissue type. Such moieties include, but are not limited to, lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N. Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Lett., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., dihexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polylamine or a polyethylene glycol chain (Mancharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmitoyl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-t-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937). Representative conjugate groups are also disclosed in International Patent Application No.
The effectiveness of the inhibitory nucleic acid may be assessed by any of a number of assays, including reverse transcriptase polymerase chain reaction or Northern blot analysis to determine the level of existing MIF mRNA, or Western blot analysis using antibodies which recognize the MIF protein, after sufficient time for turnover of the endogenous pool after new protein synthesis is repressed.

The effects of an inhibitory nucleic acid (e.g., an antisense oligonucleotide) on the level of activity or expression of MIF can be tested in vitro in CNS precursor cells, neural cells (e.g., neurons), neuronal progenitor cells, astrocytes, oligodendrocytes and glial cells. Cell types used for such analyses are available from commercial vendors (e.g. American Type Culture Collection, Manassus, Va.; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, Md.) and cells are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, Calif.).

In some embodiments, inhibitory nucleic acids (e.g., antisense oligonucleotides) are tested in animals to assess their ability to inhibit expression of a target nucleic acid and produce phenotypic changes. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, inhibitory nucleic acids are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous, and further includes intrathecal and intracerebroventricular routes of administration. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from spinal fluid and/or neural cells and changes in MIF gene expression is measured. Changes in MIF protein expression encoded by target nucleic acids may also be measured.

Stimulation in the levels of expression or activity of a MIF nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or
poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM® 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, Calif. and used according to manufacturer’s instructions.

[00191] Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM® 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, Calif.) according to manufacturer’s instructions. Methods of quantitative real-time PCR are well known in the art.

[00192] MIF protein levels can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, Mich.), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

[00193] Gene Therapy

[00194] In some aspects of the invention, polynucleotides are used/delivered to achieve MIF expression in cells (or achieve expression of an inhibitory nucleic acid that targets microRNA-451), a process that may be termed Gene Therapy.

(see, for example, U.S. Patent No. 5,856,152 incorporated herein by reference) a vaccinia viral or a herpesviral (see, for example, U.S. Patent No. 5,879,934; U.S. Patent No. 5,849,571; U.S. Patent No. 5,830,727; U.S. Patent No. 5,661,033; U.S. Patent No. 5,328,688 each incorporated herein by reference) or a lentiviral vector (see, for example, U.S. Patent Nos. 6,207,455 and 6,235,522, each of which are incorporated herein by reference). For many applications, replication-deficient strains of viruses are preferred.

Gene therapy is an emerging treatment modality for disorders affecting the central nervous system (CNS). CNS gene therapy has been facilitated by the development of viral vectors capable of effectively infecting post-mitotic neurons. The CNS is made up of the spinal cord and the brain. The spinal cord conducts sensory information from the peripheral nervous system to the brain and conducts motor information from the brain to various effectors. For a review of viral vectors for gene delivery to the central nervous system, see Davidson et al. (2003) Nature Rev. 4:353-364.

In embodiments employing a viral vector, preferred polynucleotides still include a suitable promoter and polyadenylation sequence as described above. Moreover, it will be readily apparent that, in these embodiments, the polynucleotide further includes vector polynucleotide sequences (e.g., adenoviral polynucleotide sequences) operably connected to the sequence encoding a polypeptide of the invention.

Adeno-associated virus (AAV) vectors are considered useful for CNS gene therapy because they have a favorable toxicity and immunogenicity profile, are able to transduce neuronal cells, and are able to mediate long-term expression in the CNS (Kaplitt et al. (1994) Nat. Genet. 8:148-154; Bartlett et al. (1998) Hum. Gene Ther. 9:1181-1186; and Passini et al. (2002) J. Neurosci. 22:6437-6446). One useful property of AAV vectors lies in the ability of some AAV vectors to undergo retrograde and/or anterograde transport in neuronal cells. Neurons in one brain region are interconnected by axons to distal brain regions thereby providing a transport system for vector delivery. For example, an AAV vector may be administered at or near the axon terminals of neurons. The neurons internalize the AAV vector and transport it in a retrograde manner along the axon to the cell body. Similar properties of adenovirus, HSV, and pseudo-rabies virus have been shown to deliver genes to distal structures within the brain (Soudas et al. (2001) FASEB J. 15:2283-2285; Breakefield et al. (1991) New Biol. 3:203-218; and deFalco et al. (2001) Science, 291:2608-2613).

In some embodiments, delivery of a polynucleotide encoding a MIF protein (or an inhibitory nucleic acid described herein) is mediated by a DNA viral vector, such as an adenovirus (Ad) or adeno-associated virus (AAV). Adenoviruses (Ads) are a relatively well characterized, homogenous group of viruses, including over 50 serotypes. See, e.g., International PCT Application No. WO 95/27071. Ads are easy to grow and do not require integration into the host cell genome. Recombinant Ad derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. See, International PCT Application Nos. WO 95/00655 and WO 95/11984, the disclosure of which are incorporated herein by reference in their entireties. Wild-type AAV has high infectivity and specificity integrating into the host cell’s genome. See, Hermonat and Muzyczka (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470 and Lebkowski, et al. (1988) Mol. Cell. Biol. 8:3988-3996.

AAV vectors are derived from single-stranded (ss) DNA paroviruses that are nonpathogenic for mammals (reviewed in Muzyscka (1992) Curr. Top. Microb. Immunol., 158:97-129). Briefly, AAV-based vectors have the rep and cap viral genes that account for 96% of the viral genome removed, leaving the two flanking 145-basepair (bp) inverted terminal repeats (ITRs), which are used to initiate viral DNA replication, packaging and integration. In the absence of helper virus, wild-type AAV integrates into the human host-cell genome with preferential site-specificity at chromosome 19q 13.3 or it may be maintained episomally. A single AAV particle can accommodate up to 5 kb of ssDNA, therefore leaving about 4.5 kb for a transgene and regulatory elements, which is typically sufficient. However,
trans-splicing systems as described, for example, in U.S. Pat. No. 6,544,785, may nearly
double this limit.

[00202] The use of any AAV serotype or pseudotype is contemplated. For example, AAV
vectors contemplated for delivery of a polynucleotide encoding a protein described herein (or
an inhibitory nucleic acid described herein) include, but are not limited to AAV1, AAV2,
AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV9. In some embodiments, the AAV
vector is AAV9. The delivery of polynucleotides to the CNS via an AAV vector as has been
described in U.S. Patent Application Publication No. 2010/00130594, the disclosure of which
is incorporated herein by reference in its entirety. In some embodiments, the expression
vector comprises the nucleotide sequence set forth in SEQ ID NO: 12.

[00203] Pseudotyped AAV vectors are those which contain the inverted terminal repeats
(ITRs) of one AAV serotype and the capsid of a second AAV serotype; for example, an AAV
vector that contains the AAV2 capsid and the AAV1 ITRs (i.e. AAV1/2) or an AAV vector
that contains the AAV5 capsid and the AAV2 ITRs (i.e. AAV2/5).

[00204] For viral vectors, one generally will prepare a viral vector stock. Depending on
the kind of virus and the titer attainable, one will deliver, for example, 1 X 10^4, 1 X 10^5, 1 X
10^6, 1 X 10^7, 1 X 10^8, 1 X 10^9, 1 X 10^10, 1 X 10^11 or 1 X 10^12 infectious particles to the
patient, or a dose in a range defined by any two of these values. Similar figures may be
extrapolated for liposomal or other non-viral formulations by comparing relative uptake
efficiencies. Formulation as a pharmaceutically acceptable composition is discussed below.

[00205] Other non-viral delivery mechanisms contemplated include calcium phosphate
precipitation (Graham and Van Der Eb, Virology, 52:456-467, 1973; Chen and Okayama,
direct microinjection (Harland and Weintraub, J. Cell Biol., 101:1094-1099, 1985), DNA-
loaded liposomes (Nicolaou and Sene, Biochim. Biophys. Acta, 721:185-190, 1982; Fraley et
Acad. Sci. USA, 84:8463-8467, 1987), gene bombardment using high velocity
microprojectiles (Yang et al., Proc. Natl. Acad. Sci USA, 87:9568-9572, 1990), and receptor-

[00206] The expression vector (or indeed the polynucleotides or polypeptides described herein) may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multi-lamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, In: Liver diseases, targeted diagnosis and therapy using specific receptors and ligands, Wu G, Wu C ed., New York: Marcel Dekker, pp. 87-104, 1991). The addition of DNA to cationic liposomes causes a topological transition from liposomes to optically birefringent liquid-crystalline condensed globules (Radler et al., Science, 275(5301):810-4, 1997). These DNA-lipid complexes are potential non-viral vectors for use in gene therapy and delivery.

[00207] Liposome-mediated nucleic acid delivery and expression of foreign DNA in vitro has been successful. Also contemplated in the present invention are various commercial approaches involving “lipofection” technology. In certain embodiments of the invention, the liposome may be complexed with a hemagglutinating virus (HJV). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda et al., Science, 243:375-378, 1989). In other embodiments, the liposome may be complexed or employed in conjunction with nuclear nonhistone chromosomal proteins (HMG-1) (Kato et al., J. Biol. Chem., 266:3361-3364, 1991). In yet further embodiments, the liposome may be complexed or employed in conjunction with both HJV and HMG-1. In that such expression constructs have been successfully employed in transfer and expression of nucleic acid in vitro and in vivo, then they are applicable for the present invention.

[00208] Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein et al., Nature, 327:70-73, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang et al., Proc. Natl. Acad. Sci USA, 87:9568-9572, 1990).
microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

[00209] In another embodiment, the invention provides host cells, including prokaryotic and eukaryotic cells, that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell, which are well known and routinely practiced in the art, include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. As stated above, such host cells are useful for amplifying the polynucleotides and also for expressing the polypeptides of the invention encoded by the polynucleotide. The host cell may be isolated and/or purified. The host cell also may be a cell transformed in vivo to cause transient or permanent expression of the polypeptide in vivo. The host cell may also be an isolated cell transformed ex vivo and introduced post-transformation, e.g., to produce the polypeptide in vivo for therapeutic purposes. The definition of “host cell” explicitly excludes a transgenic human being.

[00210] For expression of polypeptides of the invention, any host cell is acceptable, including but not limited to bacterial, yeast, plant, invertebrate (e.g., insect), vertebrate, and mammalian host cells. For developing therapeutic preparations, expression in mammalian cell lines, especially human cell lines, is preferred. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation, lipidation, and phosphorylation) as may be desirable to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of polypeptides are embraced by the present invention. Similarly, the invention further embraces polypeptides described above that have been covalently modified to include one or more water soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol.

[00211] In another embodiment, the invention provides a neuronal precursor progenitor cell transformed or transfected ex vivo with the gene(s) encoding a MIF polypeptide, and the transfected cells as administered to the mammalian subject.

[00212] Pharmaceutical Formulations and Routes of Administration
[00213] The agents described herein may be administered in any suitable manner using an appropriate pharmaceutically acceptable vehicle, e.g., a pharmaceutically acceptable diluent, adjuvant, excipient or carrier. Liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media are preferred. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, water, saline solutions, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl and propylhydroxybenzoate, t alc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, glycerol, calcium phosphate, mineral oil, and cocoa butter. Such formulations are useful, e.g., for administration of polypeptides or polynucleotides of the invention to mammalian (including human) subjects in therapeutic regimens.

[00214] In some embodiments, an agent for use in accordance with the methods described herein is combined with one or more pharmaceutically acceptable carriers for an injectable formulation. In some embodiments, the carriers are isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, allow the constitution of injectable solutions.

[00215] In some embodiments, the injectable preparations are a solution or suspension in a nontoxic parenterally acceptable solvent or diluent, such as saline, buffered saline, isotonic saline (e.g. monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride, or mixtures of such salts), Ringer's solution, dextrose, water, sterile water, glycerol, ethanol, and combinations thereof. 1,3-butanediol and sterile fixed oils are conveniently employed as solvents or suspending media. Any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid also find use in the preparation of injectables.

[00216] The agent is, in some embodiments, targeted to the central nervous system (CNS) of an individual suffering from a neurodegenerative disorder, such as ALS, and in particular to the regions of the CNS affected by the neurodegenerative disorder. As the blood-brain barrier is generally impermeable to various agents administered systemically, one method of providing the agent to the tissues of the CNS is via administration of the agent directly into the cerebrospinal fluid (CSF). Means for delivery to the CSF include, but are not limited to, intrathecal (IT) and intracerebroventricular (ICV) administration and lumbar puncture. In some embodiments, IT or ICV administration is achieved through the use of surgically
implanted pumps (e.g., an infusion pump) that infuse a therapeutic agent into the CSF. In some embodiments, IT, ICV or lumbar administration of an agent described herein is for a period of about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 20 hours or about 24 hours. In some embodiments, the antisense oligonucleotide is continuously infused into the CSF for the entire course of treatment; such administration is referred to as "continuous infusion" or, in the case of IT infusion, "continuous IT infusion."

[00217] In some embodiments, administering an antisense oligonucleotide to the CSF employs an infusion pump, such as Medtronic SyncroMed® II pump. The SyncroMed® II pump is surgically implanted according the procedures set forth by the manufacturer. The pump contains a reservoir for retaining a drug solution, which is pumped at a programmed dose into a catheter that is surgically implanted. For intrathecal administration of a drug, the catheter is surgically intrathecally implanted.

[00218] Kits

[00219] In another embodiment, kits are provided which contain the necessary reagents to carry out the assays or therapies described herein. In some variations, reagents are packaged together but not in admixture. In one embodiment, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising a viral vector comprising a nucleotide sequence that encodes a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity, and a promoter operably linked to the nucleotide sequence that is capable of promoting expression of the nucleotide sequence in mammalian cells; and optionally (b) a second container comprising a viral vector comprising a nucleotide sequence that is at least partly complementary to SEQ ID NO: 4 or SEQ ID NO: 5, wherein the nucleotide sequences binds to microRNA that inhibits MIF expression in cells from the CNS.

[00220] In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another.
Screening Methods

Other aspects of the invention relate to methods of screening for compounds which modulate, such as increase in MIF expression and/or MIF chaperone activity in cells from CNS. In some embodiments, the method comprises contacting a CNS cell with a test compound and determining the quantity of the MIF mRNA or protein as described herein. In some embodiments, the method comprises contacting a CNS cell with a test compound and determining the quantity of MIF mRNA or protein, MIF chaperone activity, and/or the quantity of decreased SOD1 misfolding, SOD1 self-aggregation, or SOD1 association with one or more cellular structures such as mitochondria, endoplasmic reticulum or endosomes in cells from CNS cells as described herein. The quantity of MIF mRNA or protein, SOD1 misfolding, SOD1 self-aggregation, or SOD1 association with one or more cellular structures such as mitochondria, endoplasmic reticulum or endosomes may be compared with the quantities produced in the absence of the test compound. Another aspects of the invention relate to methods of screening for MIF agonist compounds which permeate the blood brain barrier.

Another aspect of the invention relates to methods of screening for compounds that inhibit mutant SOD1 from binding to mitochondria. For example, a SOD1 protein (which may be a mutant SOD1) and mitochondria are contacted together in the presence and absence of a test molecule, and measurable decreases in the binding of mutant SOD1 to mitochondria identifies the test molecule as a molecule that inhibits binding of mutant SOD1 to mitochondria.

Stimulation in the levels of expression or activity of a MIF nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM® 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, Calif. and used according to manufacturer's instructions.

Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM® 7600, 7700, or 7900 Sequence Detection System (PE-Applied
Biosystems, Foster City, Calif.) according to manufacturer’s instructions. Methods of quantitative real-time PCR are well known in the art.

[00226] In some variations, small molecules are screened in a cell free assay. For example, a MIF protein and a SOD1 protein (which may be a mutant SOD1) are contacted together in the presence and absence of a test molecule, and measureable decreases in SOD1 misfolding, aggregation, or association with cellular structures (if mitochondria, endoplasmic reticulum, endosomes, or other structures are included in the assay) identifies the test molecule as a molecule that beneficially modulate MIF activity.

[00227] MIF protein levels can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, Mich.), or be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

[00228] The invention may be more readily understood by reference to the following examples, which are given to illustrate the invention and not in any way to limit its scope.

EXAMPLES

Example 1 - Demonstration that Macrophage Migration Inhibitory Factor Affects SOD1 Folding and has a Therapeutic Indication in Neural Cells

[00229] The experiments described below demonstrate that Macrophage Migration Inhibitory Factor (MIF) has chaperone-like activity with respect to mutant superoxide dismutase (SOD1) protein, and that this activity has a therapeutic indication in neural cells.

Materials and Methods

[00230] Transgenic Rats: Transgenic rats expressing hSOD1\textsuperscript{wt}, hSOD1\textsuperscript{G93A} and hSOD1\textsuperscript{H468R} were as originally described in Chan et al., J. Neurosci., 18:8292, 1998; Howland et al., Proc. Natl. Acad. Sci. USE, 99:1604, 2002; and Nagai et al., J. Neurosci., 21:9246, 2001, respectively. All animal procedures were consistent with the requirements of the Animal Care and Use Committee of the University of California.
Subcellular Fractionation: Mitochondria were purified as previously described (Vande Velde et al., Proc. Natl. Acad. Sci. USA, 105:4022, 2008). Tissues were homogenized on ice in 5 volumes of ice-cold homogenization buffer (HB) composed of 210 mM mannitol, 70 mM sucrose, 1 mM EDTA-(Tris) and 10 mM Tris-HCl (pH 7.2). Homogenates were centrifuged at 1000 x g for 10 min. Supernatants were recovered, and pellets were washed with ½ volume HB and centrifuged at 1000 x g. Supernatants were pooled and centrifuged at 12,000 x g for 15 min to yield a crude mitochondrial pellet. The supernatant was used to make cytosolic fractions by further centrifugation at 100,000 x g for 1 hour. The mitochondria were gently resuspended in HB and then adjusted to 1.204 g/ml Optiprep (iodixanol) and loaded on the bottom of a polycarbonate tube. Mitochondria were overlaid with an equal volume of 1.175 g/ml and 1.079 g/ml Optiprep and centrifuged at 50,000 x g for 4 hours (SW-55; Beckman). Mitochondria were collected at the 1.079/1.175 g/ml interface and washed once to remove the Optiprep. Optiprep stock solution was diluted in 250 mM sucrose, 120 mM Tris-HCl (pH 7.4), 6 mM EDTA plus protease inhibitors.

Liver was homogenized in 5 volumes of ice-cold homogenization buffer (HB) on ice. Homogenates were centrifuged at 1000 x g for 5 min. Supernatants were recovered, and centrifuged again at 1000 x g for 5 min. Supernatant was centrifuged at 12,000 x g for 10 min to yield a crude mitochondrial pellet. These mitochondria were resuspended in HB (without EDTA) and centrifuged again at 12,000 x g for 10 min. The pellet was resuspended in a small volume of HB without EDTA.

Protein Purification: Baculovirus stocks expressing human wild type and mutants for SOD1 were provided by Dr. Lawrence Hayward (University of Massachusetts). Recombinant hSOD1wt, hSOD1G93A and hSOD1G85R were expressed in sf-9 cells and purified using an Hydrophobic Interaction Chromatography (HIC) and Ion Exchange Chromatography (IEX), as described previously (Hayward et al., J. Biol. Chem., 577:15923, 2002).

Mutant SOD1 binding to mitochondria: Spinal cord or liver cytosolic fractions from hSOD1G93A or hSOD1H46R symptomatic rats or recombinant hSOD1wt, hSOD1G93A or hSOD1G85R proteins were incubated with spinal cord or liver mitochondria isolated from non-transgenic rats for 1 hour at 37°C in the presence or absence of recombinant mouse MIF (R&D Systems). Then, the mitochondrial fraction was recovered by centrifugation at 12,000 x g for 10 min at 4°C and washed twice with mitochondrial buffer. The pellet was resuspended with sample buffer and run by SDS-PAGE.
[00235] **Immunoprecipitation:** Isolated mitochondria (100 μg) were solubilized in immunoprecipitation (IP) buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40 plus protease inhibitors] and incubated overnight with DSE2 or B8H10 (Medimabs) antibodies previously crosslinked to Dynabeads protein G (Invitrogen) with dimethyl pimelimidate (Pierce) according to the manufacturer’s instructions. The beads were magnetically isolated and washed three times with IP buffer. Samples were eluted with boiling in 2.0x sample buffer.

[00236] **DSE2 Antibodies:** Disease-specific epitopes (DSE) of SOD1 were as previously described (Vande Velde et al., *supra*; Israelson et al., Neuron, 67:575, 2010). The epitope recognized by the antibodies was predicted to be exposed and unstructured during misfolding or metal depletion (Rahkt et al., Nat. Med., 13:754, 2007). Two independent IgG monoclonal clones of monoclonal antibody DSE2 (3H1 and 8D1) were selected by reactivity to the DSE2 peptide (comprising the electrostatic loop of hSOD1; residues 125-142 of SEQ ID NO: 8), to denatured and/or oxidized hSOD1 in vitro.

[00237] **Immunoblotting:** Proteins were separated on SDS-PAGE and transferred to nitrocellulose membranes and probed with various antibodies as indicated. Antibodies to wild type SOD1 have been described (Howland et al., *supra*; Clement et al., Science, 302:113, 2003). Commercial antibodies include goat anti-SOD1 (C-17), goat anti-MIF (N-18), monoclonal anti-MIF (Q-18), rabbit anti-CyPA (H-24), goat anti-AATC (V-14), goat anti-CTH (P-15), monoclonal anti-Profilin-1 (C-2), monoclonal anti ADH (A-8), rabbit anti-Arginase I (H-52), goat anti-hsp 27 (C-20) and monoclonal anti GPx-1/2 (D-12) from Santa Cruz Biotechnology. Sheep anti-SOD1 and monoclonal anti-VDAC/porin (31HL) from Calbiochem, rabbit anti calnexin (Stressgen) and rabbit anti-cytochrome c (BD Biosciences). Horseradish peroxidase-conjugated anti-mouse, anti-rabbit, or anti-goat IgG secondary antibodies (Jackson Immunochemicals) were used and detected by ECL (GE Biosciences).

[00238] **Cell culture and Plasmids:** To generate pCDNA-hMIF and pCDNA-hMIFC60S human macrophage migration inhibitory factor (MIF) cDNA (Jurgen Bernhagen, University Hospital RWTH Aachen, Germany), was amplified by PCR and inserted into pCDNA3.1(-) using BamH-I and Xba-I sites. pEGFP-hSOD1wt, pEGFP-hSOD1G93A and pEGFP-hSOD1G85R were kindly provided by Jean Pierre Julien (Laval University, Canada).

[00239] NSC-34 cells were grown at 37 °C and 5% CO2 in DMEM supplemented with 10% tetracycline-free FBS and penicillin/streptomycin. Transfection was performed using
Lipofectamine-2000 (Invitrogen) according to the manufacture’s protocol. The cells were collected 24 hours after transfection and analyzed for misfolded protein accumulation.

**Mass Spectrometry analysis**: The MS/MS data were collected by an LTQ Orbitrap Discovery and subsequently searched on Sorcerer-SEQUEST using a semitryptic monoisotopic database generated for the human IPI database, version 3.47. A 20-ppm parent mass tolerance and variable modification for lysine and arginine were included in the search. The searched data were then analyzed by TPP.

**Results and Discussion**

**[00241]** We identified misfolded SOD1 to be associated with spinal cord, but not liver, mitochondria despite comparable mutant SOD1 abundance in the two tissues (Israelson et al., *supra*). After incubation of non-transgenic mitochondria isolated either from spinal cord or liver with mutant SOD1^G93A-^ or SOD1^H46R-^ containing cytosol from either tissue (as detailed in Figure 1A), we observed that the cytosol and not the mitochondria determines mutant SOD1 mitochondrial association (Figure 1B).

**[00242]** Next, we investigated if there is some factor in spinal cord cytosol that induces that association or in contrary, some factor in the liver that prevents it. We have now established that mutant SOD1 misfolding onto mitochondria is blocked by a cytosolic factor present in unaffected tissues. Liver cytosol added to recombinant mutant SOD1^G85R-^ inhibits mutant SOD1 association with mitochondria in a proteinase K-resistant manner (Figure 1C), a property that we exploited for the purification step to eliminate almost all other proteins. Moreover, the liver cytosolic factor was found to be heat sensitive and also Ca^{2+}-, hsp70- and hsp90-independent (Figure 3).

**[00243]** After further fractionation by gel filtration and assay for ability to inhibit recombinant mutant SOD1^G85R-^ binding to mitochondria, proteins in the fraction of peak activity with the simplest protein composition, as shown by coomassie staining (Figure 4), were identified with mass spectrometry as detailed in Figure 2A. Remarkably, only 7 proteins were detected in this fraction. See Table 2 below.

**[00244]** Table 2.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Name</th>
<th>Mw (kDa)</th>
<th># Peptides</th>
<th>% Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cystathionine gamma-lyase</td>
<td>43</td>
<td>10</td>
<td>20.1</td>
</tr>
<tr>
<td>2</td>
<td>Aspartate aminotransferase</td>
<td>47</td>
<td>9</td>
<td>19.5</td>
</tr>
</tbody>
</table>
From these 7 proteins, we identified the 12 kDa macrophage migration inhibitory factor (MIF). Immunoblotting revealed that only MIF presence fractionates perfectly with inhibitory activity (Figures 2B, 2C and Figure 5).

While a proportion of MIF can be sequestered into vesicles and released extracellularly in response to a variety of signals - and in that context it does have well established inhibitory activity on macrophages as the name suggests – MIF is synthesized as a soluble, cytoplasmic protein (it has no signal sequence for co-translational incorporation into the endoplasmic reticulum). Indeed, we have validated that most MIF is found in the soluble cytoplasmic supernatant of liver after 100,000 x g centrifugation to remove even small vesicles (Figure 6). Moreover, MIF has previously been implicated in intracellular protein chaperone activity, with switching from multimeric to monomeric forms exposing a hydrophobic surface that can provide ATP-independent chaperone activity (Cherepkova et al., supra). Curiously, this parallels the well-known ATP-dependent protein chaperone Hsp70, for which dual roles as a chaperone and cytokine have been identified (Asea et al., Nat. Med., 6:435, 2000).

As a direct test of MIF inhibition of mutant SOD1 mitochondrial association, we used recombinant human MIF and demonstrated that MIF inhibits binding *in vitro* of mutant SOD1G93A and SOD1G85R to normal mitochondria (Figure 2D). This inhibition was observed in a dose-dependent manner (Figure 7) and was specific for MIF and not for other proteins as the small hsp27 chaperone, previously shown to interact with mutant SOD1 (Krishnan et al., J. Neurochem., 106:2170, 2008), cyclophilin-A (a candidate protein from our list) or glutathione peroxidase (a protein with thiol-oxidoreductase activity) (Figure 8).

In order to test if MIF inhibits the accumulation of misfolded SOD1, immunoprecipitation was performed with the conformation antibodies for misfolded SOD1 (DSE2 and B8H10) that were previously reported (Vande Velde et al., supra; Israelson et al., supra; Gros-Louis et al., J. Neurochem., 113:1188, 2010). Recombinant SOD1G93A and SOD1G85R but not SOD1WT were immunoprecipitated with the DSE2 antibody. Incubation of the proteins with recombinant MIF strongly inhibits the accumulation of misfolded SOD1, as
shown by western blot using anti SOD1 antibody (Figure 9A). A similar effect was observed when the SOD1 mutants were incubated with a liver cytosolic fraction expressing a similar amount of MIF (Figure 9A).

[00249] Using DNA transfection to express MIF and B8H10 antibodies to misfolded SOD1, we tested if increasing MIF levels can inhibit accumulation of misfolded SOD1 in NSC-34 motor neuron-like cell expressing ALS-linked SOD1 mutants hSOD1^{G93A} or hSOD1^{G85R}. The cells that were transfected to express MIF clearly show a reduction in misfolded SOD1 accumulation (Figure 9B).

[00250] To establish if the protective action of MIF is due to its enzymatic activity as a thiol-oxidoreductase, cells were transfected with a mutant version of MIF (MIF^{C60S} – SEQ ID NO: 9) that was shown before to lose completely its oxidoreductase activity (Kleemann et al., J. Molec. Biol., 280:85, 1998). Expression of MIF^{C60S} in NSC-34 cells inhibits the accumulation of misfolded SOD1 in a dose dependent manner (Figure 9C). Interestingly, expression of MIF^{C60S} in these cells inhibits also mutant SOD1 association with endosomal membranes (Figure 10) suggesting that MIF action is not due to its enzymatic activity and it has a general effect on mutant SOD1 toxicity, suppressing the accumulation of misfolded species and association to different cellular organelles. In some variations, the invention described herein is practiced with a MIF protein comprising a C60S or other mutation, wherein the MIF retains chaperone-like activity towards SOD1 but lacks thiol-oxidoreductase activity.

[00251] Collectively, these experiments establish a role for MIF as a chaperone for SOD1 (especially mutant and/or misfolded SOD1) and establish that increasing MIF in neural cells (or other cells that naturally express lower MIF levels) has a therapeutic benefit with respect to SOD1 misfolding, accumulation, and mitochondrial membrane association. These experiments demonstrate a therapeutic indication for MIF in ALS or other neurodegenerative diseases characterized by SOD1 misfolding, aggregation or membrane association.

Example 2 - Demonstration that MIF Affects Aggregation of SOD1 and has a Therapeutic Indication in Neural Cells

[00252] The experiments described in Example 1 are repeated with other SOD1 variants to demonstrate that MIF has chaperone-like activity with respect to superoxide dismutase (SOD1) protein mutants in addition to the SOD1^{G93A} and SOD1^{G85R} tested in Example 1, and
that this activity has a therapeutic indication in neural cells harboring the other SOD1 variants.

[00253] It is expected that these experiments demonstrate a therapeutic indication for MIF in ALS or other neurodegenerative diseases characterized by protein misfolding, aggregation or membrane association cause by the tested SOD1 mutants.

Example 3 - Demonstration that MIF Mutants Affect Aggregation of SOD1 and have a Therapeutic Indication in Neural Cells

[00254] The experiments described in Example 1 are repeated to demonstrate that MIF Family members (e.g., MIF2 (Merk et al., Proc. Natl. Acad. Sci. USA., early edition, pages 1-9, 2011)) or MIF mutants (e.g., P1G-MIF (Fingerle-Rowson et al., Mol. Cell. Biol., 29:1922-1932, 2009)) or various N-terminal, C-terminal, or internal deletion variants of MIF have chaperone-like activity with respect to mutant SOD1 protein and that this activity has a therapeutic indication in neural cells.

[00255] It is expected that these experiments demonstrate a therapeutic indication for MIF-2 and P1G-MIF in ALS or other neurodegenerative diseases characterized by SOD1 misfolding, aggregation or membrane association. In some variations the invention is practiced with fragments or variants of MIF that retain chaperone-like activity relative to SOD1 but lack one or more of MIF’s other biological activities.

Example 4 - Demonstration that Macrophage Migration Inhibitory Factor Affects Aggregation of TDP-43 and has a Therapeutic Indication in Neural Cells

[00256] The experiments described in Examples 1-3 are repeated to demonstrate the effect of MIF on the aggregation of TDP-43 (Neumann et al. Science, 314:130-133, 2006), in neural cells. It is expected that MIF will demonstrate a chaperone-like activity with respect to TDP-43 protein. Such activity would demonstrate a therapeutic indication for MIF in ALS or other neurodegenerative diseases (e.g., Fronto-, temporal dementia) characterized by TDP-43 aggregation in neural cells.

Example 5 - Demonstration that Macrophage Migration Inhibitory Factor Affects Aggregation of FUS/TLS and has a Therapeutic Indication in Neural Cells

[00257] The experiments described in Examples 1-3 are repeated to demonstrate the effect of MIF on the aggregation of FUS/TLS (Kwiatkowski et al.k, Science, 323:1205-1208, 2009) in neural cells. It is expected that MIF will demonstrate a chaperone-like activity with
respect to FUS/TLS protein. Such activity would demonstrate a therapeutic indication for MIF in ALS or other neurodegenerative diseases FUS/TLS aggregation in neural cells.

Example 6 - Demonstration that Macrophage Migration Inhibitory Factor Affects Aggregation of Alpha Synuclein and has a Therapeutic Indication in Neural Cells

[00258] The experiments described in Examples 1-3 are repeated to demonstrate the effect of MIF on the aggregation of alpha synuclein (Ross et al., Nat. Med., S10-S17, 2004) in neural cells or in cerebrospinal fluid. It is expected that MIF will demonstrate a chaperone-like activity with respect to alpha synuclein protein. Such activity would demonstrate a therapeutic indication for MIF in Alzheimer’s disease, Parkinson’s disease or other neurodegenerative diseases characterized by alpha synuclein aggregation in neural cells.

Example 7 - Demonstration that Macrophage Migration Inhibitory Factor Affects Aggregation of Amyloid Beta and has a Therapeutic Indication in Neural Cells

[00259] The experiments described in Examples 1-3 are repeated to demonstrate the effect of MIF on the aggregation of amyloid beta (Ross et al., Nat. Med., S10-S17, 2004) in neural cells or in cerebrospinal fluid. It is expected that MIF will demonstrate a chaperone-like activity with respect to the amyloid beta protein. Such activity would demonstrate a therapeutic indication for MIF in Alzheimer’s disease or other neurodegenerative diseases characterized by amyloid beta aggregation in neural cells.

Example 8 - Demonstration that Macrophage Migration Inhibitory Factor Affects Aggregation of Huntintin and has a Therapeutic Indication in Neural Cells

[00260] The experiments described in Examples 1-3 are repeated to demonstrate the effect of MIF on the aggregation of huntintin (Ross et al., Nat. Med., S10-S17, 2004) in neural cells. It is expected that MIF will demonstrate a chaperone-like activity with respect to the huntintin protein. Such activity would demonstrate a therapeutic indication for MIF in Huntington’s disease or other neurodegenerative diseases characterized by huntintin aggregation in neural cells.

[00261] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.
References:


P. Shi, J. Gal, D. M. Kwinter et al., Biochimica et biophysica acta 1802 (1), 45.


What is claimed is:

1. A method of treatment comprising
   administering a composition to a mammalian subject at risk for, or having, a
   neurodegenerative disorder, wherein the composition comprises at least one agent selected
   from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone
   activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and
   an agent that increases endogenous MIF expression or MIF chaperone activity.

2. The use of a composition for neurodegenerative disorder prophylaxis or
   therapy, wherein the composition comprises at least one agent selected from: a polypeptide
   that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide
   comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases
   endogenous MIF expression or MIF chaperone activity.

3. The method or use according to claims 1 or 2, wherein the composition further
   comprises a pharmaceutically acceptable excipient, adjuvant, diluents, or carrier in admixture
   with the agent.

4. The method or use according to any of claims 1-3, wherein the mammalian
   subject is human.

5. The method or use according to any one of claims 1-4, comprising
   administering the composition to a mammalian subject with the neurodegenerative disorder,
   in a therapeutically effective amount.

6. The method or use according to any one of claims 1-4, comprising
   administering the composition to a mammalian subject identified as having a familial or
   genetic increased risk for the neurodegenerative disorder, in a prophylactically effective
   amount.

7. The method or use of any one of claims 1-6, wherein the mammalian subject
   has a mutation in an superoxide dismutase 1 (SOD1) gene.

8. The method or use of claim 7, wherein the mutation is associated with SOD1
   misfolding, SOD1 self-aggregation, or SOD1 association with mitochondria, endoplasmic
   reticulum or endosomes in cells from CNS of the subject.

9. The method or use of any one of claims 7-8, wherein the SOD1 mutation is a
   missense mutation.
10. The method or use of claim 9, wherein the missense mutation codes for an amino acid alteration selected from the group consisting of SOD1<sup>G93A</sup> and SOD1<sup>G85R</sup>, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

11. The method of any one of claims 3-10, further comprising screening a subject for a SOD1 mutation prior to the administering step.

12. The method of claim 11, wherein the screening step comprising assaying a biological sample from the subject for evidence that the SOD1 mutation is present in the subject.

13. The method of claim 12, wherein the biological sample comprises spinal fluid or cells obtainable from CNS of a subject.

14. The method of claim 13, wherein the cells obtainable from CNS of a subject are selected from the group consisting of glial cells, glial cell-precursors, astrocytes, oligodendrocytes, neural cells and neuronal progenitor cells.

15. The method of claim 12, wherein the assaying comprises analyzing nucleic acid from the subject for a mutation that codes for SOD1<sup>G93A</sup> or SOD1<sup>G85R</sup>, relative to the SOD1 wild type sequence set forth in SEQ ID NO: 8.

16. The method of claim 12, wherein the assaying comprises analyzing SOD1 protein or cell from CNS of the subject for evidence of SOD1 misfolding, SOD1 self-aggregation or SOD1 association with mitochondria, endoplasmic reticulum or endosomes the cell.

17. The method of claim 12, wherein the screening comprises analyzing a medical record for evidence that the SOD1 mutation is present in the subject.

18. The method of claim 17, wherein the medical record comprises genomic nucleotide sequence information.

19. The method or use according to any one of claims 1-18, wherein the composition is administered in an amount effective to reduce accumulation of misfolded SOD1 in cells obtainable from CNS of the subject.

20. The method or use of any one of claims 1-19, wherein the agent comprises at least one substance selected from the group consisting of:
(a) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;

(b) a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(c) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 11;

(d) a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 11, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(e) a polypeptide comprising a MIF mutant, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(f) a fragment of any one of (a)-(e), wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(g) N-terminal deletion fragments of any one of (a)-(e) wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(h) C-terminal deletion fragments of any one of (a)-(e) and (g), wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(i) a variant of any one of (a)-(h) wherein the cysteine at position 60 of SEQ ID NO: 2 is deleted or replaced with another amino acid, and wherein the polypeptide (variant) exhibits chaperone activity towards a mutant SOD1 protein;

(j) a variant of any one of (a)-(i) lacking an N-terminal methionine, and wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(k) a variant of any one of (a)-(i), wherein the proline corresponding to position 2 of SEQ ID NO: 2 is deleted or replaced with another amino acid, and wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein;

(l) a polynucleotide comprising a nucleotide sequence that encodes the polypeptide of any one of (a)-(k), optionally attached to one or more heterologous coding sequences or non-coding sequences;

(m) a vector comprising the polynucleotide of (l); and
(n) a cell transformed or transfected with (l) or (m), that expresses the encoded polypeptide.

21. The method of claim 20, wherein the agent comprises an expression vector that contains a promoter sequence operatively connected to a polynucleotide that comprises nucleotide sequence that encodes a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein.

22. The method or use of claim 21, wherein the expression vector contains a promoter sequence operatively connected to a polynucleotide that comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO: 2.

23. The method or use of claim 21, wherein the expression vector contains a promoter sequence operatively connected to a polynucleotide that comprises a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or a fragment thereof that exhibits chaperone activity towards a mutant SOD1 protein.

24. The method or use of claim 21, wherein the encoded polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 2.

25. The method or use of claim 21, wherein the polynucleotide comprises a cDNA or fragment thereof that encodes a polypeptide or fragment thereof that exhibits chaperone activity towards a mutant SOD1 protein.

26. The method or use of claim 21, wherein the encoded polypeptide exhibits chaperone activity towards a mutant SOD1 protein characteristic of MIF, and exhibits reduced oxidoreductase activity compared to wild type MIF (SEQ ID NO: 2).

27. The method or use of claim 21, wherein the polynucleotide encodes a MIF polypeptide containing a $\text{MIF}^{\text{C608S}}$ mutation (SEQ ID NO: 9).

28. The method or use according to any one of claims 20-26, wherein the vector is a replication-deficient viral vector.

29. The method or use of any one of claims 20-26, wherein the vector is an adeno-associated viral (AAV) vector.

30. The method or use of claim 27, wherein the AAV vector is an AAV9 vector.
31. The method or use of any one of claims 21-30, wherein the promoter is selected from the group consisting of CMV promoter, Prp promoter, VACHt promoter, GFAP promoter, CD11b promoter and Pp promoter.

32. The method or use of claim 20, wherein the agent comprises a CNS precursor cell transformed or transfected with a polynucleotide that encodes a polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 2, wherein the polypeptide exhibits chaperone activity towards a mutant SOD1 protein.

33. The method or use of claim 32, wherein cell is isolated from CNS of the mammalian subject, wherein the transforming or transfecting is performed ex vivo, and wherein the cell or its progeny are re-administered to the same subject after the transforming or transfecting.

34. The method or use of claim 33, wherein the cell is expanded after the transforming or transfecting, and wherein progeny cells are re-administered to the subject after the expanding.

35. The method of use of claim 20, wherein the polynucleotide encodes MIF-2 or P1G-MIF.

36. The method or use of any one of claims 1-19, wherein the agent comprises an oligonucleotide that increases MIF expression or activity in cells from CNS.

37. The method or use of claim 36, wherein the oligonucleotide is an antisense oligonucleotide that binds to a nucleotide sequence that inhibits MIF expression in cells from CNS, thereby upregulating expression of the MIF protein in the CNS.

38. The method or use of claim 37, wherein the antisense oligonucleotide is at least partly complementary to the microRNA sequence of SEQ ID NO: 4 or SEQ ID NO: 5.

39. The method or use of claim 38, wherein the antisense oligonucleotide is 15 to 30 nucleotides in length.

40. The method or use of any one of claims 36-39, wherein the oligonucleotide comprises a nucleotide sequence that is complementary to bases 1 to 9 of SEQ ID NO: 4.

41. The method or use of any one of claims 1-40, wherein the neurodegenerative disorder is selected from amyotrophic lateral sclerosis (ALS), Alzheimer’s Disease, Parkinson’s Disease, and Huntington’s Disease.
42. The method or use of claim 41, wherein the neurodegenerative disorder is ALS.

43. The method or use of claim 42, wherein the ALS is sporadic ALS.

44. The method or use of claim 42, wherein the ALS is familial ALS.

45. The method or use of one of claims 1-44, wherein the composition is administered by intrathecal administration.

46. The method or use of any one of claims 1-44, wherein the composition is administered to the spinal cord of the subject.

47. The method or use of claim 46, wherein the composition is administered to the lumbar of the subject.

48. The method or use of any one of claims 1-44, wherein the composition is administered to the cerebrospinal fluid of the subject.

49. The method or use of any one of claims 1-44, wherein the composition is administered intravascularly.

50. The method or use of any one of claims 1-49, further comprising administering to the subject a standard of care therapeutic selected from the group consisting of gabapentin (Neurontin®), Myotrophin® (Insulin-like Growth Factor 1, IGF-1), brain-derived neurotrophic factor (BDNF), BFGF, Rilutek® (riluzole), SR57746A, metal chelators (e.g., D-penicillamine), erythropoietin, VEGF, creatine, cyclosporin, CoQ10, inhibitors of tubulin/filament assembly, diazepam, and various vitamins (e.g., C, E and B).

51. A method of palliating a deleterious effect of mutant SOD1 in a cell comprising contacting the cell with a composition that comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity in the cell.

52. Use of a composition for palliating a deleterious effect of a mutant SOD1 in a cell, wherein the composition comprises at least one agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone activity in the cell.
53. The method of claim 51 that comprises:
screening a mammalian subject for a SOD1 mutation, and
administering to a subject identified from the screening as having the SOD1 mutation
the composition, in an amount effective to palliate a deleterious effect of the SOD1 mutation
in CNS cells of the subject.

54. The method or use of claim 51 or 52, wherein the deleterious effect comprises
the formation or accumulation misfolded SOD1 in the cell, or inhibition of mitochondrial
activity by the SOD1.

55. The method or use of any one of claims 51-54, wherein the mutant SOD1 is
selected from the group consisting of SOD1G93A and SOD1G85R, relative to the SOD1 wild
type sequence set forth in SEQ ID NO: 8.

56. A method of treating a mammalian subject having a disorder associated with
accumulation of misfolded SOD1 in CNS cells of the subject, the method comprising
administering to the subject an effective amount of a composition that comprises at least one
agent selected from: a polypeptide that exhibits macrophage inhibitory factor (MIF) protein
chaperone activity; a polynucleotide comprising a nucleotide sequence that encodes the
polypeptide; and an agent that increases endogenous MIF expression or MIF chaperone
activity.

57. The method of claim 56, wherein the disorder is a neurodegenerative disorder.
Fig. 1
Liver cytosol G93A rat

Proteinase K digestion

Gel filtration

Measure fractions activity (inhibition of SOD1 association with mitochondria)

Mass spectrometry analysis of active fraction with simplest composition (Fig 2S)

7 candidates (Table 1S)

Overlap between activity and candidate protein distribution (C) and (Fig. 3S)

Cytosol G93A rat

Gel filtration fraction number

SC L 11 14 19 23 30 38 41

SOD1
Cyt.c
MIF

Non transgenic liver mitochondria

Recombinant proteins

+ + - - - - - hSOD1\textsuperscript{wt}
- - + + - - - hSOD1\textsuperscript{G93A}
- - - - + + hSOD1\textsuperscript{G85R}
- + - + - + mMIF

hSOD1\textsuperscript{G93A}
hSOD1\textsuperscript{G85R}
VDAC

Input (25 %)

hSOD1\textsuperscript{wt/G93A}
hSOD1\textsuperscript{G85R}

Fig. 2
Non-transgenic liver mitochondria

- + - - - - - -
- - + - - - - -
- - + + + + + +
- - - + - - - -
- - - - + - - -
- - - - - + - -
- - - - - - + -
- - - - - - - +

Spinal cord cytosol
Brain cytosol
Liver cytosol
EGTA
Proteinase K
Heated 70°C
PES (hsp70 inhibitor)
17-AAG (hsp90 inhibitor)

hSOD1\textsuperscript{G93A} rat

hSOD1\textsuperscript{G93A} rSOD1

Cyt. C

Input (25 %)

hSOD1\textsuperscript{G93A} rSOD1

Fig. 3
Fig. 4
**A** Liver cytosol G93A rat
Proteinase K digestion
Gel filtration

**B**

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**C**

Measure fractions activity (inhibition of SOD1 association with mitochondria)

Mass spectrometry analysis of active fraction with simplest composition (Fig 2S)

7 candidates (Table 1S)

Overlap between activity and candidate protein distribution (C)

Fig. 5
Non-transgenic liver mitochondria

MIF

hSOD1\textsuperscript{G85R}

VDAC

Input (25 %)

hSOD1\textsuperscript{G85R}

mMIF

0 10 50 100 250 500 (ng)

Fig. 7
Fig. 8
Microsomal membranes

- + + + - - - pEGFP-hSOD1 G93A
- - - - + + + pEGFP-hSOD1 G85R
- + + - + + + pCDNA-hMIF C60S

pEGFP-hSOD1

Calnexin

Cytosol (20 %)

hMIF C60S

Fig. 10
dsAAV CB MCS
6445 bp

Fig. 11
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Fig. 12
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K38/17  A61P25/28
ADD.

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K  A61P

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

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

EPO-Internal, BIOSIS, WPI Data, CHEM ABS Data, Sequence Search, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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X Further documents are listed in the continuation of Box C.

X See patent family annex.

* Special categories of cited documents:
  
  "A" document defining the general state of the art which is not considered to be of particular relevance
  
  "E" earlier application or patent but published on or after the international filing date
  
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  
  "O" document referring to an oral disclosure, use, exhibition or other means
  
  "P" document published prior to the international filing date but later than the priority date claimed

**Date of the actual completion of the international search**

18 June 2013

**Date of mailing of the international search report**

28/06/2013

**Name and mailing address of the ISA/Authorized officer**

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3018

Vandenbogaerde, Ann
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Form PCT/SA/210 (continuation of second sheet) (April 2005)
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